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REGULAR ARTICLE

blood advances

Biomarker analysis of the ASPEN study comparing zanubrutinib with ibrutinib for patients with Waldenström macroglobulinemia

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Key Points

- Patients with Waldenström macroglobulinemia and mutations in *CXCR4* or *TP53* had poorer prognosis after treatment with BTKis.
- Patients with *CXCR4* or *TP53* mutations had more favorable outcomes when treated with zanubrutinib vs ibrutinib.

The phase 3 ASPEN trial (NCT03053440) compared Bruton tyrosine kinase inhibitors (BTKis), zanubrutinib and ibrutinib, in patients with Waldenström macroglobulinemia (WM). Post-hoc biomarker analysis was performed using next-generation sequencing on pretreatment bone marrow samples from 98 patients treated with zanubrutinib and 92 patients treated with ibrutinib with mutated (MUT) *MYD88* and 20 patients with wild-type (WT) *MYD88* treated with zanubrutinib. Of 329 mutations in 52 genes, mutations in *CXCR4* (25.7%), *TP53* (24.8%), *ARID1A* (15.7%), and *TERT* (9.0%) were most common. $TP53^{MUT}$, $ARID1A^{MUT}$, and $TERT^{MUT}$ were associated with higher rates of $CXCR4^{MUT}$ ($P < .05$). Patients with $CXCR4^{MUT}$ (frameshift or nonsense [NS] mutations) had lower very good partial response (VGPR) and complete response rates (CR; 17.0% vs 37.2%, $P = .020$) and longer time to response (11.1 vs 8.4 months) than patients with $CXCR4^{WT}$ treated with BTKis. $CXCR4^{NS}$ was associated with inferior progression-free survival (PFS; hazard ratio [HR], 3.39; $P = .017$) in patients treated with ibrutinib but not in those treated with zanubrutinib (HR, 0.67; $P = .598$), but VGPR + CR rates were similar between treatment groups (14.3% vs 15.4%).

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All authors had access to the original data for the analyses described here. On request, and subject to certain criteria, conditions, and exceptions, BeiGene, Ltd will provide access to individual deidentified participant data from BeiGene-sponsored global interventional clinical studies conducted for medicines (1) for indications that have been approved or (2) in programs that have been terminated. BeiGene will also consider requests for the protocol, data dictionary, and statistical analysis plan. Data requests may be submitted to datadisclosure@beigene.com.

The full-text version of this article contains a data supplement.

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Compared with ibrutinib, patients with $CXCR4^{NS}$ treated with zanubrutinib had a favorable major response rate (MRR; 85.7% vs 53.8%; P = .09) and PFS (HR, 0.30; P = .093). In patients with $TP53^{MUT}$, significantly lower MRRs were observed for patients treated with ibrutinib (63.6% vs 85.7%; P = .04) but not for those treated with zanubrutinib (80.8% vs 81.9%; P = .978). In $TP53^{MUT}$, compared with ibrutinib, patients treated with zanubrutinib had higher VGPR and CR (34.6% vs 13.6%; P < .05), numerically improved MRR (80.8% vs 63.6%; P = .11), and longer PFS (not reached vs 44.2 months; HR, 0.66; P = .37). Collectively, patients with WM with $CXCR4^{MUT}$ or $TP53^{MUT}$ had worse prognosis compared with patients with WT alleles, and zanubrutinib led to better clinical outcomes.

Introduction

Waldenström macroglobulinemia (WM) is a B-cell malignancy characterized by lymphoplasmacytic bone marrow infiltration of monoclonal immunoglobulin M–secreting cells that constitutively activate the B-cell receptor signaling complex.[1] A critical component of the B-cell receptor signaling pathway is Bruton tyrosine kinase (BTK), an important regulator of B-cell proliferation and survival.[2] *Myeloid differentiation factor 88* (*MYD88*) and *C-X-C chemokine receptor type 4* (*CXCR4*) are the most frequently mutated (MUT) genes in patients with WM.[3] A point mutation in *MYD88* that switches leucine to proline at amino acid position 265 ($MYD88^{L265P}$) is present in >90% of patients, and this activating mutation triggers tumor cell growth via BTK signaling.[4] Mutations in *CXCR4* ($CXCR4^{WHIM}$) that are similar to germ line mutations detected in patients with warts, hypogammaglobulinemia, infection, and myelokathexis syndrome–like symptoms have been found in up to 40% of patients with WM and are thought to promote cell survival signaling and confer ibrutinib resistance.[5] The mutational status of *MYD88* and *CXCR4* affects the efficacy of BTK inhibitors (BTKis) in patients with WM. Patients with wild-type (WT) *MYD88* had lower major response rate (MRR) and shorter overall survival (OS) than patients with $MYD88^{L265P}$ when treated with ibrutinib.[6,7] Patients with $CXCR4^{WHIM}$ mutations treated with ibrutinib had lower response rates and shorter progression-free survival (PFS) than patients with $CXCR4^{WT}$, and patients with nonsense (NS) mutations in this gene had lower rates of major response and worse PFS than patients with frameshift (FS) mutations.[8] Additionally, mutations or deletions, often in the DNA binding domain, of *tumor protein P53* (*TP53*) have been reported in <14% of patients with WM[3,9-11] and are associated with worse survival outcomes.[9,10]

BTKis have led to substantial improvements in outcomes for patients with WM, reflected by the approval of ibrutinib and zanubrutinib by the US Food and Drug Administration. The ASPEN trial is a phase 3 randomized, open-label, multicenter study comparing zanubrutinib with ibrutinib treatment for patients with WM.[12] Although the primary efficacy end point was not met, the study's long-term follow-up confirmed that patients who received zanubrutinib treatment had a higher very good partial response and complete response (VGPR + CR) rate over time (zanubrutinib, 36% vs ibrutinib, 25%; descriptive P = .07) and lower rates of atrial fibrillation, diarrhea, hypertension, localized infection, hemorrhage, muscle spasms, pneumonia, and adverse events leading to discontinuation or death than those treated with ibrutinib. Patients with $MYD88^{WT}$ treated with zanubrutinib also demonstrated major responses, with MRRs of 63% and 65.4% in 2 separate studies,[13] respectively, compared with none reported in patients treated with ibrutinib.[14]

Although BTKis are effective therapies for many patients, not all patients may have clinical benefit. Many patients with WM and disease progression during covalent BTKi treatment acquire mutations in *BTK* at the covalent BTKi binding site (BTK^{CYS481}) or its downstream mediator, *PLCG2*.[15] These mutations lead to reactivation of downstream signaling pathways and cytokine release, resulting in resistant BTK^{WT} cells.[16] In addition to resistance mutations in *BTK* and *PLCG2*, other genetic alterations that may be associated with *BTK* include deletions on chromosomes 6q and 8p, which disrupt *BTK*, *MYD88/NF-κB*, and apoptotic signaling, as well as recurring mutations in ubiquitin ligases, innate immune signaling, and *TLR/MYD88* pathway regulators.[17,18]

The objectives of this post-hoc biomarker analysis were (1) to evaluate baseline genetic alterations in patients with WM enrolled in the ASPEN study and their association with the efficacy of ibrutinib and zanubrutinib treatment and (2) to explore acquired mutations potentially conferring resistance to BTKis.

Materials and methods

Patients

The phase 3 ASPEN study (NCT03053440) enrolled patients with $MYD88^{MUT}$ WM (confirmed by qualitative *MYD88* allele–specific polymerase chain reaction with a limit of detection [LOD] of 0.2% to 0.5%[19]) to compare responses to zanubrutinib (n = 102) vs ibrutinib (n = 99). In total, 28 patients without *MYD88* mutations ($MYD88^{WT}$) were enrolled in a separate cohort 2. Details of the study design have been described previously.[12,19] Overall, 190 patients with $MYD88^{MUT}$ (zanubrutinib, n = 98, and ibrutinib, n = 92) and 20 patients with $MYD88^{WT}$ provided residual DNA from pretreatment bone marrow aspirate (BMA) samples without B-cell enrichment for baseline genetic alterations testing. In addition, 5 patients with disease progression after zanubrutinib treatment (3 with $MYD88^{MUT}$ and 2 with $MYD88^{WT}$) gave informed consent to provide BMA samples at the time of clinical progression to explore resistance mechanisms. The trial was approved by the institutional review board or independent ethics committee at each study site and conducted in accordance with applicable regulatory requirements, the principles of the Declaration of Helsinki, and Good

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Clinical Practice guidelines of the International Conference on Harmonization. All patients provided written informed consent.

Biomarker assessments

MYD88 mutational status for patient enrollment was assessed by a qualitative allele-specific polymerase chain reaction with an LOD of 0.2% to 0.5%, as previously described.[19] To detect other baseline genetic alterations, next-generation sequencing (NGS) was performed using the PredicineCARE panel, a Clinical Laboratory Improvement Amendments–certified NGS assay, which was validated to have a high sensitivity (LOD: ~0.1%-0.25%), representing 152 genes. To examine mutations that may be associated with resistance to zanubrutinib, sequencing was performed using the PredicineHeme panel containing 106 hematologic malignancy–related genes, including whole exons of *BTK* and *PLCG2*, validated to have assay sensitivity of ~0.1% to ~0.25%. A variant was considered a true mutation only when (1) at least 3 distinct fragments (at least 1 of which is double stranded) contained the mutation and (2) the variant allele frequency (VAF) was ≥0.25% and hot-spot variants had an allele frequency of ≥0.1%. Mutations annotated as benign, likely benign, or common germ line variants were filtered out based on OncoKB,[20] 1000 genomes,[21] ExAC,[22] gnomAD,[23] and KAVIAR,[24] with population allele frequency of >0.5%. Finally, the hematopoietic expansion–related variants, including those in *DNMT3A*, *ASXL1*, and *TET2*, and specific alterations within *ATM* (residue 3008), *GNAS* (residue 201, 202), or *JAK* (residue 617) were excluded. The clinical efficacy end points used for biomarker analysis included response rate (VGPR + CR, major response), time to response, and PFS assessed by investigator according to response criteria in the National Comprehensive Cancer Network WM guidelines and modified Owen criteria[25] as of data cutoff date, 31 October 2021.

To evaluate the dosage effect of *TP53* mutations, patients were classified into 4 subgroups based on $TP53^{MUT}$ VAF and deletion status: (1) $TP53^{WT}$ (mutation and deletion not detected), (2) $TP53^{MUT}$ with VAF between 0.25% and <1%, (3) $TP53^{MUT}$ with VAF between 1% and <10%, and (4) $TP53^{MUT}$ with VAF of ≥10% or deletion present. For patients with multiple *TP53* mutations, the variant with the maximum VAF was used for classification. The clinical efficacy end points were compared among subgroups.

Statistical analysis

Fisher exact tests were used to evaluate the correlations among different mutations and between mutation status and treatment status (treatment naïve vs relapsed/refractory) or treatment arms. Univariate and multivariate logistic regression models were used to compare the response rates between the mutation statuses. Covariates in the multivariate logistic regression models include mutation status of *CXCR4* (WT, NS, and FS or WT and MUT), *TP53* (WT and MUT), and *TERT* (WT and MUT) and the treatment arm to account for treatment differences. Odds ratios (MUT vs WT) and *P* values were estimated.

The median PFS was analyzed using the Kaplan-Meier method. Hazard ratio (HR) and *P* values of PFS between mutation statuses (MUT vs WT) were estimated using a Cox regression model with treatment arm and *CXCR4*, *TP53*, and *TERT* mutation status as covariates. *P* values ≤ .05 were considered statistically significant without multiplicity adjustment.

Results

Baseline genetic alteration profiling from 210 patients with WM treated with BTKi

Across 210 patients with WM, including 190 patients with $MYD88^{MUT}$ (zanubrutinib, 98; ibrutinib, 92) and 20 patients with $MYD88^{WT}$ (all zanubrutinib), baseline genetic profiling revealed 329 genetic alterations in 52 genes from 124 patients with enrichment in genes involved in DNA damage response, cell cycle, chromatin remodeling, and kinase pathways (supplemental Table 1). In 86 patients, genetic alterations were not observed, and the percentage of bone marrow–infiltrated $CD20^{+}$ cells in these patients was not different from patients with detectable genetic alterations. Although $MYD88^{L265P}$ mutations were the most common, mutations in *CXCR4*, *TP53*, *ARID1A*, and *TERT* were also observed, with mutation rates of 25.7%, 24.8%, 15.7%, and 9.0%, respectively. Mutation rates of the remaining 48 genes were ≤4.3% (Figure 1; supplemental Table 2). Post-hoc analysis revealed FS and NS mutations were most common in *CXCR4*, and patients in the zanubrutinib arm had a higher rate of $CXCR4^{MUT}$ than patients in the ibrutinib arm (33.7% vs 21.7%; $P = .08$). Specifically, $CXCR4^{FS}$ mutations were more prevalent in patients in the zanubrutinib arm compared with those in the ibrutinib arm of the study (19.4% vs 7.6%; $P = .02$; supplemental Table 4). FS and NS mutations were also common in patients with $ARID1A^{MUT}$; $ARID1A^{MUT}$ were more often detected in patients with $CXCR4^{MUT}$ compared with in those with $CXCR4^{WT}$ (44.4% vs 5.8%; $P < .001$; supplemental Table 3). Mutations in *TP53* consisted of missense, truncation, and splice site mutations, with the majority localized in the DNA binding domain. *TP53* mutations were more common in patients with $CXCR4^{MUT}$ compared with patients with $CXCR4^{WT}$ (35.2% vs 21.2%; $P < .05$; supplemental Table 3). *TERT* mutations were localized to promoter regions, including −57A>C, −124C>T, and −146C>T, and were detected more frequently in patients with $CXCR4^{MUT}$ compared with in those with $CXCR4^{WT}$ (24.1% vs 3.9%; $P < .001$; supplemental Table 3).

Response to BTKi therapy was inferior in patients with mutations in *CXCR4* and *TP53*

To evaluate the association between genetic alterations and clinical efficacy of the BTKis zanubrutinib and ibrutinib in $MYD88^{MUT}$ WM, a pooled analysis of patients with $MYD88^{MUT}$ (n = 190) was conducted. Lower rates of VGPR + CR and longer times to response were generally observed in patients with the common mutations compared with in patients with respective WT alleles. Univariate analysis revealed that patients with $CXCR4^{MUT}$ and $TERT^{MUT}$ had significantly lower VGPR + CR and major response, respectively (Table 1, Table 2, Table 3) than patients with WT alleles; $CXCR4^{MUT}$ and $TERT^{MUT}$ cooccurred in 13 patients (supplemental Table 3). In patients with $CXCR4^{MUT}$, $TP53^{MUT}$, and $TERT^{MUT}$, a dosage-dependent effect on PFS was observed; patients harboring mutations in these genes with VAF of ≥1% trended toward less favorable outcomes than patients with the respective WT alleles (supplemental Figure 1). Because $TP53^{MUT}$ and $TERT^{MUT}$ were associated with higher rates of $CXCR4^{MUT}$, we performed multivariate analyses including *CXCR4*, *TP53*, and *TERT* mutational status and treatment as covariates (supplemental Table 3). Compared with patients with WT alleles, patients with $CXCR4^{MUT}$ had significantly lower VGPR + CR rates (17.0% vs

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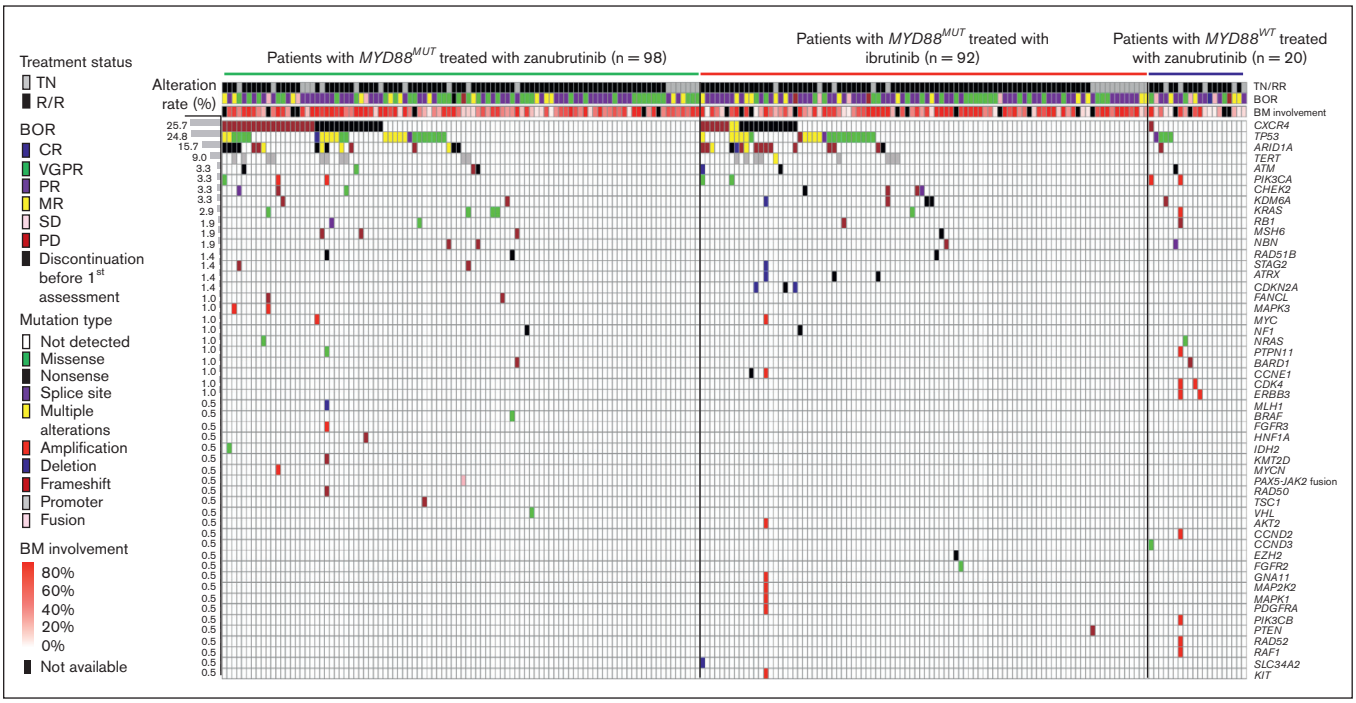

Figure 1. Baseline genetic alteration landscape in 210 patients with WM treated with BTKis. DNA mutation profile of patients with WM and the distribution of mutations among different study cohorts by mutation type and treatment status (TN and RR). Each column represents 1 patient, and each row represents 1 gene (represented by the gene symbol). Mutation rates of each gene are shown on the left. Mutation type, treatment status, and best overall response are color coded as shown in the figure legend. BM, bone marrow; BOR, best overall response; MR, minor response; PD, progressive disease; RR, relapsed/refractory; SD, stable disease; TN, treatment naïve.

37.2%; $P = .020$; Table 1, Table 2) and patients with $TP53^{MUT}$ had worse PFS ($P = .008$; Figure 2).

Of patients with $MYD88^{MUT}$ WM, 22 of 190 (11.6%) patients had $TP53^{MUT}$ at VAF of <1% whereas 26 of 190 (13.7%) patients had $TP53^{MUT}$ at VAF of ≥1% or had a deletion of *TP53*. Patients with $TP53^{MUT}$ at VAF of ≥1% or *TP53* deletion had higher rates of $CXCR4^{NS}$ (supplemental Table 5). A dosage effect of *TP53* mutations in patients treated by both zanubrutinib and ibrutinib was observed. Compared with patients with $TP53^{WT}$, patients with $TP53^{MUT}$ VAF of <1% treated with ibrutinib had lower rates of VGPR or better and major response, but patients with $TP53^{MUT}$ VAF of ≥1% or deletion had much lower rates of response and 42-month PFS (supplemental Table 6; supplemental Figure 3). Similarly, a lower 42-month PFS rate was observed in patients with $TP53^{MUT}$ VAF of <1% treated with zanubrutinib, and PFS was even lower in patients with $TP53^{MUT}$ VAF of ≥1% or deletions (supplemental Table 6; supplemental Figure 3). Because patients with $TP53^{MUT}$ VAF of ≥1% or deletion had higher rates of $CXCR4^{NS}$ and trended toward less favorable outcomes, a 1% VAF cutoff was used to assess $TP53^{MUT}$ associations with PFS. In both BTKi treatment groups, PFS in this population was inferior compared with patients with $TP53^{WT}$ or $TP53^{MUT}$ VAF of <1% (ibrutinib: HR, 3.792; $P = .008$; zanubrutinib: HR, 2.239; $P = .140$; supplemental Figure 3).

$TERT^{MUT}$ were detected in 19 patients (zanubrutinib: n = 10; ibrutinib: n = 9). Patients with $TERT^{MUT}$, especially those with PFS events, had high rates of *CXCR4* or *TP53* comutations (supplemental Table 7) and less favorable PFS compared with patients with $TERT^{WT}$ (Table 1, Table 4, Table 5).

Among 20 patients with $MYD88^{WT}$, 4 patients with $TP53^{MUT}$ had a lower MRR (50%) and none achieved VGPR or CR, compared with patients with $TP53^{WT}$ who had 62.5% MRR and a 25% VGPR + CR rate (Table 6). Generally, patients with $TP53^{MUT}$ tended to have less favorable PFS than patients with $TP53^{WT}$; the 12-month PFS for $TP53^{MUT}$ vs $TP53^{WT}$ was 25% vs 75%, respectively (supplemental Figure 4).

Zanubrutinib demonstrated deeper and faster responses, as well as favorable PFS, compared with ibrutinib in patients with $CXCR4^{NS}$, $CXCR4^{FS}$, or $TP53^{MUT}$

To evaluate the response to different BTKis in patients with $MYD88^{MUT}$ with $CXCR4^{MUT}$, $TP53^{MUT}$, and $TERT^{MUT}$, multivariate analysis was conducted separately for patients treated with zanubrutinib and those treated with ibrutinib. As has been reported previously, patients with $CXCR4^{MUT}$ in the ASPEN study had a lower VGPR + CR rate, lower MRR, and longer time to response than those with $CXCR4^{WT}$, independent of treatment. However, patients with $CXCR4^{MUT}$ treated with zanubrutinib demonstrated deeper and faster responses, as well as more favorable PFS compared with patients treated with ibrutinib.

To address whether *CXCR4* mutation type affected the patients' response to BTKis, we compared treatment responses in patients with $CXCR4^{NS}$ vs $CXCR4^{FS}$. Patients with either $CXCR4^{NS}$ or

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Table 1. Response assessment by *CXCR4*, *TP53*, *TERT*, and *ARID1A* mutational status in patients with WM with *MYD88*^{MUT}

	CXCR4^{WT} (n = 137)	*CXCR4*^{MUT} (n = 53)	*CXCR4*^{FS} (n = 26)	*CXCR4*^{NS} (n = 27)	*TP53*^{WT} (n = 142)	*TP53*^{MUT} (n = 48)	*TERT*^{WT} (n = 171)	*TERT*^{MUT} (n = 19)	*ARID1A*^{WT} (n = 158)	*ARID1A*^{MUT} (n = 32)
Best overall response, n (%)										
CR	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
VGPR	51 (37.2)	9 (17.0)	5 (19.2)	4 (14.8)	48 (33.8)	12 (25.0)	58 (33.9)	2 (10.5)	52 (32.9)	8 (25.0)
PR	64 (46.7)	30 (56.6)	15 (57.7)	15 (55.6)	71 (50.0)	23 (47.9)	85 (49.7)	9 (47.4)	77 (48.7)	17 (53.1)
MR	16 (11.7)	10 (18.9)	4 (15.4)	6 (22.2)	16 (11.3)	10 (20.8)	20 (11.7)	6 (31.6)	21 (13.3)	5 (15.6)
SD	3 (2.2)	3 (5.7)	2 (7.7)	1 (3.7)	4 (2.8)	2 (4.2)	5 (2.9)	1 (5.3)	6 (3.8)	0 (0.0)
PD	2 (1.5)	1 (1.9)	0 (0.0)	1 (3.7)	2 (1.4)	1 (2.1)	2 (1.2)	1 (5.3)	2 (1.3)	1 (3.1)
NE	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)	1 (3.1)
VGPR or better	51 (37.2)	9 (17.0)	5 (19.2)	4 (14.8)	48 (33.8)	12 (25.0)	58 (33.9)	2 (10.5)	52 (32.9)	8 (25.0)
Major response	115 (83.9)	39 (73.6)	20 (76.9)	19 (70.4)	119 (83.8)	35 (72.9)	143 (83.6)	11 (57.9)	129 (81.7)	25 (78.1)
Time to response, median (min, max), mo										
VGPR or CR	8.4 (1.9, 50.0)	11.1 (2.8, 46.0)	11.1 (2.8, 26.0)	13.9 (9.4, 46.0)	9.3 (1.9, 50.0)	11.1 (3.0, 46.9)	9.3 (1.9, 50.0)	34.1 (22.2, 46.0)	9.8 (1.9, 49.9)	10.7 (2.8, 46.0)
Major response	2.8 (0.9, 49.8)	4.6 (1.0, 49.8)	6.6 (1.8, 49.8)	3.7 (1.0, 38.7)	2.9 (0.9, 49.8)	2.9 (1.0, 13.8)	2.8 (0.9, 49.8)	5.6 (1.8, 22.2)	2.8 (0.9, 49.8)	3.0 (1.0, 38.7)

Patients with *CXCR4*^{MUT}, *TP53*^{MUT}, and *TERT*^{MUT} trended toward lower VGPR + CR rate, lower MRR, and/or longer median time to response than patients with the respective WT alleles.
max, maximum; min, minimum; MR, minor response; NE, not evaluable due to discontinuation before first assessment; PD, progressive disease; SD, stable disease.

CXCR4^{FS} had lower VGPR + CR response rates than patients with *CXCR4*^{WT}, regardless of treatment, but patients treated with zanubrutinib trended toward a higher VGPR + CR rate than those treated with ibrutinib with *CXCR4*^{FS} and *CXCR4*^{WT} (Table 4, Table 5). Furthermore, relative to patients with *CXCR4*^{WT}, patients with *CXCR4*^{NS} had lower MRR with ibrutinib treatment but not with zanubrutinib (Table 4, Table 5). A logistic regression model with treatment group and *TERT* (WT and MUT) and *TP53* (WT and MUT) mutational status as covariates was used to compare VGPR + CR rate and MRR between zanubrutinib and ibrutinib in patients with *CXCR4*^{NS} and *CXCR4*^{FS}. Compared with ibrutinib, patients with *CXCR4*^{FS} treated with zanubrutinib had a more favorable VGPR + CR rate (26.3% vs 0%; $P = .06$) although MRR was similar (73.7% vs 85.7%). In patients with *CXCR4*^{NS}, the VGPR + CR rates were similar between zanubrutinib and ibrutinib treatment groups (14.3% vs 15.4%). Patients treated with zanubrutinib had a more favorable MRR (85.7% vs 53.8%; $P = .09$). Patients with *CXCR4*^{NS} and *CXCR4*^{FS} had longer times to response to both ibrutinib and zanubrutinib than patients with *CXCR4*^{WT}, but zanubrutinib treatment led to faster response compared with ibrutinib in all *CXCR4* subgroups (Table 4, Table 5). Compared with *CXCR4*^{WT}, *CXCR4*^{NS} was significantly associated with inferior PFS (HR, 3.39; $P = .02$), and patients with *CXCR4*^{FS} treated with ibrutinib trended toward a less favorable PFS (HR, 2.08; $P = .185$). PFS was not significantly affected by *CXCR4* mutational status in patients treated with zanubrutinib (*CXCR4*^{NS}: HR, 0.67; $P = .60$; *CXCR4*^{FS}: HR, 0.62; $P = .473$; Table 5). Patients with either *CXCR4*^{NS} or *CXCR4*^{FS} treated with zanubrutinib had similar PFS, but PFS in patients with these mutations treated with ibrutinib was less favorable (supplemental Figure 2). In patients receiving ibrutinib, the median PFS (months) by *CXCR4*^{NS}, *CXCR4*^{FS}, and *CXCR4*^{WT} was 39.8, 44.2, and not reached (NR), respectively, and NR in all subpopulations receiving zanubrutinib. Compared with ibrutinib treatment, zanubrutinib treatment was associated with trends toward more favorable PFS in patients with *CXCR4*^{NS} ($P = .09$), *CXCR4*^{FS} ($P = .07$), and *CXCR4*^{WT} ($P = .32$; supplemental Figure 2).

In addition to detecting high rates of *TP53*^{MUT} in the ASPEN study population, we further observed that, in patients treated with ibrutinib, these mutations were significantly associated with lower MRR ($P = .04$) and a trend toward a lower VGPR + CR rate ($P = .20$) than *TP53*^{WT}. In patients treated with zanubrutinib, MRR ($P = .98$) and VGPR + CR rates ($P = .64$) were similar between patients with *TP53*^{MUT} and *TP53*^{WT} (Table 5). A logistic regression model with treatment group and *TERT* (WT and MUT) and *CXCR4* (WT, FS, and NS) mutational status as covariates was used to compare the VGPR + CR rate and MRR between zanubrutinib and ibrutinib in patients with *TP53*^{MUT} WM. Compared with ibrutinib, zanubrutinib treatment resulted in a better VGPR + CR rate (34.6% vs 13.6%; $P < .05$) and a more favorable MRR (80.8% vs 63.6%; $P. = .11$) in *TP53*^{MUT}. Compared with patients with *TP53*^{WT}, patients with *TP53*^{MUT} had a longer time to response among both patients treated with ibrutinib and those treated with zanubrutinib, although patients treated with zanubrutinib responded faster (Table 4, Table 5). Similarly, patients with *TP53*^{MUT} had worse PFS compared with patients with *TP53*^{WT} in both zanubrutinib and ibrutinib treatment groups. However, in patients treated with ibrutinib, *TP53*^{MUT} was associated with significantly inferior PFS vs *TP53*^{WT} (HR, 2.36; $P = .027$) compared with patients treated with

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Table 2. Statistical analysis for rates of VGPR or better by *CXCR4, TP53, TERT*, and *ARID1A* mutational status in patients with WM with *MYD88*^{MUT}

	Univariate analysis*		Multivariate analysis†		Multivariate analysis‡	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
CXCR4^{MUT}	0.345 (0.156, 0.765)	.009	0.370 (0.160, 0.855)	.020	-	-
CXCR4^{FS}	0.401 (0.143, 1.130)	.084	-	-	0.379 (0.130, 1.101)	.075
CXCR4^{NS}	0.293 (0.096, 0.896)	.031	-	-	0.360 (0.112, 1.156)	.086
TP53^{MUT}	0.653 (0.311, 1.368)	.259	0.780 (0.360, 1.690)	.529	0.781 (0.360, 1.692)	.530
TERT^{MUT}	0.229 (0.051, 1.026)	.054	0.353 (0.075, 1.671)	.189	0.355 (0.075, 1.687)	.193

Patients with *CXCR4*^{MUT}, *TP53*^{MUT}, and *TERT*^{MUT} trended toward lower VGPR + CR rate, lower MRR, and/or longer median time to response than patients with the respective WT alleles. CI, confidence interval.

*To compare the response rates, univariate logistic regression models were performed, and the odds ratio (95% CI) and the corresponding *P* values are shown.

†Odds ratio (95% CI) and *P* values were estimated using a multivariate logistic regression model with treatment arm and *CXCR4* (WT and MUT), *TERT* (WT and MUT), and *TP53* (WT and MUT) mutational status as covariates to account for the correlations among the mutations and the treatment differences between treatment arms. WT is the reference group. The same models were performed to further compare the response rates between *CXCR4*^{WT} and *CXCR4*^{NS} or *CXCR4*^{FS} (CXCR4 [WT, FS, and NS]).

‡*MYD88* status was assessed by polymerase chain reaction–based assay, with a total of 190 patients with *MYD88*^{MUT} WM.

zanubrutinib (HR, 2.20; $P = .120$; Table 4, Table 5); the median PFS in patients with *TP53*^{MUT} was not reached in the zanubrutinib treatment group and was 44.2 months in the ibrutinib treatment group (HR, 0.66; $P = .37$; Table 4, Table 5). In addition, 3 patients harbored both a *TP53* deletion and mutation. One patient treated with ibrutinib had disease progression after 2.8 months of treatment, whereas 2 patients treated with zanubrutinib achieved VGPR and PR but progressed after 35.6 months and 16.9 months of treatment, respectively.

Patients with *TP53*^{MUT} treated with ibrutinib exhibited a dosage effect on response rate by VAF; patients with *TP53*^{MUT} with VAF of ≥10% or *TP53* deletion had no VGPR + CR and a lower MRR (supplemental Table 6). This dosage effect on response rate was not observed in patients with *TP53*^{MUT} treated with zanubrutinib. PFS analysis revealed that patients with *TP53*^{MUT} at VAF of ≥1% or a *TP53* deletion had worse PFS when treated with ibrutinib (HR, 3.792; $P = .008$) relative to patients treated with zanubrutinib (HR, 0.491; $P = .22$; supplemental Figure 3). In addition, patients with *TP53*^{MUT} at VAF of ≥1% or *TP53* deletion achieved 35.3% VGPR rate with zanubrutinib vs 0% VGPR with ibrutinib treatment (supplemental Table 6). Collectively, these data demonstrate that *TP53* mutations confer a worse prognosis in patients treated with BTKi, but zanubrutinib had a more favorable outcome than ibrutinib in this higher risk population.

In summation, the adverse impact of *CXCR4*^{MUT} and *TP53*^{MUT} on response and PFS was more marked in patients treated with ibrutinib than patients treated with zanubrutinib.

Resistance mutations analysis

To begin to explore how acquired mutations may confer resistance to BTKis, we sequenced BMA samples from 5 patients with WM who progressed after achieving a response on zanubrutinib (3 with *MYD88*^{MUT} and 2 with *MYD88*^{WT}) to analyze the mutational status of *BTK* and other hematological malignancy–related genes. The median treatment duration was 27.9 months (range, 10.2-34.5 months), and paired baseline progression samples were available for 4 of 5 patients. *BTK*^{C481}, a mutation associated with resistance, was identified in 1 patient with progressive disease after zanubrutinib treatment but unknown at baseline (supplemental Table 9). Additionally, 4 of 5 (80%) of progressive patients had *TP53* mutations, and 2 of 5 (40%) had mutations within the *TERT* promoter (supplemental Table 9).

Table 3. Statistical analysis for major response rate by *CXCR4, TP53, TERT*, and *ARID1A* mutational status in patients with WM with *MYD88*^{MUT}

	Univariate analysis*		Multivariate analysis†		Multivariate analysis‡	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
CXCR4^{MUT}	0.533 (0.249, 1.142)	.106	0.718 (0.308, 1.677)	.444	-	-
CXCR4^{FS}	0.638 (0.230, 1.768)	.387	-	-	0.737 (0.251, 2.167)	.579
CXCR4^{NS}	0.454 (0.177, 1.167)	.101	-	-	0.701 (0.242, 2.027)	.512
TP53^{MUT}	0.520 (0.239, 1.132)	.100	0.625 (0.276, 1.411)	.258	0.626 (0.277, 1.415)	.260
TERT^{MUT}	0.269 (0.099, 0.729)	.010	0.345 (0.118, 1.014)	.053	0.348 (0.117, 1.038)	.058

Patients with *CXCR4*^{MUT}, *TP53*^{MUT}, and *TERT*^{MUT} trended toward lower VGPR + CR rate, lower MRR, and/or longer median time to response than patients with the respective WT alleles. Abbreviations are explained in Table 2.

*To compare the response rates, univariate logistic regression models were performed, and the odds ratio (95% CI) and the corresponding *P* values are shown.

†Odds ratio (95% CI) and *P* values were estimated using a multivariate logistic regression model with treatment arm and *CXCR4* (WT and MUT), *TERT* (WT and MUT), and *TP53* (WT and MUT) mutational status as covariates to account for the correlations among the mutations and the treatment differences between treatment arms. WT was the reference group. The same models were performed to further compare the response rates between *CXCR4*^{WT} and *CXCR4*^{NS} or *CXCR4*^{FS} (CXCR4 [WT, FS, and NS]).

‡*MYD88* status was assessed by polymerase chain reaction–based assay, with a total of 190 patients with *MYD88*^{MUT} WM.

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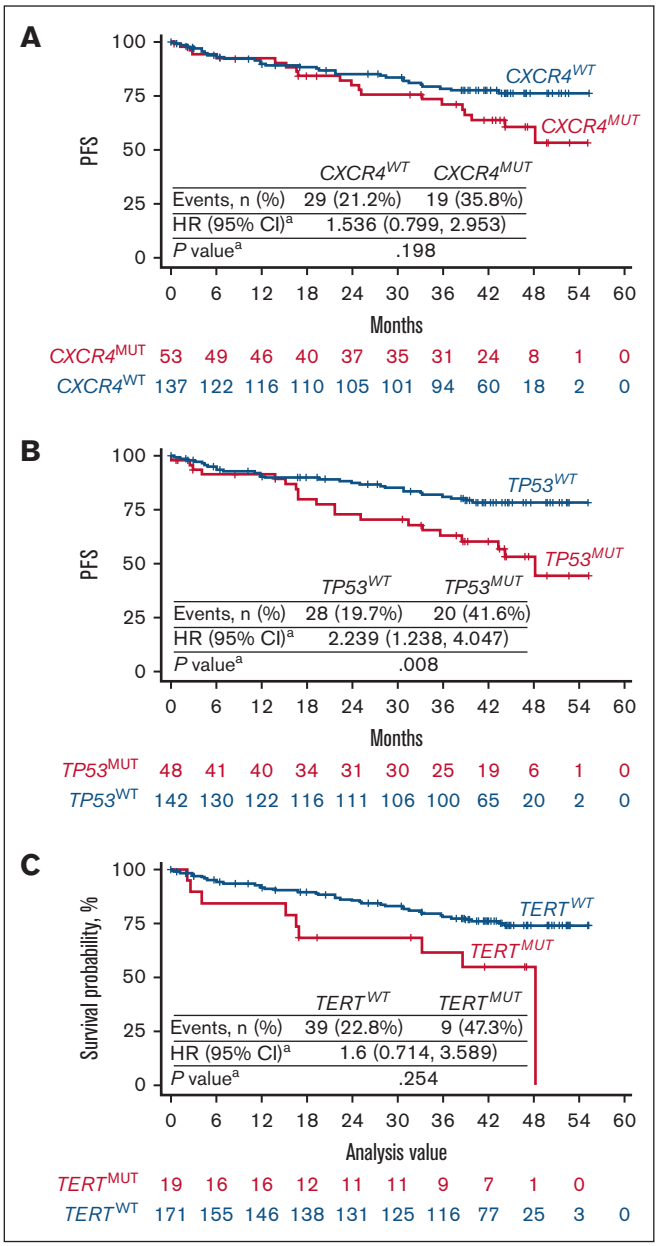

Figure 2. Kaplan-Meier curves of PFS in patients with WM with *MYD88*^{MUT} in relation to *CXCR4*, *TP53*, and *TERT* mutational status. Pooled analysis of patients with *MYD88*^{MUT}, including 98 treated with zanubrutinib and 92 treated with ibrutinib. Kaplan-Meier curves of PFS were presented according to the mutational status of (A) *CXCR4*, (B) *TP53*, and (C) *TERT*. PFS in patients with *CXCR4*^{MUT}, *TP53*^{MUT}, and *TERT*^{MUT} trended toward less favorable outcomes than in patients with the respective WT alleles. HR and *P* values were estimated using a Cox regression model with *CXCR4* (WT and MUT), *TP53* (WT and MUT), *TERT* (WT and MUT) mutational status and treatment arms as covariates. WT is the reference group.

Discussion

In this follow-up to the ASPEN study, we examined NGS data and the mutational status of 210 patients with WM to document the association of common mutations with treatment outcomes in patients treated with either ibrutinib or zanubrutinib. Data from the ASPEN study suggest that, overall, patients with *MYD88*^{MUT} with *CXCR4*^{MUT} and *TP53*^{MUT} have a worse response to BTKi treatment compared with patients with WT alleles and that patients with these mutations have generally better treatment outcomes with zanubrutinib.

In our study population, an NGS panel with sensitivity of 0.1% to 0.25% in nonenriched BMA samples identified mutation rates of *CXCR4* and *ARID1A* comparable to previous studies,[3,8] indicating that a highly sensitive NGS panel may compensate for non-enrichment of samples. However, compared with previous studies that observed a <14% mutation rate in *TP53*,[3,9-11] in our study, the *TP53* mutation rate (24.8%) was considerably higher. This observation may potentially be because the high-sensitivity assay used in this study detected a high rate (11.6%) of low-frequency (VAF < 1%) mutations compared with less sensitive assays such as Sanger sequencing, whole-exome sequencing, whole-genome sequencing, or targeted sequencing.

A previous study reported that patients with *CXCR4*^{NS} who were treated with ibrutinib had worse odds of major response and worse PFS than patients with *CXCR4*^{FS} and *CXCR4*^{WT}.[26] *CXCR4* mutations may potentially activate cell survival pathways, therefore bypassing BTK to promote ibrutinib resistance. In cell culture, *CXCR4*^{NS} and *CXCR4*^{FS} mutations reportedly cause impaired membrane internalization, leading to prolonged phosphorylation of AKT1 and MAPK1 and, thus, prolonged survival signals for cancer cells.[27] In our study, we also found that patients with *CXCR4*^{NS} mutations had worse clinical outcomes than patients with *CXCR4*^{FS} or *CXCR4*^{WT} in treatment with ibrutinib, but response to zanubrutinib was not affected by *CXCR4* mutation type. This may suggest that zanubrutinib, a more potent BTKi because of prolonged exposure to, or sustained occupancy on, BTK, can potentially offset the deleterious effects of *CXCR4* mutations.[28]

Our current study, consistent with a previous report,[9] found that *TP53*^{MUT} is commonly associated with *MYD88* and *CXCR4* mutations. We demonstrated that patients with *TP53* VAF <1% have similar PFS to patients with *TP53*^{WT}, whereas patients with *TP53* VAF between 1% and 10% or >10% fared worse. This observation suggests that low-frequency (VAF<1%) *TP53* mutations may be small subclones detected with a highly sensitive NGS panel and of less clinical significance. The NGS assay used in this study has a validated limit of detection of 0.1% to 0.25% VAF. Furthermore, variant calls were made using a strict bioinformatic analysis pipeline defined by at least 3 distinct fragments containing mutations and VAF of ≥0.25% or hot-spot VAF of ≥0.1%. Thus, the low-frequency mutations observed in this study are unlikely to be false positives. Three patients harbored both *TP53* deletions and mutations and all of them had disease progression after 16.9 months (range, 2.8-35.6 months) after BTKi treatment (2 treated with zanubrutinib). These data indicate that deficiencies in *TP53* lead to a poor prognosis in patients with WM treated with BTKis, although validation in additional WM populations is warranted. Our finding that *TP53* mutations have a significant negative impact on MRR and PFS in patients treated with ibrutinib, but not in patients treated with zanubrutinib, suggests that more potent BTKis, such as zanubrutinib, could improve the suboptimal response of patients with WM with *TP53*^{MUT}.

Although mutations in *CXCR4*, *ARID1A*, and *TP53* have previously been reported in WM, we also identified *TERT* promoter mutations in 19 patients with *MYD88*^{MUT}. Although patients with *TERT*^{MUT}

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Table 4. Response assessment by *CXCR4, TP53,* and *TERT* mutational statuses in patients treated with ibrutinib with $MYD88^{MUT}$

	Patients with $MYD88^{MUT}$ treated with ibrutinib (n = 92)						
	$CXCR4^{WT}$ (n = 72)	$CXCR4^{FS}$ (n = 7)	$CXCR4^{NS}$ (n = 13)	$TP53^{WT}$ (n = 70)	$TP53^{MUT}$ (n = 22)	$TERT^{WT}$ (n = 83)	$TERT^{MUT}$ (n = 9)
VGPR or better, n (%)*	22 (30.6)	0	2 (15.4)	21 (30.0)	3 (13.6)	23 (27.7)	1 (11.1)
OR (95% CI)	-	0.14 (0.00, 3.23)	0.64 (0.13, 3.08)	-	0.44 (0.12, 1.55)	-	0.52 (0.07, 3.79)
P value	-	.223	.579	-	.202	-	.525
Major response, n (%)*	61 (84.7)	6 (85.7)	7 (53.8)	60 (85.7)	14 (63.6)	70 (84.3)	4 (44.4)
OR (95% CI)	-	1.06 (0.10, 10.36)	0.33 (0.07, 1.41)	-	0.29 (0.09, 0.95)	-	0.23 (0.04, 1.22)
P value	-	.958	.135	-	.040	-	.085
Time to VGPR or better, median (min, max), mo	11.3 (2.0, 49.9)	-	31.3 (16.6, 46.0)	11.4 (2.0, 49.9)	24.9 (5.6, 46.9)	11.4 (2.0, 49.9)	46.0 (46.0, 46.0)
Time to major response, median (min, max), mo	2.8 (0.9, 49.8)	7.0 (2.8, 41.5)	2.9 (1.2, 13.6)	2.9 (0.9, 49.8)	3.0 (1.0, 13.8)	2.8 (0.9, 49.8)	10.3 (2.9, 13.8)
PFS†							
Event-free rate at 42 months, %	74.6	57.1	43.5	72.1	57.9	68.4	74.0
Median, mo	NE	44.2	39.8	NE	44.2	NE	48.2
HR (95% CI)	-	2.08 (0.70, 6.16)	3.39 (1.23, 9.31)	-	2.36 (1.10, 5.09)	-	0.44 (0.10, 1.81)
P value		.185	.017		.027	-	.257

Response rates, time to response, and PFS were compared according to the mutational status of *CXCR4*, *TP53*, and *TERT* genes in 92 patients with $MYD88^{MUT}$ WM treated with ibrutinib and 98 patients with $MYD88^{MUT}$ WM treated with zanubrutinib, respectively. *MYD88* status was assessed by a polymerase chain reaction–based assay, with a total of 190 patients with $MYD88^{MUT}$ WM.

CI, confidence interval; max, maximum; min, minimum; NE, not estimable; OR, odds ratio.

*Odds ratio and *P* values were estimated using a logistic regression model with *CXCR4* (WT, NS, and FS), *TP53* (WT and MUT), and *TERT* (WT and MUT) mutational status as covariates.

†Median PFS was estimated by Kaplan-Meier method; HR and *P* values were estimated using a Cox regression model with *CXCR4* (WT, FS, and NS), *TP53* (WT and MUT), and *TERT* (WT and MUT) mutational statues as covariates. WT is the reference group.

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Table 5. Response assessment by *CXCR4*, *TP53*, and *TERT* mutational statuses in patients treated with zanubrutinib with *MYD88*^{MUT}

	Patients with *MYD88*^{MUT} treated with zanubrutinib (n = 98)						
	CXCR4^{WT} (n = 65)	*CXCR4*^{FS} (n = 19)	*CXCR4*^{NS} (n = 14)	*TP53*^{WT} (n = 72)	*TP53*^{MUT} (n = 26)	*TERT*^{WT} (n = 88)	*TERT*^{MUT} (n = 10)
VGPR or better, n (%)*	29 (44.6)	5 (26.3)	2 (14.3)	27 (37.5)	9 (34.6)	35 (39.8)	1 (10.0)
OR (95% CI)	-	0.51 (0.16, 1.66)	0.24 (0.04, 1.26)	-	1.27 (0.46, 3.52)	-	0.25 (0.02, 2.26)
P value	-	.269	.093	-	.636	-	.219
Major response, n (%)*	54 (83.1)	14 (73.7)	12 (85.7)	59 (81.9)	21 (80.8)	73 (83.0)	7 (70.0)
OR (95% CI)	-	0.66 (0.18, 2.36)	1.52 (0.25, 9.01)	-	1.01 (0.29, 3.47)	-	0.47 (0.09, 2.39)
P value	-	.524	.639	-	.978	-	.362
Time to VGPR or better, median (min, max), mo	6.5 (1.9, 42.0)	11.1 (2.8, 26.0)	10.3 (9.4, 11.1)	6.5 (1.9, 42.0)	11.1 (3.0, 26.0)	6.7 (1.9, 49.8)	22.2 (22.2, 22.2)
Time to major response, median (min, max), mo	2.8 (0.9, 28.5)	2.9 (1.8, 49.8)	4.1 (1.0, 38.7)	2.8 (0.9, 49.8)	2.8 (1.0, 5.6)	2.8 (0.9, 49.8)	3.7 (1.8, 22.2)
PFS†							
Event-free rate at 42 months, %	81.3	76.4	66.7	84.6	62.0	83.4	37.5
Median, mo	NE	NE	NE	NE	NE	NE	25.0
HR (95% CI)	-	0.62 (0.17, 2.25)	0.67 (0.15, 2.88)	-	2.20 (0.81, 5.98)	-	5.78 (1.75, 19.13)
P value		.473	.598		.120	-	.004

Response rates, time to response, and PFS were compared according to the mutational status of *CXCR4*, *TP53*, and *TERT* genes in 92 patients with *MYD88*^{MUT} WM treated with ibrutinib and 98 patients with *MYD88*^{MUT} WM treated with zanubrutinib, respectively. *MYD88* status was assessed by a polymerase chain reaction–based assay, with a total of 190 patients with *MYD88*^{MUT} WM.

CI, confidence interval; max, maximum; min, minimum; NE, not estimable; OR, odds ratio.

*Odds ratio and *P* values were estimated using a logistic regression model with *CXCR4* (WT, NS, and FS), *TP53* (WT and MUT), and *TERT* (WT and MUT) mutational status as covariates.

‡Median PFS was estimated by Kaplan-Meier method; HR and *P* values were estimated using a Cox regression model with *CXCR4* (WT, FS, and NS), *TP53* (WT and MUT), and *TERT* (WT and MUT) mutational statues as covariates. WT is the reference group.

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Table 6. Response assessment by *TP53* mutation status in patients with *MYD88*^{WT}

	Total (n = 20)	*TP53*^{WT} (n = 16)	*TP53*^{MUT} (n = 4)
Best overall response, n (%)			
CR	1 (5.0)	1 (6.3)	0 (0.0)
VGPR	3 (15.0)	3 (18.8)	0 (0.0)
PR	8 (40.0)	6 (37.5)	2 (50.0)
MR	4 (20.0)	3 (18.8)	1 (25.0)
SD	3 (15.0)	2 (12.5)	1 (25.0)
PD	1 (5.0)	1 (6.3)	0 (0.0)
VGPR or better, n (%)	4 (20.0)	4 (25.0)	0 (0.0)
Major response, n (%)	12 (60.0)	10 (62.5)	2 (50.0)
Time to response, median (min, max), mo			
VGPR or CR	9.6 (2.8, 22.1)	9.6 (2.8, 22.1)	-
Major response	3.4 (1.8, 44.6)	3.3 (1.8, 44.6)	4.3 (3.0, 5.5)

Among 20 patients with *MYD88*^{WT}, 4 patients with *TP53*^{MUT} had a lower major response rate (50%), and none achieved VGPR or CR, compared with *TP53*^{WT} patients.

max, maximum; min, minimum; MR, minor response; PD, progressive disease, SD, stable disease.

had high rates of *CXCR4* or *TP53* comutations, no associations between *TERT*^{MUT} and clinical response were observed (Table 1, Table 2, Table 3; Figure 2). Although more PFS events were observed in patients with *TERT*^{MUT} treated with zanubrutinib, this may be because of a higher rate of cooccurring *CXCR4*^{MUT} and *TP53*^{MUT} in these patients than in those in the ibrutinib treatment arm (60% vs 22.2%). Because of unsorted BMA samples used in this study, we are not able to confirm whether the *TERT* mutations that we observed were derived from tumor cells or clonal hematopoiesis of indeterminate potential. Subsequent studies to validate *TERT* mutations in additional WM populations or using single-cell sequencing to elucidate its functional role in WM pathogenesis are needed to determine whether these mutations represent a risk factor or have prognostic value in WM.

The main limitation of this study is the small sample size of patients available for analysis with each mutation type. Further comparisons of zanubrutinib and ibrutinib in patients with these risk mutations from other clinical studies or real-world data are warranted. A second potential limitation is that baseline BMA samples were not enriched for tumor cells before NGS sequencing. A third limitation is that, because paired baseline and disease progression samples were only available for 4 patients treated with zanubrutinib, comprehensive analysis of potential zanubrutinib resistance mechanisms in patients with WM is limited. Finally, it is unclear whether *TERT* mutations are pathogenic, and additional studies to validate and elucidate their function in WM pathogenesis are needed.

In conclusion, patients with WM harboring mutations in *CXCR4* and *TP53* were found to have poorer prognosis after treatment with BTKis than those without mutations in these genes, and patients with these mutations who received zanubrutinib had favorable outcomes compared with those who received ibrutinib. Thus, inclusion of the mutational status of *MYD88*, *CXCR4*, and *TP53* in genomic-based treatment algorithms may be valuable when assessing BTKi treatment options for patients with WM. Our data further suggest that zanubrutinib can be effective independent of *MYD88*, *CXCR4*, and *TP53* mutational status.

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Authorship

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