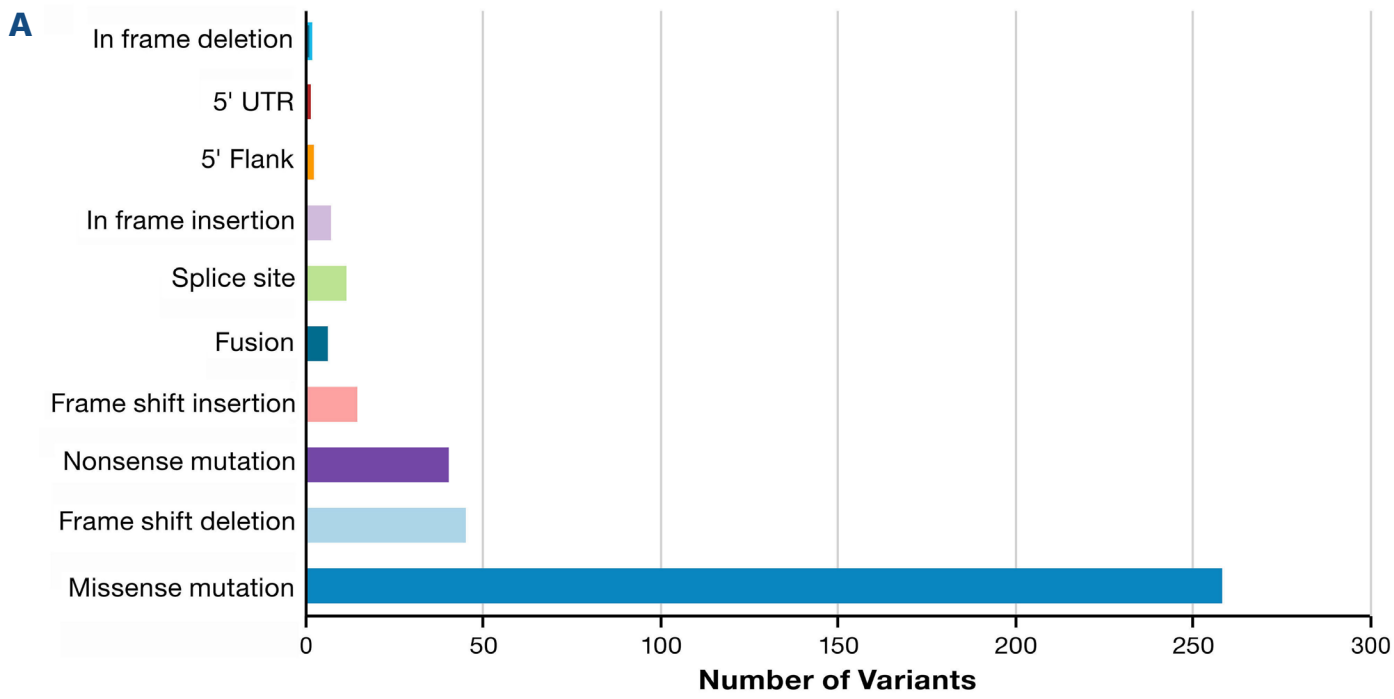
Genomic landscape of patients in a phase II study of zanubrutinib in ibrutinib- and/or acalabrutinib-intolerant patients with B-cell malignancies

Bruton tyrosine kinase (BTK) inhibitors have shown remarkable efficacy in the treatment of B-cell malignancies, yet many patients develop intolerance to these drugs leading to treatment discontinuation. Ibrutinib, the first-in-class BTK inhibitor, can cause adverse effects including cardiotoxicities, bleeding, and cytopenias leading to discontinuation in up to 16% of patients, largely due to off-target activity.[1] The second-generation, more selective BTK inhibitor, acalabrutinib, also leads to adverse effects and treatment discontinuation in up to 23% of patients.[2] Zanubrutinib is a next-generation covalent, irreversible BTK inhibitor that has been approved world-wide for the treatment of patients with B-cell malignancies including chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), previously treated mantle cell lymphoma (MCL), Waldenström macroglobulinemia, and relapsed or refractory marginal zone lymphoma. Preclinical and clinical studies have demonstrated that zanubrutinib has superior potency, selectivity, efficacy, and a more favorable toxicity profile compared to ibrutinib.[3-6] Results from BGB-3111-215 (NCT04116437), an ongoing phase II study evaluating the safety and efficacy of zanubrutinib monotherapy, demonstrate that zanubrutinib could be a safe treatment option for patients with B-cell malignancies who exhibited intolerance to prior treatment with ibrutinib (cohort 1) or to ibrutinib and/or acalabrutinib (cohort 2).[6]

To date, it is unclear whether the genomic profile of patients with B-cell malignancies who are intolerant to ibrutinib and/or acalabrutinib is associated with intolerance or response to subsequent therapy. In this *post-hoc* analysis, a highly sensitive, targeted, next-generation sequencing panel (PredicineHEME™; Predicine, Hayward, CA, USA) with full exon coverage of 106 genes commonly mutated in hematologic malignancies was used to explore the genetic profiles of patients enrolled in study BGB-3111-215.[7] This study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. Written informed consent was obtained from each patient and institutional review board approval was obtained at each study site.

Samples were sequenced to a median depth of >20,000 reads, with a validated sensitivity of 0.25% mutant allele frequency for all genomic regions and 0.1% for mutational hotspots. Variant allele frequency (VAF) <0.1% for hotspot mutations and VAF <0.25% for non-hotspot mutations were excluded from analysis. Germline and clonal hematopoiesis of indeterminate potential mutations were also excluded from analysis. Baseline blood samples from 95.9% (71/74) of all patients enrolled in the study and from 77.8% (7/9) of patients with disease progression (CLL: n=5; SLL: n=1; MCL: n=1) at data cutoff were available for analysis. Baseline demographics and disease characteristics were similar between cohorts and are summarized in *Online Supplementary Table S1*. Of note, most patients enrolled in this study had CLL or SLL (n=54).

We identified mutations in 91.5% (65/71) of baseline samples and in all (7/7) samples from patients with progressive disease (median = 4 mutations per sample; range, 1-14). The types of mutations identified as well as mutation frequencies are shown in Figure 1. Across all patients intolerant to BTK inhibitors, the most common baseline mutations were in *TP53* (32%), *SF3B1* (23%), *ATM* (18%), *NOTCH1* (17%), and *CHEK2* (15%) (Figure 1B). The mutation spectra of these genes are visualized as lollipop plots (*Online Supplementary Figure S1*) and are similar to those that have been observed in other studies.[8] As expected, mutation prevalence at baseline differed among diseases and was mostly consistent with the findings in previous studies of relapsed or refractory patients with various B-cell malignancies (Figure 1B).[8] In patients with CLL or SLL, the most frequently mutated genes were *TP53* (16/54, 30%), *SF3B1* (15/54, 28%), *ATM* (13/54, 24%), and *NOTCH1* (11/54, 20%) – all within cell signaling pathways (e.g., DNA damage response and NOTCH signaling) known to be associated with disease susceptibility and/or poor prognosis in these patients. Observed rates of baseline *TP53* (5/8 *vs.* 11/46, *P*=0.04), *ATM* (4/8 *vs*. 9/46, *P*=0.08), and *SF3B1* (5/8 *vs*. 10/46, *P*=0.03) mutations were higher in patients who later developed progressive disease (n=8) than in patients who did not (n=46) (Figure 2A). Patients with these mutations also had a shorter progression-free survival compared to that of patients without mutations in these genes, as evidenced by the observed hazard ratios (and 95% confidence intervals): *TP53* 3.2 (0.84-11.8), *SF3B1* 5.9 (1.48-23.77), or *ATM* 5.5 (1.47-20.72) (Figure 2B-D), which is consistent with previous reports.[9,10] However, the mutation frequency of *NOTCH1* was similar in patients with and without disease progression (Figure 2A) and *NOTCH1* mutation status was not correlated with progression-free survival (Figure 2E), a finding inconsistent with that of a previous study of patients with relapsed or refractory CLL treated with ibrutinib in whom *NOTCH1* mutations were strongly associated with shorter progression-free survival (*P*=0.00002) and overall survival (*P*=0.0001).[11] This discrepant finding was not due to the increased sensitivity of the PredicineHeme panel as patients with *NOTCH1* VAF <1% (45.5% [5/11]) exhibited a similar



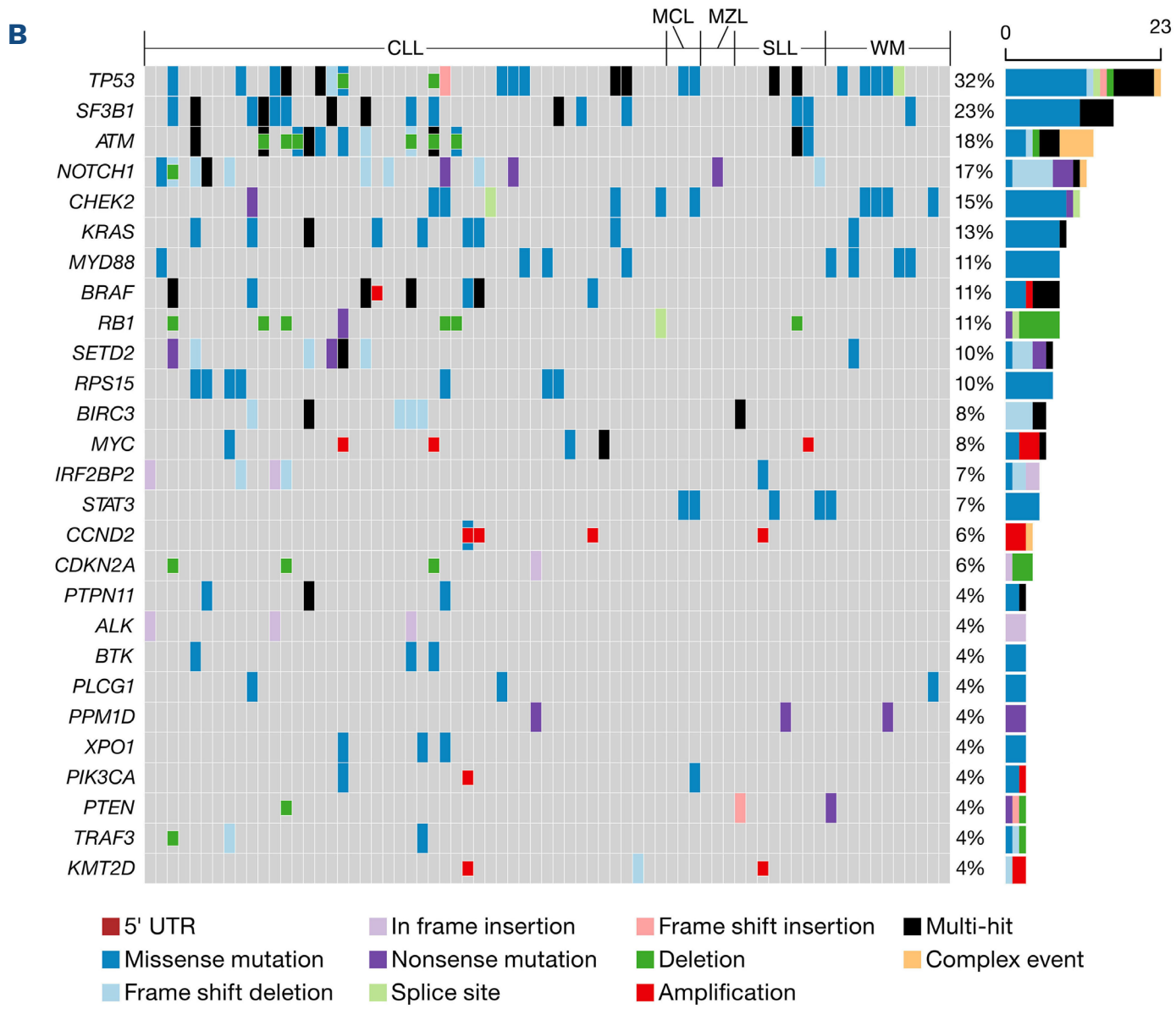


Figure 1. Variant classification and heatmap representation of mutations detected at baseline in at least three patients with B-cell malignancies. Samples were sequenced to a median depth of >20,000 reads, with a validated sensitivity of 0.25% mutant allele frequency for all genomic regions, and 0.1% for mutational hotspots. Variant allele frequency (VAF) <0.1% for hotspot mutations and VAF <0.25% for non-hotspot mutations were excluded from the analysis. Germline and clonal hematopoiesis of indeterminate potential mutations were also excluded from the analysis. (A) The number of variants is shown on the X-axis. (B) DNA mutation profile of patients and the distribution of mutations among different study cohorts by mutation type and treatment status. Each column represents one patient, and each row represents one gene (indicated by the gene symbol on the left). Mutation rates of each gene are shown on the right. Mutation type is color-coded as shown in the figure. UTR: untranslated region; CLL: chronic lymphocytic leukemia; MCL: mantle cell lymphoma; MZL: marginal zone lymphoma; SLL: small lymphocytic lymphoma; WM: Waldenström macroglobulinemia.

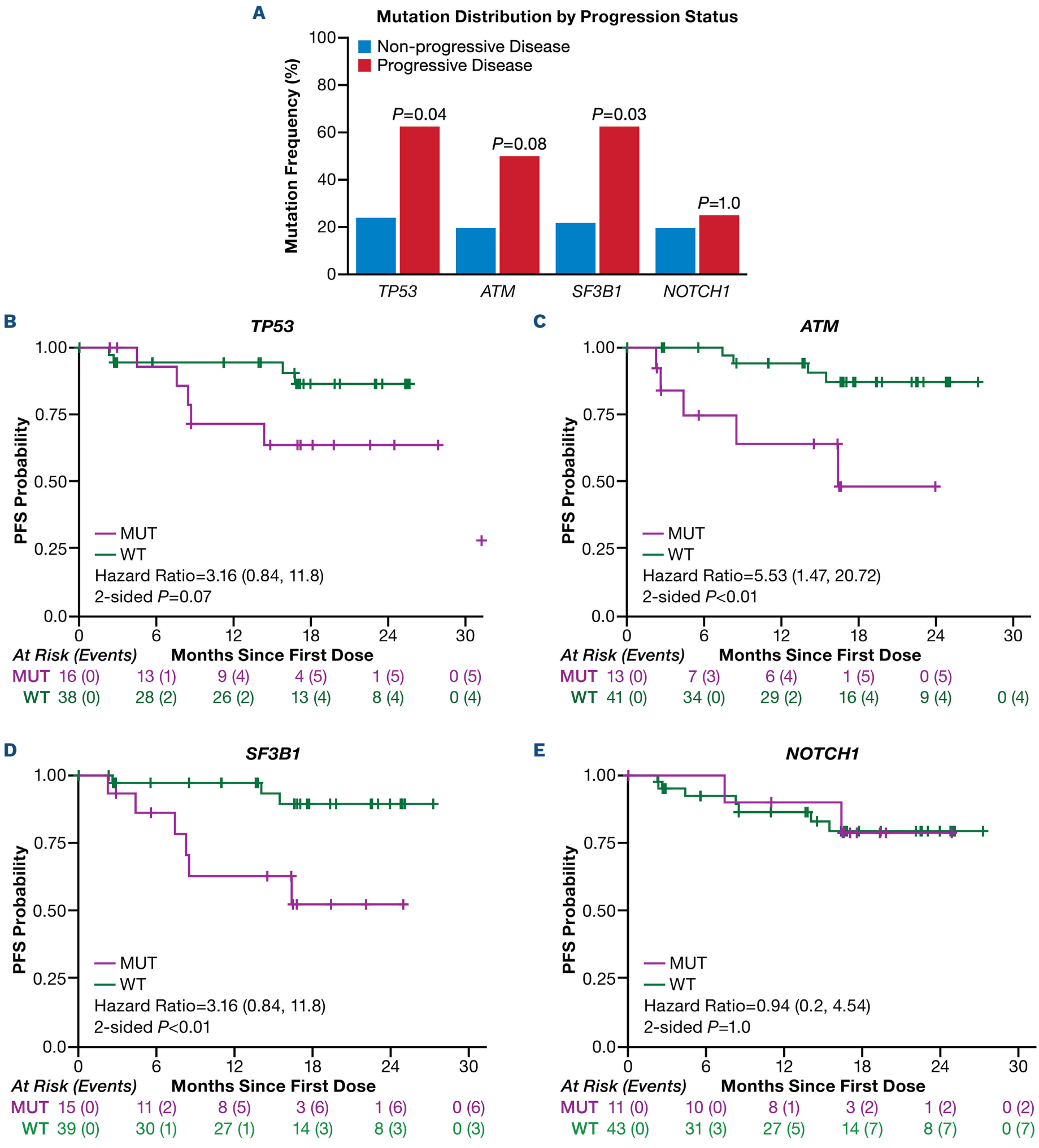


Figure 2. The presence of *TP53, SF3B1*, or *ATM* mutations is associated with inferior outcomes in patients with chronic lymphocytic leukemia or small lymphocytic lymphoma. (A) Distributions of baseline mutations between patients who developed progressive disease and those who did not were compared using proportions. (B-E) The association between genetic mutations and progression-free survival (PFS, defined as time from the date of first zanubrutinib dose to date of first progression of disease or death) was quantified by the log-rank test and hazard ratio and summarized by the Kaplan-Meier method. As of June 6, 2022, the data cutoff, patients were censored for PFS if: (i) they had no documented disease progression or death; (ii) they initiated subsequent anti-cancer therapy prior to progressive disease; or (iii) they progressed or died after more than one consecutive missed disease assessment. All analyses were performed using either R or SAS (version 9.4). Patients with mutations in *TP53*, *SF3B1*, and *ATM*, but not *NOTCH1*, showed shorter PFS. MUT: mutant; WT, wild type; PFS: progression-free survival.

progression-free survival to that of patients with *NOTCH1* VAF >1%. Instead, the finding suggests the possibility that, in contrast to ibrutinib, zanubrutinib may uniquely suppress the outgrowth of clones with *NOTCH1* mutations. Although the numbers of patients with Waldenström macroglobulinemia, MCL, and marginal zone lymphoma enrolled in our study were small and association analyses could not be performed, mutation profiles of these patients are reported for reference (Figure 1).

At the data cutoff for this analysis, nine patients (12.7%; 9/71) who were intolerant to ibrutinib and/or acalabrutinib and who were subsequently treated with zanubrutinib developed progressive disease. We assessed BTK inhibitor resistance mutations in *BTK* and *PLCG2* in these patients (Table 1). At baseline, *BTK* C481S mutations were detected in 4.2% (3/71) of patients (Figure 1B). Of these, two patients progressed on zanubrutinib; patients n. 3 and n. 9 progressed at 4.6 months and 17.7 months of zanubrutinib treatment, respectively (Table 1). A third patient (*data not shown*) with a baseline *BTK* C481S mutation died from COVID-19 shortly after enrollment before disease assessments could be made. Patient n. 3, with CLL, had a high frequency (VAF=60.9%) of *BTK* C481S mutations at baseline. This patient had long exposure to ibrutinib treatment (~65 months) prior to enrolling in the study and had disease progression after 4.6 months of zanubrutinib treatment (Table 1). Patient n. 9, with CLL, who also had long prior exposure to ibrutinib (51.9 months), initially presented with a low frequency *BTK* C481S mutation (VAF=0.9%) that increased in frequency at the time of disease progression (VAF=20.4%) (Table 1). This patient also had new *PLCG2* mutations (L845F and D993H; VAF<1% for both mutations) at disease progression (Table 1). It is worth noting that this patient stayed on zanubrutinib treatment for 17.7 months before disease progression, with stable disease as best overall response. This suggests that *BTK* C481S mutations at low VAF did not prevent zanubrutinib efficacy in this patient. At the time of disease progression, patients n. 1 (CLL) and n. 2 (SLL) had developed new *BTK* and *PLCG2* mutations. Patient n. 1 acquired high frequencies of *BTK* C481S mutations (VAF=19.2%) as well as low frequency mutations in *PLCG2* (L845F, N750D, and R665W; VAF<1% for all three *PLCG2* mutations) that had not been observed at baseline (Table 1). This patient was on zanubrutinib treatment for 9.2 months before disease progression. Patient n. 2 acquired *BTK* mutations (C481S: VAF=3.8%; C481Y: VAF=14.0%) and *PLCG2* mutations (S707F, L845V, M1141K, and E1139del; VAF <6% for all *PLCG2* mutations) at disease progression (Table 1). This patient was on zanubrutinib treatment for 17.9 months prior to disease progression. All *BTK* mutations detected in this study were located at the BTK-inhibitor binding site (C481S or C481Y).

The remaining five patients with disease progression had no *BTK* or *PLCG2* mutations detected at baseline or at disease progression. Patient n. 5, with MCL, had a *CCND1-IGH*

Table 1. Relapse on zanubrutinib was associated with known Bruton tyrosine kinase inhibitor resistance mutations.

Patient	Disease	Cohort	Duration of prior ibrutinib, months	Duration of prior acalabrutinib, months	Time on zanubrutinib, months	*BTK* mutations at baseline (VAF)	*BTK* mutations at/after PD (VAF)	*PLCG2* mutations at baseline	*PLCG2* mutations at/after PD (VAF)
1	CLL	2	6.7	10.1	9.2	ND[a]	C481S, 1442G>C (19.2%) C481S, 14421T>A (1.1%)	ND	L845F, 2535A>C (1.0%) N750D, 2248A>G (0.8%) R665W, 1993C>T (0.3%)
2	SLL	1	17.3	NA	17.9	ND	C481S, 1442G>C (0.3%) C481S, 14421T>A (3.8%) C481Y, 1442G>C (14.0%)	ND	S707F, 2120C>T (5.8%) L845V, 2533T>G (1.7%) E1139del, 3417_3419del (4.7%) M1141Lys, 3422T>A (0.9%)
3	CLL	1	64.8	NA	4.6	C481S, 1442G>C (60.9%)	C481S, 1442G>C (69.1%)	ND	ND
4	CLL	2	3.1	1.2	13.4	ND	ND	ND	ND
5	MCL	1	6.5	NA	8.7	ND[b]	ND	ND[b]	ND
6	CLL	1	4.0	NA	12.8	ND	No sample available	ND	No sample available
7	CLL	1	7.8	NA	7.7	ND	ND	ND	ND
8	CLL	1	1.5	NA	5.5	ND	No sample available	ND	No sample available
9	CLL	1	51.9	NA	17.7	C481S, 1442G>C (0.9%)	C481S, 1442G>C (20.4%)	ND	L845F, 2535A>C (0.4%) D993H, 2977G>C (0.6%)

[a]Initial sample collected on study day 87. [b]Initial sample collected on study day 141. *BTK*: Bruton tyrosine kinase gene; VAF: variant allele frequency; PD: progressive disease; *PLCG2*: phospholipase C gamma 2 gene; CLL: chronic lymphocytic leukemia; MCL: mantle cell lymphoma; SLL: small lymphocytic lymphoma; NA: not applicable; ND: not detected.

fusion mutation at both baseline and disease progression; *CCND1-IGH* fusions have been reported to be associated with ibrutinib resistance in MCL patients.[13] Four other patients with CLL harbored mutations in genes associated with poor prognosis, including *TP53, ATM*, and *SF3B1* (*Online Supplementary Table S2*).[9,10]

Here we show that the gene mutational profile of patients with B-cell malignancies who were intolerant to ibrutinib and/or acalabrutinib is comparable to that of patients with relapsed or refractory disease. For example, patients with mutations in *TP53, SF3B1*, or *ATM* genes had a less favorable prognosis in this study and similar to that previously observed in patients treated with BTK inhibitors.[9,10] Furthermore, progression-free survival was comparable between zanubrutinib-treated patients with or without *NOTCH1* mutations. Lastly, four of seven intolerant patients who progressed on zanubrutinib acquired new *BTK* mutations and/or had an increase in the frequency of *BTK* mutations. Although there are limitations to this study (e.g., small sample size, short follow-up times, and a lack of direct comparison to non-intolerant patients), this is the first study to describe the genomic landscape of patients with B-cell malignancies who were intolerant to ibrutinib and/or acalabrutinib and were switched to treatment with zanubrutinib.

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Disclosures

The study protocol was developed by BeiGene, Ltd. in collaboration with the study investigators. BeiGene was also involved in data collection, analysis, and interpretation of results. Statistical analyses were performed by statisticians at BeiGene. LX, RC, KB, AC, AI, and VR are employees of and hold stock/shares in BeiGene Co. MS is a consultant for AbbVie, Genentech, AstraZeneca, Sound Biologics, Pharmacyclics, BeiGene, Bristol Myers Squibb, Morphosys/Incyte, TG Therapeutics, Innate Pharma, Kite Pharma, Adaptive Biotechnologies, Epizyme, Eli Lilly, Adaptimmune, Mustang Bio, Regeneron, Merck, Fate Therapeutics, MEI Pharma, and Atara Biotherapeutics, and receives research funding from Mustang Bio, Celgene, Bristol Myers Squibb, Pharmacyclics, Gilead, Genentech, AbbVie, TG Therapeutics, BeiGene, AstraZeneca, Sunesis, Atara Biotherapeutics, Genmab, and Morphosys/Incyte. IF is a member of a board of directors or advisory committees at Vincerx. ML receives consulting fees from AbbVie, Amgen, AstraZeneca, BeiGene, Bristol Myers Squibb, Genmab, GSK, Incyte, Janssen, Karyopharm, Kite, Lilly, Sanofi, Seagen, and Takeda, payment or honoraria from AbbVie, Amgen, AstraZeneca, BeiGene, Bristol Myers Squibb, Genmab, GSK, Incyte, Janssen, Karyopharm, Kite, Lilly, Sanofi, Seagen, and Takeda, travel support from AbbVie, Amgen, AstraZeneca, BeiGene, Bristol Myers Squibb, Genmab, GSK, Incyte, Janssen, Karyopharm, Kite, Lilly, Sanofi, Seagen, and Takeda, and has a leadership or fiduciary role from Sellas. JMB receives consulting fees from AbbVie, Adaptive Biotechnologies, AstraZeneca, BeiGene, Bristol Myers Squibb, Constellation, Eli Lilly, Epizyme, Foresight, Genentech, Genmab, Kura, Kymera, Morphosys, Novartis, Nurix, TG Therapeutics, Verastem, and X4 Pharmaceuticals and receives payments/honoraria from Seagen and BeiGene. SFZ receives honorarium from Bristol Myers Squibb, Immunosome, and AbbVie. MDG receives honoraria from Karyopharm, TG Therapeutics, Janssen, and GSK. RC has equity with Pfizer and GSK and individual stocks in SAGA Diagnostics. D-YC has equity with BeiGene. JPS receives consulting fees from TG Therapeutics, Genentech, AbbVie, AstraZeneca, BeiGene, Bristol Myers Squibb, and Merck, and research funds from Genentech, Celgene, Gilead Sciences, TG Therapeutics, Merck, and Takeda.

Contributions

MS, IWF, MYL, RP, JMB, SFZ, JLC, JM, ECK, HAY, BF, AC, PKT, MDG, SM, and JPS enrolled patients and collected clinical data. LX, RC, KB, AC, D-YC, AI, and VR contributed to the intellectual content, conception, and design of the study. LX and KB processed data, analyzed statistics, generated figures, and wrote the manuscript. All authors contributed to data interpretation and revised the manuscript.

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Data-sharing statement

BeiGene voluntarily shares anonymous data on completed studies responsibly and provides qualified scientific and medical researchers access to anonymous data and supporting clinical trial documentation for clinical trials in dossiers for medicines and indications after submission and approval in the USA, China, and Europe. Clinical trials supporting subsequent local approvals, new indications, or combination products are eligible for sharing once corresponding regulatory approvals are achieved. BeiGene shares data only when permitted by applicable data privacy and security laws and regulations. In addition, data can only be shared when it is feasible to do so without compromising the privacy of study participants. Qualified researchers may submit data requests/research proposals for BeiGene review and consideration through BeiGene's clinical trial webpage at https://www.beigene.com/our-science-and-medicines/our-clinical-trials/.

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