Acquired Mutations in Patients With Relapsed/Refractory Chronic Lymphocytic Leukemia (CLL) That Progressed in the ALPINE Study

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INTRODUCTION

- Patients administered covalent Bruton tyrosine kinase (cBTK) inhibitors for chronic lymphocytic leukemia (CLL) can develop acquired drug resistance, leading to disease progression
- Often, cBTK inhibitor resistance results from the emergence of subclones with *BTK* mutations at the cBTK inhibitor binding site (C481) and/or *PLCG2* mutations
- Less frequently, non-C481 BTK mutations, including gatekeeper residue T474 and kinase-impaired L528 mutations, have been reported in patients with progression on cBTK inhibitors
- Most previous reports of cBTK inhibitor resistance mutations have been retrospective or in small patient populations
- Here, to gain further insight into the genetic mechanisms of cBTK inhibitor resistance in a randomized population of patients with CLL, next-generation sequencing (NGS) was performed on samples from patients who progressed on zanubrutinib or ibrutinib in the phase 3 ALPINE study (NCT03734016)[1]

METHODS

- Progressive disease (PD) was determined by an independent review committee (n=139) and/or by investigator (n=132) using Hallek et al criteria[2]
- A total of 57 patients with PD assessed by either investigator and/or the independent review committee (40.2% based on investigator assessment [53/132]) had PD samples collected for this post hoc biomarker analysis. PFS final analysis data cutoff: August 8, 2022 (**Table 1**)
- Peripheral blood samples were collected at baseline and at or after PD and prior to subsequent therapy. A total of 52 patients with paired baseline and PD samples and without Richter transformation as assessed at PD were included in this analysis (**Table 2**)

Table 1. Baseline Characteristics of Patients With PD

	Zanubrutinib (n=26)	Ibrutinib (n=31)
Number of prior treatments, median (range)	1 (1-3)	1 (1-7)
Study follow-up time, median (range), mo	25.4 (10.6-40.5)	28.1 (5.8-42.3)
Duration of treatment, median (range), mo	19.9 (4.3-39.3)	16.6 (3.4-35.7)
del(17p) and/or *TP53* mutation, n (%)	5 (19.2)	6 (19.4)
IGHV unmutated, n (%)	22 (84.6)	26 (83.9)

Table 2. Blood Samples Available for Biomarker Analysis

Patients, n		Zanubrutinib (n=26)	Ibrutinib (n=31)	Total (N=57)
No RT at PD	Paired baseline and PD sample	24	28	52
	Without baseline but had PD sample	0	1[a]	1[a]
RT at PD	Paired baseline and PD sample	2[a]	0	2[a]
	Without baseline but had PD sample	0	2[a]	2[a]

RT reported as of data cutoff (August 8, 2022).
RT, Richter transformation.
[a] No acquired *BTK/PLCG2* mutations were detected.

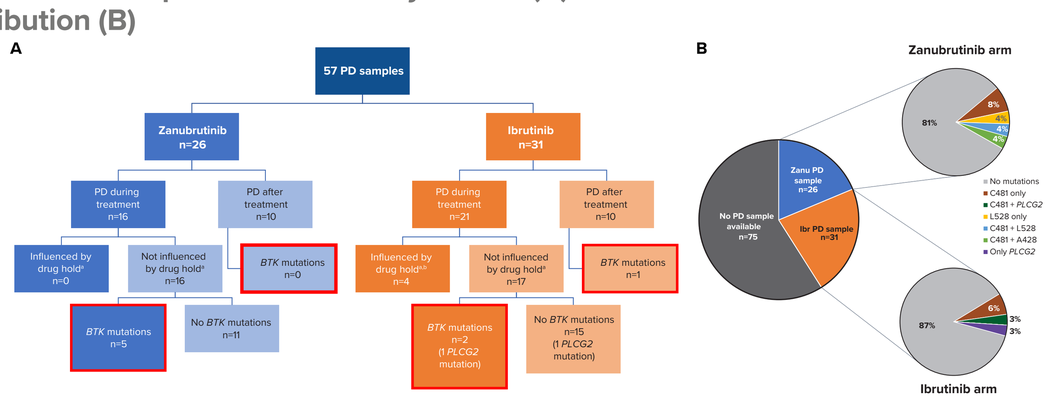
- NGS was performed using a 106-gene PredicineHEME panel (the limit of detection was 0.1% for hotspot mutations and 0.25% for non-hotspot mutations); 27 CLL driver genes identified by Knisbacher et al[3] were represented in this panel. Data reported include all *BTK* and *PLCG2* mutations with a variant allele frequency (VAF) of ≥0.25%. For all other genes, pathogenic mutations with a VAF ≥1% were reported
- Other assessments included fluorescence in situ hybridization for chromosome abnormalities; cytogenetic analysis for complex karyotype (CK ≥3); and NGS for IGHV gene mutation per the European Research Initiative on CLL

RESULTS

BTK/PLCG2 Mutations

- No *BTK* mutations were identified at baseline. At PD, 8 patients had acquired mutations in *BTK*, with half of these patients having 2 or more *BTK* mutations (**Figure 1**); 77.8% (14/18) of *BTK* mutations were at C481. One patient had a sole *PLGC2* mutation at PD (**Table 3**)

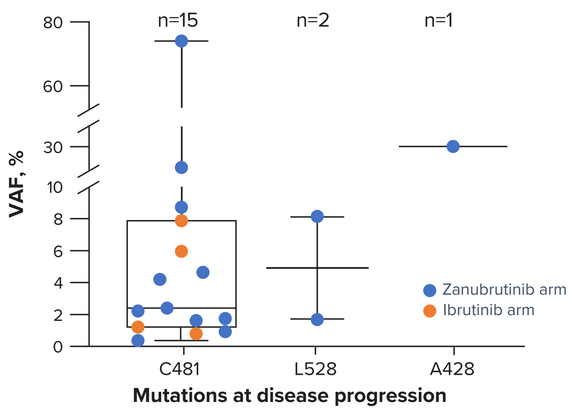
Figure 1. PD Samples for Each Study Cohort (A) and the *BTK* and/or *PLCG2* Mutation Distribution (B)



[a]Hold ≥7 days within 6 weeks before progressive disease. [b]No *BTK* or *PLCG2* mutations.

- The VAF of the 2 *BTK* L528 mutations was similar to that of the *BTK* C481 mutations (**Figure 2**)
- Overall median treatment duration was 17.0 months (range, 5.0-34.5 months)
- Among the 24 patients in this analysis who progressed on zanubrutinib, 5 acquired *BTK* mutations (L528W only, n=1; C481 only, n=2; L528W and C481, n=1; A428D and C481, n=1) (**Figure 1A**; **Table 3**)
- Among the patients with *BTK* mutations at PD (zanubrutinib, n=5; ibrutinib n=3), median treatment duration was 29.7 months (range, 18.4-34.2 months) in those treated with zanubrutinib vs 30.8 months (range, 11.8-34.5 months) in those treated with ibrutinib (**Table 3**)
  - Compared to these patients, median treatment duration at disease progression was shorter in patients with wild-type *BTK* in both the zanubrutinib (n=19, 16.8 months [range, 5.0-33.3 months], *P*<.01) and ibrutinib (n=25, 15.9 months [range, 5.9-29.4 months], *P*=.21) treatment arms (**Figure 3**)

Figure 2. VAF of Acquired *BTK* Mutations



VAF, variant allele frequency.

Table 3. Acquired *BTK* and *PLCG2* Mutations by Patient

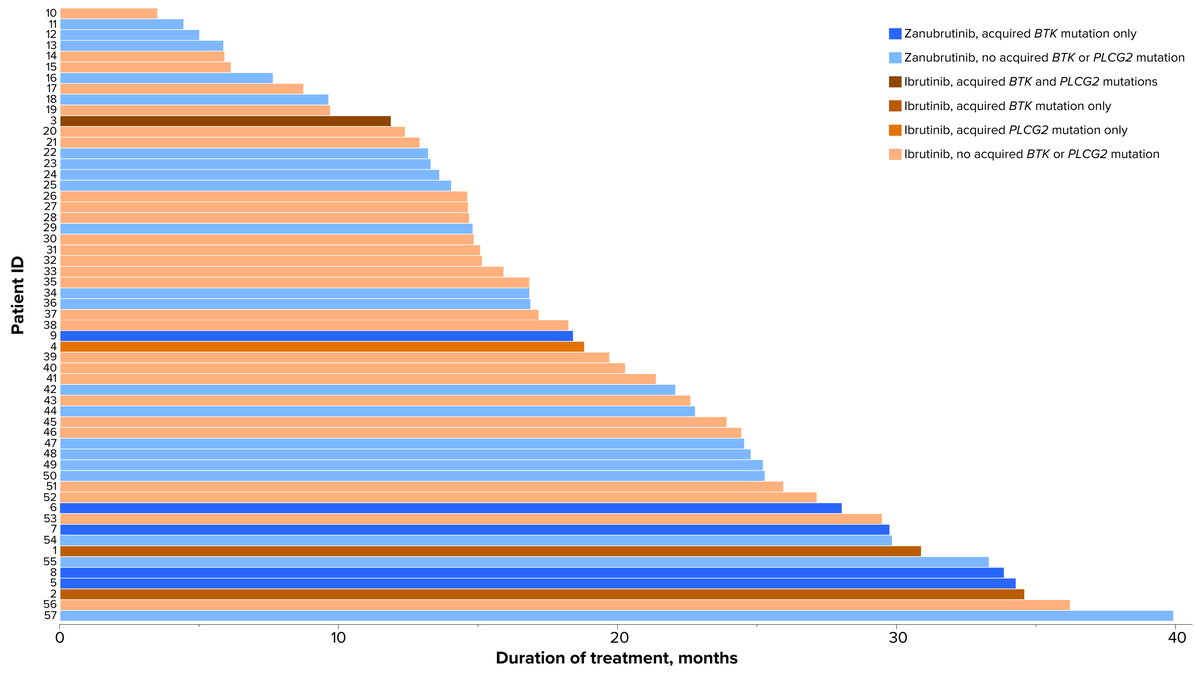
Patient ID	Treatment Arm	Acquired *BTK* Mutation at PD: Coding DNA Description (VAF, %)	Acquired *BTK* Mutation at PD: Protein Description	Acquired *PLCG2* Mutation at PD: Coding DNA Description (VAF, %)	Acquired *PLCG2* Mutation at PD: Protein Description	Duration of Treatment, Months
1	Ibrutinib	1442G>C (1.29)	C481S	Not detected	Not detected	30.8
2	Ibrutinib	1442G>C (7.95)	C481S	Not detected	Not detected	34.5
3	Ibrutinib	1442G>C (0.88)	C481S	2535A>C (0.60)	L845F	11.8
		127G>C (0.51)	D43H			
4	Ibrutinib	Not detected	Not detected	3422T>A (5.69)	M1141K	18.8
5	Zanubrutinib	1442G>C (8.80)	C481S	Not detected	Not detected	34.2
6	Zanubrutinib	1283C>A (31.10)	A428D	Not detected	Not detected	28.0
		1442G>C (4.72)	C481S			
		1441T>A (2.48)	C481S			
7	Zanubrutinib	1442G>C (16.22)	C481S	Not detected	Not detected	29.7
		1583T>G (8.22)	L528W			
		1441T>A (4.28)	C481S			
		1442G>A (1.83)	C481Y			
		1442G>T (1.70)	C481F			
		1441T>C (1.01)	C481R			
8	Zanubrutinib	1583T>G (1.76)	L528W	Not detected	Not detected	33.8
9	Zanubrutinib	1442G>C (74.39)	C481S	Not detected	Not detected	18.4
		1441T>C (2.30)	C481R			
		1441T>A (0.45)	C481S			

VAF, variant allele frequency.

CONCLUSIONS

- Of the patients who progressed in ALPINE and were included in this analysis, most (82.6%) did not acquire *BTK* or *PLCG2* mutations
- Among the 24 patients in this analysis who progressed on zanubrutinib, 5 acquired *BTK* mutations
- These data suggest that *BTK* and/or *PLCG2* mutations are not the main factors driving PD in this population
- Given the low incidence to date of non-C481 mutations in patients with PD in ALPINE, patients with CLL who have been treated with cBTK inhibitors are likely to remain sensitive to other BTK-targeting therapies

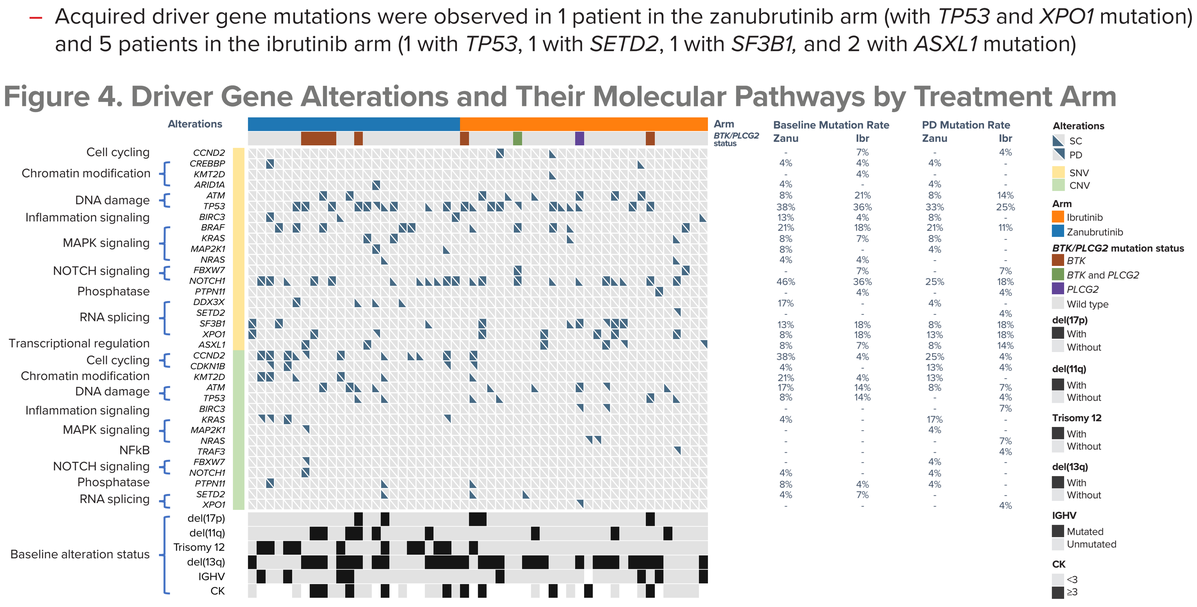
Figure 3. Treatment Duration Stratified by Treatment Arm and *BTK* and/or *PLCG2* Mutation Status



Driver Gene Mutations

- Among the 48 patients who had baseline CLL driver gene mutations, 18 mutated driver genes were identified; the median number of driver genes mutated per patient was 3 (range, 1-5) (**Figure 4**)
  - Mutations were most frequently observed in *NOTCH1* (n=21), *TP53* (n=19), *BRAF* (n=10), *SF3B1* (n=8), and *ATM* (n=8) at baseline
  - Acquired driver gene mutations were observed in 1 patient in the zanubrutinib arm (with *TP53* and *XPO1* mutation) and 5 patients in the ibrutinib arm (1 with *TP53*, 1 with *SETD2*, 1 with *SF3B1,* and 2 with *ASXL1* mutation)

Figure 4. Driver Gene Alterations and Their Molecular Pathways by Treatment Arm



CK, complex karyotype; CNV, copy number variant; SC, screening; SNV, single-nucleotide variant.

- No associations between driver gene mutations and *BTK* mutational status were detected
- Driver gene mutations at either baseline or PD were not associated with del(17p), IGHV mutation, or CK status (**Figure 4**, bottom)

Next Line of Treatment

- The majority of patients in this study population received additional treatment following study treatment discontinuation (zanubrutinib, 18/26 [69.2%]; ibrutinib, 21/31 [67.7%]), including all patients with acquired *BTK* and/or *PLCG2* mutations (**Table 4**)

Table 4. Next Line of Treatment After Discontinuation of Study Treatment

Next Line of Treatment After Discontinuing Study Treatment	Zanubrutinib (n=26) Patients, n	Zanubrutinib (n=26) Outcome	Ibrutinib (n=31) Patients, n	Ibrutinib (n=31) Outcome
Chemotherapy	1	Ongoing/completed	0	N/A
Chemoimmunotherapy[a]	4	Ongoing/completed, n=2 (*BTK* C481 mutation, n=1; RT when completing study treatment, n=1); discontinued due to AE, n=2	3	Ongoing/completed
cBTK inhibitor therapy	2	Ongoing/completed, n=1; PD, n=1	5	Ongoing/completed, n=3; PD, n=1 (*BTK* C481 mutation); discontinued due to AE, n=1
Noncovalent BTK inhibitor therapy[b]	2	Ongoing/completed, n=1; PD, n=1 (*BTK* C481 and L528 mutations)	2	Ongoing/completed, n=1; death, n=1
BCL2i monotherapy	3	Ongoing/completed, n=2; discontinued due to AE, n=1	5	Ongoing/completed, n=2 (*BTK* C481 mutation, n=1); PD, n=1; discontinued due to AE, n=1; death, n=1
BCL2i plus mCD20Ab therapy	3	PD, n=1 (*BTK* L528 mutation); discontinued due to AE, n=1 (*BTK* C481 mutation); death, n=1	2	Ongoing/completed, n=1; PD, n=1 (*PLCG2* mutation)
BCL2i plus BTK inhibitor therapy[c]	0	N/A	3	Ongoing/completed, n=1; PD, n=1 (RT when completing study treatment); discontinued due to AE, n=1 (*BTK* C481 and *PLCG2* mutations)
mCD20Ab plus BCL2i plus noncovalent BTK inhibitor	1	Ongoing/completed	0	N/A
Other[d]	2	Ongoing/completed, n=1; unknown, n=1 (*BTK* C481 and A428 mutations)	1	PD
No known treatment after study treatment discontinuation	8	(RT when completing study treatment, n=1)	10	(RT when completing study treatment, n=1)

AE, adverse event; BCL2i, B-cell lymphoma 2 inhibitor; mCD20Ab, monoclonal CD20 antibody; N/A, not applicable; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; RT, Richter transformation.
[a] One patient in the zanubrutinib arm was co-administered venetoclax. [b] One patient in the ibrutinib arm was co-administered mCD20Ab. [c] Two patients were co-administered a cBTK inhibitor and 1 patient a noncovalent BTK inhibitor. [d] Two patients (1 in each arm) were treated with a spleen tyrosine kinase inhibitor and 1 patient with rituximab plus a PI3K-δ inhibitor.

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