Acquired Mutations in Patients With Relapsed/Refractory Chronic Lymphocytic Leukemia Who 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 cut-off: 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 cut (August 8, 2022).
RT, Richter transformation.
[a] No acquired *BTK*/*PLCG2* mutations were detected.

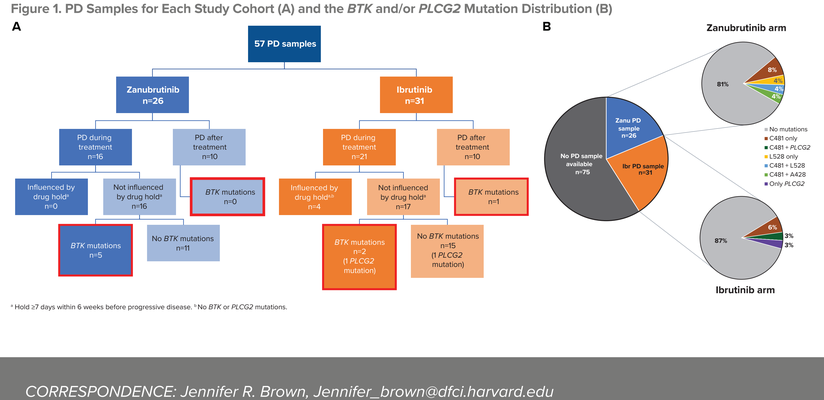
- NGS was performed using a 106-gene PredicineHEME panel (the limit of detection was 0.1% for hotspot mutations and 0.25% for non-hotspot mutations); 27 CLL driver genes identified by Knisbacher et al[3] were represented in this panel. Data reported include all *BTK* and *PLCG2* mutations with a variant 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 *PLCG2* mutation at PD (**Table 3**)

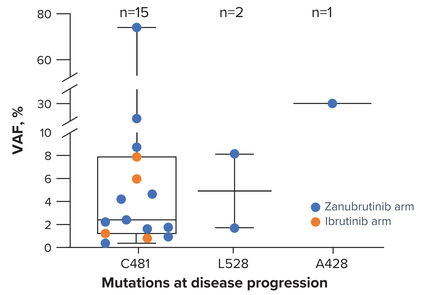
Figure 1. PD Samples for Each Study Cohort (A) and the *BTK* and/or *PLCG2* Mutation Distribution (B)



[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



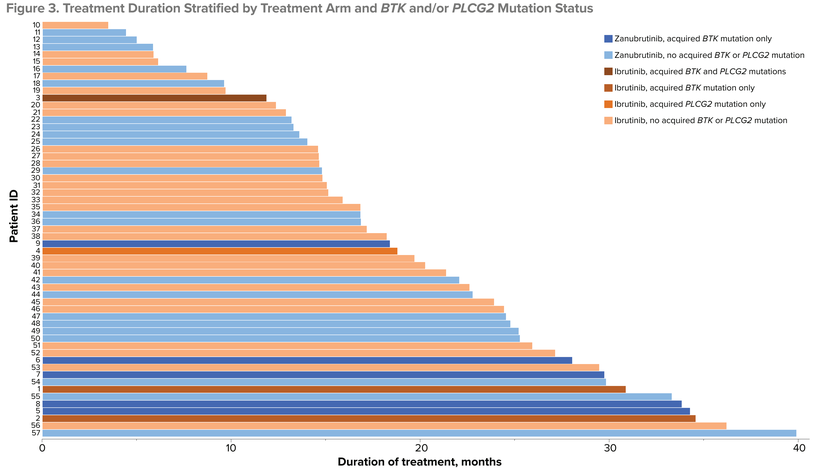
VAF, variant allele frequency.

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) 127G>C (0.51)	C481S D43H	2535A>C (0.60)	L845F	11.8
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) 1442G>C (4.72) 1441T>A (2.48)	A428D C481S C481S	Not detected	Not detected	28.0
7	Zanubrutinib	1442G>C (16.22) 1583T>G (8.22) 1441T>A (4.28) 1442G>A (1.83) 1442G>T (1.70) 1441T>C (1.01)	C481S L528W C481S C481Y C481F C481R	Not detected	Not detected	29.7
8	Zanubrutinib	1583T>G (1.76)	L528W	Not detected	Not detected	33.8
9	Zanubrutinib	1442G>C (74.39) 1441T>C (2.30) 1441T>A (0.45)	C481S C481R C481S	Not detected	Not detected	18.4

VAF, variant allele frequency.

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



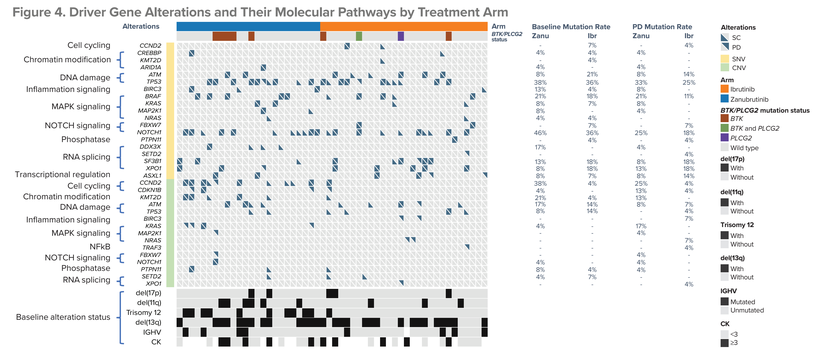
CONCLUSIONS

- 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

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.

REFERENCES

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ACKNOWLEDGMENTS

We would like to thank the investigators, site support staff, and especially the patients for participating in this study. This study was sponsored by BeiGene, Ltd. Editorial assistance was provided by Jenna M. Gaska, PhD, CMPP, of Nucleus Global, an Inizio Company, and was supported by BeiGene.

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Presented at the 65th ASH Annual Meeting and Exposition; December 9-12, 2023, San Diego, CA