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2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

Mutational profile of previously treated chronic lymphocytic leukemia patients progressing on acalabrutinib or ibrutinib

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Jennifer Woyach (The Ohio State University Comprehensive Cancer Center, United States) Daniel Jones (The Ohio State University Comprehensive Cancer Center; Department of Pathology, The Ohio State University, United States) Wojciech Jurczak (Maria Sklodowska-Curie National Research Institute of Oncology, Poland) Tadeusz Robak (Medical University of Lodz, and Copernicus Memorial Hospital, Poland) Arpad Illes (Division of Hematology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Hungary) Arnon Kater (Amsterdam University Medical Centers, Cancer Center Amsterdam, University of Amsterdam, on behalf of HOVON, Netherlands) Paolo Ghia (Università Vita-Salute San Raffaele, Italy) John Byrd (The University of Cincinnati, United States) John Seymour (Peter MacCallum Cancer Centre and The Royal Melbourne Hospital, Australia) Susan Long (Ohio State University Wexner Medical Center James Molecular Laboratory, United States) Nehad Mohamed (The Ohio State University Wexner Medical Center, United States) Samon Benrashid (The Ohio State University Comprehensive Cancer Center, United States) Tzung-Huei Lai (The Ohio State University, United States) Gary De Jesus (AstraZeneca, United States) Richard Lai (AstraZeneca, United States) Gerjan de Bruin (Acerta Pharma BV, a member of the AstraZeneca Group, Netherlands) Simon Rule (AstraZeneca, Canada) Veerendra Munugalavadla (AstraZeneca, United States)

Abstract:
Chronic lymphocytic leukemia (CLL) progression during Bruton tyrosine kinase (BTK) inhibitor treatment is typically characterized by emergent B-cell receptor pathway mutations. Using peripheral blood samples from relapsed/refractory CLL patients in ELEVATE-RR (NCT02477696) (median 2 prior therapies), we report clonal evolution data for patients progressing on acalabrutinib or ibrutinib (median follow-up 41 months). Paired (baseline and progression) samples were available for 47 (excluding 1 Richter) acalabrutinib-treated and 30 (excluding 6 Richter) ibrutinib-treated patients. At progression, emergent BTK mutations were observed in 31 (66%) acalabrutinib-treated and 11 (37%) ibrutinib-treated patients (median variant allele fraction [VAF]: 16.1% vs 15.6%). BTK C481S mutations were most common in both groups; T474I (n = 9; 8 co-occurring with C481) and the novel E41V mutation within the pleckstrin homology domain of BTK (n = 1) occurred with acalabrutinib, while neither mutation occurred with ibrutinib. L528W and A428D co-mutations presented in one ibrutinib-treated patient. Pre-existing TP53 mutations were present in 25 (53.2%) acalabrutinib-treated and 16 (53.3%) ibrutinib-treated patients at screening. Emergent TP53 mutations occurred with acalabrutinib and ibrutinib (13% vs 7%; median VAF: 6.0% vs 37.3%, respectively). Six acalabrutinib-treated patients and one ibrutinib-treated patient had emergent TP53/BTK co-mutations. Emergent PLCG2 mutations occurred in 3 (6%) acalabrutinib-treated and 6 (20%) ibrutinib-treated patients. One acalabrutinib-treated patient and 4 ibrutinib-treated patients had emergent BTK/PLCG2 co-mutations. While common BTK C481 mutations were observed with both treatments, patterns of mutation and co-mutation frequency, mutation VAF, and uncommon BTK variants varied with acalabrutinib (T474I and E41V) and ibrutinib (L528W, A428D) in this patient population.

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Short title (right running head): Acalabrutinib and ibrutinib mutational profile

Left running head: Woyach JA *et al*

Jennifer A. Woyach, MD[1]; Daniel Jones, MD, PhD[1,2]; Wojciech Jurczak, MD, PhD[3]; Tadeusz Robak, MD, PhD[4]; Árpád Illés, PhD[5]; Arnon P. Kater, MD, PhD[6]; Paolo Ghia, MD, PhD[7,8]; John C. Byrd, MD[9]; John F. Seymour, MBBS, PhD[10]; Susan Long, BA, MB(ASCP)CM[11]; Nehad Mohamed, MS, MB (ASCP)CM[2]; Samon Benrashid, BS[1]; Tzung-Huei Lai, PhD[1]; Gary De Jesus, BS[12]; Richard Lai, BS[12]; Gerjan de Bruin, PhD[13]; Simon Rule, MD[14]; Veerendra Munugalavadla, PhD[12]

[1]The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA; [2]Department of Pathology, The Ohio State University, Columbus, OH, USA; [3]Maria Sklodowska-Curie National Research Institute of Oncology, Krakow, Poland; [4]Medical University of Lodz, and Copernicus Memorial Hospital, Lodz, Poland; [5]Division of Hematology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [6]Amsterdam University Medical Centers, Cancer Center Amsterdam, University of Amsterdam, on behalf of HOVON, Amsterdam, the Netherlands; [7]Università Vita-Salute San Raffaele, Milano, Italy; [8]IRCCS Ospedale San

Raffaele, Milano, Italy; [9]Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, USA; [10]Peter MacCallum Cancer Centre, Royal Melbourne Hospital & University of Melbourne, Victoria, Australia; [11]Ohio State University Wexner Medical Center James Molecular Laboratory, Columbus, OH, USA; [12]AstraZeneca, South San Francisco, CA, USA; [13]Acerta Pharma BV, a member of the AstraZeneca Group, Oss, Netherlands; [14]AstraZeneca, Mississauga, Ontario, Canada

Corresponding Author:
Jennifer A. Woyach, MD
Professor, Division of Hematology
The Ohio State University Comprehensive Cancer Center
455A Wiseman Hall 410 W 12th Avenue
Columbus, OH 43210 Columbus, OH, USA
Phone: (614) 685-5667
Fax: (614) 293-6420
E-mail: woyach.2@osu.edu

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KEY POINTS

- *BTK* C481S was most common in both groups; in the acalabrutinib arm, low-VAF T474I (n = 9/47; 8 co-occurring with C481S) but no L528W was seen
- More patients receiving acalabrutinib acquired *BTK* mutations, though overall, patients with *BTK* mutation did not fare worse vs those without

Explanation of Novelty

Further characterization of the mutational profile at progression of acalabrutinib and ibrutinib from the head-to-head ELEVATE-RR trial confirmed that the most common mutation was C481S. The patterns of mutation frequency, variant allele frequencies, and uncommon variants differed between acalabrutinib and ibrutinib. A novel BTK-activating E41V mutation, previously observed only in vitro, was seen in one acalabrutinib-treated patient. *BTK* L528W was observed in one ibrutinib-treated patient.

ABSTRACT

Chronic lymphocytic leukemia (CLL) progression during Bruton tyrosine kinase (BTK) inhibitor treatment is typically characterized by emergent B-cell receptor pathway mutations. Using peripheral blood samples from relapsed/refractory CLL patients in ELEVATE-RR (NCT02477696) (median 2 prior therapies), we report clonal evolution data for patients progressing on acalabrutinib or ibrutinib (median follow-up 41 months). Paired (baseline and progression) samples were available for 47 (excluding 1 Richter) acalabrutinib-treated and 30 (excluding 6 Richter) ibrutinib-treated patients. At progression, emergent *BTK* mutations were observed in 31 (66%) acalabrutinib-treated and 11 (37%) ibrutinib-treated patients (median variant allele fraction [VAF]: 16.1% vs 15.6%). *BTK* C481S mutations were most common in both groups; T474I (n = 9; 8 co-occurring with C481) and the novel E41V mutation within the pleckstrin homology domain of *BTK* (n = 1) occurred with acalabrutinib, while neither mutation occurred with ibrutinib. L528W and A428D co-mutations presented in one ibrutinib-treated patient. Pre-existing *TP53* mutations were present in 25 (53.2%) acalabrutinib-treated and 16 (53.3%) ibrutinib-treated patients at screening. Emergent *TP53* mutations occurred with acalabrutinib and ibrutinib (13% vs 7%; median VAF: 6.0% vs 37.3%, respectively). Six acalabrutinib-treated patients and one ibrutinib-treated patient had emergent *TP53*/*BTK* co-mutations. Emergent *PLCG2* mutations occurred in 3 (6%) acalabrutinib-treated and 6 (20%) ibrutinib-treated patients. One acalabrutinib-treated patient and 4 ibrutinib-treated patients had emergent *BTK*/*PLCG2* co-mutations. While common *BTK* C481 mutations were observed with both treatments, patterns of mutation and co-mutation

frequency, mutation VAF, and uncommon *BTK* variants varied with acalabrutinib (T474I and E41V) and ibrutinib (L528W, A428D) in this patient population.

Keywords: Bruton tyrosine kinase, mutation, disease progression

INTRODUCTION

Covalent Bruton tyrosine kinase inhibitors (BTKis) are highly effective in the treatment of chronic lymphocytic leukemia (CLL) and have resulted in a paradigm shift in the management of the disease.[1] However, disease progression in patients receiving covalent BTKis eventually occurs in most patients and is often characterized by B-cell receptor pathway mutations at relapse, which commonly occur in the *BTK* and *PLCG2* genes.[2-8] *BTK* mutations often occur at the C481 residue and disrupt binding to, and inactivation of, *BTK* by all covalent BTKis.[9-11] C481 mutations preclude irreversible binding of ibrutinib to BTK, resulting in a greatly reduced drug potency; these mutations were subsequently associated with resistance to acalabrutinib and zanubrutinib.[10,12,13] This has led to the development of non-covalent BTKis designed to avoid the resistance mechanisms associated with these mutations.[14] Mutations in *PLCG2*, which acts downstream of BTK, also allow for B-cell receptor signaling irrespective of BTK inhibition,[10] which also may affect efficacy of non-covalent BTKis.[15]

Ibrutinib is a first-generation covalent BTKi first approved in 2013 for relapsed/refractory (R/R) mantle cell lymphoma (MCL), and, subsequently, other B-cell malignancies (chronic lymphocytic leukemia [CLL], Waldenström macroglobulinemia, and marginal zone lymphoma).[16,17] Acalabrutinib is a selective next-generation covalent BTKi first approved for R/R MCL in 2017 and for CLL in 2019.[18,19] In the head-to-head ELEVATE-RR trial (NCT02477696), which comprised a population of patients with R/R CLL and higher-risk genetic features [del(17p) and/or del(11q)], acalabrutinib demonstrated noninferior progression-free survival (PFS) with an improved safety and tolerability profile, including fewer cardiovascular adverse events, vs ibrutinib. However,

to our knowledge, no data to date have directly compared the mutational profiles of patients who progress on acalabrutinib and ibrutinib. Herein, we report comparative clonal evolution of genes implicated in resistance, including but not limited to *BTK* and *PLCG2,* in patients with CLL progression on acalabrutinib or ibrutinib in the ELEVATE-RR clinical trial.

METHODS

Study design

The study design and primary results of ELEVATE-RR have been published previously.[20] Briefly, in this phase 3, randomized, multicenter, open-label, noninferiority trial, eligible patients were adults with previously treated CLL, an Eastern Cooperative Oncology Group performance status ≤2, and presence of del(17p) and/or del(11q). Cytogenic testing of peripheral blood was performed by a central laboratory using fluorescence in situ hybridization (FISH) and stimulated karyotyping. Complex karyotype was defined based on the patient having ≥3 chromosomal abnormalities and ≥1 structural abnormalities. The study was conducted in accordance with local laws, the protocol, the Declaration of Helsinki, and International Conference on Harmonisation Guidelines for Good Clinical Practices. All patients provided written informed consent. Patients were randomly assigned to receive acalabrutinib 100 mg twice daily or ibrutinib 420 mg once daily until disease progression or unacceptable toxicity.

Mutational analysis

Peripheral blood samples were collected at baseline and at relapse. DNA was extracted from enriched CD19+ cells (RoboSep) and subjected to a 50-gene AmpliSeq next-generation sequencing assay (LifeTech)[6] covering the full *BTK* and *PLCG2* coding region with a mean depth of 2000−4000 reads, producing a validated sensitivity cutoff of 0.5% variant allele fraction (VAF) for resistance–associated mutations. Forty-eight other genes associated with CLL were assessed at 1–2% VAF (based on call depth/quality), including full coding regions of *ASXL1*, *B2M*, *BCL2*, *BCOR*, *BCORL1*, *BIRC3*, *BRAF*, *CARD11*, *CXCR4*, *DDX41*, *DNMT3A*, *ELANE*, *EZH2*, *ETV6*, *FBXW7*, *GATA2*, *GNA13*, *KLF2*, *KRAS*, *MAP2K1*, *MEF2B*, *NOTCH1*, *NOTCH2*, *NRAS*, *PIM1*, *POT1*, *PTEN*, *PTPRD*, *SAMHD1*, *SETD2*, *SF3B1*, *SH2B3*, *STAT6*, *TERC*, *TERT*, *TET2*, *TP53*, and *ZRSR2* and the recurrently mutated regions (from public variant databases) of *CD79B*, *CREBBP*, *KIT*, *MYD88*, *PIK3CA*, *PIK3CD*, *PIK3CG*, *RPS15*, *U2AF1*, and *XPO1*. Mutational data were examined in relation to PFS, which was defined as the time from random assignment to disease progression or death from any cause. Data cutoff was the same as the published primary analysis (September 15, 2020).

Statistical analysis

Survival analysis using Kaplan-Meier analysis was performed to determine the median time of PFS between acalabrutinib and ibrutinib for patients who developed mutations during the trial. Proportional-hazards Cox regression analysis was used to calculate the hazard ratio and corresponding *P*-value to assess whether a significantly increased risk

of developing a mutation in one treatment arm vs the other existed. Median VAFs were calculated as the median of the maximum VAF values derived by gene mutation and by subject. For genes with mutations present in both treatments in ≥ 2 subjects, *P*-values were calculated using Wilcoxon rank sum test to determine significant difference at 95% confidence.

The study protocol and informed consent were approved by the appropriate Institutional Review Board (IRB)/Independent Ethics Committee (IEC) at each of the study sites before initiation of the study and during the study.

RESULTS

Patients

In total, 268 and 265 patients were randomly assigned to receive acalabrutinib and ibrutinib, respectively. Demographics and baseline characteristics were reported previously.[20] At baseline, 45.1% and 45.3% of acalabrutinib- and ibrutinib-treated patients, respectively, had del(17p), 62.3% and 66.0% had del(11q), 37.3% and 42.3% had *TP53* mutations, 82.1% and 89.4% had unmutated immunoglobulin heavy chain variable region genes (IGHV), and 46.3% and 47.2% had a complex karyotype.[20,21]

Mutation analysis

Paired (baseline and progression) samples were available and included in the analysis for 47 of 82 (57.3%) and 30 of 68 (44.1%) patients who experienced disease progression while receiving acalabrutinib and ibrutinib, respectively (**Figure S1**). One

additional acalabrutinib-treated patient and 6 additional ibrutinib-treated patients who had Richter transformation as their mode of progression were excluded from analysis; mutational analysis for these patients can be found in **Figures S2** and **S3**. Full data (excluding patients with Richter transformation) including mutations at screening and emergent mutations for both treatment arms are presented in **Figure 1**. The most common mutations at screening were *TP53* mutations in both groups (**Figure S4**). Baseline cytogenetics for patients included in this analysis are summarized in **Table 1**. A summary of the change in mutations by the end of treatment is presented in **Figure S5**. Among patients with paired samples, the median time to progression was numerically longer for acalabrutinib vs ibrutinib (32.9 vs 21.9 months, respectively), but the PFS hazard ratio was not significantly different between treatment groups (**Figure S6**).

No *BTK* mutations were observed at screening. Acquired *BTK* mutations were observed in 31 (66.0%) acalabrutinib-treated patients and 11 (36.7%) ibrutinib-treated patients at time of progression (**Table 2**). The median VAF for *BTK* mutations was not significantly different in the acalabrutinib group (16.1%) vs the ibrutinib group (15.6%; **Table S1**). When analyzed by *BTK* mutation status in both treatment arms combined, time to progression was significantly (P = .03) longer in patients with vs without a *BTK* mutation (**Figure 2**). Among those with acquired *BTK* mutations, 29 of 31 (93.5%) acalabrutinib-treated and 10 of 11 (90.9%) ibrutinib-treated patients had C481S mutations; 2 (6.5%) and 2 (18.2%) had C481F mutations; 2 (6.5%) and 1 (9.1%) had C481Y mutations; and 1 (3.2%) and 1 (9.1%) had C481R mutations (**Figure 3A**). The VAF for C481S mutations ranged from 0.7% to 95.6% with acalabrutinib and from 2.0%

to 67.3% with ibrutinib (**Figure 3B**). Nine of 31 (29.0%) acalabrutinib-treated patients had T474I mutations (gatekeeper mutation), only one of which did not co-occur with *BTK* C481 mutations; VAF ranged from 0.5% to 4.5%. A novel E41V mutation within the pleckstrin homology (PH) domain of *BTK* was seen in one acalabrutinib-treated patient, with a VAF of 16.1% (**Figure 3A** & **Figure 3B**). Initial preclinical investigation in TMD8 cells suggests this E41V mutation may not independently confer resistance to acalabrutinib (see methodology and results in **Figure S7**). In the ibrutinib group, L528W (kinase-dead mutation) and A428D co-mutations were observed in one patient (and did not co-occur with C481S mutation), with VAFs of 4.6% and 8.7%, respectively, and one patient had C481W mutation (VAF 4.2%; **Figure 3A** & **Figure 3B**). No statistical difference was seen in the proportions of acalabrutinib- or ibrutinib-treated patients who acquired *BTK* mutations among patients with del(17p), del(11q), complex karyotype, unmutated IGHV, or trisomy 12 positivity (**Table 2**).

No *PLCG2* mutations were observed at screening. Emergent *PLCG2* mutations occurred in 3 (6.4%) acalabrutinib-treated and 6 (20.0%) ibrutinib-treated patients (*P* = .142; **Table 2**), with median VAFs of 1.9% and 9.5%, respectively (**Table S1**); only one acalabrutinib-treated patient had co-occurrence of *BTK* and *PLCG2* mutations vs 4 ibrutinib-treated patients (**Figure 3A**). One patient in the acalabrutinib group and 3 in the ibrutinib group had ≥4 co-occurring *PLCG2* mutation variants. The most common acquired *PLCG2* variants in acalabrutinib- and ibrutinib-treated patients, respectively, were M1141K (1 vs 3 patients), S707F (0 vs 3 patients), D993H (0 vs 3 patients), and R665W (2 vs 2 patients; **Figure 3A**). All other variants occurred in one patient each.

Pre-existing *TP53* mutations were present in 25 (53.2%) and 16 (53.3%) patients at screening, among whom 1 acalabrutinib-treated patient and 2 ibrutinib-treated patients lost *TP53* mutation by end of treatment (all 3 had del(17p) at baseline; **Figure S5**). After *BTK*, *TP53* mutations were the next most frequent emergent mutation in the acalabrutinib arm (12.8%; n=6); 2 patients (6.7%) in the ibrutinib arm had emergent *TP53* mutations (**Table 2**); the median VAF was 6.0% in the acalabrutinib arm and 37.3% in the ibrutinib arm (**Table S1**). The VAF for *TP53* mutations at screening and end of treatment for each patient is shown in **Figure S8**. Six acalabrutinib-treated patients and one ibrutinib-treated patient had *TP53* and *BTK* co-mutations (the one ibrutinib-treated patient had co-occurring *TP53*, *BTK*, and *PLCG2* mutations) (**Figure S9**).

Additional emergent mutations observed with both acalabrutinib and ibrutinib were *DNMT3A* and *TET2* (**Table 2**); among the few patients who had mutations in these genes at screening, these mutations were lost by end of treatment (**Figures S4** and **S5**). Data regarding additional mutations and associated VAF at screening and end of treatment are shown for the *SF3B1* gene in **Figure S10** and for the *RPS15*, *BIRC3*, and *NOTCH1* genes in **Figure S11**.

DISCUSSION

This analysis of the ELEVATE-RR study provided an opportunity to further characterize the profile of mutations observed in patients who progress on acalabrutinib and ibrutinib within a well-defined cohort of patients. Overall, the most common emergent mutation with both treatments, *BTK* C481S, was similar; however, the distribution of other

mutations, including their respective VAFs, varied between treatment arms. Not all patients who progressed had a mutation in *BTK* C481, but in those who did, the VAF was highly variable; therefore, other mechanisms of resistance may exist. Baseline cytogenetics did not appear to result in differences between treatment arms in the proportion of patients with *BTK* mutations. No particular pattern regarding the proportion of patients with non-*BTK* mutations was observed in either treatment arm. Emergent *BTK* mutation was associated with longer time to progression compared with absence of *BTK* mutation.

The rate of emergent *BTK* mutations reported with acalabrutinib at relapse in our study (66%) was consistent with the rate of *BTK* mutations at relapse reported previously with acalabrutinib in a single-center study of patients with treatment-naive or R/R CLL (69%).[12] However, the proportion of emergent *BTK* mutations seen in the ibrutinib arm (37%) at relapse in our study was much lower than previously reported in the literature (49%−67%), including real-world evidence.[11,22] A greater number of samples were available for analysis in the acalabrutinib arm vs ibrutinib arm (n = 47 vs n = 30) and a greater number of patients in the ibrutinib arm (n = 6) were also excluded vs the acalabrutinib arm (n = 1) from the analysis due to Richter transformation, resulting in a greater proportion of paired samples being included for acalabrutinib (57% of progressed patients) compared with ibrutinib (44% of progressed patients), which could have impacted the results from our analysis. With covalent BTKis, C481 mutations are typically the most common resistance mutations encountered,[11] and the majority (> 50%) of *BTK* mutations in both treatment groups of the current study were C481 mutations.

Mutations occurring at codon T474 are considered a gatekeeper change because they often interfere with BTKi (both covalent and non-covalent) binding to BTK, allowing for normal B-cell signaling.[23] In patients with ibrutinib resistance, the T474I mutation has been previously observed co-occurring with the C481S mutation.[7] Co-occurring mutations in *BTK* have been observed as a potential additional escape mechanism for BTKis based on preclinical data.[24] Both of these mutations are considered kinase proficient, still allowing BTK kinase activity in the presence or absence of BTKis.[25] T474I mutations were observed in 9 acalabrutinib-treated patients in our study, albeit at low VAF. In all but one of these patients, *BTK* C481S was also present.

The L528W mutation results in a kinase-dead BTK, hindering BTK catalytic activity; however, B-cell signaling is thought to continue via a BTK scaffolding mechanism that recruits other kinases for B-cell signaling.[25,26] A recent study of the covalent BTKi zanubrutinib also identified *BTK* C481 mutations in 5 of 8 patients with zanubrutinib resistance, one of whom also harbored an L528W mutation.[13] Another study showed that the L528W mutation was more prevalent in patients with CLL who had disease progression while receiving zanubrutinib compared with those receiving ibrutinib.[27] Similarly, mutational analysis of the phase 3 ALPINE study showed L528W mutation in 2 of 5 patients with *BTK* mutations treated with zanubrutinib, and none of the 3 patients with *BTK* mutations treated with ibrutinib.[28] In the current study, L528W mutation was observed in one ibrutinib-treated patient and no acalabrutinib-treated patients. We also observed a novel E41V mutation at relapse in one acalabrutinib-treated patient but no ibrutinib-treated patients. A previous in vitro study in murine NIH

3T3 cells demonstrated the E41K mutation to be a BTK-activating mutation.[29] Mutations at this location in the PH domain of BTK have resulted in higher binding affinity for inositol 1,2,3,4,5,6-hexakisphosphate (IP_6), which may be involved in hematopoietic cell differentiation by activating the BTK/Tec/ITK family.[30] To our knowledge, our study is the first time a *BTK* mutation at this residue has been observed in a treated population. The clinical relevance of the VAF of the specific mutations discussed above (C481, T474I, L528W, and E41K), however, is not well understood and is an area for further research.

Regarding other gene mutations observed in our study, *PLCG2* mutations have been shown previously to confer resistance to ibrutinib by promoting B-cell receptor signaling despite continued inhibition of BTK by ibrutinib.[10] *PLCG2* mutations were the second most frequent emergent mutations observed with ibrutinib after *BTK* mutations in our study, although the difference between treatment arms was not significant (P = .142). Certain *PLCG2* variants have been shown previously to be associated with ibrutinib resistance[31] and appeared with both acalabrutinib and ibrutinib in our analysis; *PLCG2* mutations R665W and M1141K were reported in both arms, while other mutations were seen only in the ibrutinib arm (S707F, D993H, D993Y, L845F, L845V) or only in the acalabrutinib arm (S707Y, D1140N). *TP53* mutations were the second most common emergent mutations in acalabrutinib-treated patients after *BTK* mutations. While *TP53* mutation typically predisposes patients to relapse, *TP53* mutation is not a known cause of disease progression with BTKi therapy, whereas mutated *BTK* is often associated with relapse.[32] There were 3 patients whose pre-existing *TP53* mutations were no longer detectable at progression (1 treated with acalabrutinib and 2 treated with ibrutinib) and all 3 patients had 17p deletion. Loss of

pre-existing *TP53* mutations has been observed previously in ibrutinib-treated patients.[22]

Despite shared resistance mutations such as C481S, differing patterns of mutation frequency, mutation VAFs, and uncommon *BTK* variants were observed with acalabrutinib vs ibrutinib in this R/R CLL population. For example, T474I occurred with acalabrutinib, but not with ibrutinib. This analysis established a mutational profile in this population utilizing a unique comparative data set; however, because of the limited sample size, the clinical significance of the mutations data reported herein is not known. In addition, for approximately 33% of patients with disease progression, no emergent mutations in *BTK* were detected, and these patients had shorter PFS, suggesting additional research is needed to better understand mechanisms of resistance that may occur outside the B-cell receptor pathway. It is also not clear whether the higher-risk genomic features of patients in ELEVATE-RR contributed to genetic instability or impacted the generalizability of the results. With limited data, the mechanism of resistance to BTKi is becoming increasingly complex. The ability to clinically sequence covalent to non-covalent BTKis may be dependent on the combination of co-occurring mutations that impart resistance to covalent and non-covalent BTKis and the VAF of each mutational clone in the tumor. It will become increasingly important to further understand the patterns of mutations commonly observed with the various BTKis in order to best optimize and appropriately sequence these important drugs for their maximal clinical benefit.

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Authorship

Contribution:

Study design: JCB

Study investigator: JAW, WJ, TR, ÁI, APK, PG, JCB, JFS

Provided patients or study materials: JAW, WJ, TR, ÁI, APK, PG, JCB, JFS, RL

Collection and assembly of data: DJ, WJ, TR, ÁI, JCB, SL, SB, TL, GD, RL

Data analysis: DJ, JCB, SL, SB, NM, GD

Data interpretation: JAW, DJ, ÁI, APK, PG, JCB, JFS, SB, TL, NM, GD

Manuscript preparation: JAW, DJ, WJ, ÁI, PG, JCB, TL, NM, GD

All authors participated in the critical review and revision of this manuscript and provided approval of the manuscript for submission.

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Tables

Table 1. Baseline genetics

Baseline cytogenetics, n (%)	Acalabrutinib 100 mg BID (n = 47)	Ibrutinib 420 mg QD (n = 30)	*P*-value
11q deletion	32 (68.1)	16 (53.3)	.232
17p deletion	20 (42.6)	19 (63.3)	.103
IGHV unmutated	42 (89.4)	28 (93.3)	.699
Complex karyotype	25 (53.2)	16 (53.3)	1
TP53 mutation	24 (51.1)	16 (53.3)	1
Trisomy 12 positive	2 (4.3)	5 (16.7)	.103

BID, twice daily; IGHV, immunoglobulin heavy chain variable region genes; QD, once daily.

Table 2. Emergent mutations summary

Gene, n (%)	Acalabrutinib 100 mg BID (n = 47)	Ibrutinib 420 mg QD (n = 30)	*P*-value
BTK	31 (66.0)	11 (36.7)	.0185
By baseline cytogenetics*			
11q deletion	24 (77.4)	5 (45.5)	.0664
17p deletion	12 (38.7)	7 (63.6)	.18
IGHV unmutated	28 (90.3)	11 (100.0)	.554
Complex karyotype	18 (58.1)	8 (72.7)	.485
Trisomy 12 positive	1 (3.2)	2 (18.2)	.163
TP53	6 (12.8)	2 (6.7)	.472
DNMT3A	5 (10.6)	1 (3.3)	.395
PLCG2	3 (6.4)	6 (20.0)	.142
ASXL1	1 (2.1)	0	–
KRAS	1 (2.1)	0	–
NRAS	1 (2.1)	0	–
PPM1D	1 (2.1)	0	–
RPS15	1 (2.1)	0	–
SAMHD1	1 (2.1)	0	–
TET2	1 (2.1)	1 (3.3)	1
XPO1	1 (2.1)	0	–
FBXW7	0	1 (3.3)	–
NOTCH2	0	1 (3.3)	–
NOTCH1	0	2 (6.7)	–
POT1	0	1 (3.3)	–
Any emergent non-BTK mutation†	14 (29.8)	10 (33.3)	.804
Any emergent mutation‡	36 (76.6)	16 (53.3)	.0464

*Percentages based on patients with *BTK* mutation.
†Patients who had any newly emergent mutation during treatment in non-*BTK* genes (ie, a new mutation that was not present at baseline, excluding patients with *BTK* mutations).
‡Patients who had any newly emergent mutation during treatment in any gene including *BTK* (ie, a new mutation that was not present at baseline).
BID, twice daily; BTK, Bruton tyrosine kinase; IGHV, immunoglobulin heavy chain variable region genes; QD, once daily.

Figure Legends

Figure 1. Mutations by treatment arm. BID, twice daily; BTK, Bruton tyrosine kinase; IGHV, immunoglobulin heavy chain variable region genes; QD, once daily.

Figure 2. Progression-free survival by *BTK* mutation status. BTK, Bruton tyrosine kinase.

*Kaplan-Meier survival estimate.

Figure 3. Emergent *BTK* and *PLCG2* variants (A) and emergent *BTK* mutation variant allele frequency (B). BID, twice daily; BTK, Bruton tyrosine kinase; IGHV, immunoglobulin heavy chain variable region genes; QD, once daily; VAF, variant allele frequency.

Figure 1

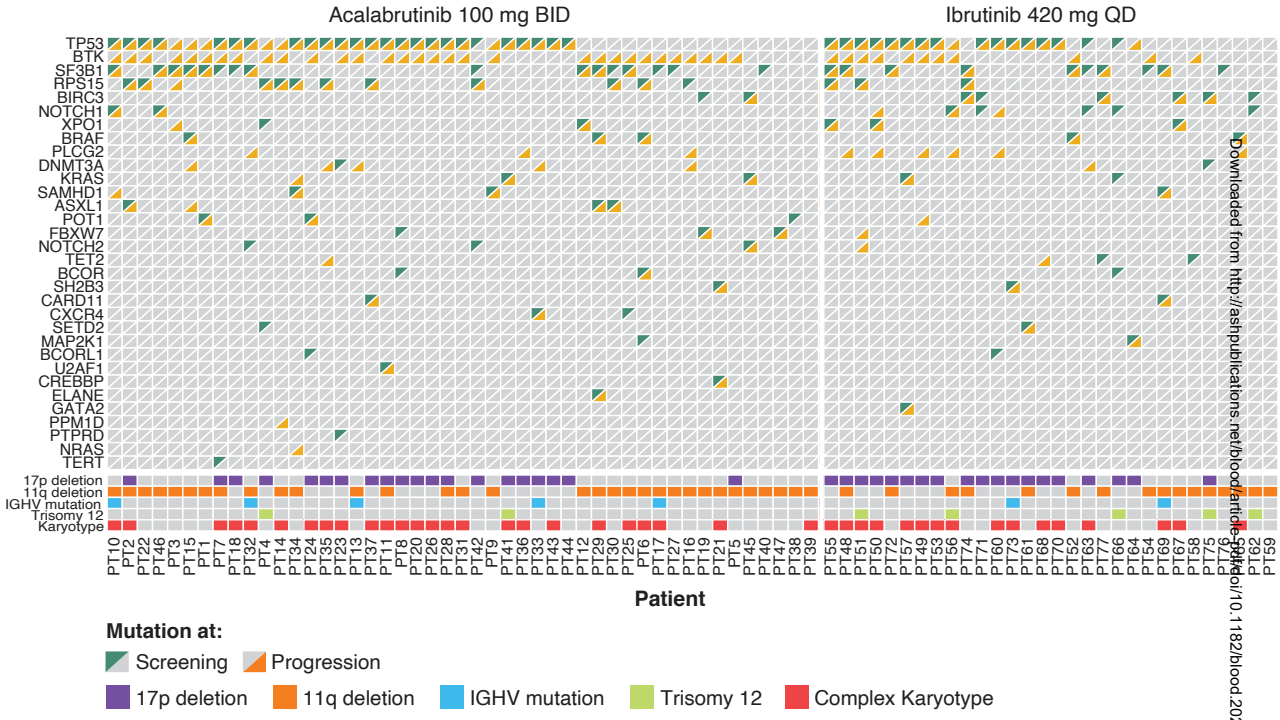


Figure 2

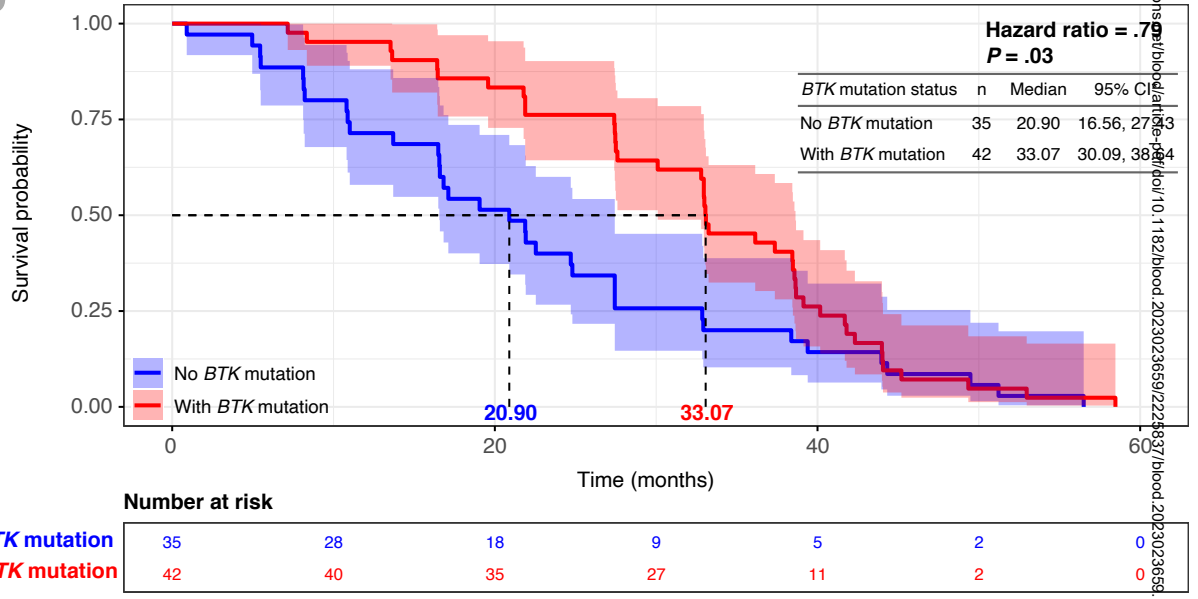


Figure 3

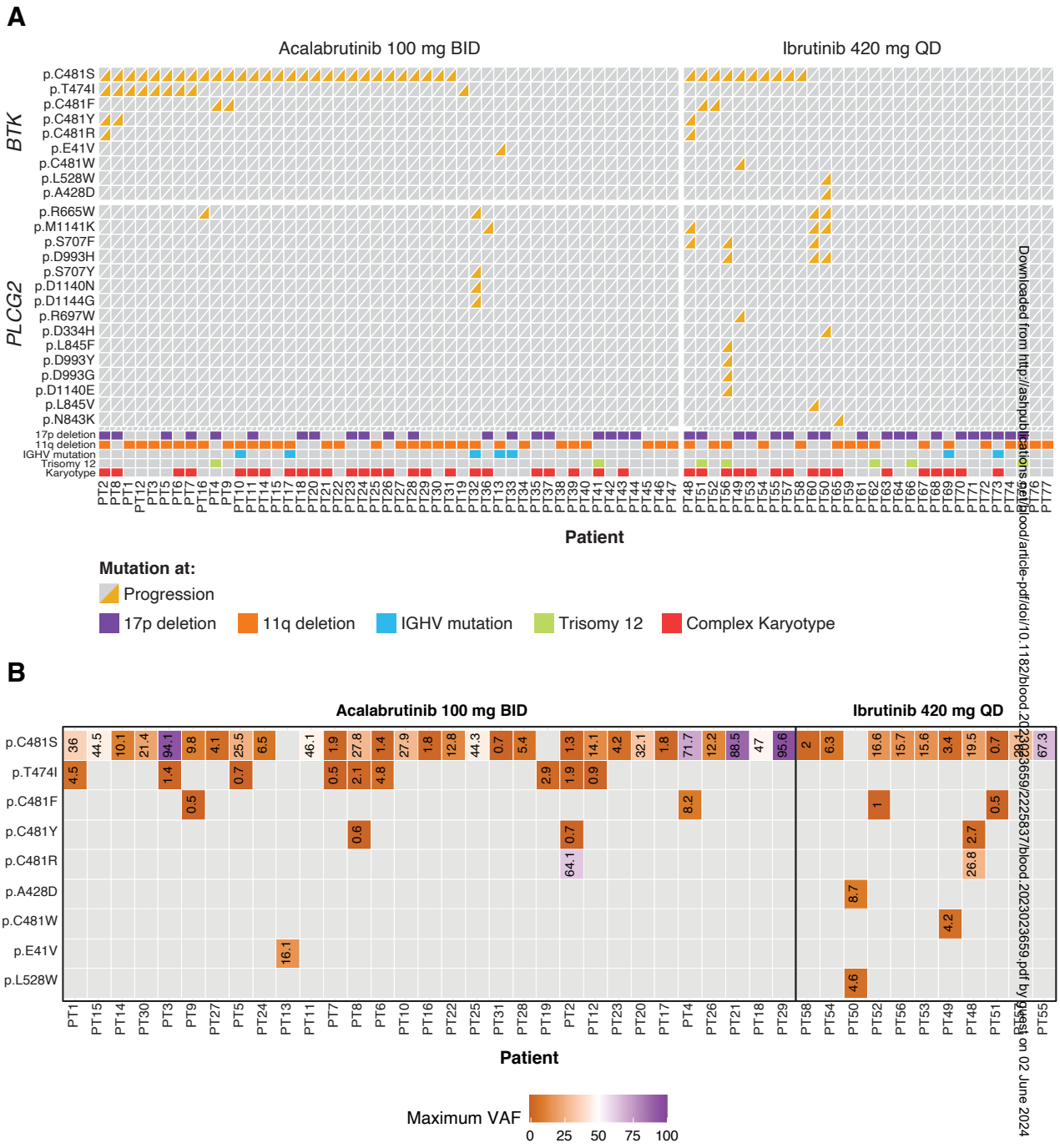


Figure 3

Mutational Profile of Patients With Previously Treated CLL Who Progressed on Acalabrutinib or Ibrutinib in ELEVATE-RR

Acalabrutinib 100 mg PO BID n=268 OR **Ibrutinib** 420 mg PO QD n=265

Mutational analysis
Paired samples
(baseline/progression)

Acalabrutinib n=47 | **Ibrutinib** n=30

Overall, median time to progression was numerically longer for acalabrutinib vs ibrutinib (32.9 vs 21.9 mo)

Pooling data from both treatment groups, time to progression was significantly longer in patients with vs without a *BTK* mutation (33.1 vs 20.9 mo)

***BTK* mutation (acalabrutinib vs ibrutinib)**

66% (31/47) vs 37% (11/30)

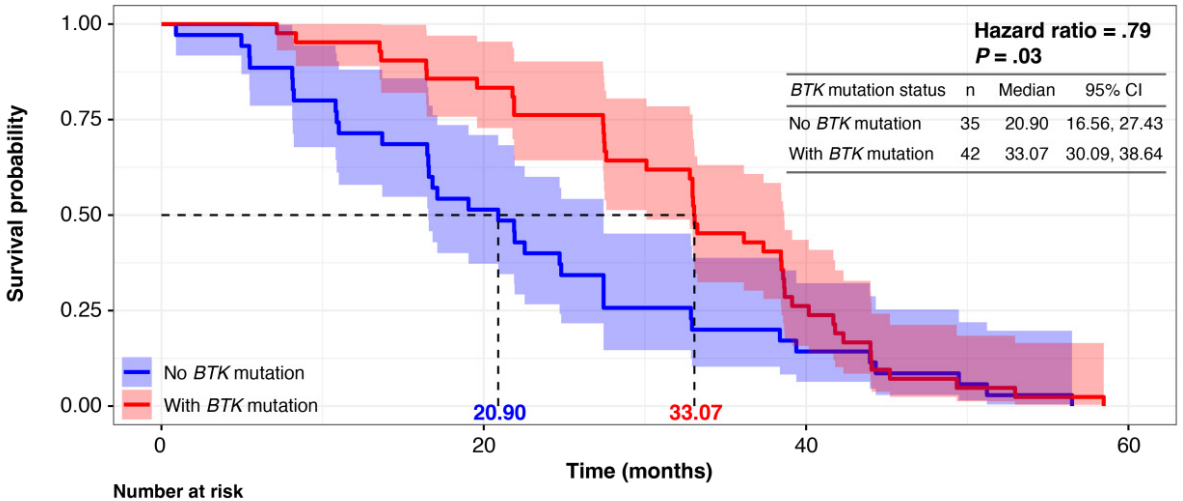
Among *BTK* mutations

***BTK* C481S**
94% (29/31) vs 91% (10/11)

***BTK* E41V**
2% (1/31) vs 0%

***BTK* T474I (gatekeeper)**
29% (9/31) vs 0%

***BTK* L528W (kinase-dead)**
0% vs 3% (1/11)



Number at risk

No *BTK* mutation	35	28	18	9	5	2	0
With *BTK* mutation	42	40	35	27	11	2	0

BID, twice daily; BTK, Bruton tyrosine kinase; CI, confidence interval; CLL, chronic lymphocytic leukemia; QD, once daily; PO, orally; VAF, variant allele fraction.

***BTK* C481S was most common in both groups; in the acalabrutinib arm, low-VAF T474I (n=9/47, 8 co-occurring with C481S) but no L528W was seen. More patients receiving acalabrutinib acquired *BTK* mutations, though overall, patients with *BTK* mutations had longer PFS vs those without**

Ref: Woyach JA et al, Blood, DOI, PMID