

## **Acalabrutinib Plus Obinutuzumab in Treatment-Naïve and Relapsed/Refractory Chronic Lymphocytic Leukemia**

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### **Conflict of Interest**

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VM is employed by Acerta Pharma, a member of the AstraZeneca Group and an immediate family member is employed by Gilead Sciences; and has stock or other ownership with AstraZeneca and Gilead Sciences.

MG, RI, CQ, and MHW are employed by Acerta Pharma, a member of the AstraZeneca Group; and have stock or other ownership with AstraZeneca.

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### **Abstract (150/150 words)**

Acalabrutinib is a selective irreversible Bruton tyrosine kinase inhibitor that does not affect interleukin-2 associated tyrosine kinase or antibody-dependent cellular cytotoxicity, making it an attractive candidate for combination therapy with anti-CD20 antibodies. We investigated acalabrutinib plus obinutuzumab in a phase 1b/2 study (NCT02296918) of patients with treatment-naïve or relapsed/refractory chronic lymphocytic leukemia (CLL). Nineteen treatment-naïve and 26 relapsed/refractory patients were treated with acalabrutinib (100 mg twice daily) until progression and obinutuzumab (cycle 1: 100 mg day 1, 900 mg day 2, 1000 mg days 8 and 15; cycles 2-6: 1000 mg day 1). Grade 3/4 adverse events occurred in 71% of patients. Overall response rates were 95% (treatment-naïve) and 92% (relapsed/refractory). Thirty-two percent of treatment-naïve and 8% of relapsed/refractory patients achieved complete remission. At 36 months, 94% (treatment-naïve) and 88% (relapsed/refractory) were progression free. Acalabrutinib plus obinutuzumab was well tolerated, producing high and durable responses in treatment-naïve and relapsed/refractory CLL.

### **Statement of significance (50/50 words)**

Rituximab plus the less selective BTK inhibitor ibrutinib has not shown benefit in CLL; however, the selective BTK inhibitor acalabrutinib plus antibody-dependent cellular cytotoxicity-enhanced antibody obinutuzumab yielded durable responses that deepened over time in treatment-naïve and relapsed/refractory CLL, supporting the evaluation of this approach in larger, comparative studies in CLL.

## Introduction

Treatment of chronic lymphocytic leukemia (CLL) has been transformed by the introduction of targeted agents that inhibit the B-cell receptor signaling pathway. Bruton tyrosine kinase (BTK), an essential component of B-cell receptor signaling, is constitutively active in CLL and is essential for multiple survival pathways that are important in CLL (1). Inhibition of BTK by the oral agent ibrutinib has been shown to lead to abrogation of CLL cell signaling, proliferation, homing, and adhesion in vitro and in patients (1-7). In patients, this has translated to high response rates and durable remissions. Clinical trials have shown improved overall response rates (ORRs), progression-free survival (PFS), and overall survival (OS) with ibrutinib compared with standard therapy in the relapsed (8) and frontline settings (9-11).

Despite these promising results, real-world data suggest that many patients discontinue ibrutinib due to toxicity (12). Common causes for discontinuation are arthralgias, atrial fibrillation, and bleeding. These adverse effects are hypothesized to be the result of alternative irreversible and reversible targets of ibrutinib because they are not reported in high frequency in patients with x-linked agammaglobulinemia (13,14). In addition to their potential roles in toxicity, alternative targets of ibrutinib may diminish the drug's ability to be successfully combined with anti-CD20 antibodies, a mainstay of CLL therapy (15).

Acalabrutinib is a highly selective, potent, covalent inhibitor of BTK with minimal off-target activity (16,17). Due to the short half-life, the drug can safely be dosed twice daily, resulting in sustained high occupancy of BTK that is hypothesized to lead to longer remission durations (16). The reported outcomes in patients with CLL are promising, with an ORR of 93% (including partial response with lymphocytosis [PRL]) and an 18-month PFS rate of 90% (95% CI, 83%-94%) (18). The high selectivity of acalabrutinib may reduce the likelihood of off-target effects leading to toxicity. Although the results of a comparative trial between acalabrutinib and ibrutinib are not yet available, single-agent studies of acalabrutinib have shown lower rates of atrial fibrillation, hypertension, and arthralgias than similar studies with ibrutinib (18,19). Additionally, the greater selectivity of acalabrutinib leads to less interference with antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC) as interleukin 2-associated tyrosine kinase (ITK) is not inhibited. This may allow more effective combination with anti-CD20 antibodies (17,20).

Given the promising clinical data with acalabrutinib monotherapy, we conducted the phase 1b/2 ACE-CL-003 trial of acalabrutinib in combination with obinutuzumab in patients with relapsed/refractory and treatment-naïve CLL. Here we present the results of this study at a median follow-up of 3.5 years.

## **Results**

### *Patients and Baseline Characteristics*

A total of 45 patients were enrolled and treated at a single center, including 19 patients with treatment-naïve CLL (1 patient had small lymphocytic lymphoma) and 26 patients with relapsed/refractory CLL. Full clinical characteristics can be found in **Table 1**. Among patients with treatment-naïve CLL, the median age was 61 years (range, 42-75 years). Fifty-three percent of patients were advanced Rai stage, and 53% (9/17) had unmutated immunoglobulin heavy-chain variable region gene (*IGHV*). Twenty-two percent (4/18) of patients had del(17)(p13.1), and 28% (5/18) had del(11)(q22.3) without or missing del(17)(p13.1). Baseline tumor protein p53 gene (*TP53*) mutation was seen in 28% (5/18) of patients, and 21% (4/19) of patients had del(17)(p13.1) and *TP53* mutation. Forty-two percent (8/19) had a complex karyotype, defined as 3 or more independent abnormalities. CLL International Prognostic Index (CLL-IPI) scores were low or intermediate risk in 37%, high risk in 21%, and very high risk in 21%.

Among patients with relapsed/refractory CLL, the median age was 63 years (range, 42-76 years). The median number of prior therapies was 1 (range, 1-9). No patients had previously received a BTK inhibitor or a BCL2 inhibitor; 1 patient had received a PI3K inhibitor (**Supplemental Table 1**). Forty-six percent of patients had previously received a nucleoside analog, and 1 patient had a previous allogeneic stem cell transplant. Sixty-five percent (17/26) had unmutated *IGHV*, 20% (5/25) had del(17)(p13.1), and 35% (9/26) had del(11)(q22.3) without or missing del(17)(p13.1). *TP53* mutation was observed in 25% (6/24) of patients, and 12% (3/26) patients had del(17)(p13.1) and *TP53* mutation. Complex karyotype was observed in 56% (14/25) of patients. CLL-IPI scores were low or intermediate risk in 42%, high risk in 23%, and very high risk in 12%.

#### *Patient Disposition*

In patients with treatment-naïve CLL, the median follow-up was 39 months (range, 1-45 months). Eighty-nine percent of patients in this cohort completed all planned doses of obinutuzumab, and 89% remain on acalabrutinib therapy, with 58% requiring at least 1 dose interruption of acalabrutinib for an adverse event (AE). Two patients required dose de-escalation for grade 3 toxicity (grade 3 neutropenia [n=1] and grade 3 peripheral edema [n=1]). Two patients discontinued acalabrutinib, with 1 discontinuation due to Richter's transformation, and 1 due to an AE.

In patients with relapsed/refractory CLL, the median follow-up was 42 months (range, 20-49 months). All patients completed all doses of obinutuzumab, and 69% remain on acalabrutinib. Sixty-two percent of relapsed/refractory patients required a dose

interruption of acalabrutinib for an AE. One patient required dose de-escalation for grade 2 neutropenia. Eight patients discontinued acalabrutinib, with 2 discontinuations due to Richter's transformation, 4 due to AEs, 1 due to progressive disease, and 1 due to death.

Richter's transformation occurred in 1 treatment-naïve patient at 15.8 months and in 2 relapsed/refractory patients at 18.4 and 24.0 months. Two of 3 patients (1 treatment-naïve and 1 relapsed/refractory) had del(17)(p13.1) and unmutated *IGHV*. All 3 patients had complex karyotype, and none had del(11)(q22.3).

### *Pharmacodynamics*

Dosing with 100 mg acalabrutinib twice daily provided greater target coverage overall (96%-99%) compared with 200 mg once daily (87%-95%;  $P < 0.05$  for all timepoints compared; **Figure 1A**). Acalabrutinib treatment resulted in pronounced inhibition of BTK phosphorylation and a reduction of total BTK protein levels in the tumor CD19+CD5+ cell subset (**Figures 1B and C**). Obinutuzumab did not affect acalabrutinib binding to BTK, downstream kinase function, or protein levels. No clinically significant changes in T cell (CD4+, CD8+), natural killer cell (CD16+/CD56+), or monocyte (CD14+) numbers were observed in any treated patients (**Figure 2A-D**); similar results were noted for the treatment-naïve and relapsed/refractory cohorts. Immunoglobulin A and G levels did not change appreciably over time, while immunoglobulin M levels gradually decreased (**Supplemental Figure 1A-C**).

## *Efficacy*

Using International Workshop on CLL (iwCLL) 2008 guidelines, the ORR was 95% (95% CI, 74%-100%) for patients with treatment-naïve CLL and 92% (95% CI, 75%-99%) for those with relapsed/refractory CLL. ORR including PRL was 100% in relapsed/refractory CLL, and no PRLs were observed in treatment-naïve CLL. Complete responses (CRs) were achieved by 32% of patients with treatment-naïve CLL and 8% of patients with relapsed/refractory CLL. All responding treatment-naïve patients (18/19) achieved at least partial response (PR) by the first disease assessment (2.8 months). The median time to  $\geq$ PR in relapsed/refractory patients was also 2.8 months (range, 2.7-21.4). The median time to  $\geq$ CR was 18.4 months (range, 5.6-32.3) in treatment-naïve patients and 12.9 months (range, 10.2-15.7) in relapsed/refractory patients.

Bone marrow biopsies were performed at 12 months and repeated thereafter only for patients with minimal residual disease (MRD)–negative peripheral blood assessed by flow cytometry with evidence of CR on computed tomography (CT) scan. At 12 months, MRD negativity in the bone marrow was attained by 3 of the 6 patients (50%) in the treatment-naïve group who had a CR and by none of the 2 patients in the relapsed/refractory group who had a CR. Interestingly, 2 of the 12 treatment-naïve patients who achieved PR (17%) and 4 of the 22 relapsed/refractory patients who achieved PR (18%) achieved bone marrow MRD negativity at 12 months. CR rates may have been underestimated, as MRD was monitored in peripheral blood every 3 cycles, and some patients with low-level blood disease ( $>0.01\%$  to  $<1\%$  CLL cells) would likely have had an MRD-positive CR rather than a PR if MRD had been evaluated by bone

marrow. At the time of last evaluation, 69% of patients in the total population (31/45) had lymph nodes >1.5 cm on CT scan; however, 47% (21/45) had lymph nodes <2 cm.

Median PFS (**Figure 3A**) and median OS have not been reached for either cohort (**Figure 3B**). For patients with treatment-naïve CLL, PFS was 94.4% (95% CI, 66.6%-99.2%) and OS was 100% (95% CI, 100%-100%) at 39 months. For patients with relapsed/refractory CLL, PFS was 72.7% (95% CI, 43.8%-88.4%) and OS was 82% (95% CI, 57%-93%) at 42 months. PFS by CLL-IPI risk score and complex karyotype status is presented in **Supplemental Figure 2A and B**.

*TP53* mutation, *TP53* loss of copy number, or del(17)(p13.1) was present at baseline in 5/19 treatment-naïve (26%; 1/6 CRs, 3/11 PRs, and 1 patient whose responses were not assessed due to early discontinuation) and 8/26 relapsed/refractory patients (31%; 1/2 CRs and 7/22 PR/PRLs; **Figure 4A**). No enrichment of *TP53* loss (mutation or chromosome deletion) was observed in any response category or with any MRD status.

#### *Minimal Residual Disease*

At 12 months, 5/19 patients (26%) in the treatment-naïve cohort and 4/26 patients (15%) in the relapsed/refractory cohort had achieved MRD negativity in bone marrow assessed by 10-color flow cytometry using  $10^{-4}$  CLL cells/leukocyte as cut-off (**Figure 4B**). Peripheral blood MRD was measured longitudinally every 3 cycles, and MRD-based responses in the peripheral blood generally deepened over time. A trend toward

deeper MRD negativity in treatment-naïve patients was observed (**Figure 4C**), although it appears that the discontinuation of obinutuzumab temporarily increased peripheral blood tumor burden.

### *Safety*

**Supplemental Table 2** shows all treatment-emergent AEs occurring in  $\geq 20\%$  of patients. Eight patients (42%) in the treatment-naïve group and 17 patients (65%) in the relapsed/refractory group experienced headache of any grade. Infusion-related reactions of any grade occurred in 6 patients (32%) in the treatment-naïve group and 13 patients (50%) in the relapsed/refractory group, and all of these events were grade 1 or 2. There was 1 event of tumor lysis syndrome (grade 3), which occurred in the relapsed/refractory group. Grade  $\geq 3$  AEs were observed in 63% of treatment-naïve and 77% of relapsed/refractory patients (**Table 2**). Grade  $\geq 3$  AEs observed in  $>5\%$  of patients included neutrophil count decreased (n=11 [24%]), syncope (n=5 [11%]), platelet count decreased (n=4 [9%]), cellulitis (n=4 [9%]), weight increased (n=4 [9%]), hypertension (n=3 [7%]), and hypophosphatemia (n=3 [7%]). Serious AEs (SAEs) occurred in 16% of treatment-naïve and 46% of relapsed/refractory patients. The most commonly reported SAEs ( $\geq 2$  patients) were cellulitis (n=4 [9%]), diarrhea (n=2 [4%]), dyspnea (n=2 [4%]), pneumonia (n=2 [4%]), pyrexia (n=2 [4%]), and syncope [n=2 [4%]]. One patient in the treatment-naïve cohort discontinued acalabrutinib and obinutuzumab due to an AE of metastatic squamous cell carcinoma during the first week of therapy, and 4 patients discontinued acalabrutinib in the relapsed/refractory cohort due to AEs that included grade 1 vomiting (n=1), grade 3 maculopapular rash

(n=1), lung adenocarcinoma (n=1), and grade 3 diarrhea (n=1). No grade 5 AEs were reported. Four patient deaths occurred from Richter's transformation (n=2), lung adenocarcinoma (n=1), and disease progression (n=1).

Of particular interest were toxicities commonly associated with the BTK inhibitor ibrutinib, including atrial fibrillation and bleeding (12). Atrial fibrillation was observed in 1 patient (2%; grade 3). No serious or severe ventricular arrhythmias or sudden deaths have been reported. Hypertension of any grade (either new or worsening from baseline) was seen in 40% of patients, including 3 patients (7%) with grade  $\geq 3$  events. Bleeding events (any grade) occurred in 71% of patients, most commonly ( $\geq 20\%$  of patients) contusion (n=19 [42%]), and petechiae (n=11 [24%]). Two relapsed/refractory patients (4%) had grade 3 bleeding events (hematuria [n=1] and muscle hemorrhage [n=1]).

#### *Incidence of BTK C481S Mutations*

One treatment-naïve patient developed a BTK C481S mutation (0.2% variant allele frequency) 48 months after the initiation of therapy but has no sign of clinical progression. In the relapsed/refractory cohort, 1 patient who progressed with CLL developed a BTK C481S mutation 3 months before clinical progression, and 1 patient who developed Richter's transformation developed a BTK C481S mutation at progression. Phospholipase C $\gamma$ 2 mutations were not assessed in this study.

#### *Predictors of Outcome*

We used baseline genomic mutation profiles and cytogenetic status to explore predictors of clinical response or molecular MRD responses. While there are no predictors of clinical/molecular outcome, of the 3 patients with Richter's transformation, 2 had *TP53* mutation, and 2 had unmutated *IGHV* (**Figure 3A**).

### *Quality of Life*

The European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life questionnaire (QLQ) and the Short Form-36 Health Survey (SF-36) were used to assess patient-reported quality of life. There were no significant effects of time or cohort, or significant interactions between time and cohort in quality of life measures between baseline and 12 months (**Supplemental Tables 3, 4 and 5**). Across the study period from baseline to 24 months, there were no significant differences between relapsed/refractory and treatment-naïve patients (**Table 3**). Overall, the analyses suggested patients generally perceived improvement with time, with significant gains on the physical component scale of the SF-36 ( $P=0.01$ ). Patients reported a 7-point improvement on the 0-100 global health scale of the EORTC ( $P=0.03$ ). For the 5 functional items, patients reported improvements in physical functioning ( $P=0.01$ ), which may have resulted from gains reported by relapsed/refractory patients by 24 months (93.33) compared with their baseline score (83.81). Improvements were found for 3 of the 9 symptom items, including reductions in appetite loss ( $P=0.04$ ), diarrhea ( $P=0.01$ ), and dyspnea ( $P=0.01$ ). No significant change from baseline was observed for the remaining functional and symptom scores.

## Discussion

Here we show for the first time that the combination of acalabrutinib with obinutuzumab resulted in high response rates and durable remissions in patients with relapsed/refractory or treatment-naïve CLL. These results were observed in patients with poor risk factors, and although 3 patients experienced Richter's transformation, only 1 patient had CLL progression with the current follow-up of 3.5 years. This regimen was also well tolerated. AEs were generally manageable, with few patients discontinuing the drug due to toxicity (11%).

Acalabrutinib monotherapy can produce high response rates and durable remissions in patients with CLL; obinutuzumab was added to produce deeper responses and to potentially prolong PFS. Although the combination of a CD20 antibody with ibrutinib does not improve outcomes over ibrutinib monotherapy in randomized studies (10), this question has not been addressed with BTK inhibitors optimized for selectivity or CD20 antibodies engineered to optimize ADCC. Ibrutinib, through inhibition of ITK and other non-BTK kinases, abrogates natural killer cell ADCC and ADCP *in vitro* (20-22) and may reduce the effectiveness of the CD20 antibody in patients. Acalabrutinib is highly selective for BTK and is not a potent inhibitor of most other kinases (15,23), suggesting that this BTK inhibitor might be more appropriate for combination therapy with antibodies. Similarly, several groups have demonstrated that the CD20 engineered antibody obinutuzumab mediates improved ADCC over rituximab (24,25). Indeed, although our study was small, the results suggest that this combination of BTK inhibitor plus immune therapy (BTI) may have improved outcomes compared with previously

published data for acalabrutinib alone (16). Additional data will come from the ongoing phase 3 study (NCT02475681) comparing chlorambucil plus obinutuzumab, acalabrutinib alone, and acalabrutinib plus obinutuzumab in previously untreated CLL.

Despite the addition of obinutuzumab, CR rates with the combination remained relatively low, though responses tended to deepen over time. Lower MRD negativity rates were seen in bone marrow due to MRD testing in bone marrow being conducted only on C12D1. Although patients are currently treated to progression, it is unclear whether continuous treatment is necessary or whether treatment for specific patients could be discontinued; these issues must be addressed in prospective clinical trials.

This regimen appears quite tolerable, with a low rate of discontinuation due to AEs, despite the long follow-up. Real-world studies of BTK inhibitors have highlighted that a favorable safety profile in clinical trials does not guarantee that patients outside of trials will tolerate drugs well (12); however, toxicities that generally led to discontinuation of ibrutinib, such as atrial fibrillation and arthralgias, did not lead to acalabrutinib discontinuation in this study. Moreover, the overall rate of atrial fibrillation in this study was low, with only 1 patient experiencing an atrial fibrillation event. This also suggests that patients who develop or are at high risk for developing these specific adverse effects might benefit from acalabrutinib if BTK inhibitor treatment is indicated.

Historically, chemotherapy treatment for hematologic malignancies was associated with significant numbers or severity of AEs and quality of life burdens (26,27). Targeted

therapies like acalabrutinib have the potential for more favorable AE profiles and are less likely to be associated with quality of life decrements (12,28). Our data show that the combination of acalabrutinib plus obinutuzumab did not decrease patients' quality of life. Instead, some improvements were found, with patients viewing their physical health as generally improved, and physical symptoms were less disruptive to their quality of life. The source for the latter may derive from improvements in 3 common symptoms: diarrhea, dyspnea, and appetite loss. During the course of treatment, all 3 common symptoms were of sufficient frequency/intensity to rise to the level of AE ratings (any grade: 64%, 24%, and 36%, respectively). But despite this, patients reported symptoms as having improved by cycle 24. This affirms that this regimen is tolerable and can be given safely without impairing patients' overall quality of life.

In conclusion, the combination of acalabrutinib and obinutuzumab leads to high response rates and prolonged remission duration. In this study, acalabrutinib plus obinutuzumab was tolerable, with low rates of discontinuation due to AEs. Further study of this combination is warranted in CLL and potentially in other lymphoid malignancies.

## **Methods**

### *Patients*

Eligible patients had a diagnosis of intermediate or high-risk Rai stage CLL or small lymphocytic lymphoma by iwCLL 2008 criteria (29); patients in the previously untreated cohort were  $\geq 65$  years old or  $< 65$  and refused or were ineligible for chemoimmunotherapy, and patients in the relapsed/refractory cohort had  $\geq 1$  prior

therapy for CLL. All patients were required to be  $\geq 18$  years of age with an Eastern Cooperative Oncology Group performance status of  $\leq 2$  and active disease requiring therapy per iwCLL 2008 guidelines (29). Patients were required to have measurable nodal disease, adequate organ function defined as creatinine clearance  $\leq 2.5\times$  the upper limit of normal (ULN), alanine aminotransferase and aspartate aminotransferase  $\leq 3.0\times$  ULN, and bilirubin  $\leq 2.5\times$  ULN. Platelets had to be  $\geq 50\times 10^9/L$  and absolute neutrophil count (ANC)  $\geq 750/mm^3$ ; for patients with bone marrow involvement by CLL, platelets had to be  $\geq 30\times 10^9/L$  and ANC  $\geq 500/mm^3$ . Prior BTK inhibitor treatment was permitted if the reason for discontinuation was not disease progression during treatment.

### *Study Design*

In this phase 1b study, both cohorts received the same regimen of acalabrutinib plus obinutuzumab. Acalabrutinib was started on cycle 1 day 1 and administered in 28-day cycles until disease progression or unacceptable toxicity. In the first 15 patients, the dose of acalabrutinib was 200 mg once daily; however, upon observing that BTK occupancy was higher with twice-daily dosing (16), all patients subsequently received 100 mg twice daily. Obinutuzumab was given by intravenous infusion starting with cycle 2: 100 mg on day 1, 900 mg on day 2, and then 1000 mg on days 8 and 15 of cycle 2, and 1000 mg on day 1 of cycles 3-7.

This was a single-center phase 1b/2 study conducted at The Ohio State University Comprehensive Cancer Center (NCT02296918). All patients provided written informed consent. The study was coordinated by Acerta Pharma, a member of the AstraZeneca

Group and was approved by the Institutional Review Board of The Ohio State University. The study was conducted in accordance with the principles of the Declaration of Helsinki, and safety was monitored every 3 months by The Ohio State University Data Safety Monitoring Board.

### *Endpoints and Assessments*

The primary endpoints were ORR (CR + PR) and safety with acalabrutinib plus obinutuzumab. Secondary endpoints included, but were not limited to, analysis of MRD negativity, PFS, OS, time to next treatment (data not shown), pharmacodynamics, and correlative assessments of mutational data and efficacy endpoints. Patients underwent bone marrow biopsy at screening, on day 1 of cycle 12, at the time of presumed CR defined by computed tomography scans and MRD negative peripheral blood, and at relapse. Disease assessments were conducted every 3 cycles for the first 24 cycles, and then every 6 cycles thereafter. Evaluations included toxicity assessment, physical exam, laboratory studies, and CT scans. Bone marrow biopsy was performed at day 1 of cycle 12 and thereafter only if criteria for CR were met and peripheral blood was MRD negative by flow cytometry. Toxicities were evaluated using Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 or higher for nonhematologic toxicities and iwCLL 2008 guidelines (29) for hematologic toxicities.

Responses were determined according to iwCLL 2008 guidelines. Patients were screened for BTK C481S mutations using digital droplet PCR every visit starting 12 cycles after treatment began; all patients had >1 year of screening. MRD was assessed

using flow cytometry in the peripheral blood every 3 cycles, and in the bone marrow at cycle 12 and anytime CR criteria were met by CT scans and when peripheral blood MRD by flow cytometry was negative. A cutoff of <1 cell in 10,000 lymphocytes was used to define MRD negativity, in accordance with standard definitions.

### *Correlative Analyses*

Baseline *TP53* mutation analysis was performed in the Experimental Hematology Laboratory at The Ohio State University as previously described (10).

MRD analysis was performed using 10-color flow cytometry in the clinical flow cytometry laboratory at The Ohio State University as described previously (10) (combination of antibodies listed in **Supplemental Table 3**). The method allows reliable and reproducible detection of CLL MRD at the level of 0.01% ( $10^{-4}$ ) of all events, which corresponds with 1 CLL cell in 10,000 total cells analyzed. Criteria used for CLL MRD status determination for each sample were described in detail previously (30,31).

### *Pharmacodynamics*

Occupancy of BTK by acalabrutinib (using a validated Enzyme-Linked Immunosorbent Assay [ELISA]) was measured in peripheral blood mononuclear cells (PBMCs) with the aid of a drug-analogue probe at predose on day 1 of cycles 1-3 and predose on day 2 of cycles 1 and 2. PBMCs were also used for flow cytometry–based signaling assays to measure phosphorylated BTK (Y223) and total BTK protein levels. Additional details on the methods for pharmacodynamic analyses were reported previously<sup>16</sup>.

### *Quality of Life*

A repeated measures design was used to obtain quality of life data. Quality of life assessments were obtained from patients on screening/day 1 (cycle 1) and day 1 of cycles, 5, 9, and 12, and then on day 1 of cycles 18 and 24. Excepting individuals leaving the trial, there were no missing data at each timepoint.

With the aid of an assistant, patients completed self-report measures of quality of life on Teleforms that were subsequently scanned; there was no hand entry of data. Two measures were used: the SF-36 Health Survey and the EORTC-QLQ. The SF-36 is a 26-item questionnaire that uses 2 summary scales — the physical component and mental component scales (32). The EORTC-QLQ is a 30-item questionnaire designed to assess health-related quality of life in patients with cancer participating in clinical trials.

Cohort, time, and cohort x time interactions were assessed. Prior to analysis, Spearman correlations between SF-36 and EORTC-QLQ scores at baseline, as well as sex and age were evaluated for significant associations ( $P < 0.05$ ) to determine the need for inclusion as covariates in the analyses. Linear mixed effects (LME) models were used for primary analyses and were fitted using an autoregressive covariance structure to account for correlations between proximate timepoints. A repeated measures ANOVA was used to assess quality of life across the study period, using baseline and 24-month outcomes. Cohort (treatment-naïve and relapsed/refractory) was the factor used among

patients to assess time-related outcomes and to determine any cohort, time, and interaction effects for individual patients.

### *Statistical Considerations*

Data are presented as of February 12, 2019. Efficacy and safety analyses were performed on all enrolled patients who received  $\geq 1$  dose of acalabrutinib. Safety analyses include both cohorts combined except as indicated and include all treatment-emergent AEs regardless of attribution to study drugs. Time-to-event endpoints, including duration of response (DOR), PFS, and OS were estimated using the Kaplan-Meier method. DOR and time to response analyses were conducted on patients who achieved CR or PR as their best overall response. Descriptive statistics were used to summarize the findings.

### **Author contributions**

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## Tables

**Table 1.** Demographic and baseline characteristics

Characteristic	Treatment-Naïve (n=19)	Relapsed/Refractory (n=26)
Median age (range), y	61 (42-75)	63 (42-76)
Male sex, n (%)	11 (58)	21 (81)
ECOG PS ≤1, n (%)	19 (100)	26 (100)
Bulky disease ≥5 cm, n (%)	10 (53)	13 (50)
Rai Stage III-IV, n (%)	10 (53)	7 (27)
CLL-IPI score, n (%)		
0-3 (Low/intermediate risk)	7 (37)	11 (42)
4-6 (High risk)	4 (21)	6 (23)
7-10 (Very high risk)	4 (21)	3 (12)
B2-microglobulin >3 mg/L, n (%)	13 (68)	17 (65)
No. prior therapies, median (range)	0	1 (1-9)
Histology, n (%)		
CLL	18 (95)	26 (100)
SLL	1 (5)	0
Genomic status, n/n (%)		
Del(17)(p13.1)	4/18 (22)	5/25 (20)
Del(11)(q22.3) without or missing del(17)(p13.1)	5/18 (28)	9/26 (35)
Unmutated <i>IGHV</i>	9/17 (53)	17/26 (65)
<i>TP53</i> mutation	5/18 (28)	6/24 (25)
<i>TP53</i> mutation + del(17)(p13.1)	4/19 (21)	3/26 (12)

CLL = chronic lymphocytic leukemia; ECOG PS = Eastern Cooperative Oncology Group performance status; *IGHV* = immunoglobulin heavy-chain variable region gene; IPI = International Prognostic Index; SLL = small lymphocytic lymphoma; *TP53* = tumor protein p53 gene.

**Table 2.** Grade  $\geq 3$  adverse events observed in  $>2$  patients

Grade 3/4 Adverse event, n (%)	All Patients (N=45)	
	Grade 3	Grade 4
Neutrophil count decreased	5 (11)	6 (13)
Syncope	5 (11)	0 (0)
Platelet count decreased	3 (7)	1 (2)
Weight increased	4 (9)	0 (0)
Cellulitis	4 (9)	0 (0)
Hypophosphatemia	3 (7)	0 (0)
Hypertension	3 (7)	0 (0)
Pneumonia	2 (4)	0 (0)
Anemia	2 (4)	0 (0)
Diarrhea	2 (4)	0 (0)
Fall	2 (4)	0 (0)
Dyspnea	2 (4)	0 (0)

**Table 3.** SF-36 and EORTC scores at baseline and cycle 24.

Outcome	Treatment-Naïve Patients		Relapsed/Refractory Patients		Repeated Measures ANOVA			
	Baseline	Cycle 24	Baseline	Cycle 24	Time		Cohort	
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	F	P	F	P
<b>SF-36</b>								
PCS	43.73 (2.39)	48.70 (1.83)	42.16 (2.85)	46.98 (2.18)	<b>8.01</b>	<b>0.01</b>	0.34	0.56
MCS	52.61 (2.21)	53.02 (2.15)	49.02 (2.64)	50.37 (2.57)	0.80	0.38	0.93	0.34
<b>EORTC</b>								
Global Health	71.67 (4.73)	78.33 (4.30)	70.24 (5.65)	77.98 (5.13)	<b>5.04</b>	<b>0.03</b>	0.02	0.88
<b>EORTC symptom domains<sup>1</sup></b>								
Financial Difficulties	10.00 (5.17)	6.67 (4.35)	11.91 (6.18)	4.76 (5.20)	0.86	0.36	0.00	1.00
Diarrhea	10.00 (4.49)	5.00 (3.30)	19.05 (5.37)	4.76 (3.94)	<b>7.36</b>	<b>0.01</b>	0.77	0.39
Constipation	3.33 (3.27)	3.33 (4.43)	7.14 (3.91)	16.67 (5.29)	1.67	0.21	3.16	0.09
Appetite Loss	5.00 (3.22)	5.00 (2.72)	14.29 (3.85)	4.76 (3.25)	<b>4.43</b>	<b>0.04</b>	1.24	0.27
Insomnia	31.58 (7.18)	19.30 (6.78)	26.19 (8.36)	28.57 (7.90)	0.70	0.41	0.05	0.83
Dyspnea	8.33 (3.99)	3.33 (2.69)	19.05 (4.77)	7.14 (3.21)	<b>6.94</b>	<b>0.01</b>	2.96	0.10
Pain	22.50 (5.69)	20.00 (5.23)	16.67 (6.80)	19.05 (6.27)	0.00	0.99	0.20	0.66
Nausea/Vomiting	1.67 (1.70)	3.33 (1.55)	4.76 (2.03)	3.57 (1.86)	0.05	0.83	0.54	0.47
Fatigue	26.67 (5.27)	25.00 (4.19)	32.54 (6.29)	26.19 (5.01)	0.82	0.37	0.35	0.56
<b>EORTC functional domains<sup>2</sup></b>								
Social Functioning	90.00 (3.73)	92.50 (2.97)	89.29 (4.46)	92.86 (3.56)	0.95	0.34	0.00	0.97
Cognitive Functioning	86.67 (3.78)	83.33 (5.15)	80.95 (4.52)	77.38 (6.16)	1.64	0.21	0.80	0.38
Emotional Functioning	87.92 (3.86)	87.92 (3.74)	85.12 (4.61)	83.33 (4.47)	0.15	0.70	0.46	0.50
Role Functioning	85.00 (5.18)	87.50 (4.23)	83.33 (6.19)	91.67 (5.06)	3.09	0.09	0.04	0.85
Physical Functioning	92.83 (3.47)	96.67 (2.13)	83.81 (4.15)	93.33 (2.54)	<b>7.50</b>	<b>0.01</b>	2.69	0.11

Significant time effects are noted in bold text.

ANOVA = analysis of variance; EORTC = European Organisation for Research and Treatment of Cancer; F = variation between sample means divided by the variation within samples; MCS = mental component score; PCS = physical component score; SE = standard error; SF-36 = Short Form-36 Health Survey. <sup>1</sup> Score range 0-100, with a higher score indicative of a higher level of symptoms. Correction for 9 multiple comparisons is  $P=0.006$ . <sup>2</sup> Score range 0-100, with a higher score indicative of a higher level of functioning. Correction for 5 multiple comparisons is  $P=0.01$

## Figure Legends

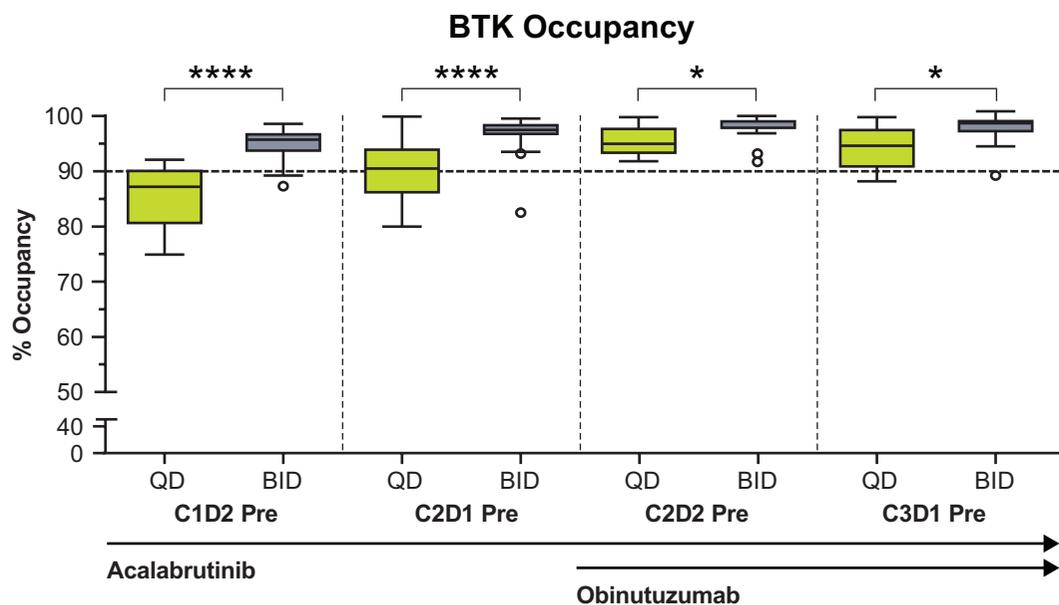
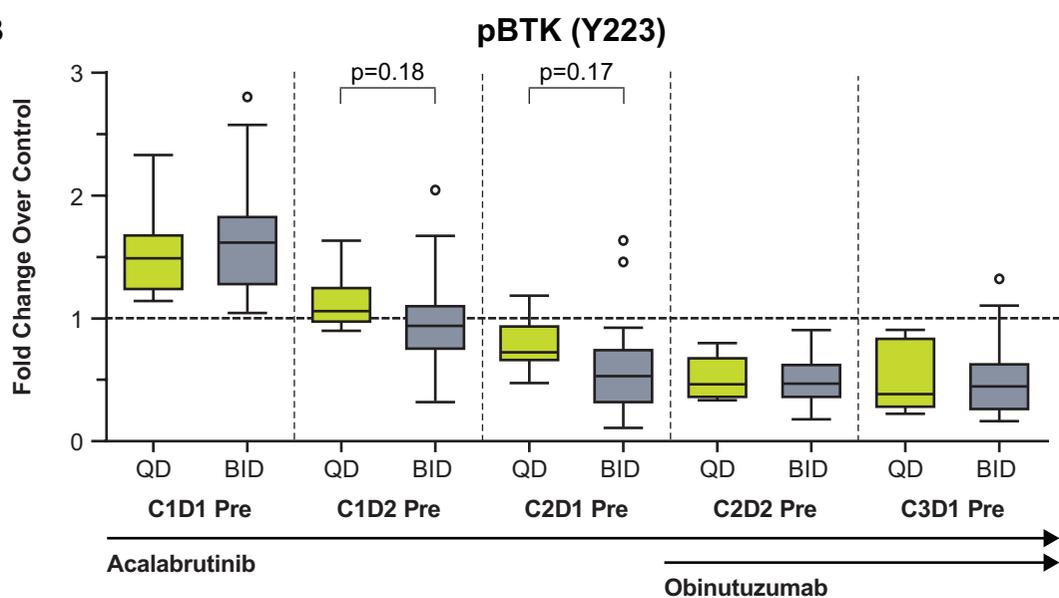
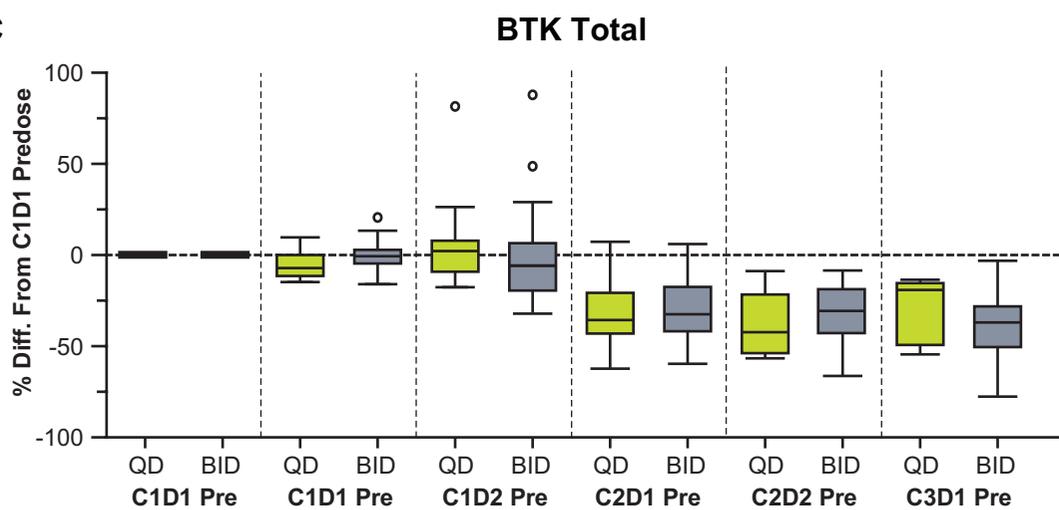
**Figure 1.** Pharmacodynamics of acalabrutinib. (A) The percentage of BTK occupancy by acalabrutinib at each timepoint for patients with a signal to noise  $\geq 5$  for the day 1 presample; (B) the pBTK fold change over control (C1D1 pre + 1  $\mu\text{M}$  exogenous acalabrutinib) for each timepoint; (C) the BTK fold change over control (C1D1 pre + 1  $\mu\text{M}$  exogenous acalabrutinib) for each timepoint. For each figure, the horizontal line in the center of the box represents the median. Significance was determined using a paired, 2-tailed parametric t-test: \* $P < 0.5$ , \*\*\*\* $P < 0.0001$ . BID = twice a day; BTK = Bruton tyrosine kinase; pBTK = phosphorylated BTK; QD = once a day.

**Figure 2.** T cells, monocytes, and natural killer cell counts in peripheral blood from all acalabrutinib-treated patients over time. Absolute counts of CD4+ (A) and CD8+ T cells (B), CD14+ monocytes (C), and natural killer cells (CD16+/CD56) (D). Cells were measured by flow cytometry. The normal reference range for each subset is shown with dashed lines. All plots include patients being assessed at baseline assessment and the respective visit.  $P$  values are from the Wilcoxon signed-rank test. NK = natural killer.

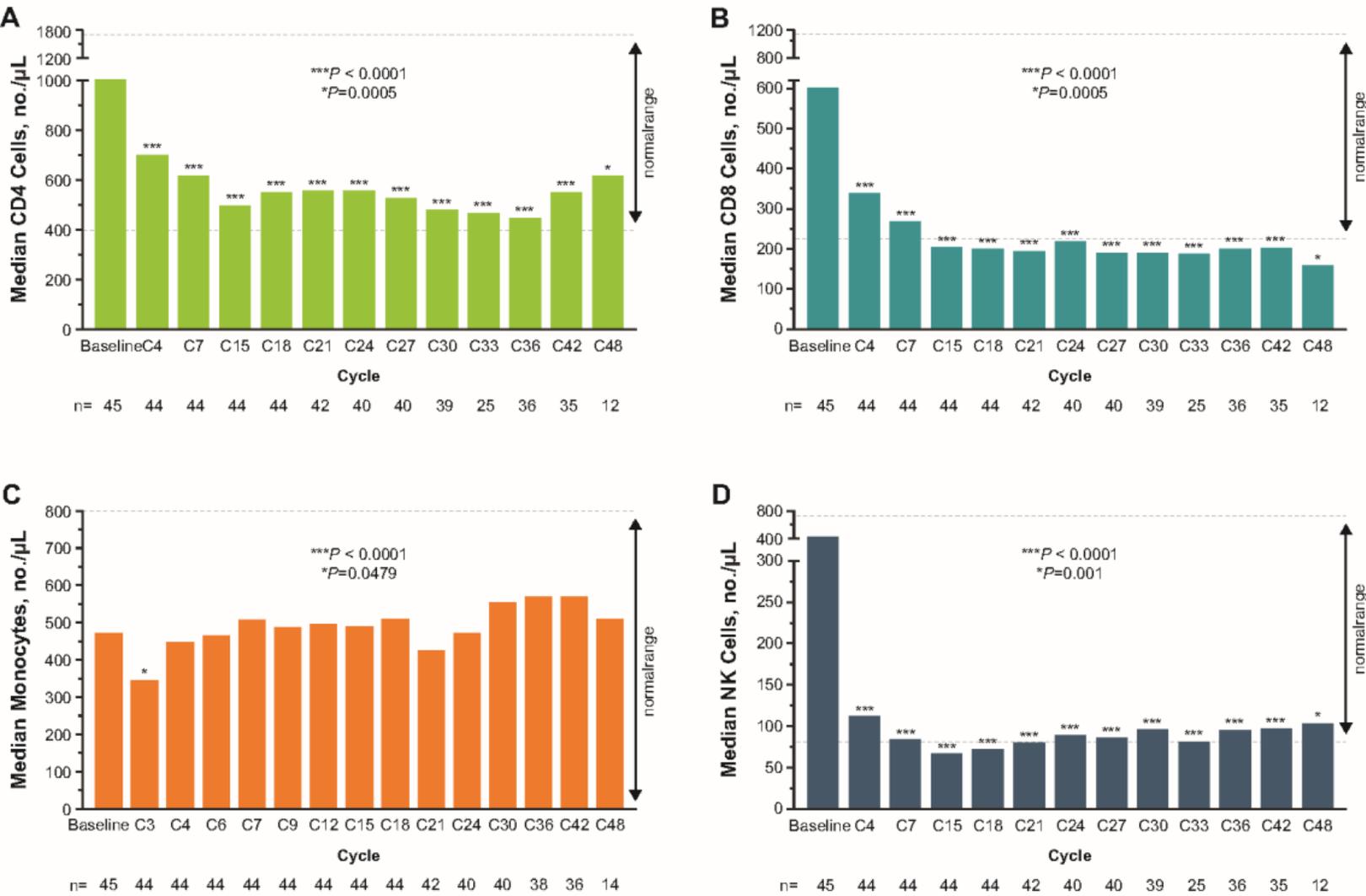
**Figure 3.** Kaplan-Meier curves for (A) progression-free survival and (B) overall survival. mo = months; OS = overall survival; PFS = progression-free survival; R/R = relapsed/refractory; TN = treatment-naïve.

**Figure 4.** (A) Response and minimal residual disease status by baseline genomic alterations. (B-C) Minimal residual disease in (B) bone marrow and (C) peripheral blood.

CLL = chronic lymphocytic leukemia; CR = complete response; FISH = fluorescence in situ hybridization; *IGHV* = immunoglobulin heavy-chain variable region gene; LOH = loss of heterozygosity; MRD = minimal residual disease; mut = mutated; PR = partial response; PRL = partial response with lymphocytosis; *TP53* = tumor protein p53 gene; TN = treatment-naïve; R/R = relapsed/refractory.

**Figure 1****A****B****C**

**Figure 2**



**Figure 3****A****Progression-Free Survival**

Proportion Progression-Free

**TN** 39-mo PFS rate (95% CI): 94.4% (66.6%, 99.2%)  
**RR** 42-mo PFS rate (95% CI): 72.7% (43.8%, 88.4%)

+ Censored



At Risk

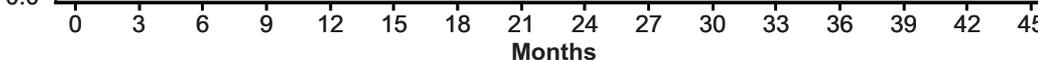
Months	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45
<b>TN</b>	19	18	18	18	18	18	17	17	17	17	17	15	15	4	4	0
<b>RR</b>	26	26	26	26	26	26	26	25	23	23	23	22	21	10	9	1

**B****Overall Survival**

Proportion Surviving

**TN** 39-mo OS rate (95% CI): 100% (100%, 100%)  
**RR** 42-mo OS rate (95% CI): 82% (57%, 93%)

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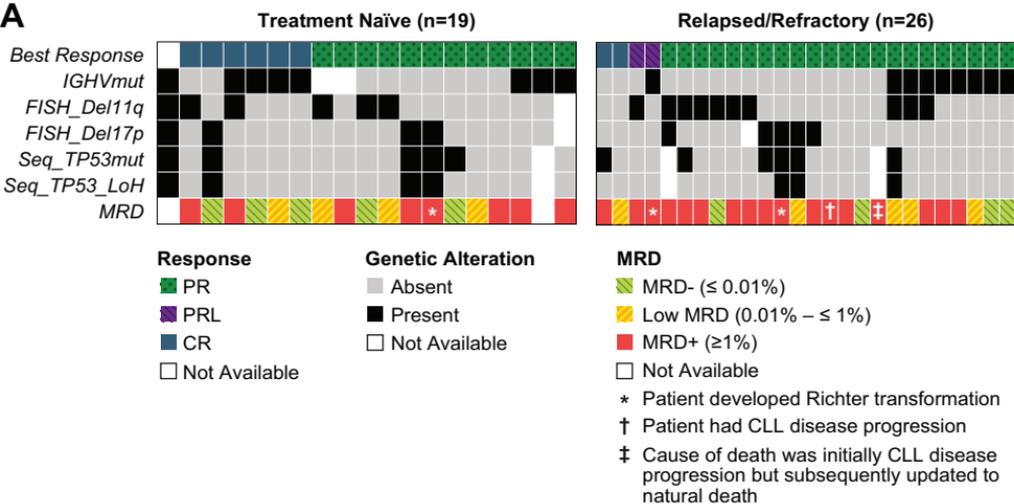


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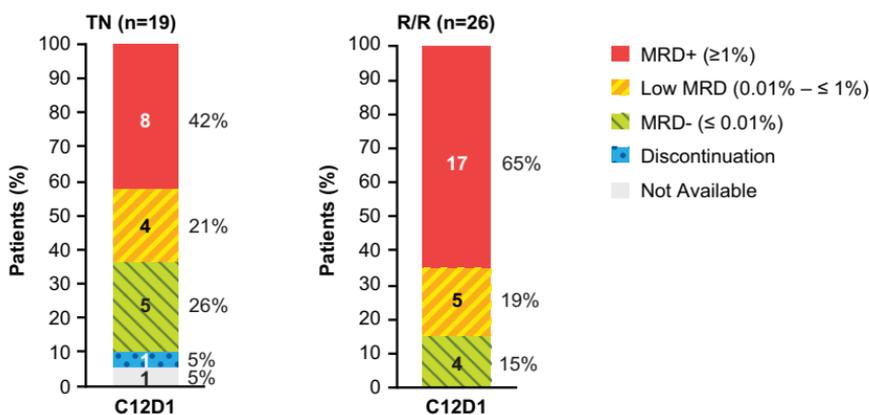
Months	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45
<b>TN</b>	19	18	18	18	18	18	17	17	17	17	17	16	16	6	6	0
<b>RR</b>	26	26	26	26	26	26	26	25	25	25	24	23	21	12	11	4

# Figure 4

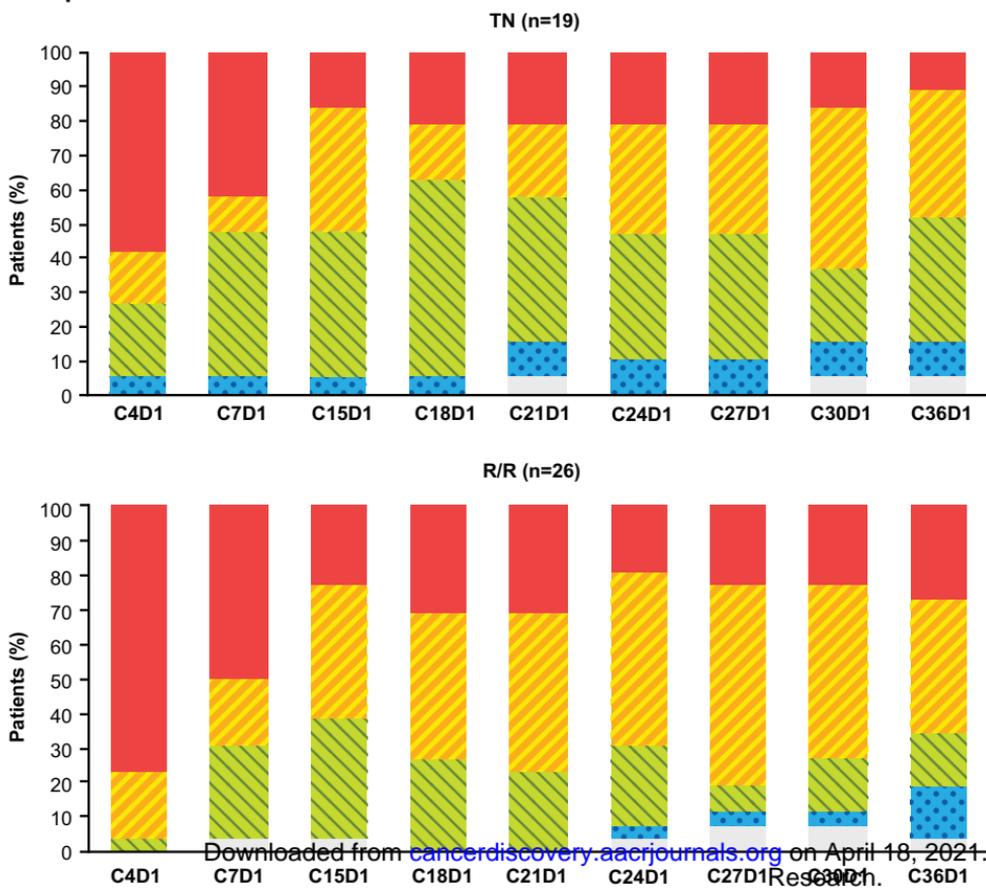
**A**



**B Bone Marrow**



**C Peripheral Blood**



# CANCER DISCOVERY

## Acalabrutinib Plus Obinutuzumab in Treatment-Naive and Relapsed/Refractory Chronic Lymphocytic Leukemia

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