THERAPEUTIC ADVANCES in
Hematology

Original Research

Phase I/II results of ceralasertib as monotherapy or in combination with acalabrutinib in high-risk relapsed/refractory chronic lymphocytic leukemia

Ther Adv Hematol

2023, Vol. 14: 1–14

DOI: 10.1177/20406207231173489

Wojciech Jurczak, Nagah Elmusharaf, Christopher P. Fox, William Townsend, Amanda G. Paulovich, Jeffrey R. Whiteaker, Fanny Krantz, Chuan-Chuan Wun, Graeme Parr, Shringi Sharma, Veerendra Munugalavadla, Richa Manwani, Emma Dean and Talha Munir

Abstract

Background: Patients with relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL) have limited treatment options. Ceralasertib, a selective ataxia telangiectasia and Rad-3-related protein (ATR) inhibitor, demonstrated synergistic preclinical activity with a Bruton tyrosine kinase (BTK) inhibitor in *TP53*- and *ATM*-defective CLL cells. Acalabrutinib is a selective BTK inhibitor approved for treatment of CLL.

Objectives: To evaluate ceralasertib ± acalabrutinib in R/R CLL.

Design: Nonrandomized, open-label phase I/II study.

Methods: In arm A, patients received ceralasertib monotherapy 160 mg twice daily (BID) continuously (cohort 1) or 2 weeks on/2 weeks off (cohort 2). In arm B, patients received acalabrutinib 100 mg BID continuously (cycle 1), followed by combination treatment with ceralasertib 160 mg BID 1 week on/3 weeks off from cycle 2. Co-primary objectives were safety and pharmacokinetics. Efficacy was a secondary objective.

Results: Eleven patients were treated [arm A, $n = 8$ (cohort 1, $n = 5$; cohort 2, $n = 3$); arm B, $n = 3$ (acalabrutinib plus ceralasertib, $n = 2$; acalabrutinib only, $n = 1$)]. Median duration of exposure was 3.5 and 7.2 months for ceralasertib in arms A and B, respectively, and 15.9 months for acalabrutinib in arm B. Most common grade ⩾3 treatment-emergent adverse events (TEAEs) in arm A were anemia (75%) and thrombocytopenia (63%), with four dose-limiting toxicities (DLTs) of grade 4 thrombocytopenia. No grade ⩾3 TEAEs or DLTs occurred in arm B. Ceralasertib plasma concentrations were similar when administered as monotherapy or in combination. At median follow-up of 15.1 months in arm A, no responses were observed, median progression-free survival (PFS) was 3.8 months, and median overall survival (OS) was 16.9 months. At median follow-up of 17.2 months in arm B, overall response rate was 100%, and median PFS and OS were not reached.

Conclusion: Ceralasertib alone showed limited clinical benefit. Acalabrutinib plus ceralasertib was tolerable with preliminary activity in patients with R/R CLL, though findings are inconclusive due to small sample size.

Registration: NCT03328273

Keywords: acalabrutinib, ceralasertib, chronic lymphocytic leukemia, molecular targeted therapy

Received: 25 October 2022; revised manuscript accepted: 17 April 2023.

Correspondence to:
Wojciech Jurczak
Maria Sklodowska-Curie National Institute of Oncology, Garncarska 11, 31-115 Krakow, Poland.
wojciech.jurczak@lymphoma.edu.pl

Nagah Elmusharaf
University Hospital of Wales, Cardiff, UK

Christopher P. Fox
Nottingham University Hospitals, Nottingham, UK

William Townsend
NIHR Biomedical Research Centre, University College London Hospitals NHS Foundation Trust, London, UK

Amanda G. Paulovich
Jeffrey R. Whiteaker
Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, WA, USA

Fanny Krantz
Chuan-Chuan Wun
Shringi Sharma
Veerendra Munugalavadla
AstraZeneca, South San Francisco, CA, USA

Graeme Parr
Richa Manwani
Emma Dean
Oncology R&D, AstraZeneca, Cambridge, UK

Talha Munir
St. James's University Hospital, Leeds, UK

Introduction

Chronic lymphocytic leukemia (CLL) is a mature clonal B-cell malignancy and the most common type of leukemia in adults.[1,2] Recent advances with targeted therapies remain at the forefront as treatment options for patients with CLL.[2,3] Bruton tyrosine kinase (BTK) inhibitors and anti-apoptotic protein B-cell lymphoma 2 (BCL2) inhibitors have demonstrated improved efficacy and safety compared with standard chemoimmunotherapy agents.[1–3] However, the majority of patients with CLL who are treated with currently available, targeted treatments are not cured, and those who relapse on BTK inhibitor or BCL2 inhibitor therapy have limited treatment options and often have a poor prognosis.[3] Additional therapeutic options are therefore needed for these patients.[4]

CLL is often initiated by chromosomal abnormalities, such as del(13q), del(11q), and trisomy 12, and additional mutations may arise as the disease progresses that render the disease increasingly aggressive.[4] Certain genomic features, such as *TP53* aberrations (4–37% of patients), del(17p) (5–8%) and del(11q) (~25%), are associated with poor survival.[3,4]

TP53 and ataxia telangiectasia mutated (*ATM*) are genes that govern the cellular response of CLL cells to DNA damage, resulting in cell cycle arrest or apoptosis. Disruption of these genes in CLL through gene deletion [del(17p) in *TP53* or del(11q) in *ATM*] and/or mutation results in genomic instability, chemoresistance, and an adverse prognosis.[5] Ataxia telangiectasia and Rad-3-related protein (ATR) expression is dysregulated in various cancer types, including leukemia. The ATR kinase initiates the DNA repair process in response to persistent single-stranded DNA damage; therefore, ATR inhibitors may disrupt downstream signaling pathways, leading to cell death.[6] In ATR inhibition, genomic integrity becomes dependent upon functional p53 and ATM. ATR is therefore a particularly attractive synthetically lethal target in p53 or ATM deficiency.[5] Ceralasertib, a selective, oral ATR inhibitor, has been shown to induce synthetic lethality, overcome chemoresistance, and to demonstrate synergistic preclinical activity with a BTK inhibitor in *TP53*- and *ATM*-defective CLL cells.[5] ATR inhibitors, such as ceralasertib, are in clinical development in a variety of solid tumor and hematological cancer indications, as monotherapy and in combination with chemotherapy and/or immunotherapy.

Acalabrutinib is a potent, highly selective, irreversible oral BTK inhibitor approved for the treatment of CLL/small lymphocytic lymphoma and relapsed/refractory (R/R) mantle cell lymphoma.[7] In the pivotal ELEVATE-TN and ASCEND studies, the acalabrutinib arms showed significant progression-free survival (PFS) benefit in treatment-naive CLL and R/R CLL, respectively, *versus* the comparator arms, including in patients with high-risk genomic features, such as del(17p)/*TP53* mutation.[8–11]

Here, we report the results from a phase I/II proof-of-concept study investigating ceralasertib as monotherapy or in combination with acalabrutinib in patients with high-risk R/R CLL.

Methods

Study design and patient population

This multicenter, nonrandomized, open-label phase I/II study (NCT03328273; ACE-CL-110) evaluated ceralasertib as monotherapy or in combination with acalabrutinib in patients with high-risk R/R CLL requiring treatment per International Workshop on Chronic Lymphocytic Leukemia criteria.[12]

The study was divided into two parts: dose escalation (part 1) and dose expansion (part 2). The two treatment arms of part 1 were staggered, with arm A initiated first to test ceralasertib monotherapy. In part 1, cohorts of up to six patients were planned to be enrolled in arms A and B each. Patients in arm A received ceralasertib monotherapy in two cohorts. Patients in cohort 1 were given ceralasertib 160 mg twice daily (BID) continuously; if ⩾2 dose-limiting toxicities (DLTs) occurred in cohort 1, ceralasertib 160 mg BID 2 weeks on and 2 weeks off was to be explored in cohort 2. Patients in arm B received acalabrutinib monotherapy 100 mg BID continuously during cycle 1 (28 days); patients then received acalabrutinib 100 mg BID continuously plus ceralasertib 160 mg BID 1 week on and 3 weeks off from cycle 2 onward (28 days). Part 2 was not opened for either arm because enrollment was discontinued for both arms in part 1.

Eligible patients were ⩾18 years of age who had received ⩾1 prior therapy for treatment of their disease and had adequate hematologic function (hemoglobin ⩾ 9.0 g/dL, platelet count $>75 \times 10^9$/L, independent of transfusion and growth factor support for ⩾14 days before screening). Presence of high-risk genomic features was also a key inclusion criterion. Patients in arm A (part 1) must have had ⩾1 of the following prognostic factors: del(17p), *TP53* mutation, or del(11q); patients also had to have exhausted available treatment options. Patients in arm B (parts 1 and 2) were required to have del(11q) and be deemed suitable to receive a BTK inhibitor and ceralasertib per investigator's assessment. Key exclusion criteria included diagnosis of ataxia telangiectasia, prior exposure to an ATR inhibitor, and known cardiovascular (CV) conditions or procedures currently or within the last 6 months. See the Data Supplement for additional exclusion criteria. The study was conducted in accordance with the Good Clinical Practice guidelines. The protocol and all amendments were approved by the institutional review board and independent ethics committee. All patients provided written informed consent.

This report has been written following the CONSORT extension for pilot and feasibility trials and its guidelines.[13]

Outcomes

The co-primary objectives were safety and pharmacokinetics (PK). The secondary objective was to evaluate preliminary activity of ceralasertib as monotherapy and in combination with acalabrutinib, measured by overall response rate [ORR; complete response (CR) + partial response (PR)], CR rate, duration of response (DOR), PFS, and overall survival (OS). Exploratory objectives included pharmacodynamic (PD) biomarker assessments.

Safety was assessed based on adverse events (AEs), serious AEs (SAEs), DLTs, and AEs leading to treatment discontinuation. A DLT analysis was performed during the first 28 days of treatment (i.e. during cycle 1 for arm A and during cycle 2 for arm B). Blood samples for PK analysis were collected at prespecified times for acalabrutinib and ceralasertib. Additional details on DLT assessments and PK blood sampling are described in the Data Supplement.

Efficacy outcomes were assessed by investigators according to the response criteria for CLL.[12] In the exploratory PD analyses for arm B, blood samples were taken predose and 1 hour postdose on cycle 1, day 1 and cycle 2, day 1; predose on other indicated days; and as soon as possible after disease progression (which may have been at the safety follow-up visit). BTK occupancy by acalabrutinib with or without ceralasertib was assessed in peripheral blood mononuclear cells (PBMCs) using an enzyme-linked immunosorbent assay (ELISA). The percentage of occupied BTK was calculated in each PBMC sample relative to the patient's baseline sample (cycle 1, day 1 predose). PD changes, including phosphorylated ATM (pATM), monoubiquitylation of proliferating cell nuclear antigen (PCNA), and phosphorylation of nibrin (pNibrin) were explored using novel multiple reaction monitoring (MRM)-targeted mass spectrometry.[14] High-throughput single telomere length analysis (HT-STELA) assay was utilized to measure telomere length.[15] HT-STELA cutoffs for shorter, intermediate, and longer telomere lengths were <2.169 kb, between 2.169 and 3.650 kb, and >3.650 kb, respectively.

Statistical analysis

Safety was evaluated using the safety analysis set, which included all patients who took ⩾1 dose of study drug. PK was evaluated using the PK analysis set, which included patients with reportable plasma concentrations and no important AEs or protocol deviations that could impact PK. Response, PFS, and DOR were evaluated using the tumor response analysis set, which included all patients with baseline tumor assessment who received ⩾1 dose of study drug. OS was assessed using the safety analysis set.

Descriptive statistics for continuous and discrete variables were used to summarize data as appropriate. Investigator-assessed response rates were reported with corresponding two-sided 80% confidence intervals (CIs) based on exact binomial test. PFS, DOR, and OS were estimated using the Kaplan–Meier (KM) method and the median with the corresponding two-sided 95% CIs were reported.

Results

Patients

Between January 31, 2018 and October 19, 2020, 16 patients were screened and 11 eligible patients

were enrolled in the study (Supplemental Figure 1). Arm A (ceralasertib monotherapy) enrolled a total of eight patients (cohort 1, $n=5$; cohort 2, $n=3$); arm B (acalabrutinib plus ceralasertib) enrolled three patients. Of the 11 patients treated, the median age was 64 years (range, 53–74) and the median number of prior therapies was 3 (Table 1). Seven patients (88%) in arm A had received prior BTK inhibitor therapy, whereas all three patients in arm B were BTK inhibitor naive. Notably, no patients in arm B had a *TP53* mutation.

As of the data cutoff date (September 7, 2021), the median follow-up was 15.1 months for patients in arm A and 17.2 months for patients in arm B. Only two of the three patients in arm B were treated with acalabrutinib plus ceralasertib; the third patient received acalabrutinib monotherapy only. For the latter patient, the initiation of combination treatment was delayed for at least a cycle due to suspected COVID-19 exposure; the investigator's decision was to continue the patient on acalabrutinib monotherapy. Efficacy analyses excluded the one patient in arm B who received acalabrutinib monotherapy only. At the data cutoff date, all patients had discontinued ceralasertib therapy, but all three patients in arm B who received acalabrutinib were still on treatment with acalabrutinib monotherapy.

Safety

Median duration of exposure was 3.7 months (range, 0.5–9.5) overall [arm A, 3.5 months (range, 0.5–7.7); arm B, 7.2 months (range, 4.9–9.5)] for ceralasertib and 15.9 months (range, 9.7–18.4) for acalabrutinib (arm B). In arm A, four DLTs of grade 4 thrombocytopenia were reported in three patients (three DLTs in two patients in cohort 1; one DLT in one patient in cohort 2); no DLTs were reported in arm B. All 11 patients experienced at least one treatment-emergent AE (TEAE) of any grade (Table 2 and Supplemental Table 1). The most common TEAEs in arm A were anemia ($n=7$; 88%), thrombocytopenia ($n=6$; 75%), and upper respiratory tract infection ($n=3$; 38%). In arm B, no TEAE was reported in ⩾2 patients.

All eight patients in arm A had at least one grade ⩾3 TEAE; no patients in arm B had a grade ⩾3 TEAE. The most common grade ⩾3 TEAEs in arm A were anemia ($n=6$; 75%) and thrombocytopenia ($n=5$; 63%). The most common grade ⩾3 TEAEs considered related to ceralasertib in arm A were anemia ($n=5$; 63%) and thrombocytopenia ($n=4$; 50%). No patients discontinued treatment due to AEs in either arm. No AEs with a fatal outcome were reported.

Overall, SAEs were reported in seven patients: six patients in arm A and one patient in arm B (Table 3). In arm A, SAEs reported in >1 patient were anemia ($n=5$; 63%) and thrombocytopenia ($n=3$; 38%). In arm B, an SAE of COVID-19 (grade 2) was reported in one patient, which was considered to be unrelated to either study drug by the investigator. All SAEs resolved in both arms.

In arm A, TEAEs leading to dose modifications and interruptions were reported in five (63%) patients and four (50%) patients, respectively (all in cohort 1), which were considered related to ceralasertib. In arm B, a TEAE leading to dose interruption was reported in one (33%) patient, which was considered related to both acalabrutinib and ceralasertib therapies. There were no dose modifications in arm B.

Deaths were reported in five patients during this study in arm A (ceralasertib monotherapy), all of which were considered unrelated to ceralasertib treatment by the investigator. All deaths occurred more than 30 days after the last treatment dose; all five of these patients died due to disease progression.

Pharmacokinetics

Plasma concentrations of acalabrutinib and ceralasertib were evaluated across collection time points and study visits (Figures 1 and 2). PK results for acalabrutinib and ACP-5862 (active metabolite of acalabrutinib) were comparable with historical controls from the acalabrutinib monotherapy arm of the ELEVATE-TN study (data on file, AstraZeneca). The six patient samples for acalabrutinib/ACP-5862 represent the total number of PK assessments available in a total of two patients. Plasma concentrations for ceralasertib were similar when administered as monotherapy or in combination with acalabrutinib, suggesting that there are no interactions between acalabrutinib and ceralasertib. The

Table 1. Patient demographics and baseline characteristics.

Parameter	Arm A: ceralasertib (*n* = 8)			Arm B: acalabrutinib + ceralasertib (*n* = 3)	Total (*N* = 11)
	Cohort 1 (*n* = 5)	Cohort 2 (*n* = 3)	Total (*n* = 8)		
Median age (range), years	64.0 (53.0–67.0)	61.0 (57.0–74.0)	62.5 (53.0–74.0)	68.0 (64.0–73.0)	64.0 (53.0–74.0)
Male	4 (80)	3 (100)	7 (87.5)	3 (100)	10 (90.9)
Number of prior anticancer systemic regimens					
<3	2 (40.0)	1 (33.3)	3 (37.5)	0	3 (27.3)
⩾3	3 (60.0)	2 (66.7)	5 (62.5)	3 (100)	8 (72.7)
Relapsed	4 (80.0)	2 (66.7)	6 (75.0)	3 (100)	9 (81.8)
Refractory[a]	1 (20.0)	1 (33.3)	2 (25.0)	0	2 (18.2)
Refractory to last therapy	1 (20.0)	1 (33.3)	2 (25.0)	0	2 (18.2)
Rai stage at study entry					
I	1 (20.0)	1 (33.3)	2 (25.0)	1 (33.3)	3 (27.3)
II	0	1 (33.3)	1 (12.5)	1 (33.3)	2 (18.2)
III	2 (40.0)	0	2 (25.0)	1 (33.3)	3 (27.3)
IV	2 (40.0)	0	2 (25.0)	0	2 (18.2)
Missing	0	1 (33.3)	1 (12.5)	0	1 (9.1)
Binet stage at study entry					
A	2 (40.0)	0	2 (25.0)	1 (33.3)	3 (27.3)
B	0	2 (66.7)	2 (25.0)	2 (66.7)	4 (36.4)
C	3 (60.0)	0	3 (37.5)	0	3 (27.3)
Missing	0	1 (33.3)	1 (12.5)	0	1 (9.1)
Genomic features					
del(11q)	3 (60.0)	0	3 (37.5)	3 (100)	6 (54.5)
del(17p)	3 (60.0)	0	3 (37.5)	0	3 (27.3)
TP53 mutation	2 (40.0)	3 (100)	5 (62.5)	0	5 (45.5)
Prior therapies					
Alkylating agent	5 (100)	2 (66.7)	7 (87.5)	3 (100)	10 (90.9)
Anti-CD20 mAb	5 (100)	3 (100)	8 (100)	3 (100)	11 (100)
BTK inhibitor	4 (80.0)	3 (100)	7 (87.5)	0	7 (63.6)
Purine analogue	5 (100)	2 (66.7)	7 (87.5)	3 (100)	10 (90.9)
BCL-2 inhibitor	1 (20.0)	2 (66.7)	3 (37.5)	1 (33.3)	4 (36.4)
Allogeneic stem cell transplant	0	1 (33.3)	1 (12.5)	0	1 (9.1)

(Continued)

Table 1. (Continued)

Parameter	Arm A: ceralasertib ($n=8$)			Arm B: acalabrutinib + ceralasertib ($n=3$)	Total ($N=11$)
	Cohort 1 ($n=5$)	Cohort 2 ($n=3$)	Total ($n=8$)		
Labs					
Mean platelets (*SD*), 10^9/L	150.4 (82.8)	159.0 (79.9)	153.6 (75.9)	171.3 (47.5)	158.5 (67.5)
Baseline platelets $< 100 \times 10^9$/L	2 (40.0)	1 (33.3)	3 (37.5)	0	3 (27.3)
Mean Hb (*SD*), g/dL	11.6 (2.5)	12.1 (0.5)	11.8 (1.9)	13.5 (2.2)	12.3 (2.1)
Baseline Hb < 10 g/dL	1 (20.0)	0	1 (12.5)	0	1 (9.1)
Mean ALC count (*SD*), 10^9/L	32.6 (52.9)	63.7 (88.5)	44.2 (64.0)	36.0 (44.1)	42.0 (57.2)
Baseline ALC $< 25 \times 10^9$/L	4 (80.0)	1 (33.3)	5 (62.5)	2 (66.7)	7 (63.6)

ALC, absolute lymphocyte count; BCL-2, B-cell lymphoma 2; BTK, Bruton tyrosine kinase; Hb, hemoglobin; mAb, monoclonal antibody; *SD*, standard deviation.
Data are *n* (%) unless otherwise specified.
[a]Data missing for one patient in arm B.

Table 2. TEAEs reported in ⩾2 patients.

Events, *n* (%)	Arm A: ceralasertib ($n=8$)						Arm B: acalabrutinib + ceralasertib ($n=3$)		Total ($N=11$)	
	Cohort 1 ($n=5$)		Cohort 2 ($n=3$)		Total ($n=8$)					
	Any grade	Grade ⩾ 3	Any grade	Grade ⩾ 3	Any grade	Grade ⩾ 3	Any grade	Grade ⩾ 3	Any grade	Grade ⩾ 3
Any TEAE	5 (100)	5 (100)	3 (100)	3 (100)	8 (100)	8 (100)	3 (100)	0	11 (100)	8 (72.7)
Anemia	5 (100)	5 (100)	2 (66.7)	1 (33.3)	7 (87.5)	6 (75.0)	0	0	7 (63.6)	6 (54.5)
Thrombocytopenia	4 (80.0)	4 (80.0)	2 (66.7)	1 (33.3)	6 (75.0)	5 (62.5)	0	0	6 (54.5)	5 (45.5)
Cough	2 (40.0)	0	0	0	2 (25.0)	0	1 (33.3)	0	3 (27.3)	0
Diarrhea	1 (20.0)	0	1 (33.3)	0	2 (25.0)	0	1 (33.3)	0	3 (27.3)	0
Fatigue	0	0	2 (66.7)	0	2 (25.0)	0	1 (33.3)	0	3 (27.3)	0
Nausea	0	0	2 (66.7)	0	2 (25.0)	0	1 (33.3)	0	3 (27.3)	0
Upper respiratory tract infection	2 (40.0)	0	1 (33.3)	0	3 (37.5)	0	0	0	3 (27.3)	0
Constipation	0	0	1 (33.3)	0	1 (12.5)	0	1 (33.3)	0	2 (18.2)	0
Contusion	0	0	1 (33.3)	0	1 (12.5)	0	1 (33.3)	0	2 (18.2)	0
Decreased appetite	0	0	1 (33.3)	0	1 (12.5)	0	1 (33.3)	0	2 (18.2)	0
Dyspnea	0	0	2 (66.7)	0	2 (25.0)	0	0	0	2 (18.2)	0
Epistaxis	1 (20.0)	0	0	0	1 (12.5)	0	1 (33.3)	0	2 (18.2)	0
Insomnia	0	0	1 (33.3)	0	1 (12.5)	0	1 (33.3)	0	2 (18.2)	0
Neutropenia	2 (40.0)	2 (40.0)	0	0	2 (25.0)	2 (25.0)	0	0	2 (18.2)	2 (18.2)
Oropharyngeal pain	2 (40.0)	0	0	0	2 (25.0)	0	0	0	2 (18.2)	0
Vomiting	0	0	1 (33.3)	0	1 (12.5)	0	1 (33.3)	0	2 (18.2)	0

TEAE, treatment-emergent adverse event.

Table 3. Serious adverse events.

Events, *n* (%)	Arm A: ceralasertib (*n* = 8)			Arm B: acalabrutinib + ceralasertib (*n* = 3)	Total (*N* = 11)
	Cohort 1 (*n* = 5)	Cohort 2 (*n* = 3)	Total (*n* = 8)		
Any SAE	4 (80.0)	2 (66.7)	6 (75.0)	1 (33.3)	7 (63.6)
Anemia	4 (80.0)	1 (33.3)	5 (62.5)	0	5 (45.5)
Thrombocytopenia	2 (40.0)	1 (33.3)	3 (37.5)	0	3 (27.3)
COVID-19	0	0	0	1 (33.3)	1 (9.1)
Febrile neutropenia	1 (20.0)	0	1 (12.5)	0	1 (9.1)
Gastrointestinal hemorrhage	0	1 (33.3)	1 (12.5)	0	1 (9.1)
Hypogammaglobulinemia	1 (20.0)	0	1 (12.5)	0	1 (9.1)
Pneumonia	1 (20.0)	0	1 (12.5)	0	1 (9.1)
Splenic rupture	1 (20.0)	0	1 (12.5)	0	1 (9.1)

SAE, serious adverse event.

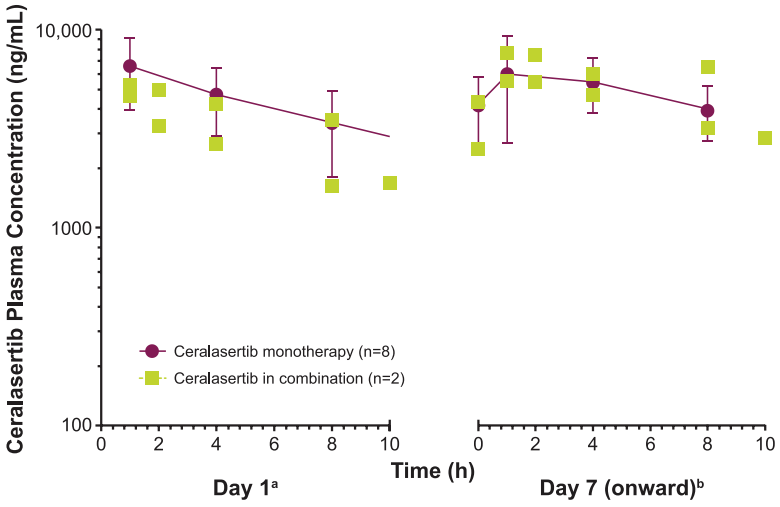

Figure 1. Plasma concentration by visit for ceralasertib.
n represents the total number of patients.
Circles represent mean (±*SD*) concentrations (monotherapy) and the squares represent individual plasma concentrations (combination).
C, cycle; D, day; h, hour; *SD*, standard deviation.
[a]Day 1 represents C1D1 for monotherapy and C2D1 for combination treatment.
[b]Day 7 (onward) represents C1D15, C1D22, and C2D15 for monotherapy and C2D7 for combination treatment.

data from 10 patients for ceralasertib represent the total number of patients in all treatment arms.

Efficacy

At the time of data cutoff, no responses were observed in arm A; two patients treated with ceralasertib monotherapy had a best response of stable disease, and six had progressive disease. In arm B, the ORR was 100% (80% CI, 32–100) in the two patients treated with acalabrutinib plus ceralasertib combination, both of whom had partial responses (Table 4). The median DOR in arm B was not reached. In arm A, median PFS was 3.8 months (95% CI, 0.7–4.6). Median PFS

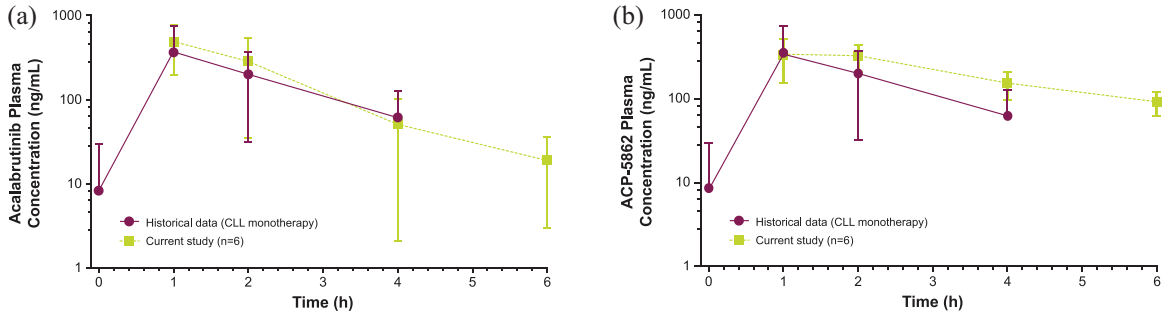

Figure 2. Mean plasma concentration for acalabrutinib (a) and ACP-5862 (b) compared with historical data.
For acalabrutinib and ACP-5862, *n* represents the total number of PK assessments available in a total of two patients at C1D7. Historical data are from PK assessments at C1D1 from the ELEVATE-TN study (data on file, AstraZeneca). The data represent mean (±*SD*) concentrations.
C, cycle; CLL, chronic lymphocytic leukemia; D, day; h, hour; PK, pharmacokinetics; *SD*, standard deviation.

Table 4. Response rates.

Response	Arm A: ceralasertib (*n* = 8)			Arm B: acalabrutinib + ceralasertib (*n* = 2)	Total (*N* = 10)
	Cohort 1 (*n* = 5)	Cohort 2 (*n* = 3)	Total (*n* = 8)		
Best response, *n* (%)					
CR	0	0	0	0	0
CRi	0	0	0	0	0
PR	0	0	0	2 (100)	2 (20.0)
SD	1 (20.0)	1 (33.3)	2 (25.0)	0	2 (20.0)
PD	4 (80.0)	2 (66.7)	6 (75.0)	0	6 (60.0)
CR rate (CR + CRi), % (80% CI)	0 (0–36.9)	0 (0–53.6)	0 (0–25.0)	0 (0–68.4)	0 (0–20.6)
ORR (CR + CRi + PR), % (80% CI)	0 (0–36.9)	0 (0–53.6)	0 (0–25.0)	100 (31.6–100)	20.0 (5.5–45.0)
mDOR, months	N/A	N/A	N/A	NR	N/A
mPFS, months	4.4 (3.2–NE)	1.6 (0.7–NE)	3.8 (0.7–4.6)	NR	4.4 (0.7–NE)
mOS, months	15.1 (7.1–NE)	23.2 (6.6–NE)	16.9 (6.6–NE)	NR	23.2 (6.6–NE)

CI, confidence interval; CR, complete response; CRi, complete response with incomplete hematologic recovery; mDOR, median duration of response; mOS, median overall survival; mPFS, median progression-free survival; N/A, not applicable; NE, not estimable; NR, not reached; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

was not reached in arm B. Median OS was 16.9 months (95% CI, 6.6–not estimable) in arm A and was not reached in arm B.

Exploratory analysis

PBMCs from two patients in arm B demonstrated BTK occupancy of 97–99% with acalabrutinib monotherapy, which was not affected by the addition of ceralasertib (Figure 3).

Blood samples from three patients receiving ceralasertib monotherapy were assessed to determine the impact of treatment on DNA repair response based on levels of pATM, monoubiquitinated PCNA, and pNibrin. Upon treatment with

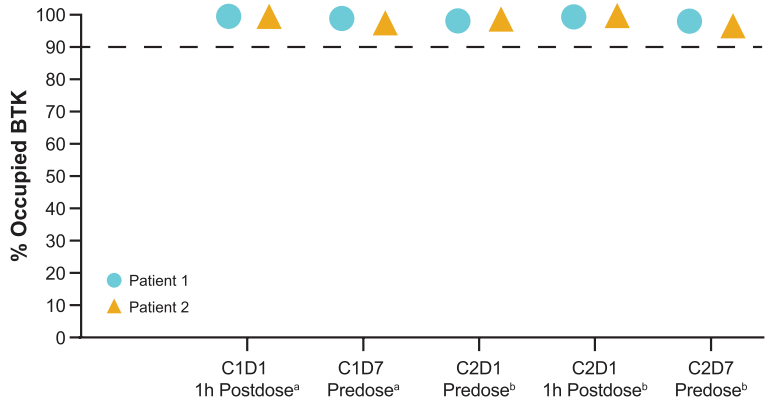

Figure 3. BTK target occupancy of samples from patients receiving acalabrutinib plus ceralasertib in arm B.
BTK, Bruton tyrosine kinase; C, cycle; D, day.
[a]Acalabrutinib monotherapy was administered during C1.
[b]Acalabrutinib + ceralasertib were administered during C2.

ceralasertib monotherapy, the levels of pATM, PCNA, and pNibrin were elevated (Figure 4).

Telomere length was evaluated in patients in arm A (Figure 5). Based on HT-STELA classification, four patients (50%) had shorter telomeres (<2.169 kb) and four patients (50%) had intermediate telomere lengths (2.169–3.650 kb). Of the four patients with shorter telomeres, three patients (75%) had Rai stage III/IV. All samples in arm A had telomere lengths (<3.650 kb) that were in the 'fusogenic' range (i.e. the range in which fusion occurs: <2.26–3.81 kb).

Discussion

Despite emerging novel therapies in CLL, patients who relapse on B-cell receptor (BCR) antagonists, including ibrutinib (BTK inhibitor) and venetoclax (BCL2 inhibitor), have limited treatment options. Based on *in vitro* and *in vivo* data supporting ATR inhibition as a potential therapeutic approach in CLL,[5] this proof-of-concept study investigated the clinical potential of ATR inhibition alone or a dual BTK and ATR inhibition approach by evaluating ceralasertib as monotherapy or in combination with acalabrutinib, in patients with high-risk R/R CLL. Given the proven safety and efficacy of acalabrutinib in patients with R/R CLL, the addition of ceralasertib was a rational approach in the attempt to deepen response to therapy, especially in the del(11q) population. PK findings were consistent with another acalabrutinib monotherapy clinical study (ELEVATE-TN; data on file, AstraZeneca). Based on preliminary efficacy data, patients had limited clinical benefit with ceralasertib monotherapy.

In the ELEVATE-TN study, the most common grade ⩾3 TEAE in patients treated with acalabrutinib monotherapy was neutropenia (10%).[8] In the ASCEND study, the most common grade 3/4 AEs were neutropenia (16%) and anemia (12%) in patients treated with acalabrutinib monotherapy.[10] In the current study, hematological toxicity was common in arm A (ceralasertib monotherapy); the most common grade ⩾3 TEAEs in arm A were anemia (75%) and thrombocytopenia (63%). No grade ⩾3 TEAE was reported in patients treated with acalabrutinib plus ceralasertib (1 week on/3 weeks off) in arm B. These data suggest that a shorter dosing duration of ceralasertib may be better tolerated, and that ceralasertib did not appear to cause additive adverse effects when used in combination with acalabrutinib. However, these findings must be interpreted with caution as arm B comprised three patients only, one of whom received acalabrutinib monotherapy.

Patients were heavily pretreated (median of three prior lines of therapies); almost all patients in arm A had been previously exposed to BTK inhibition, whereas no patients in arm B had received a prior BTK inhibitor. This suggests that the responses observed in arm B may be due to acalabrutinib. In patients treated with ceralasertib monotherapy (arm A, $n=8$), at a median follow-up of 15.1 months, there were no treatment

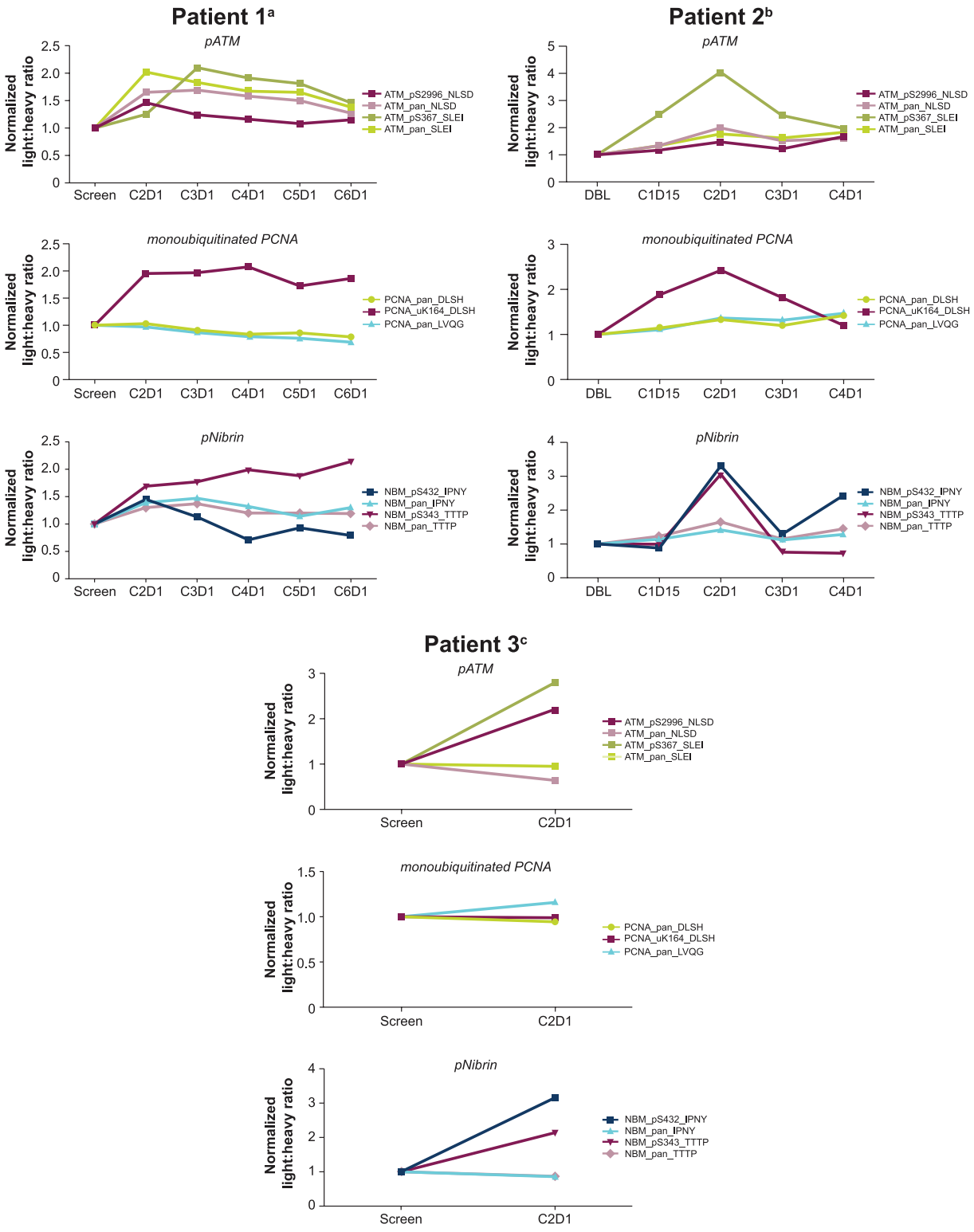

Figure 4. PD changes in blood with ceralasertib monotherapy.
Normalized light: heavy ratio measures endogenous (light) peptide relative to the stable isotope-labeled internal standard (heavy) peptide spiked into each sample.
BID, twice daily; C, cycle; D, day; DBL, double baseline; pATM, phosphorylated ataxia telangiectasia mutated; PCNA, proliferating cell nuclear antigen; PD, pharmacodynamic.
[a]Patient with del(11q)/del(17p) and *TP53* and C481S mutation was treated with ceralasertib 160 mg BID continuously.
[b]Patient with del(11q)/del(17p) was treated with ceralasertib 160 mg BID continuously.
[c]Patient with *TP53* and C481S mutation was treated with ceralasertib 160 mg BID for 2 weeks on and 2 weeks off.

responses. Two patients had a best response of stable disease, and the remainder had progressive disease. The median PFS was 3.8 months and median OS was 16.9 months. In patients treated with acalabrutinib plus ceralasertib (arm B, $n = 2$), at a median follow-up of 17.2 months, the ORR was 100%, with both patients having partial responses. The median DOR, median PFS, and median OS were not reached.

Overall, the mean plasma concentrations of acalabrutinib and its active metabolite ACP-5862 were comparable with those observed in historical control samples (ELEVATE-TN study) following monotherapy. The mean plasma concentrations of ceralasertib were similar when administered as monotherapy or in combination with acalabrutinib, suggesting no interaction between the two drugs in combination.

Preliminary PD data suggest that the addition of ceralasertib does not affect BTK occupancy of acalabrutinib. Functional assays quantifying the phosphosignaling of DNA damage response (DDR) proteins in response to DNA damage may have clinical implications for patients in identifying potential novel PD biomarkers.[14,16] The DDR proteins ATM, PCNA, and nibrin[16] are of interest because, in response to DNA damage, autophosphorylation of ATM pS367/pS2996, monoubiquitylation of PCNA, and phosphorylation of nibrin (pNibrin) pS343 are seen.[16] Ceralasertib is a highly specific inhibitor of ATR, but not ATM, which is a closely related apical kinase in the DDR pathway.[17] In response to DNA damage, ATR inhibition leads to compensatory mechanisms of the ATM pathway.[17] Therefore, with ceralasertib therapy, levels of pATM, PCNA, and pNibrin would be expected to increase. Indeed, in our study, treatment with ceralasertib monotherapy increased levels of pATM, monoubiquitinated PCNA, and pNibrin.

Telomere erosion and subsequent telomere fusion are key contributors to disease progression of CLL.[15] Patients with telomeres within the 'fusogenic' range have demonstrated significantly shorter PFS, and telomere length and dysfunction were shown to be strong independent predictors of PFS and OS.[15] Therefore, telomere 'fusogenic' range (<2.26–3.81 kb) has emerged as a prognostic tool to predict survival.[15] In the present study, all patients treated with

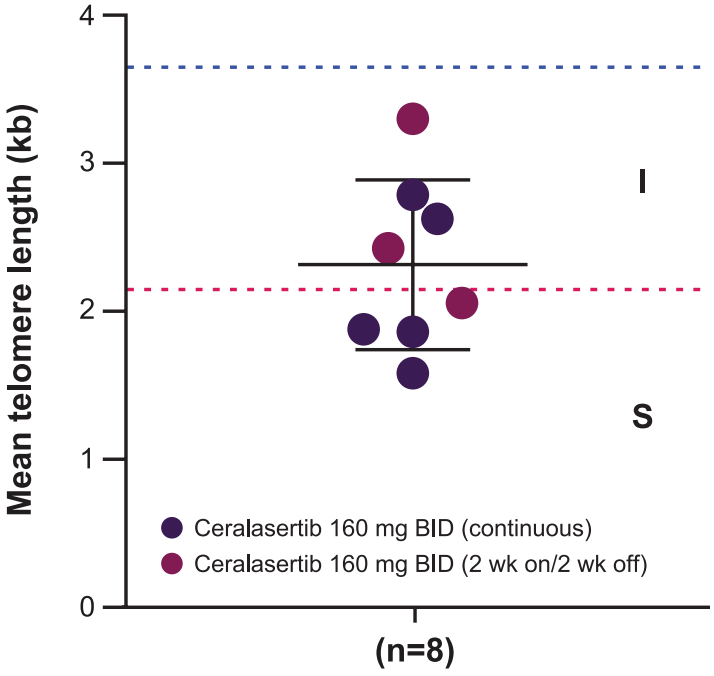

Figure 5. Telomere length for patients on ceralasertib monotherapy.
BID, twice daily; I, intermediate; S, short; w, week.

ceralasertib monotherapy had baseline telomere lengths (<3.650 kb) that were within the 'fusogenic' range, which suggests that the patients were at increased risk of poor outcomes.

The study is limited by the small number of patients treated with ceralasertib monotherapy and with the combination of ceralasertib and acalabrutinib overall, as well as the small number of patients with del(11q) enrolled in arm A. At the time of study (enrollment period: January 31, 2018–October 19, 2020), there were many other treatment options available for R/R CLL, which affected patient enrollment into this study. Only 38% of patients had del(11q) in arm A and only two of the three patients in arm B with del(11q) received combination therapy. Of note, 87.5% of patients in arm A had previously received BTK inhibitor therapy. These factors may have contributed to the low clinical efficacy observed with ceralasertib monotherapy. A decision to stop enrollment into the study was based on the operational feasibility of the study and the changing CLL landscape that impacted the execution of this study. Another limitation is the lack of data in patients with del(17p) or *TP53* mutations treated with ceralasertib and acalabrutinib in arm B. Preclinically, ceralasertib sensitized *TP53*- and *ATM*-defective CLL cells to BTK inhibitor

therapy, which was a rationale for this combination treatment approach in this study.[5] While arm B was initially limited to patients with del(11q) only, potential expansion of arm B to include patients with del(17p) or *TP53* mutations was not possible given the premature study termination. Therefore, it remains unknown whether ATR inhibition in combination with BTK inhibition may improve depth of response in *TP53*-mutated CLL. No safety concerns were observed during the study. Although no further studies of ceralasertib in CLL are in development, this agent is currently being investigated in various solid tumors, including a phase III study (NCT05450692) in combination with durvalumab in patients with non-small cell lung cancer.

Conclusion

Findings from this phase I/II proof-of-concept study demonstrated little clinical benefit of ceralasertib monotherapy in patients with high-risk CLL who had previously received BTK inhibitor treatment. Acalabrutinib in combination with ceralasertib was tolerable and demonstrated limited preliminary clinical activity in two patients with BTK inhibitor–naive CLL and del(11q). However, findings are inconclusive due to the small sample size, and it is possible that responses in the combination arm were due to acalabrutinib. PK findings suggest that there are no interactions between ceralasertib and acalabrutinib and several ATR pathway PD markers were altered. The study terminated prematurely due to the evolving treatment landscape.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Good Clinical Practice guidelines. The protocol and all amendments were approved by the institutional review board and independent ethics committee; all patients provided written informed consent (UK REC reference: 17/YH/0289; POL EC reference: 193/KBL/OIL/2017).

Consent for publication

Not applicable.

Author contributions

Wojciech Jurczak: Conceptualization; Formal analysis; Investigation; Writing – review & editing.

Nagah Elmusharaf: Resources; Writing – review & editing.

Christopher P. Fox: Investigation; Resources; Writing – review & editing.

William Townsend: Investigation; Resources; Writing – review & editing.

Amanda G. Paulovich: Formal analysis; Investigation; Writing – review & editing.

Jeffrey R. Whiteaker: Formal analysis; Investigation; Writing – review & editing.

Fanny Krantz: Investigation; Writing – review & editing.

Chuan-Chuan Wun: Formal analysis; Methodology; Validation; Writing – review & editing.

Graeme Parr: Formal analysis; Funding acquisition; Project administration; Resources; Validation; Writing – review & editing.

Shringi Sharma: Data curation; Investigation; Methodology; Visualization; Writing – review & editing.

Veerendra Munugalavadla: Formal analysis; Methodology; Writing – review & editing.

Richa Manwani: Writing – review & editing.

Emma Dean: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Validation; Writing – review & editing.

Talha Munir: Investigation; Writing – review & editing.

Acknowledgements

The authors thank all patients who participated in this study, their families, and the study investigators. They would like to thank Benedetta Lombardi for her assistance in analyzing the MRM data, and Rafael While for facilitating biosample collections. William Townsend acknowledges support and funding from the NIHR University College London Hospitals Biomedical Research Center.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was sponsored by AstraZeneca. Medical writing support was provided by Sarah Huh, PharmD, and Cindy Gobbel, PhD, of Peloton Advantage, LLC (Parsippany, NJ, USA), an OPEN Health company, and funded by AstraZeneca. Manuscript submission support was provided by Kelly Montenegro of Peloton Advantage, LLC, funded by AstraZeneca and authorized by the authors. Fred Hutchinson Cancer Center would like to acknowledge infrastructural funding for targeted proteomic assay analyses and part of Fred Hutchinson staff time to work on the manuscript from the National Cancer Institute (NCI) grant no. U01CA271407 (NCI Clinical Proteomic Tumor Analysis Consortium program) and grant no. R01CA235575 to AGP, and the NCI Research Specialist program (grant no. R50CA211499) to JRW.

Competing interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article:

WJ: Contract/grant – AstraZeneca, Janssen, and Lilly.

NE: Conference registration grants – AbbVie; speakers fees – Roche and AstraZeneca.

CPF: Consultancy and honoraria – AbbVie, Acerta Pharma/AstraZeneca, Atara Biotherapeutics, Celgene/BMS, GenMab, Gilead/Kite, Incyte, Lilly, Janssen, MorphoSys, Ono, Roche, and Takeda; research funding – BeiGene.

WT: Consultancy and honoraria – BMS, Gilead, Incyte, and Roche; travel expenses – Gilead and Takeda.

AGP: Consultancy – CellCarta; founder – Precision Assays.

JRW: Consultancy – CellCarta.

FK: Employment and stock ownership – AstraZeneca.

CW: Employment – AstraZeneca.

GP: Employment and stock ownership – AstraZeneca.

SS: Employment and stock ownership – AstraZeneca.

VM: Employment and stock ownership – AstraZeneca (a family member is an employee and stock owner of Gilead).

RM: Employment and stock ownership – AstraZeneca.

ED: Employment and stock ownership – AstraZeneca.

TM: Honoraria – AstraZeneca, Alexion, Gilead, Novartis, Roche, Janssen, AbbVie; advisory board – AstraZeneca, AbbVie, Janssen, MorphoSys, Alexion.

Availability of data and materials

Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure. Data for studies directly listed on Vivli can be requested through Vivli at www.vivli.org. Data for studies not listed on Vivli could be requested through Vivli at https://vivli.org/members/enquiries-about-studies-not-listed-on-the-vivli-platform/. AstraZeneca Vivli member page is also available outlining further details: https://vivli.org/ourmember/astrazeneca/.

ORCID iDs

Wojciech Jurczak https://orcid.org/0000-0003-1879-8084

Christopher P. Fox https://orcid.org/0000-0002-6322-9254

William Townsend https://orcid.org/0000-0003-3975-2448

Amanda G. Paulovich https://orcid.org/0000-0001-6532-6499

Jeffrey R. Whiteaker https://orcid.org/0000-0001-5042-8580

Veerendra Munugalavadla https://orcid.org/0000-0002-2390-3822

Talha Munir https://orcid.org/0000-0002-2901-0769

Supplemental material

Supplemental material for this article is available online.

References

1. Hallek M, Cheson BD, Catovsky D, *et al*. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood* 2018; 131: 2745–2760.
2. Forconi F, Lanham SA and Chiodin G. Biological and clinical insight from analysis of the tumor b-cell receptor structure and function in chronic lymphocytic leukemia. *Cancers* 2022; 14: 663.
3. Dreger P, Ghia P, Schetelig J, *et al*. High-risk chronic lymphocytic leukemia in the era of pathway inhibitors: integrating molecular and cellular therapies. *Blood* 2018; 132: 892–902.
4. Hallek M and Al-Sawaf O. Chronic lymphocytic leukemia: 2022 update on diagnostic and therapeutic procedures. *Am J Hematol* 2021; 96: 1679–1705.
5. Kwok M, Davies N, Agathanggelou A, *et al*. ATR inhibition induces synthetic lethality and overcomes chemoresistance in TP53- or ATM-defective chronic lymphocytic leukemia cells. *Blood* 2016; 127: 582–595.
6. Wang LW, Jiang S, Yuan YH, *et al*. Recent advances in synergistic antitumor effects exploited from the inhibition of ataxia telangiectasia and RAD3-related protein kinase (ATR). *Molecules* 2022; 27: 2491.
7. AstraZeneca Pharmaceuticals. Calquence (package insert). Wilmington, DE: AstraZeneca Pharmaceuticals, 2022.
8. Sharman JP, Egyed M, Jurczak W, *et al*. Acalabrutinib with or without obinutuzumab versus chlorambucil and obinutuzmab for treatment-naive chronic lymphocytic leukaemia (ELEVATE TN): a randomised, controlled, phase 3 trial. *Lancet* 2020; 395: 1278–1291.
9. Sharman JP, Egyed M, Jurczak W, *et al*. Efficacy and safety in a 4-year follow-up of the ELEVATE-TN study comparing acalabrutinib with or without obinutuzumab versus obinutuzumab plus chlorambucil in treatment-naïve chronic lymphocytic leukemia. *Leukemia* 2022; 36: 1171–1175.
10. Ghia P, Pluta A, Wach M, *et al*. ASCEND: phase III, randomized trial of acalabrutinib versus idelalisib plus rituximab or bendamustine plus rituximab in relapsed or refractory chronic lymphocytic leukemia. *J Clin Oncol* 2020; 38: 2849–2861.
11. Jurczak W, Pluta A, Wach M, *et al*. Three-year follow-up of the ASCEND trial: acalabrutinib vs rituximab plus idelalisib or bendamustine in relapsed/refractory chronic lymphocytic leukemia [abstract]. *Blood* 2021; 138: 393.
12. Hallek M, Cheson BD, Catovsky D, *et al*. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood* 2008; 111: 5446–5456.
13. Eldridge SM, Chan CL, Campbell MJ, *et al*. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. *BMJ* 2016; 355: i5239.
14. Whiteaker JR, Wang T, Zhao L, *et al*. Targeted mass spectrometry enables quantification of novel pharmacodynamic biomarkers of ATM kinase inhibition. *Cancers* 2021; 13: 3843.
15. Lin TT, Norris K, Heppel NH, *et al*. Telomere dysfunction accurately predicts clinical outcome in chronic lymphocytic leukaemia, even in patients with early stage disease. *Br J Haematol* 2014; 167: 214–223.
16. Whiteaker JR, Zhao L, Saul R, *et al*. A multiplexed mass spectrometry-based assay for robust quantification of phosphosignaling in response to DNA damage. *Radiat Res* 2018; 189: 505–518.
17. Jones GN, Rooney C, Griffin N, *et al*. pRAD50: a novel and clinically applicable pharmacodynamic biomarker of both ATM and ATR inhibition identified using mass spectrometry and immunohistochemistry. *Br J Cancer* 2018; 119: 1233–1243.