Original Study

Venetoclax and Cobimetinib in Relapsed/Refractory AML: A Phase 1b Trial

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Abstract

Treatment options for patients who have acute myeloid leukemia (AML) which has come back (relapsed) or stopped responding to treatment (refractory) are limited. In this study, 30 patients with relapsed/refractory AML received 2 drugs (venetoclax and cobimetinib). Venetoclax-cobimetinib had limited responses with unwanted side effects, compared with venetoclax alone. However, these findings will help future trials of similar drug combinations.

Background: Therapies for relapsed/refractory acute myeloid leukemia remain limited and outcomes poor, especially amongst patients who are ineligible for cytotoxic chemotherapy or targeted therapies. **Patients and Methods:** This phase 1b trial evaluated venetoclax, a B-cell lymphoma-2 (BCL-2) inhibitor, plus cobimetinib, a MEK1/2 inhibitor, in patients with relapsed/refractory acute myeloid leukemia, ineligible for cytotoxic chemotherapy. Two-dimensional dose-escalation was performed for venetoclax dosed daily, and for cobimetinib dosed on days 1-21 of each 28-day cycle. **Results:** Thirty patients (median [range] age: 71.5 years [60-84]) received venetoclax-cobimetinib. The most common adverse events (AEs; in ≥40.0% of patients) were diarrhea (80.0%), nausea (60.0%), vomiting (40.0%), febrile neutropenia (40.0%), and fatigue (40.0%). Overall, 66.7% and 23.3% of patients experienced AEs leading to dose modification/interruption or treatment withdrawal, respectively. The composite complete remission (CRc) rate (complete remission [CR] + CR with incomplete blood count recovery + CR with incomplete platelet recovery) was 15.6%; antileukemic

Abbreviations: AE, adverse event; AHD, antecedent hematologic disorder; AML, acute myeloid leukemia; BCL-2, B-cell lymphoma-2; BCL-xL, B-cell lymphoma-extra large; CI, confidence interval; C_{max}, maximum plasma concentration at steady state; CR, complete remission; CRc, composite CR; CRi, CR with incomplete blood count recovery; CRp, CR with incomplete platelet count recovery; HSCT, hematopoietic stem cell transplant; MAPK, mitogen-activated protein kinase; MEK1/2, MAPK kinases 1 and 2; MLFS, morphologic leukemia-free state; MR, minor responses; MTD, maximum tolerated dose; OS, overall survival; PD, pharmacodynamic; pERK, phosphorylated ERK; PK, pharmacokinetic; PR, partial response; pS6, phosphorylated S6; R/R, relapsed or refractory; RP2D, recommended phase 2 dose; SAE, serious adverse event; sAML, secondary AML; SD, stable disease; TLS, tumor lysis syndrome; VAF, variant allele frequency; ven-cobi, venetoclax and cobimetinib; ven-idasa, venetoclax in combination with idasanutlin.

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Submitted: Nov 14, 2023; Revised: Jan 15, 2024; Accepted: Jan 15, 2024; Epub: 18 January 2024

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https://doi.org/10.1016/j.clml.2024.01.007

response rate (CRc + morphologic leukemia-free state/partial remission) was 18.8%. For the recommended phase 2 dose (venetoclax: 600 mg; cobimetinib: 40 mg), CRc and antileukemic response rates were both 12.5%. Failure to achieve an antileukemic response was associated with elevated baseline phosphorylated ERK and MCL-1 levels, but not BCL-xL. Baseline mutations in ≥1 signaling gene or *TP53* were noted in nonresponders and emerged on treatment. Pharmacodynamic biomarkers revealed inconsistent, transient inhibition of the mitogen-activated protein kinase (MAPK) pathway. **Conclusion:** Venetoclax-cobimetinib showed limited preliminary efficacy similar to single-agent venetoclax, but with added toxicity. Our findings will inform future trials of BCL-2/MAPK pathway inhibitor combinations.

Clinical Lymphoma, Myeloma and Leukemia, Vol. 24, No. 6, 364–374 © 2024 The Authors. Published by Elsevier Inc.

Keywords: B-cell lymphoma-2, Biomarker, Mitogen-activated protein kinase pathway, Targeted therapy

Introduction

Acute myeloid leukemia (AML) is an aggressive malignancy, and largely a disease of older patients, with a median age at diagnosis of 68 years.[1] Therapies for patients with relapsed or refractory (R/R) AML remain limited and outcomes poor, especially for patients who are ineligible for cytotoxic chemotherapy or targeted therapies.[2-6] Therapies that target survival pathways known to be dysregulated or aberrant in AML may improve outcomes, particularly if they are less toxic.

Venetoclax, an oral B-cell lymphoma-2 (BCL-2) inhibitor,[7] has shown limited activity as a monotherapy in R/R AML, with a complete remission (CR)/CR with incomplete blood count recovery (CRi) rate of 19%.[8] Cobimetinib, a small molecule inhibitor of mitogen-activated protein kinase (MAPK) kinases 1 and 2 (MEK1/2),[9] is approved for use in melanoma[10,11] but has not been evaluated in AML. Other MEK1/2 inhibitors, such as trametinib, selumetinib, and binimetinib, however, have shown limited single agent activity in patients with *RAS*-mutated R/R AML and myelodysplastic syndromes, with response rates (with variable definitions) of 20%, 17%, and 8%, respectively.[12-14]

Concomitant BCL-2 and MAPK/MEK blockade has demonstrated synergistic induction of apoptosis in *in vitro* AML models, including those resistant to single agents, and reduced leukemia burden in *in vivo* xenograft models.[15-18] For instance, the combination of venetoclax and cobimetinib (ven-cobi) significantly enhanced cell death, and suppressed cell growth and the clonogenic potential of myeloid progenitors, compared with venetoclax or cobimetinib alone.[15] This synergy has also been associated with MCL-1 downregulation, upregulation of pro-apoptotic BIM, and suppression of phosphorylated S6 (pS6).[15-18]

Overexpression of MCL-1 has been identified as a major acquired mechanism of venetoclax resistance, as well as a contributor to AML progression.[19,20] Thus, a venetoclax combination targeting MAPK, and indirectly MCL-1, may be a potential option to improve AML therapy. We therefore initiated a phase 1 study to assess the safety, tolerability, and preliminary efficacy of ven-cobi in older patients with R/R AML, who were ineligible for cytotoxic chemotherapy.

Methods

Study Design

This open-label, multicenter phase 1b trial evaluated ven-cobi and venetoclax in combination with idasanutlin (ven-idasa; ClinicalTrials.gov identifier: NCT02670044). Here, we present results for the ven-cobi arm. Details of the ven-idasa arm have been previously published.[21] Primary objectives included: safety profile evaluation and determination of the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D). Secondary objectives were to evaluate preliminary efficacy and pharmacokinetics (PK). Exploratory objectives included assessing biomarkers related to drug targets, cobimetinib exposure, and disease biology.

Enrollment was nonrandomized, with multiple cohorts open simultaneously, and patients were only treated with 1 combination arm (ven-cobi or ven-idasa). There were 4 ven-cobi cohorts in total: venetoclax 400 mg + cobimetinib 40 mg (Cohort 1); venetoclax 600 mg + cobimetinib 40 mg (Cohort 2); venetoclax 800 mg + cobimetinib 40 mg (Cohort 3); and venetoclax 400 mg + cobimetinib 60 mg (Cohort 4). Venetoclax initiation included a 3- to 5-day ramp-up to 400, 600, or 800 mg daily on days 1-28, with cobimetinib (40 or 60 mg daily) on days 1-21 of each 28-day cycle; dose-escalation followed a 2-dimensional, 3 + 3 + 3 design (Supplemental Figure S1). Starting doses were established based on the clinical experience with each drug. The starting dose of venetoclax was 400 mg daily, half the venetoclax monotherapy dose tested in a phase 2 study of R/R or previously untreated AML (M14-212), in which doses of up to 1200 mg daily were well tolerated.[8] The starting dose of cobimetinib was 40 mg daily, 1 dose level below the dose of cobimetinib used in the phase 3 study of cobivemurafenib in metastatic melanoma (60 mg once daily; coBRIM study, GO28141).[22] Patients were hospitalized during ramp-up in cycle 1 and received tumor lysis syndrome (TLS) prophylaxis. Safety monitoring criteria and stopping rules for toxicity were protocol-defined.

The study protocol was approved by the institutional review board or ethics committees at participating institutions in accordance with the International Conference on Harmonization guidelines, including good clinical practice and the ethical principles originating from the Declaration of Helsinki. Informed consent was obtained from all patients. Authors had access to and reviewed the clinical trial results.

Patient Population

Eligible patients had R/R AML by World Health Organization criteria[23] or newly diagnosed secondary AML (sAML) after prior treatment for an antecedent hematologic disorder (AHD).

Patients were deemed ineligible for cytotoxic chemotherapy based on age (≥60 years) and investigator opinion. Key exclusion criteria included the use of strong or moderate cytochrome P450-3A inducers or inhibitors ≤7 days before study drug administration; prior use of a BCL-2 inhibitor; or prior exposure to experimental treatment targeting the Raf, MEK, or MAPK pathways.

Assessments

Adverse events (AEs) were reported by the treating physician throughout the study and for ≥30 days after the last dose or initiation of another anticancer therapy. MTD was the highest dose at which less than one-third of ≥6 patients experienced a dose-limiting toxicity.

Response was assessed by routine laboratory tests and bone marrow examinations and evaluated per the International Working Group 2003 AML response criteria.[24] Composite CR (CRc) was defined as CR + CRi + CR with incomplete platelet count recovery (CRp), and antileukemic response as CRc + morphologic leukemia-free state (MLFS)/partial response (PR). CRp and CRi were considered mutually exclusive.

Pharmacokinetic Assessments

Plasma concentration-versus-time data for venetoclax and cobimetinib were analyzed using noncompartmental analysis. Summary statistics of PK parameters, such as maximum plasma concentration at steady state (C_{max}) and trough/minimum plasma concentration at steady state, of venetoclax and cobimetinib were computed.

Biomarker Assessments

Mutation analysis was performed on bone marrow-derived mononuclear cells at baseline, at the end of the study and, in a few cases, during treatment, using the FoundationOne Heme Panel (465 gene mutation panel, Roche Foundation Medicine, Grenzach-Wyhlen, Germany) as previously published.[25]

Intracellular protein expression of BCL-2, MCL-1, BCL-xL, phosphorylated ERK (pERK), and pS6 was evaluated on blast cells, myeloid/monocytes, and lymphocytes using surface backbone lineage markers to identify cell populations (CD45, CD34, CD117, HLA-DR). Baseline expression of BCL-2, BCL-xL, and MCL-1 in blast cells was evaluated for association with response; since high MCL-1 and/or high BCL-xL are common resistance mechanisms for venetoclax,[26] ratios of BCL-2:BCL-xL and BCL-2:MCL-1 were evaluated.

Additional details of further assessments performed are provided in the Supplementary Methods.

Statistical Methods

Safety and efficacy were summarized by descriptive statistics. The efficacy population was the intent-to-treat population; the safety-evaluable population included all patients who received one or more dose(s) of study drug. Time-to-event analyses were conducted using the Kaplan–Meier method, and the Fisher's exact test compared response rates between treatment groups.

Results

A total of 32 patients were enrolled in the ven-cobi arm across 17 centers (United States, Italy, Canada, and France) between March 2016 and July 2020 (Supplemental Figure S2). Data cutoff was December 10, 2020, and median (range) duration of follow-up was 2.7 months (0.0-14.8). Overall, 30 patients received one or more dose(s) of study drug, and the median (range) number of cycles received was 2 (1-8).

Patient Characteristics

At baseline, median (range) patient age was 71.5 years (60-84), 63.3% of patients had refractory disease, and 36.7% had relapsed disease; no patients had newly diagnosed AML transformed from a previously treated AHD. Half (50.0%) of patients had sAML. Patients had received a median (range) of 2 prior lines of therapy (1-10), which included azacitidine (36.7% of patients) and decitabine (16.7% of patients) (Supplemental Table S1); 20.0% of patients had received a prior hematopoietic stem cell transplant (HSCT); and 33.3% of patients were adverse risk by European LeukemiaNet 2010 criteria. Baseline cytogenetics included 23.3% of patients with complex karyotype, and mutation analysis identified *NRAS* and/or *KRAS* mutations in 23.3% of patients and *TP53* mutations in 13.3% of patients (Table 1).

Safety

The most common (occurring in ≥40.0% of patients) all-grade treatment-emergent AEs were diarrhea (80.0%), nausea (60.0%), vomiting (40.0%), febrile neutropenia (40.0%), and fatigue (40.0%) (Supplemental Table S2A). The most common (occurring in ≥20.0% of patients) grade 3-4 AEs included febrile neutropenia (40.0%), diarrhea (36.7%), and pneumonia (30.0%). Diarrhea (grade 3 only; no grade 4) was most commonly experienced at higher doses: 25.0% of patients in Cohort 1; 28.6% of patients in Cohort 2; 33.3% of patients in Cohort 3; and 57.1% of patients in Cohort 4 (Supplemental Table S2B). Nearly all cases (49/52) resolved completely. Due to the frequent occurrence of diarrhea, mandatory antidiarrheal prophylaxis was implemented as a protocol amendment; however, no patients were treated with ven-cobi following the amendment due to the Sponsor's decision to not pursue further investigation of the combination given the overall benefit-risk profile. Although the number of patients in each cohort was small, there were also higher rates of febrile neutropenia in Cohorts 3 (66.7%) and 4 (42.9%) compared with Cohorts 1 (0%) and 2 (14.3%). Serious AEs (SAEs) were reported in 80.0% of patients. The most commonly reported SAEs (occurring in ≥20.0% of patients) were febrile neutropenia (30.0%), pneumonia (30.0%), and sepsis (20.0%) (Supplemental Table S3). Fatal AEs were reported in 6 (20.0%) patients and consisted of: lung infection (n = 2), sepsis (n = 2), respiratory failure (n = 1), and lung disorder (n = 1).

Overall, 66.7% of patients experienced an AE that resulted in a treatment modification or interruption, most commonly (≥10.0% of patients) due to diarrhea (26.7%), febrile neutropenia (13.3%), and thrombocytopenia (13.3%) (Supplemental Table S4 and Supplemental Figure S3A-B). Treatment withdrawal due to

Table 1 Patient Demographics and Baseline Characteristics of the Safety Population.

	n = 30
Median age, y (range)	71.5 (60-84)
Male sex, n (%)	16 (53.3)
ECOG PS, n (%)	
0	5 (16.7)
1	20 (66.7)
2	5 (16.7)
Disease status, n (%)	
Refractory	19 (63.3)
Relapsed	11 (36.7)
Newly diagnosed, transformed from AHD (previously treated)	0 (0.0)
AML type, n (%)	
De novo	15 (50.0)
Secondary	15 (50.0)
ELN 2010 classification, n (%)	
Favorable	1 (3.3)
Intermediate-I	9 (30.0)
Intermediate-II	10 (33.3)
Adverse	10 (33.3)
Median prior therapies, n (range)	2 (1-10)
Prior HSCT, n (%)	6 (20.0)
WBC at baseline, 10^9/L, median (range)	2.1 (0.5-23.2)
Aspirate BM blast %, median (aspirate)	30 (7.0-96.0)
Mutations and cytogenetics, n (%)	
NRAS/KRAS mutation status[a]	
NRAS and/or *KRAS* mutation detected	7 (23.3)
NRAS mutation detected	6 (20.0)
KRAS mutation detected	3 (10.0)
NRAS and *KRAS* mutation undetected	21 (70.0)
Not evaluable	2 (6.7)
TP53 mutation status[b]	
Mutation detected	4 (13.3)
Mutation undetected	24 (80.0)
Not evaluable	2 (6.7)
t(9;11)(p22;q23);*MLLT3-MLL*	1 (3.3)
inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*	1 (3.3)
−5 or del(5q)	1 (3.3)
−7	4 (13.3)
Cytogenetic abnormalities not classified as favorable or adverse	10 (33.3)
Complex karyotype	7 (23.3)

Abbreviations: AHD = antecedent hematologic disorder; AML = acute myeloid leukemia; BM = bone marrow; ECOG PS = Eastern Cooperative Oncology Group performance status; ELN = European LeukemiaNet; HSCT = hematopoietic stem cell transplantation; WBC = white blood cells.
[a] Using FM Heme cutoff of 5%.
[b] Using FM Heme cutoff of 1%.

an AE or SAE occurred in 23.3% and 20.0% of patients, respectively; specific treatment-emergent AEs leading to any treatment withdrawal are available in Supplemental Table S5. No AEs, including diarrhea, resulted in treatment discontinuation in more than 1 patient (Supplemental Table S6).

Laboratory TLS and clinical TLS were reported in 1 patient each; the clinical TLS (grade 3) event was associated with an increase in creatinine in the setting of pneumonia and was managed with standard measures. No TLS events resulted in treatment discontinuation. Overall, 30- and 60-day Kaplan–Meier estimated mortality rates were 6.7% and 34.3%, respectively. Causes of 60-day mortality included: progressive disease (6/30 patients); respiratory failure (1/30; preceded by pneumonia in the resistant disease setting, assessed as unrelated to study treatment); sepsis (2/30; gram-negative sepsis, assessed as unrelated to study treatment; sepsis, assessed as related to study treatment); and lung disorder (1/30; lung infection in the resistant disease setting, assessed as related to study treatment).

Dose-limiting toxicities reported were diarrhea (2 patients [6.7%]: 1 each in Cohorts 2 and 4), and ejection fraction decreased (2 patients [6.7%]: 1 each in Cohorts 3 [assessed as related to both venetoclax and cobimetinib] and 4 [assessed as unrelated to venetoclax and cobimetinib]). Cohort 2 (venetoclax 600 mg + cobimetinib 40 mg) was determined to be the MTD and RP2D. Although the MTD was not exceeded in Cohort 3, Cohort 2 was established as the RP2D due to the overall safety profile.

Efficacy

CRc and antileukemic response rates across all dose cohorts were 15.6% (5/32) and 18.8% (6/32), respectively. At the RP2D, the CRc and antileukemic response rates were both 12.5% (1/8). The median (range) time to best CRc response was 1.8 months (1.4-3.0), and the median (range) duration of response was 4.2 months (1.0-10.4) (Table 2). Blast count reduction was seen across all ven-cobi dose cohorts (Figure 1). Responses were seen in patients aged ≥75 years, patients with sAML, and patients with AML who had received prior treatment with a hypomethylating agent (Supplemental Figure S4).

The median (95% confidence interval [CI] overall survival [OS] was 3.6 months [2.0-8.6] among all patients, and 7.5 months (4.2 months-not evaluable) among patients with a CRc response (Supplemental Figure S5). No evaluable patients with CRc achieved minimal residual disease negativity ($<10^{-3}$ threshold). No patients underwent a subsequent HSCT.

While minimal residual disease was not achieved, molecular responses (reductions in baseline mutation variant allele frequency [VAF]) in responders were noted when evaluating on-treatment changes to baseline mutation frequencies: VAF reductions were observed in patients who achieved CR (mean change: −11.3%) compared with patients with stable disease (SD; mean change: +2.8%) or refractory disease (mean change: +11.7%) (Supplemental Figure S6A-B). Changes were similar in genes coding for signaling or nonsignaling proteins.

Pharmacokinetics

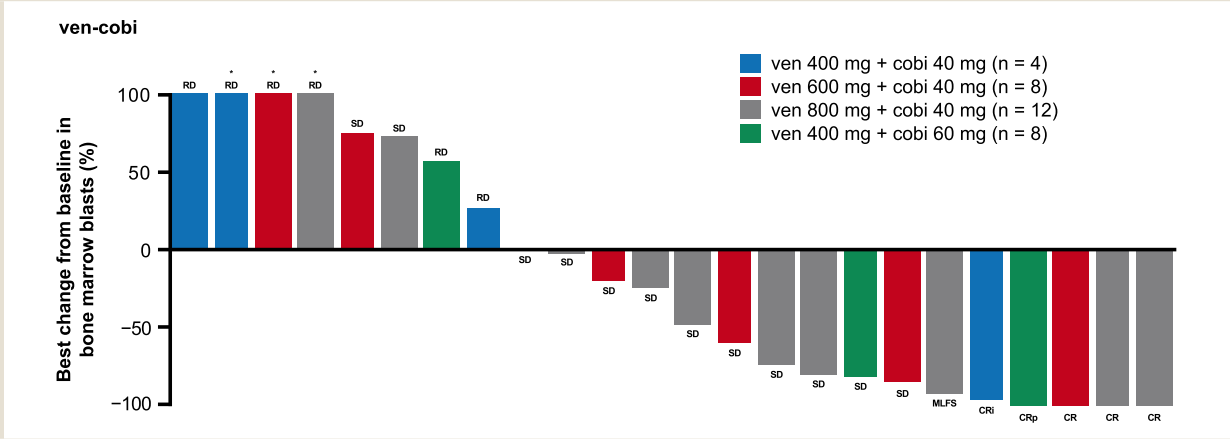
PK parameters were available for 22 patients across the 4 cohorts. In Cohorts 1 (n = 4), 2 (n = 7), 3 (n = 8), and 4 (n = 3), respectively, the mean C_{max} for venetoclax was 1.34, 1.48, 1.64, and 1.16 µg/mL, and the mean C_{max} for cobimetinib was 0.32, 0.20, 0.39, and 0.64 µg/mL (Supplemental Table S7).

Table 2 Response Outcomes for Ven-Cobi.

n (%)	Cohort 1: ven 400 mg + cobi 40 mg (n = 4)	Cohort 2: ven 600 mg + cobi 40 mg (n = 8)	Cohort 3: ven 800 mg + cobi 40 mg (n = 12)	Cohort 4: ven 400 mg + cobi 60 mg (n = 8)	Total (N = 32)
Antileukemic responders (CRc/PR/MLFS)	1 (25.0)	1 (12.5)	3 (25.0)	1 (12.5)	6 (18.8)
CRc (CR/CRi/CRp)	1 (25.0)	1 (12.5)	2 (16.7)	1 (12.5)	5 (15.6)
CR	0 (0.0)	1 (12.5)	2 (16.7)	0 (0.0)	3 (9.4)
CRi	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.1)
CRp	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	1 (3.1)
PR	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
MLFS	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)	1 (3.1)
Time to best CRc response (months), median (range)	2.8 (2.8-2.8)	1.4 (1.4-1.4)	2.3 (1.6-3.0)	1.8 (1.8-1.8)	1.8 (1.4-3.0)
Duration of response (months), median (range)	2.0 (2.0-2.0)	4.4 (4.4-4.4)	2.6 (1.0-4.2)	10.4 (10.4-10.4)	4.2 (1.0-10.4)
Duration of follow-up (months), median (range)	4.7 (1.6-8.7)	4.8 (0.0-14.2)	3.0 (0.6-14.8)	1.1 (0.0-12.3)	2.7 (0.0-14.8)

Abbreviations: cobi = cobimetinib; CR = complete remission; CRc = composite complete remission; CRi = complete remission with incomplete blood count recovery; CRp = complete remission with incomplete platelet recovery; MLFS = morphologic leukemia-free state; PR = partial response; ven = venetoclax.

Figure 1 Best percentage bone marrow blast reduction and individual patient responses. Efficacy data are presented for the intent-to-treat population for whom data were available.



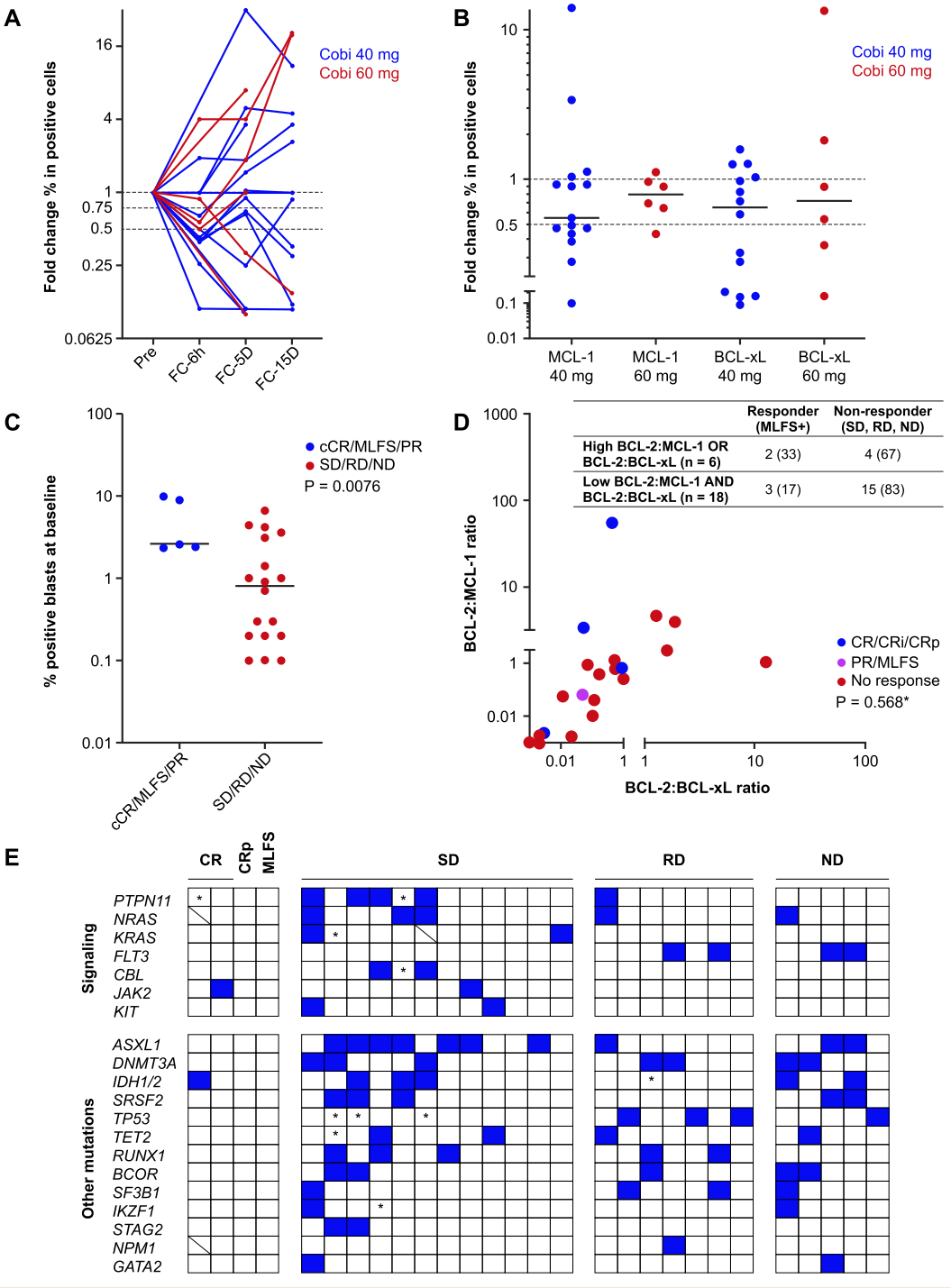
*Best % change from baseline in bone marrow blasts is >100. Abbreviations: cobi = cobimetinib; CR = complete remission; CRi = complete remission with incomplete blood count recovery; CRp = complete remission with incomplete platelet recovery; MLFS = morphologic leukemia-free state; RD = refractory disease; SD = stable disease; ven = venetoclax.

Pharmacodynamic (PD) Changes

Reductions in MEK downstream proteins pERK and pS6 (>30% reduction [or 0.7-fold change] in expressing cells) were observed in most patients (13/20 for both). pERK reductions were highly variable with 50.0% median reduction at 6-hour and 62.5% median "best" reduction at any timepoint (Supplemental Figure S7A-B). Furthermore, pERK reductions were often observed early, at the 6-hour timepoint (9/14 patients with 6-hour data), but often rebounded by day 5 to baseline values or higher (5/9 patients; Figure 2A and Supplemental Figure S7C); in contrast, pS6 reductions occurred later, with >30% reductions observed at either days 5 or 15 in most patients (11/21) (Supplemental Figure S7D-E). Notably, there were no dose interruptions associated with the observed rebound of pERK levels.

No significant PK/PD correlations were identified. Neither pERK nor pS6 reductions were associated with dose, cobimetinib exposure, or response (Figure 2A and Supplemental Figures S7D, S8A-D, and S7C and S7E, respectively). Similarly, reductions in MCL-1 and BCL-xL were not associated with dose, exposure, or response (Figure 2B and Supplemental Figures S9A, S8E-H, and S9B, respec-

Figure 2 Pharmacodynamic and correlative biomarker data: (A) pERK pharmacodynamic data by cobi dose; (B) MCL-1 and BCL-xL pharmacodynamic data by cobi dose; (C) baseline pERK data by response; (D) scatter plot of BCL-2:BCL-xL and BCL-2:MCL-1 status for responders and nonresponders; (E) baseline molecular mutations.



Mutations detected at baseline are indicated by shaded boxes, mutation loss by a diagonal line, and emergent mutations by an asterisk. Abbreviations: BCL-2 = B-cell lymphoma 2; BCL-xL = B-cell lymphoma-extra large; cobi = cobimetinib; cCR = clinical complete response; CR = complete remission; CRi = complete remission with incomplete blood count recovery; CRp = complete remission with incomplete platelet recovery; D = day; FC = fold change; h = hour; MCL-1 = myeloid leukemia 1; MLFS = morphologic leukemia-free state; ND = no data; pERK = phosphorylated ERK; PR = partial response; RD = refractory disease; SD = stable disease.

tively). Further evaluation of longitudinal MCL-1 on treatment revealed inefficient suppression over time (Supplemental Figure S9C).

Correlative Biomarker Analysis

At baseline, pERK was significantly elevated in responders (MLFS+; $P = .0076$), whereas pS6 was not (Figure 2C and Supplemental Figure S7F). Antileukemic responses were doubled in patients with high (>1.5) BCL-2:BCL-xL and/or BCL-2:MCL-1 (33.3%; 2/6) versus low (≤1.5) BCL-2:BCL-xL and BCL-2:MCL-1 (16.7%; 3/18) ratios (Figure 2D). Analysis of each individual BCL-2 family protein in nonresponders revealed high MCL-1 levels, but not BCL-xL (Supplemental Figure S10).

Baseline mutation profiling was available for most patients (28/30); out of the patients that achieved MLFS or better (n = 6), sequencing data were available for only 4 patients. Baseline *TP53* mutations were associated with nonresponse (antileukemic response rate: 0%, 0/4). The antileukemic response rate was 14.3% (1/7) in patients with both baseline *IDH1/2* and *NRAS*/*KRAS* mutations; 12.5% (2/16) in patients with any signaling mutation (*NRAS, KRAS, FLT3, PTPN11, CBL, JAK2,* or *KIT)*; and 0% (0/4) in patients with 2 or more signaling mutations (Figure 2E and Supplemental Table S8).

Emergent mutations, detected on treatment but not at baseline, were also analyzed. At discontinuation, emergent mutations were detected in *TP53* (16.7% of patients, 3/18) and signaling genes (16.7% of patients, 3/18 harboring 4 mutations: *PTPN11* [11.1%, 2/18], *KRAS* [5.6%, 1/18], and *CBL* [5.6%, 1/18]) (Figure 2E and Supplemental Figure S6C). Notably, *CBL* and *PTPN11* mutations both emerged in the same patient, who also harbored a stable *NRAS* mutation and achieved SD. Emergent *TET2* and *IKZF1* mutations were also noted in 1 patient each (5.6% of patients, 1/18) (Figure 2E and Supplemental Figure S6C). Clearance of *NRAS* (and *NPM1)* mutations was observed at the same time as an emergent *PTPN11* mutation (noted above) in a patient that achieved a CR. In addition, *KRAS* mutation clearance occurred in a patient with SD that harbored 3 other stable signaling mutations (Figure 2E and Supplemental Figure S6C).

Gene expression profiling using RNA sequencing was performed on bone marrow aspirates in some patients at baseline (n = 17) and on treatment (n = 26). On-treatment timepoints varied from end of cycle 1 to treatment discontinuation. The "hallmark *KRAS* signaling Up" gene signature[27] was significantly increased in patients on or after ven-cobi therapy but not ven-idasa therapy (Supplemental Figure S11).

Discussion

In this phase 1b trial in patients with R/R AML who were ineligible for cytotoxic chemotherapy, ven-cobi showed limited efficacy comparable to single-agent venetoclax,[8] but with additional toxicity. The safety profile of ven-cobi was as anticipated, with no new safety signals identified for either venetoclax or cobimetinib. The observed dose-limiting toxicities (diarrhea and ejection fraction decreased) have been previously identified as risks with venetoclax and cobimetinib treatment: diarrhea has been associated with both venetoclax and cobimetinib, and ejection fraction decreased has been associated with cobimetinib. Overall, the observed PK profiles of venetoclax and cobimetinib were similar to previous findings.[10,28]

Diarrhea was the most common AE and was observed at higher rates than previously observed with either cobimetinib[29] or venetoclax monotherapy[8] (80.0% vs 61% vs 56% for all-grade diarrhea, and 36.7% vs 7% vs 6% for grade ≥3 diarrhea, respectively). However, there are caveats which make cross-trial comparisons difficult, such as different diseases (eg, advanced solid tumors in the cobimetinib monotherapy study), and different dosing levels/schedules. The observed difference in rates of diarrhea should therefore be viewed cautiously. Although limited by small patient numbers, diarrhea appeared to be cobimetinib dose-related, with grade ≥3 diarrhea observed at higher rates in Cohort 4, which evaluated cobimetinib 60 mg (as opposed to cobimetinib 40 mg in Cohorts 1-3) and exceeded the cobimetinib MTD and the RP2D. While diarrhea was frequent, dose modifications/interruptions due to diarrhea were infrequent, particularly at the RP2D (all dose cohorts: 26.7%; RP2D: 14.3%); similarly, only 1 patient (3.3%; patient was in Cohort 2 [RP2D]) withdrew from treatment due to diarrhea. Though the majority of events were manageable with supportive measures, mandatory antidiarrheal prophylaxis is encouraged for future studies to improve tolerability. The frequency of other AEs, including hematologic toxicities and infections, was consistent with the known myelosuppressive effects of venetoclax and cobimetinib, and within the previously reported ranges for a similar R/R AML population.[2,4-6,8,30,31] Clinical TLS was infrequent, as reported in other venetoclax AML trials,[8,28] but was noted in 1 patient, where it was managed and did not lead to treatment discontinuation; it is therefore recommended that TLS prophylaxis and monitoring should continue when using venetoclax-based combinations in AML. At the proposed RP2D (venetoclax 600 mg + cobimetinib 40 mg), the safety profile was deemed manageable with the addition of risk mitigation measures for gastrointestinal toxicity.

The clinical activity of ven-cobi was limited across all dose cohorts evaluated (all dose cohorts CRc: 15.6%; RP2D CRc: 12.5%). Ven-cobi activity was comparable to that observed with single-agent venetoclax (CR/CRi 19%)[8] or MEK inhibitors (trametinib: CR/CRi/MLFS/PR 20%; selumetinib: CR/CRi/PR/minor responses [MR]/unconfirmed MR 17%; binimetinib: CR/CRi 8%).[12-14] Despite encouraging preclinical rationale,[15] the response rates suggest no added benefit with this BCL-2/MEK inhibitor combination therapy in the clinical setting. Similarly, a previous study evaluating the combination of venetoclax-trametinib-azacitidine in R/R AML demonstrated little benefit over single agent trametinib.[32] However, these cross-trial comparisons are limited by differences in patient populations, such as the inclusion of patients with *RAS* mutations only, high-risk myelodysplastic syndromes, or unfit newly-diagnosed AML, and differences in response definitions.[12-14] Further, in the current study, the design limited the ability to draw conclusions regarding achievement of transfusion independence for red blood cells or platelets; capturing this information is therefore recommended to complement efficacy data in future R/R AML studies. Overall, the observed response rates and OS reported with ven-cobi were within the expected range for an elderly or unfit R/R AML population.[5,6,33]

While an RP2D was determined, due to the overall risk-benefit profile, expansion was not pursued. Of note, a short median duration of therapy (2 cycles [range 1-8]) was observed; this reflects the risk-benefit profile, including the limited quality of the responses and the added toxicity of the combination. It is not clear whether dosing optimization, such as continuous MEK inhibition or intermittent BCL-2 dosing as investigated in the ven-idasa arm, and mandatory gastrointestinal prophylaxis could improve the benefit-risk profile. Additionally, this study was conducted in an unfit R/R AML population, characterized by frailty and poor outcomes; as such, this may have had a negative impact on the risk-benefit profile.

Further, 1 potential explanation for the limited efficacy observed is insufficient target or pathway inhibition. For instance, a lack of exposure dependency or response effect in pERK and pS6 was noted. Baseline levels of pERK, which is proximal to MEK and therefore likely best reflects MEK activity,[34] was associated with antileukemic responses to ven-cobi. However, pERK was only partially reduced by cobimetinib in most patients, and transiently suppressed in some patients, suggesting that MAPK inhibition was not deep nor durable. In contrast, pS6, which is known to act downstream of multiple signaling pathways, so may be a less reliable assessment of MEK/MAPK activity,[35] was inhibited at later timepoints. Furthermore, while MEK inhibition has been reported to downregulate MCL-1, this was not clearly observed;[18] it is unclear whether this was related to feedback loops that reactivate MAPK[36,37] or inadequate dosing, and is difficult to conclude given the limited number of responders. Previously described resistance mechanisms such as activation of parallel signaling pathways, including PI3K/AKT, JAK/STAT, or NFKB, or upregulation of receptor tyrosine kinases may have contributed to the limited clinical activity.[38-40] Alternatively, sample timepoints and/or collection may have affected the ability to detect PD effects.

Similar to ven-idasa,[21] patients with low (≤1.5) baseline BCL-2:BCL-xL and BCL-2:MCL-1 ratios had a lower response rate to ven-cobi compared with patients with high (>1.5) baseline ratios. MCL-1 expression was most associated with nonresponse to ven-cobi, indicating that MCL-1, a known venetoclax resistance factor,[26] was not sufficiently inhibited by cobimetinib. Targeting MCL-1 in AML remains an unmet need, and thus alternative strategies are being explored.[41,42]

Although an all-comer population was enrolled, rather than a *RAS* mutant population, this did not appear to drive the activity profile. Antileukemic activity was noted both in patients with (14.3%) and without (11.1%) *RAS* mutations (1/7 and 3/21 patients with baseline mutation data available, respectively). Similar results were observed with a broader definition of "signaling mutations" known to activate RAS/MAPK signaling, suggesting that RAS signaling as defined by mutational status did not define ven-cobi efficacy. However, it is important to note that as sequencing data were limited (available in 4/6 patients that achieved a response), it is difficult to delineate mutations associated with response. Importantly, pERK protein levels were elevated in patients achieving an MLFS or better response, suggesting that pretreatment MAPK activation may confer responsiveness to therapy.

Emergent *TP53* mutations were observed in 16.7% of patients treated with ven-cobi, which is fewer than the 33.3% noted with ven-idasa.[21] This suggests that the effect of ven-idasa on *TP53* mutation emergence may only be partially observed with ven-cobi, and may be a common occurrence in R/R AML, even with non-targeted therapy.[43] However, the small number of responders and the short ven-cobi treatment duration may have contributed to the observed difference in the frequency of emergent *TP53* mutations. In addition, emergent signaling mutations were observed in 3 patients, and clearance in 2 patients. Interestingly, clearance was observed in the context of 3 other stable signaling mutations in 1 patient, and both clearance of *NRAS* and emergence of *PTPN11* were noted at the same timepoint in the other patient. These data indicate selective pressure to maintain MAPK signaling, through various mutations upstream of MEK. This is further supported by the observed increase in the "hallmark *KRAS* signaling Up" gene signature observed with ven-cobi therapy. Together these data indicate that there is strong pressure to drive RAS signaling networks in AML, particularly in the context of MEK inhibition, and suggest that effective strategies may need to target further downstream, such as MCL-1 directly, rather than indirectly through complex signaling networks. Furthermore, it was observed that pERK levels were associated with response. This, along with the increase in "hallmark *KRAS* signaling Up" gene signature and the emergence of signaling mutations on therapy, suggests that there may be an optimal level of pathway activation needed to achieve a response, with too little or too much RAS signaling impairing the efficacy of ven-cobi.

Although limited, analyses of VAF dynamics showed an overall decrease in VAF in responders, slight increases in patients with SD, and more extensive increases in patients with resistant disease, in line with molecular changes tracking clinical response. Inconsistent effects on *RAS* pathway mutations were similarly observed in R/R AML patients responding to triplet trametinib-ven-azacitidine therapy.[32] Our findings suggest that AML clones harboring a signaling mutation are not uniformly sensitive to cobimetinib, and may be influenced by other factors such as the specific mutated gene, co-occurring mutations, number of signaling mutations, nongenetic factors, or extracellular signals. Notably, coculture of AML blasts with mesenchymal stromal cells consistently induced MAPK signaling in AML cells – a mechanism that contributes to mutation-agnostic resistance to multiple treatment modalities.[44] However, low numbers of antileukemic responders and incomplete serial mutation data in the current study limited the ability to draw conclusions on the effect of cobimetinib on mutation dynamics, and the mechanisms of response/resistance to ven-cobi. Therefore, future studies of RAS/MAPK inhibitors in AML should carefully evaluate clonal dynamics at serial timepoints to better inform sensitivity and resistance patterns using mutations, and nongenetic approaches to assess pathway activation.

As this study was designed before venetoclax was approved for newly diagnosed unfit AML patients, all patients were venetoclax-naïve. Thus, a limitation of this study is the inability to make conclusions regarding venetoclax retreatment. While it has been suggested that venetoclax retreatment may still be effective due to unique synergies that may exist with different venetoclax combinations,[45] future studies of BCL-2 inhibitor combinations are needed to address this data gap. Additionally, a challenge of the current study is the ability to dissect specific correlates for cobime-

tinib versus venetoclax, as some patients may have been responding to venetoclax only; a key example of this is the responses observed in patients either lacking signaling mutations or harboring *IDH* mutations. Furthermore, the correlative findings observed in the current study, such as responses in patients without signaling mutations, may not translate to patients with R/R AML previously treated with venetoclax.

Targeting RAS-activated AML clones is an area of high unmet need. Not only is the RAS/RAF/MEK/ERK signaling pathway frequently dysregulated in AML, but mutations in *RAS* signaling genes have been linked to resistance to newer targeted therapies including FLT3 inhibitors, IDH inhibitors, and venetoclax.[20,46-52] Outgrowth of *RAS*-mutated clones has been observed at a high frequency in the relapsed setting after these agents.[46-52] As patients are likely to be treated with one of these agents earlier in their disease course, it is anticipated that there will be an increased prevalence of *RAS* signaling mutations in the relapsed setting; approaches that target *RAS* mutant clones in the R/R setting, or suppress the outgrowth of clones with RAS-activated signaling via combination therapy in the frontline setting, are therefore needed. Further, in preclinical studies, MEK inhibition led to cytostatic effects and less pronounced cytotoxic effects in comparison with downstream inhibition of the RAS-MAPK pathway.[53] Other approaches that may be effective include: alternative RAS-MAPK pathway targets, such as specific *RAS, ERK,* or *RAF* mutation inhibitors[53-55]; inhibition of alternative signaling pathways[56]; co-inhibition of multiple signaling molecules[57]; or MEK inhibition in conjunction with sensitizing agents.[58]

In conclusion, despite encouraging preclinical rationale, ven-cobi showed limited preliminary efficacy, similar to single-agent venetoclax, but with added toxicity in patients with R/R AML ineligible for cytotoxic chemotherapy. The results of this study therefore suggest no added benefit with this BCL-2/MEK inhibitor combination therapy in the clinical setting. Biomarker correlatives suggest that high MCL-1 levels, *TP53* mutations, and multiple signaling mutations may contribute to resistance to ven-cobi. Future trials combining venetoclax with other MAPK pathway inhibitors are anticipated to further expand on these findings. The safety, dosing, molecular, and PD learnings from this study may therefore optimize the design and patient selection for future trials of BCL-2 and MAPK pathway inhibitor combinations.

Clinical Practice Points

- Current treatment options for patients with R/R AML are limited and are associated with poor survival outcomes.
- The combination of B-cell lymphoma-2 and MAPK/MEK inhibitors, such as venetoclax and cobimetinib, respectively, has demonstrated a promising preclinical rationale in *in vitro* AML models and *in vivo* xenograft models.
- This phase 1b trial of the venetoclax and cobimetinib combination demonstrated limited preliminary efficacy, similar to single-agent venetoclax, but with added toxicity in patients with R/R AML.
- However, the safety, dosing, molecular and pharmacodynamic learnings from this study may optimize patient selection and the design of anticipated future trials of venetoclax in combination with MAPK pathway inhibitors.

Disclosure

MYK is a consultant for AbbVie, Inc., Genentech, Inc., and F. Hoffmann La-Roche; is an advisory board member for F. Hoffmann La-Roche; holds shares from Reata Pharmaceuticals; has received honoraria from Amgen, AbbVie, Inc., and Genentech, Inc.; and has received research funding from AbbVie, Inc., Genentech, Inc., Eli Lilly, Cellectis, Calithera, Stemline, Threshold, Flexus Biosciences, Novartis, Ablynx, and Agios. MD, MO, JW, WH, DD, and HH are employees of Genentech, Inc. and may hold Roche stock or stock options. NGD has received research funding from Daiichi Sankyo, Bristol-Myers Squibb, Pfizer, Gilead, Sevier, Genentech, Inc., Astellas, Daiichi Sankyo, AbbVie, Inc., Hanmi, Trovagene, Fate Therapeutics, Amgen, Novimmune, GlycoMimetics, Trillium, Kite Pharma, Aptose, Shattuck Labs, KAHR, ArcellX, Sanofi, Sumitomo, and ImmunoGen; and has served in a consulting or advisory role for Daiichi Sankyo, Bristol-Myers Squibb, Arog Pharmaceuticals, Pfizer, Novartis, Jazz Pharmaceuticals, Celgene, AbbVie, Inc., Astellas, Genentech, Inc., Immunogen, Servier, Syndax, Trillium, Gilead, Amgen, Shattuck Labs, ArcellX, Kite Pharma, Sumitomo, Caribou Biosciences, Sanofi, Rigel, Aptose, KURA, GlycoMimetics, and Agios. JSG has received research funding (for trials) from AbbVie, Inc., Genentech, Inc., Pfizer, Prelude, and AstraZeneca; and has served on advisory boards for AbbVie, Inc., Astellas, Bristol-Myers Squibb and Servier. BAJ is a consultant/advisor for AbbVie, Inc., Bristol-Myers Squibb, Daiichi Sankyo, Genentech, Inc., Gilead, GlycoMimetics, Jazz Pharmaceuticals, Kymera, Pfizer, Rigel, Servier, and Takeda; protocol steering committee for GlycoMimetics; data monitoring committee for Gilead; travel reimbursement/support from Rigel; and research funding to his institution from AbbVie, Inc., Amgen, Arog Pharmaceuticals, Aptose, BMS, Celgene, Daiichi Sankyo, F. Hoffmann-La Roche, Forma Therapeutics, Forty-Seven, Genentech, Inc./Roche, Gilead, GlycoMimetics, Hanmi, Immune-Onc, Incyte, Jazz Pharmaceuticals, Loxo Oncology, Pfizer, Pharmacyclics, Sigma Tau, and Treadwell. KWLY is a consultant for Astex, Bristol-Myers Squibb/Celgene, F. Hoffmann-La Roche, Novartis, Otsuka, Paladin, Pfizer, Shattuck Labs, Taiho, and Takeda; has received honorarium from AbbVie, Inc. and Novartis; and has received research funding from Astex, Forma Therapeutics, F. Hoffmann-La Roche, Genentech, Inc., Geron, Janssen, Jazz Pharmaceuticals, MedImmune, Novartis, Onconova, and Tolero. KRK, NV, SP, AT, AP, PF, and GV declare no competing financial interests. SA reports non-financial support from Roche/Genentech, Inc. during the conduct of the study; and has received personal fees from Roche Canada, Pfizer, Bristol-Myers Squibb, Palladin, and Lundbeck outside of the submitted work. GJR consulted for AbbVie, Inc., Amgen, Argenx, AstraZeneca, Bluebird Bio, Blueprint Medicines, Bristol-Myers Squibb, Caribou Biosciences, Celgene, Daiichi Sankyo, Ellipses Pharma, GlaxoSmithKline, Janssen, Jasper Pharmaceuticals, Jazz Pharmaceuticals, Molecular Partners, Novartis, Pfizer, Roche, Syndax, Takeda (IRC Chair), and Telix Pharma; and has received research funding from Janssen. DAP has received research funding from AbbVie, Inc. and has served as a consultant or advisory board member for AbbVie, Inc. and Genen-

tech, Inc. JMB consulted for AbbVie, Inc., Bristol-Myers Squibb, Astex, Pfizer, Astellas, Taiho, and Jazz Pharmaceuticals. BLP has received research funding from Ambit Biosciences, Genentech, Inc., F. Hoffmann-La Roche, Jazz Pharmaceuticals, Novartis, Pfizer, and Cornerstone Pharmaceuticals; and is a consultant for Cornerstone Pharmaceuticals. RLO has received research support for trials from Astellas, Pfizer, Genentech, Inc., Daiichi Sankyo, and Cellectis; and consulted for AbbVie, Inc., Astellas, and Actinium. GM is a consultant for AbbVie, Inc., Celgene, Roche, Janssen, Astellas, Pfizer, and Incyte. W-JH is a former employee of Genentech, Inc. and may hold Roche stock; and is a current employee of Prelude Therapeutics. MGO is an employee of F. Hoffmann-La Roche and may hold stock or stock options. MA consulted for Amgen, AstraZeneca, Daiichi Sankyo, Syndax, GlycoMimetics, Oncoceutics, and Aptose; has received research funding from F. Hoffmann-La Roche, AstraZeneca, Amgen, Daiichi Sankyo, Jazz Pharmaceuticals, GlycoMimetics; and holds stock from Reata, Oncoceutics/Chimerix and Aptose.

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Acknowledgments

Venetoclax is being developed in a collaboration between Genentech, Inc. and AbbVie, Inc. Genentech, Inc. provided financial support for the study and participated in the design, study conduct, analysis, and interpretation of data, as well as the writing, review, and approval of the manuscript. We thank the participating patients and their families, investigators, and trial staff. Denise Cheung, B. Pharm, of Genentech, Inc., provided support in analysis of the data, and Cherie Green and Connie Ma provided flow cytometry and data analysis support. Third-party medical writing assistance, under the direction of Maika Onishi, was provided by Roisin Weaver, MSc, of Ashfield MedComms, an Inizio company, and was funded by F. Hoffmann-La Roche Ltd.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.clml.2024.01.007.

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