

Efficacy and Biological Correlates of Response in a Phase II Study of Venetoclax Monotherapy in Patients with Acute Myelogenous Leukemia

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ABSTRACT

We present a phase II, single-arm study evaluating 800 mg daily venetoclax, a highly selective, oral small-molecule B-cell leukemia/lymphoma-2 (BCL2) inhibitor in patients with high-risk relapsed/refractory acute myelogenous leukemia (AML) or unfit for intensive chemotherapy. Responses were evaluated following revised International Working Group (IWG) criteria. The overall response rate was 19%; an additional 19% of patients demonstrated antileukemic activity not meeting IWG criteria (partial bone marrow response and incomplete hematologic recovery). Twelve (38%) patients had isocitrate dehydrogenase 1/2 mutations, of whom 4 (33%) achieved complete response or complete response with incomplete blood count recovery. Six (19%) patients had BCL2-sensitive protein index at screening, which correlated with time on study. BH3 profiling was consistent with on-target BCL2 inhibition and identified potential resistance mechanisms. Common adverse events included nausea, diarrhea and vomiting (all grades), and febrile neutropenia and hypokalemia (grade 3/4). Venetoclax demonstrated activity and acceptable tolerability in patients with AML and adverse features.

SIGNIFICANCE: Venetoclax monotherapy demonstrated clinical activity in patients with AML (relapsed/refractory or unfit for intensive chemotherapy) with a tolerable safety profile in this phase II study. Predictive markers of response consistent with BCL2 dependence were identified. Clinical and preclinical findings provide a compelling rationale to evaluate venetoclax combined with other agents in AML. *Cancer Discov*; 6(10); 1106-17. ©2016 AACR.

See related commentary by Pullarkat and Newman, p. 1082.

INTRODUCTION

Acute myelogenous leukemia (AML) is a heterogeneous disease characterized by impaired differentiation of hematopoietic stem cells and subsequent clonal expansion of myeloid blasts in the bone marrow, peripheral blood, and extramedullary tissue. Patients older than 65 years derive less benefit from standard intensive chemotherapy and poorly tolerate toxicities (1, 2).

B-cell leukemia/lymphoma-2 (BCL2), an antiapoptotic protein commonly expressed in hematologic malignancies, has been shown to be involved in tumor survival and chemoresistance (3). BH3 mimetics are an emerging group of agents that bind and inhibit antiapoptotic proteins, freeing proapoptotic proteins to initiate apoptosis (4). Venetoclax (ABT-199/GDC-0199) is a highly selective, orally bioavailable BCL2 inhibitor that has shown activity in BCL2-dependent leukemia and lymphoma cell lines (5, 6). Venetoclax induced cell death in AML cell lines and primary patient samples *in vitro* and in mouse xenograft models (7, 8). BCL2 inhibition induced cell death in AML cell lines and in leukemic blasts,

progenitor cells, and stem cells from patients with AML (9, 10). The unique role of BCL2 in leukemia stem cell survival suggests that BCL2 inhibition has the potential to eliminate chemotherapy-resistant leukemia stem cells while sparing normal hematopoietic stem cells (9–11). These data provide a rationale for targeted BCL2 inhibition with venetoclax in AML.

Venetoclax has demonstrated promising clinical activity as a single agent in the treatment of chronic lymphocytic leukemia (CLL; ref. 12). This is the first clinical study of venetoclax monotherapy in patients with relapsed/refractory AML or untreated AML unfit for intensive therapy. The primary objective was efficacy; secondary objectives included safety and pharmacokinetics. Exploratory objectives evaluated biomarkers, including protein expression patterns of BCL2, BCL2-like 1 (BCL-X_L) and myeloid cell leukemia sequence 1 (MCL1), and BH3 profiling. BCL2 family protein expression and BH3 profiling using synthetic oligopeptides derived from BH3 domains of prodeath BH3 peptides that are applied to mitochondria (4) have been retrospectively evaluated as potential biomarkers of clinical response to venetoclax.

RESULTS**Patient Characteristics and Disposition**

Thirty-two patients received at least 1 dose of venetoclax, and 26 patients had at least 4 weeks of therapy. Thirteen (41%) were reported to have an antecedent hematologic disorder or myeloproliferative neoplasms, and 4 (13%) patients had therapy-related AML (from prior malignancies) with complex cytogenetics. Two previously untreated patients were considered unfit for intensive chemotherapy by the treating physician and were treatment-naïve at study entry. Thirty (94%) patients had received at least 1 prior therapy,

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Note: Supplementary data for this article are available at Cancer Discovery Online (<http://cancerdiscovery.aacrjournals.org/>).

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doi: 10.1158/2159-8290.CD-16-0313

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and 13 (41%) patients had received at least 3 prior treatment regimens. Seventeen (53%) had received standard 7+3 induction therapy (cytarabine + anthracycline), and 23 (72%) had received at least 1 hypomethylating agent. The median age was 71 years (19–84 years); baseline characteristics are summarized in Table 1.

Molecular markers and cytogenetics were evaluated locally for all patients. In addition, AML-associated genetic mutations were assessed in pretreatment specimens using two next-generation sequencing panels: the Trusight Myeloid Panel for 31 of 32 patients and the FoundationOne Heme Panel, which assesses genomic aberrations in over 400 genes, for 15 of 32 patients. Key molecular markers and cytogenetics data are highlighted in Table 1. Mutations in isocitrate dehydrogenase (*IDH*) 1 or 2 were identified in 12 (38%) patients ($n = 2$ for *IDH1* and $n = 10$ for *IDH2*). Eleven of the 12 *IDH1/2* mutations were in a known site that leads to production of (*R*)-2-hydroxyglutarate (e.g., *IDH1*-R132, *IDH2*-R140, and *IDH2*-R172; refs. 13–15). One patient had a mutation in exon 3 of *IDH2* (D76 frameshift); the functional significance of this mutation is unknown. FMS-like tyrosine kinase-3–internal tandem duplication (*FLT3*-ITD) mutations were reported in 4 (13%) patients (3 of which were confirmed in pretreatment specimens). These 3 patients had concomitant mutations in *IDH*. Ten (31%) patients had chromosome 7q deletions [*del*(7q)] and 10 (31%) had complex cytogenetics.

Overall Activity

The objective response rate by International Working Group (IWG) criteria was 19% (6/32), with 6% ($n = 2$) achieving a complete response (CR) and 13% ($n = 4$) achieving a CR with incomplete blood count recovery (CRi; Table 2). Except for 1 CRi, all objective responses were achieved by the week 4 assessment. All IWG-defined responses were observed in patients who had been previously treated for AML. Three of the 6 responders had an antecedent hematologic disorder. Of the 24 patients who received prior hypomethylating agents, 25% (6/24) achieved CR/CRi. Among the 12 patients with *IDH1/2* mutations, 4 (33%) achieved CR/CRi. One patient with an *IDH2* mutation (not in a putative hotspot) achieved a CRi at week 24 following a 20-day dose interruption after week 4. The median duration of venetoclax therapy in responders was 144.5 days (83–256 days), and the median duration of CR was 48 days (Table 3). Thirty-four percent (11/32) of patients escalated to 1,200 mg daily venetoclax after lack of objective response at the first assessment on 800 mg and 9% (3/32) after relapse following CR/CRi on 800 mg. The 1,200-mg dose did not demonstrate additional activity. Median time on study was 63.5 days (14–256 days). All patients have discontinued venetoclax: 29 discontinued due to progressive disease, 1 discontinued due to an adverse event (AE; terminal ileitis), 1 withdrew consent, and 1 proceeded to allogeneic hematopoietic stem cell transplant after achieving stable disease.

Antileukemic activity evidenced by bone marrow blast reduction from baseline and incomplete hematologic recovery, which did not meet IWG response criteria, was observed in an additional 19% (6/32) of patients. Two patients had persistent antileukemic activity: 1 patient had $\geq 50\%$ bone marrow blast reduction at week 5 and recovery of hemoglobin and platelets (until discontinuing venetoclax after 247 days),

Table 1. Patient demographics and baseline characteristics

Characteristic	N = 32
Median age (range), years	71 (19–84)
Sex, n (%)	
Female	16 (50)
Male	16 (50)
Diagnosis, n (%)	
Relapsed/refractory	30 (94)
Newly diagnosed	2 (6)
Ethnicity, n (%)	
White	25 (78)
Black	4 (13)
Asian	3 (9)
ECOG performance score, n (%) ^a	
0	3 (9)
1	14 (44)
2	14 (44)
Missing	1 (3)
Any prior therapy, n (%)	30 (94)
Prior regimens ≥ 3	13 (41)
Prior standard induction (3+7) therapy	17 (53)
Prior hypomethylating agents	24 (75)
Prior allogeneic stem cell transplant	4 (13)
Treatment naïve	2 (6)
Prior myeloid disorder, n (%)	
Prior myelodysplastic syndrome ^b	11 (35)
Prior myeloproliferative neoplasm	2 (6)
Molecular markers ^c , n (%)	
<i>IDH</i> mutations ^d	12 (38)
<i>FLT3</i> -ITD ^e	4 (13)
BCR-ABL	1 (3)
JAK2	1 (3)
KRAS	1 (3)
MLL	1 (3)
NPM1	4 (13)
CEBP α	2 (6)
Cytogenetics, n (%)	
<i>del</i> (7q)	10 (31)
Complex	10 (31)
None	2 (6)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

^aPercentage based on known values.

^bIncludes $n = 4$ with therapy-related myelodysplastic syndrome with transformation to AML.

^cCytogenetics were evaluated for all patients at the investigator sites. Not all patients had molecular marker analysis; 8 of 32 did not have *IDH* mutational analysis performed at the sites. Data included patients with expression of more than one marker.

^dTwo were *IDH1* mutations and 10 were *IDH2* mutations. Of the 12, 11 were in a known site that leads to (*R*)-2-hydroxyglutarate, and 1 mutation was not in the putative hotspot (exon 3; D76 frameshift).

^eThree with *FLT3*-ITD and a concomitant *IDH* mutation were confirmed at study entry, and one site reported *FLT3*-ITD mutation was not detected at study entry (assay sensitivity was 0.1%).

Table 2. Overall activity of venetoclax in patients with AML

	All N = 32 (%)	IDH mutation N = 12 (%)
Objective response rate (CR + CRi) by IWG criteria	6 (19)	4 (33)
CR	2 (6)	2 (17)
CRi ^a	4 (13)	2 (17)
Antileukemic activity that did not meet IWG criteria	6 (19)	2 (17)
≥50% bone marrow blast reduction with two cell line recovery and transfusion independence ^b	2 (6)	0
≥50% bone marrow blast reduction with one cell line recovery ^c	2 (6)	2 (17)
≥50% bone marrow blast reduction with no hematologic recovery	2 (6)	0
Treatment failure ^d	20 (63)	6 (50)
Overall activity ^e	12 (38)	6 (50)

^aOne with *IDH* mutation in exon 3; dose interruption for 20 days after week 4 and achieved CRi at week 24.

^bOne with ≥50% bone marrow blast reduction at week 5 and recovery of hemoglobin and platelets, and one with ≥50% bone marrow blast reduction at week 12 and recovery of neutrophils and hemoglobin.

^cBoth with ≥50% bone marrow blast reduction and recovery of a single cell line (hemoglobin).

^dTreatment failure includes progressive disease and less than partial response by IWG criteria.

^eOverall activity includes objective responses by IWG criteria and antileukemic activity that does not meet IWG criteria.

and the other patient had ≥50% bone marrow blast reduction at week 12 and recovery of neutrophils and hemoglobin (until discontinuing venetoclax after 143 days). Two patients with *IDH1/2* mutations achieved ≥50% bone marrow blast reduction and recovery of a single cell line (hemoglobin), and 2 patients achieved ≥50% bone marrow blast reduction

without evidence of hematologic recovery (Table 2). The median duration of venetoclax therapy was 117 days (86–247 days) in the 6 patients with reductions in bone marrow blasts and transfusion independence not meeting IWG response criteria, versus 30 days (14–145 days) in patients who had treatment failure (Table 3 and Supplementary Table S1). Five

Table 3. Overall activity and predictors of response

Response	Time on venetoclax, days	Cytogenetics ^a	<i>IDH</i> mutation	<i>FLT3</i> -ITD	<i>BCL2</i> family protein index at screening
Response that met IWG criteria					
CR	247	Normal	-	-	-
CRi	256	Normal	Y	-	Sensitive
CRi	170	Complex; del(7q)	-	-	Resistant
CRi	119	Normal	Y	-	-
CR	107	Normal	Y	-	-
CRi	83	Normal	Y	-	-
Median (range) = 144.5 (83–256)					
Antileukemic activity that did not meet IWG criteria					
SD ^b	247	Normal	-	-	-
SD ^c	143	Complex; del(7q)	-	-	-
SD ^d	106	del(7q)	Y	-	Sensitive
SD ^e	104	Normal	Y	-	Sensitive
SD	128	Complex; t(8;12)	-	-	Resistant
SD ^f	86	Normal	-	Y	Sensitive
Median (range) = 117 (86–247)					

^aMost common cytogenetic abnormalities were evaluated for all patients at the investigator sites.

^b≥50% bone marrow blast reduction at week 5 and recovery of hemoglobin and platelets.

^c≥50% bone marrow blast reduction at week 12 and recovery of neutrophils and hemoglobin.

^dBoth with ≥50% bone marrow blast reduction and recovery of a single cell line (hemoglobin).

^e≥50% bone marrow blast reduction with no hematologic recovery.

^f*FLT3*-ITD mutation not confirmed at study entry; assay sensitivity was less than 0.1%.

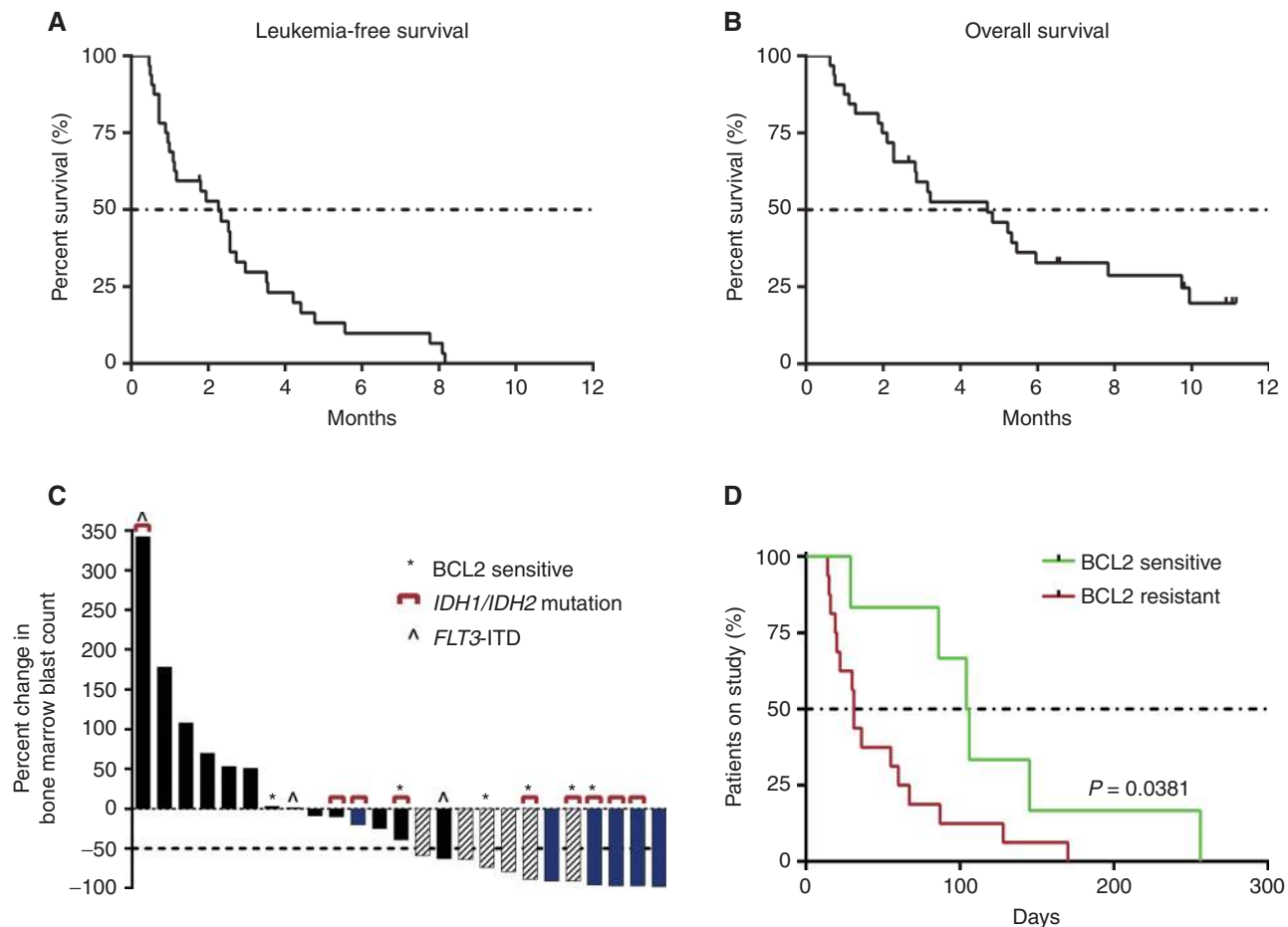


Figure 1. Biological activity of venetoclax. **A** and **B**, the median leukemia-free survival was 2.3 months (range, 1.0–2.7), and the median overall survival was 4.7 months (range, 2.3–6.0), in patients with relapsed/refractory AML or those unfit for intensive chemotherapy treated with venetoclax monotherapy. **C**, twenty-five (78%) patients had bone marrow blast counts evaluable at the first assessment (week 4). The best percent change in bone marrow counts at the first assessment (week 4) is shown. Six patients came off study before the first assessment, and 1 patient had unevaluable bone marrow (aplasia). Patients who achieved an objective response by the IWG criteria (complete response/complete response with incomplete blood count recovery) are indicated with blue, and patients who had antileukemic activity that did not meet IWG criteria are indicated with hashed bars. Patients with a BCL2 family protein sensitive index at screening and/or *IDH1/2* mutations are annotated with asterisks and brackets, respectively. **D**, six patients with a BCL2 family sensitive index (green) achieved longer durations on venetoclax therapy than the 16 patients with a BCL2 family resistant index (red; $P = 0.0381$ by Wilcoxon signed rank test). The median number of days on venetoclax was 96 days for patients with a BCL2 sensitive index versus 31 days for patients with a BCL2 resistant index.

of the patients with observed antileukemic activity not meeting IWG response criteria had relapsed/refractory AML, and 1 patient was treatment-naïve. None of the 4 patients with therapy-related AML experienced antileukemic activity on venetoclax. The median time to progression was 2.5 months (1–3 months). The 6-month leukemia-free survival rate was 10% [95% confidence interval (CI), 2.5–23.3], and the median leukemia-free survival was 2.3 months (1.0–2.7; Fig. 1A). The 6-month overall survival estimate was 36% (95% CI, 20–53), and median overall survival was 4.7 months (2.3–6.0; Fig. 1B). One patient, who is in follow-up for survival as of March 4, 2016, proceeded to transplant after achieving stable disease on venetoclax.

Twenty-five (78%) patients had bone marrow blast counts evaluable at the first assessment after 4 weeks of therapy (6 patients came off study before the first assessment and 1

patient had unevaluable bone marrow; Fig. 1C). Twelve (38%) patients had $\geq 50\%$ blast count reduction at the week 4 assessment. Of these, 5 patients achieved a CR/CRi as a best objective response (3/5 patients had *IDH1/2* mutation and none had *FLT3-ITD* mutation).

Biomarker Correlates

Peripheral blood specimens were collected from 31 of the 32 patients at screening for biomarker studies. BCL2 family protein expression in myeloblasts (identified as in Supplementary Fig. S1A and S1B) was evaluated before first dose in the 22 patients who had samples of sufficient quality for analysis. Specimens from the other 9 patients failed analysis owing to either low quality ($n = 6$, 3 of whom were CR/CRi patients) or lack of tumor cells present ($n = 3$, 1 of whom was a CR/CRi patient). The percentage of tumor cells expressing

BCL2, BCL-X_L, and the ratio of BCL2/BCL-X_L in the blasts were retrospectively assessed with time on study and reduction in bone marrow blasts with the goal of identifying BCL2 family sensitive or resistant indices based on protein expression. Patients were categorized as having a BCL2 family sensitive protein index if $\geq 35\%$ of tumor cells expressed BCL2 and $< 40\%$ of tumor cells expressed BCL-X_L protein (Supplementary Fig. S1). Patients with $< 35\%$ of tumor cells having detectable BCL2 protein expression and/or $> 40\%$ having detectable BCL-X_L protein expression were categorized as having a BCL2 family resistant protein index. Six of 22 (27%) patients had a BCL2 family sensitive protein index; of these, 1 patient achieved CRi (the other CRi patient with an evaluable specimen had a BCL2 resistant profile) and 3 patients achieved antileukemic activity not meeting IWG response criteria (Fig. 1C and Table 3). Patients with a BCL2 family sensitive protein index at screening experienced longer durations on venetoclax therapy ($P = 0.0381$ by Wilcoxon signed rank test; Fig. 1D and Table 3).

BH3 profiling is a functional assay with utility for probing interactions between antiapoptotic BCL2 family proteins and their proapoptotic counterparts to determine BCL2 family dependence (4). We assessed whether BH3 profiling results would correlate with clinical response to venetoclax (16). BH3 profiling with a series of BH3 peptides was performed on 18 pretreatment bone marrow biopsies or peripheral blood samples; 12 samples passed quality-control criteria for data analysis ($> 50\%$ viability on thaw; $> 5\%$ AML blasts; Fig. 2). Mitochondrial cytochrome *c* release in response to HRK peptide indicated BCL-X_L dependence, in response to MS1 peptide indicated MCL1 dependence, and in response to BAD peptides indicated BCL2 and/or BCL-X_L dependence. Subtracting cytochrome *c* release caused by HRK from that caused by BAD (BAD-HRK) provides a rough index of selective BCL2 dependence (17). Mitochondrial cytochrome *c* release in response to venetoclax correlated with release caused by BAD-HRK ($P = 0.0002$; Fig. 2A), indicating on-target activity within myeloblast mitochondria for venetoclax. Cytochrome *c* release caused by direct *ex vivo* exposure of myeloblast mitochondria to venetoclax correlated weakly with duration on venetoclax ($P = 0.0607$; Fig. 2B). Although not statistically significant, this result is consistent with a mitochondrial mechanism of action for venetoclax *in vivo*. Negative correlation was observed between AML blast dependence on the antiapoptotic protein BCL-X_L (using the HRK peptide, $P = 0.0072$; Fig. 2C) or the antiapoptotic protein MCL1 (using the MS1 peptide, $P = 0.0231$; Fig. 2D) correlated with days on venetoclax. Because dependence on MCL1 or BCL-X_L predicted short duration of venetoclax therapy, we asked whether we could make a superior predictor by arithmetically adding response to HRK to that of MS1 ($P = 0.0049$; Fig. 2E). This *post hoc* metric was a good binary predictor of staying on venetoclax more than 30 days; the AUC of the receiver operating characteristic was 1.0 (Fig. 2F).

Safety

Venetoclax monotherapy was generally well tolerated in patients with AML. Treatment-emergent AEs were reported for all patients and are summarized in Table 4. The most common AEs of any grade were nausea, diarrhea, hypokalemia, vomiting, and headache. The most common grade

3/4 AEs were febrile neutropenia, hypokalemia, pneumonia, hypotension, and urinary tract infection. Serious AEs were reported in 27 (84%) patients, with febrile neutropenia being the most common (10/32, 31%; Table 4). There were no events of tumor lysis syndrome (TLS). The incidence of infection was consistent with expectations for this patient population. Electrolyte abnormalities were related to concomitant supportive therapy for TLS prophylaxis and antifungal agents.

There were no AEs that required reduction of venetoclax dose. Venetoclax interruptions occurred in 8 of 32 (25%) patients. Of these, 6 patients had AEs: 4 were grade 3/4 and 2 were grade 1/2. There were 2 grade 3/4 events of diarrhea and 2 grade 3/4 events of febrile neutropenia managed by interruption of venetoclax dosing. In addition, 1 patient had bone marrow failure, and 1 patient had disease progression. Other events of febrile neutropenia were managed by standard of care ($n = 9$).

Pharmacokinetics

Venetoclax plasma concentration–time profile at week 6 day 1 from 13 patients who received the 800-mg dose is shown in Supplementary Fig. S2. Following multiple-dose administrations of 800 mg venetoclax, plasma concentrations peaked at 6 hours (4–8 hours), and the mean (coefficient of variation %) maximum plasma concentration (C_{max}), trough plasma concentration, and AUC over a 24-hour dose interval (AUC_{0-24}) were 3.74 (48) $\mu\text{g/mL}$, 1.43 (134) $\mu\text{g/mL}$, and 61.6 (69) $\mu\text{g}\cdot\text{h/mL}$, respectively. Venetoclax exposures in the current study are consistent with those observed in the first-in-human venetoclax studies in patients with relapsed/refractory CLL or non-Hodgkin lymphoma (12, 18). Venetoclax IC_{50} was < 10 nmol/L (0.0087 $\mu\text{g/mL}$) in primary AML patient myeloblast samples and < 100 nmol/L (0.087 $\mu\text{g/mL}$) in sensitive AML cell lines (7).

DISCUSSION

Single-agent venetoclax demonstrated a 19% objective response rate by IWG criteria in patients with heavily pretreated AML. An additional 19% derived had antileukemic activity demonstrated by partial bone marrow response and incomplete hematologic recovery that did not meet standard response criteria. For the majority of patients, activity was observed at the first assessment (end of week 4). Activity was observed in patients pretreated with standard induction therapy and hypomethylating agents; 25% of those previously treated with hypomethylating agents achieved an objective response.

Venetoclax was tolerable in these heavily pretreated patients with AML, with AEs consistent with expectations in this population (19, 20). There were no events of TLS, and no new safety signals were identified compared with other venetoclax monotherapy trials in hematologic malignancies (12, 18).

Mutations in *IDH1/2* are present in approximately 15% to 20% of patients with AML (15, 21); in the current study, 12 (38%) patients had *IDH1/2* mutations. These mutations are acquired early in progression to leukemia (22, 23) and appear stable during disease evolution (24, 25). Mutant *IDH1/2* proteins catalyze production of the oncometabolite (*R*)-2-hydroxyglutarate and elicit epigenetic changes, dysregulated mitochondrial function, and increased BCL2 dependence in

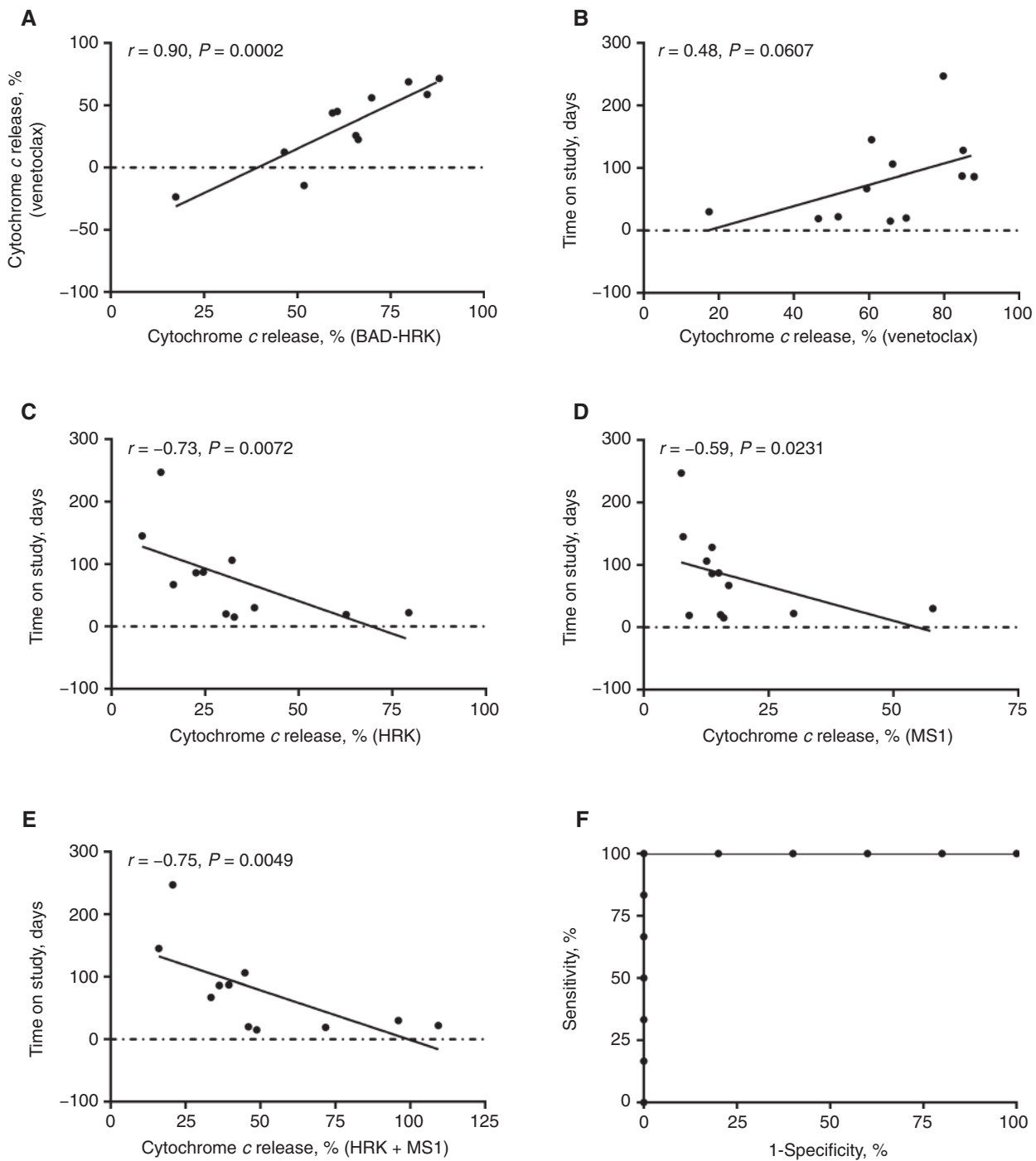


Figure 2. Venetoclax acts through on-target BCL2 inhibition. Twelve samples were analyzed (>50% viability on thaw; >5% AML blasts). **A**, mitochondrial BCL2 dependence correlated with release caused by BAD-HRK. Background BCL- X_L dependence was removed by subtraction of HRK for a specific measure of mitochondrial BCL2 dependence. **B**, mitochondrial response to venetoclax was weakly related to days on venetoclax therapy. **C** and **D**, AML blast dependence on the antiapoptotic protein BCL- X_L (using the HRK peptide) or the antiapoptotic protein MCL1 (using the MS1 peptide) negatively correlated with days on venetoclax. **E**, an index combining AML blast dependence on the antiapoptotic protein BCL- X_L and the antiapoptotic protein MCL1 negatively correlated with days on venetoclax therapy. **F**, ROC analysis of dependence on MCL1 or BCL- X_L , by arithmetically adding response to HRK peptide to that of MS1 peptide, was a perfect binary predictor of staying on venetoclax therapy more than 30 days (AUC of the ROC = 1.0). Correlations (r) and P values based on one-tailed Spearman rank-order correlation tests.

Table 4. Treatment-emergent AEs

AE, n (%) ^a	Any grade AE	Grade 3/4 AE
Any AE	32 (100)	26 (81)
Nausea	19 (59)	2 (6)
Diarrhea	18 (56)	2 (6)
Hypokalemia	13 (41)	7 (22)
Vomiting	13 (41)	0
Fatigue	11 (34)	0
Headache	11 (34)	1 (3)
Hypomagnesemia	11 (34)	0
Febrile neutropenia	10 (31)	10 (31)
Hypophosphatemia	10 (31)	2 (6)
Abdominal pain	9 (28)	2 (6)
Cough	9 (28)	0
Epistaxis	8 (25)	2 (6)
Hyperphosphatemia	8 (25)	0
Hypocalcemia	8 (25)	3 (9)
Pneumonia	8 (25)	6 (19)
Dyspnea	7 (22)	0
Hypotension	7 (22)	4 (13)
Peripheral edema	7 (22)	0
Pyrexia	7 (22)	1 (3)
Insomnia	5 (16)	1 (3)
Urinary tract infection	5 (16)	4 (13)

Serious AE, n (%) ^b	Total
Any serious AE	27 (84)
Febrile neutropenia	9 (28)
Pneumonia	5 (16)
Abdominal pain	2 (6)
Acute renal failure	2 (6)
Failure to thrive	2 (6)
Hypotension	2 (6)
Sepsis	2 (6)
Urinary tract infection	2 (6)

^aAEs occurring in $\geq 10\%$ of patients regardless of cause; excludes 9 AEs of disease progression of AML.

^bSerious AEs occurring in at least 2 patients regardless of cause; excludes serious AEs of disease progression of AML.

AML cells (26). Four (33%) patients with *IDH1/2* mutations achieved objective responses with venetoclax. Three patients with *IDH1/2* mutations had concomitant *FLT3*-ITD mutations, a known adverse prognostic factor; these patients did not achieve response and had shorter durations on venetoclax (Supplementary Table S1). Our data provide evidence that AML with *IDH1/2* mutations exhibits BCL2 dependence and validates preclinical data that suggest suppression of cytochrome *c* oxidase activity in *IDH1/2* mutant AML lowers the mitochondrial threshold to trigger apoptosis upon BCL2 inhibition (7, 8, 26). However, activity observed in patients with wild-type *IDH1/2* suggests targeting BCL2 with venetoclax should not be restricted to patients with mutations in *IDH1/2*. The number of patients in our study is relatively small, and further studies are required to draw full conclusions regarding the clinical activity of venetoclax in patients with *IDH1/2* mutations.

BCL2 family protein expression has been previously evaluated as a predictor of clinical response to BH3 mimetics with mixed success (27–30). We analyzed the predictive capacity of protein expression of BCL2, BCL-X_L and MCL1, and BH3 profiling, and evaluated putative predictors of response. The findings of the current study provide evidence that venetoclax acts as originally described, through on-target BCL2 inhibition and subsequent induction of apoptosis (Fig. 3; ref. 5). BCL2 family protein expression at screening identified patients with a BCL2 family sensitive or resistant index. Four patients with a BCL2 family sensitive index experienced clinical benefit, by achieving either an objective response, anti-leukemic activity that did not meet IWG criteria, or longer durations on venetoclax therapy.

BH3 profiling has been used in AML to predict response to novel agents (31, 32) and to identify primary cells from patients with AML sensitive to BCL2 inhibition (7, 11). In this study, BH3 profiling identified patient samples most sensitive to BCL2 inhibition by venetoclax. The performance of the *post hoc* receiver operating characteristic analysis indicates that BH3 profiling warrants further testing as a predictive assay for venetoclax sensitivity. Dependence on BCL2 does not seem to be sufficient for prolonged clinical sensitivity to venetoclax; rather, AML blasts also need to lack dependence on the resistance factors BCL-X_L and MCL1. This suggests that the best predictor of sustained response to a BCL2 inhibitor as a single agent is the lack of readily accessible resistance mechanisms provided by BCL-X_L and MCL1. These results also suggest that dependence on individual antiapoptotic proteins is more heterogeneous in AML than in CLL, in which dependence on BCL2 is relatively homogeneous (33).

Relapsed/refractory AML in the elderly population has an extremely poor prognosis, with overall survival less than 6 months. The 19% objective response rate plus additional examples of myeloblast reduction observed in this study, with a relatively well-tolerated oral single agent, is a promising clinical achievement. Response rates in this population vary greatly based on prognostic factors, ranging from 10% to 85%. However, remission rates greater than 20% are rarely obtained without the application of intense cytotoxic chemotherapy regimens requiring extended inpatient care (34–36). There is potential for improvement in the response rate with the use of predictive biomarkers to identify and select patients responsive to venetoclax therapy.

In summary, this phase II study demonstrated pharmacologic activity of venetoclax as a single agent in AML with an acceptable safety profile. These results, along with preclinical data with venetoclax in combination with other agents (9, 37, 38) and observations that AML stem cells may be dependent on BCL2 (7, 9, 10), support evaluating venetoclax in combination with other agents in patients with AML. Venetoclax is currently being investigated in phase I studies in combination with low-dose cytarabine (NCT02287233) and decitabine or azacitidine (NCT02203773). Preliminary results from clinical trials in treatment-naïve elderly patients with AML with combinations of venetoclax and hypomethylating agents suggest that response rates comparable to standard induction therapy can be obtained with tolerable toxicity (39).

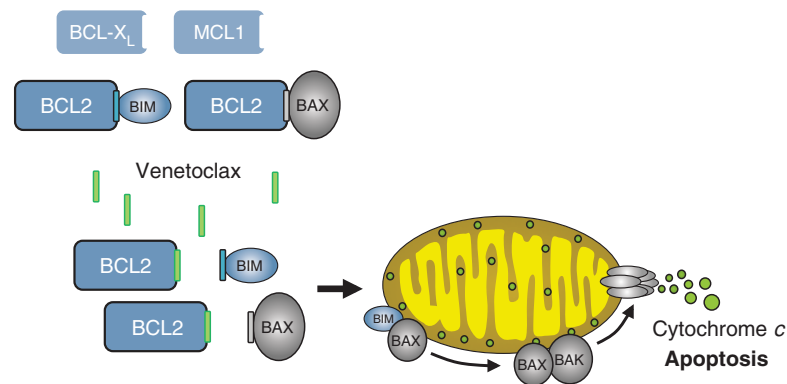


Figure 3. Mechanism of action of venetoclax. Venetoclax acts as a specific inhibitor of BCL2 and upon binding, releases proapoptotic proteins to induce apoptosis. BIM, BCL2-like 11; BAX, BCL2-associated X protein; BAK, BCL2 antagonist/killer 1.

METHODS

Patients

Eligible patients had relapsed/refractory AML by the World Health Organization classification or untreated AML unfit for intensive therapy; Eastern Cooperative Oncology Group Performance score 0–2; adequate organ function [creatinine clearance ≥ 50 mL/min, aspartate aminotransferase and alanine aminotransferase $\leq 3.0 \times$ ULN (upper limit of normal), bilirubin $\leq 1.5 \times$ ULN]; and did not meet exclusion criteria including white blood cell count $>25 \times 10^9/L$ (hydroxyurea permitted to lower count), unresolved \geq grade 2 clinically significant nonhematologic toxicities from prior anticancer therapy, other active malignancy within 1 year before study entry, major organ dysfunction, active infections, or pregnancy or breastfeeding.

This study was conducted in accordance with the Declaration of Helsinki and was approved by an Institutional Review Board at each participating institution. Informed consent was obtained from all patients.

Study Design and Treatment

Phase II, open-label, single-arm, multicenter study of venetoclax monotherapy in patients with AML enrolled between December 31, 2013, and April 5, 2014 (NCT01994837). The study used a Simon two-stage optimal design (Supplementary Fig. S3). Nineteen patients were to be enrolled and treated with venetoclax monotherapy in the first stage. If ≥ 5 patients achieved an objective response by revised IWG criteria (CR, CRi, or partial response; ref. 40) in an interim analysis after at least 12 weeks of therapy, the second stage would begin and enroll an additional 35 patients. The sample size was determined with 90% power at a type 1 error rate of 0.05 with an uninteresting response rate of 20% and an interesting response rate of 40%. During execution of the trial, 13 eligible patients were in the screening process when the interim analysis of stage one began. Patients were allowed to initiate treatment before completion of the stage one interim analysis due to the disease severity and prognosis of these patients without available options for therapy. A total of 32 patients were enrolled. Enrollment stopped after the first stage, and no additional patients were screened or treated after the interim analysis was completed.

To mitigate risk of TLS (12), a stepwise ramp-up of venetoclax dosing was used to achieve the target venetoclax dose (800 mg). Oral venetoclax was administered daily beginning with 20 mg on week 1 day 1, and escalated daily to 50 mg on day 2, 100 mg on day 3, 200 mg on day 4, 400 mg on day 5, and 800 mg on day 6 (and 800 mg daily thereafter). Patients were hospitalized in the first week of therapy (day 1 until at least 24 hours after reaching the target dose) and received hydration (oral and intravenous) and treatment with a

uric acid-reducing agent. Blood chemistries were performed on the first day of dosing and each day of a new dose at 0 (within 4 hours before dosing), 8, and 12 hours, and at 24, 48, and 72 hours after the first 800-mg dose.

Dose interruption, reduction, or continuation at the current level was permitted if toxicities were observed. Dose escalation to 1,200 mg daily was allowed if CR or CRi was not achieved at first assessment (end of week 4) and 800 mg was tolerable. Patients continued daily venetoclax until unacceptable toxicity or progression. Supportive treatment, including anti-infection prophylaxis and growth factor support, was allowed at the investigator's discretion.

Assessments

Responses were evaluated using the revised IWG guidelines for AML; hematologic responses were also evaluated (40). The overall response rate by IWG criteria included CR/CRi. Patients without clinical or cytologic progressive disease who did not achieve IWG response were considered to have stable disease. Bone marrow aspirate and biopsy were performed at screening, end of week 4, and every 8 weeks thereafter. AEs were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE; version 4.0). Physical exam, vital signs, and clinical laboratory tests were performed at screening and throughout the study. Clinical and laboratory evidence of AEs was monitored routinely throughout the study and for 30 days following discontinuation of study drug.

Pharmacokinetic samples were collected 8 hours post-dose after the first dose and the first day of each new dose level. Intensive venetoclax pharmacokinetic samples (0–8 hours) were collected at week 6 day 1. For the intensive pharmacokinetic days, venetoclax C_{max} , the time to C_{max} (peak time), and AUC_{0-24} were determined using non-compartmental methods.

BCL2 Family Protein Analysis

Pretreatment bone marrow aspirates or peripheral blood samples for BCL2 family protein mutational analysis were collected and analyzed. The presence of mutations in genes associated with hematologic malignancies was determined in baseline specimens using two next-generation sequencing panels: the Trusight Myeloid panel (Illumina) for 31 of 32 patients and the FoundationOne Heme panels (Foundation Medicine) for 15 patients. Two milliliters of peripheral blood were added to freshly prepared 1 \times Lyse/Fix buffer (BD Biosciences) in a 1:40 ratio. The sample was inverted 5 to 8 times, incubated at 37°C for 10 minutes, and frozen at -70°C . Prior to testing, the specimens were thawed at 37°C, filtered through a 45- μm filter, centrifuged at 1,500 rpm for 5 minutes,

and resuspended in PBS, and the number of peripheral blood mononuclear cells (PBMC) recovered were determined. Twenty-five specimens had sufficient integrity and cell number to determine the expression of BCL2, BCL-X_L, and MCL1 protein in the tumor cells. The PBMCs were permeabilized with 1× Phosflow Perm Wash buffer 1 (BD Biosciences) and incubated with fluorescently labeled antibodies to cell surface markers (CD45, BD Biosciences) and intracellular proteins (BCL2 and MCL1, BD Biosciences; and BCL-X_L, Cell Signaling Technology).

BH3 Profiling

Pretreatment bone marrow aspirates or peripheral blood samples were obtained, and mononuclear cells were isolated using a Ficoll gradient and viably frozen at a central repository. Twelve of 18 specimens were sufficient for analysis. Thawed cells were washed once with PBS and stained with 1:100 Invitrogen Live/Dead – aqua stain (#423102; BioLegend) in PBS for 20 minutes on ice, washed with PBS, and subsequently stained with 1:100 CD45-BV421 (clone HI30, #563879; BD Biosciences) and 1:100 CD33-PE (clone WM53, #561816; BD Biosciences) in FACS buffer (2% FBS in PBS) on ice for 20 minutes with 1:100 human FcR block (#130-059-901; Miltenyi Biotec). Cells were BH3 profiled as described in Pan and colleagues (7), and samples were analyzed using the LSRII flow cytometer and data analysis performed using BD FACSDiva Software (BD Biosciences). Cytochrome *c* loss was measured by a gating strategy, in which a gate was drawn around the DMSO-negative control to depict 100% cytochrome *c* retention. DMSO was used as a negative control for cytochrome *c* retention, whereas a control without the cytochrome *c* antibody was used as a positive control for 100% cytochrome *c* release. Cytochrome *c* loss was calculated using the following equation: [Cytochrome *c* loss = 100 – (% of cells within cytochrome *c* retention gate)].

AML blasts were identified by CD45^{lo-mid}/CD33^{mid-hi}/SSC-A^{lo}. Cells from the DHL4 cell line were BH3 profiled with the AML patient samples as an internal control for peptide function.

Statistical Analysis

The data cutoff for this report was January 22, 2015, when all patients had discontinued. Analyses were specified in a statistical analysis plan. Descriptive statistics including medians, ranges, and SDs were calculated. Efficacy, safety, and pharmacokinetic analyses were performed on all patients who received at least 1 dose of venetoclax. Assessments at screening served as baseline unless repeated on week 1 day 1 before dosing. All statistical analyses were performed using SAS version 9, and all plots were generated using GraphPad Prism Software version 6.05. The Kaplan–Meier method was used for time-to-event analyses. Data for leukemia-free survival were censored at the date of last disease assessment or date of first dose plus 1 day for patients without any disease assessments. Overall survival was censored at the date of last study visit or last known date to be alive, whichever was later. Correlations were analyzed with one-tailed Spearman rank-order correlation tests.

Study Oversight and Role of Funding Source

The study protocol was designed by the sponsors (AbbVie and Genentech) in collaboration with the investigators and approved by local Institutional Review Boards. The study was conducted according to the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice. Each patient provided signed informed consent. Study investigators and their research teams collected clinical data; AbbVie confirmed and compiled the data. Authors had access to the data and agreed to submit the manuscript for publication. Manuscript drafts were prepared with assistance from a professional medical writer employed by AbbVie.

Disclosure of Potential Conflicts of Interest

M. Konopleva reports receiving commercial research grants from AbbVie and Genentech and is a consultant/advisory board member for the same. D.A. Pollyea is a consultant/advisory board member for Alexion, Ariad, Celgene, and Pfizer. B. Chyla has ownership interest (including patents) in AbbVie. T. Busman has ownership interest (including patents) in AbbVie. E. McKeegan has ownership interest (including patents) in AbbVie. A.H. Salem has ownership interest (including patents) in AbbVie. J.L. Ricker has ownership interest (including patents) in AbbVie. W. Blum is a consultant/advisory board member for Genentech/Roche. C.D. DiNardo is a consultant/advisory board member for Agios. T. Kadia is a consultant/advisory board member for Novartis, Pfizer, and Sunesis. J. Levenson has ownership interest (including patents) in AbbVie. M. Mabry has ownership interest (including patents) in AbbVie. R. Stone is a consultant/advisory board member for AbbVie. A. Letai reports receiving commercial research support from AbbVie and is a consultant/advisory board member for the same. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

We thank the patients who participated in this trial and their families; the study coordinators and the support staff at the clinical sites; and the AbbVie and Genentech venetoclax team members. We also thank Jingwen Jia, Vinay Tavva, and Ruiling Zhang for statistical programming support; Relja Popovic for genetic mutations analysis support; and Leanne Lash for writing assistance; all are employees of AbbVie. Editorial and administrative assistance was provided by Evidence Scientific Solutions and funded by AbbVie, Inc.

Grant Support

AbbVie and Genentech provided financial support for this study and participated in the design, study conduct, and analysis and interpretation of data, as well as the writing, review, and approval of the manuscript. Venetoclax (ABT-199/GDC-0199) is being developed in collaboration between AbbVie and Genentech. A. Letai gratefully acknowledges grant support from a Leukemia and Lymphoma Society Translational Award and NIH P01 CA066996.

Received March 15, 2016; revised July 12, 2016; accepted July 22, 2016; published OnlineFirst August 12, 2016.

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