Phase I study of obatoclax mesylate (GX15-070), a small molecule pan–Bcl-2 family antagonist, in patients with advanced chronic lymphocytic leukemia

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Obatoclax mesylate is a small molecule pan–Bcl-2 antagonist with in vitro activity against chronic lymphocytic leukemia (CLL) cells. Obatoclax was administered to patients with advanced CLL at doses ranging from 3.5 to 14 mg/m² as a 1-hour infusion and from 20 to 40 mg/m² as a 3-hour infusion every 3 weeks. Twenty-six patients received a total of 74 cycles. Dose-limiting reactions were neurologic (somnolence, euphoria, ataxia) and associated with the infusion. The maximum tolerated dose (MTD) was 28 mg/m² over 3 hours every 3 weeks. One (4%) of 26 patients achieved a partial response. Patients with anemia (3/11) or thrombocytopenia (4/14) experienced improvements in hemoglobin and platelet counts. Circulating lymphocyte counts were reduced in 18 of 26 patients with a median reduction of 24%. Overall, the maximum plasma concentration (C_{max}) and area under the curve (AUC) values of obatoclax were dose proportional. Activation of Bax and Bak was demonstrated in peripheral blood mononuclear cells, and induction of apoptosis was related to overall obatoclax exposure, as monitored by the plasma concentration of oligonucleosomal DNA/histone complexes. Obatoclax mesylate has biologic activity and modest single-agent activity in heavily pretreated patients with advanced CLL. Further evaluation in less heavily pretreated patients and in combination with other therapeutic agents is warranted. This trial has been registered with http://clinicaltrials.gov under identifier NCT00600964. (Blood. 2009; 113:299-305)

Introduction

The Bcl-2 family of antiapoptotic proteins share a conserved binding site for the BH3 domain of BH3-only or multidomain proapoptotic proteins Bax and Bak, whose sequestration prevents the initiation of the apoptotic cascade through the mitochondrial pathway.[1] Small molecule mimics of the BH3 peptidic domain, such as obatoclax mesylate,[2] can inhibit these protein-protein interactions (Figure 1) and facilitate initiation of programmed cell death.[3]

Obatoclax has been shown to overcome Bcl-2–, Bcl-xl–, Bcl-w–, and Mcl-1–mediated resistance to Bax or Bak.[2] It potently interferes with the direct interaction between Mcl-1 and Bak in intact outer mitochondrial membrane and inhibits the association between Mcl-1 and Bak in intact cells. Mcl-1 has been shown to confer resistance to the Bcl-2–, Bcl-xl–, and Bcl-w–selective antagonist ABT-737.[4] Obatoclax overcomes this resistance.[2]

Chronic lymphocytic leukemia (CLL) cells express high levels of members of the Bcl-2 family of antiapoptotic proteins, namely Bcl-2, Bcl-xl, and Mcl-1, accounting for their noted resistance to apoptosis.[5,6] Obatoclax induces apoptosis of human B-CLL cells treated ex vivo and was additive with the cytotoxic agents fludarabine and chlorambucil.[7] Recent reports have emphasized its potential to synergize with cytotoxic and targeted therapies in preclinical models systems including B-cell lymphoid malignancies.[8,9]

The single-agent phase I trial reported here was the first trial of obatoclax completed and showed biologic and clinical activity in heavily pretreated patients with CLL.

Methods

Trial design

This phase I, open-label study used an accelerated titration design, with allowance for intrapatient dose escalation. Intravenous obatoclax mesylate (GeminX Biotechnologies, Montreal, QC) was administered every 3 weeks, initially using a 1-hour infusion and later a 3-hour infusion duration. Patients continued treatment for up to 8 cycles, as long as obatoclax mesylate was well tolerated and there was no disease progression.

Patients

Eligible patients had to have a diagnosis of B-CLL and been previously treated with standard systemic chemotherapy, including fludarabine. There were no limitations on the amount of prior therapy, but acute toxicities from prior treatment must have resolved to grade 1 or lower. Patients were required to be at least 18 years of age; to have an Eastern Cooperative Oncology Group performance status of 0 to 1; to have a life expectancy of at least 8 weeks; to have adequate hepatic and renal function (total bilirubin $\leq$ 2 mg/dL unless resulting from hemolysis; to have a ratio of serum aspartate aminotransferase [AST] to serum alanine aminotransferase [ALT] $\leq$ 2.5 $\times$ the institutional upper limit of normal [ULN]; creatinine within ULN or calculated creatinine clearance $\geq$ 50 mL/min/1.73 m²). In view of the fact that cytopenias are frequently seen with advanced CLL and that obatoclax was devoid of myelosuppression in animal toxicology experiments, requirements for bone marrow function were not specified.

Patients were excluded if they were receiving any other therapies administered with the intent to treat their malignancy; had a history of

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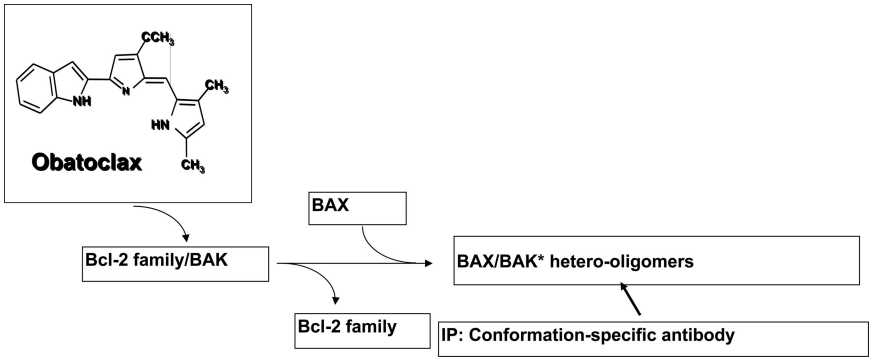


Figure 1. Structure of obatoclax and schematic of release of activated Bak (Bak*) from Bcl-2 following exposure to obatoclax and recruitment of Bax to form hetero-oligomers detectable by a conformation-specific antibody.

allergic reactions to polyethylene glycol 300 or polysorbate 20; had a history of seizure disorders; had uncontrolled, intercurrent illness including, but not limited to, symptomatic neurologic illness, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements; if they were HIV-positive receiving combination antiretroviral therapy; or if they were pregnant, breastfeeding, or unable to practice effective contraception.

The study protocol was approved by the institutional review boards of the participating institutions, and all patients gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki and its amendments.

Dose escalation

An initial accelerated titration design was planned with single patient cohorts and doubling of the dose until higher than grade 1 toxicity was observed. The starting dose was 3.5 mg/m^2. Subsequent cohorts were to include 3 to 6 patients with 40% dose increments between levels. Toxicities observed through day 15 of the first treatment cycle determined when dose escalation occurred. Single intrapatient dose escalation was allowed following administration of 2 cycles at the initial dose.

Toxicities were graded based on the National Cancer Institute (NCI) Common Toxicity Criteria, version 3.0. A dose-limiting toxicity (DLT) was defined as ataxia or somnolence/depressed level of consciousness interfering with the ability to discharge the patient from the treatment center, grade 4 neutropenia of any duration with fever, grade 4 neutropenia without fever for at least 7 days, grade 4 thrombocytopenia, or grade 3 or 4 nonhematologic toxicity not ameliorated by symptom-directed therapy. If at least 2 of 3 to 6 assessable patients had a DLT, the dose escalation was stopped, and the prior dose level was the recommended phase II dose.

Evaluation of patients

Safety assessments were performed during each treatment cycle until 30 days after the last dose. For day 1 of cycle 1 and any subsequent cycle in which an intrapatient dose escalation had occurred, additional safety assessments included the recording of the level of consciousness, respiratory rate, and pulse oxymetry every 15 minutes (for the 1-hour infusion) or 30 minutes (for the 3-hour infusion) from the start of the infusion up to 1 hour after the end of the infusion or to continue until resolution of findings, if present.

Tumor evaluations were performed after every 2 cycles. Patients were evaluable for response if they had received at least one dose of obatoclax. Response and progression was evaluated using the criteria proposed by the NCI-sponsored Working Group Guidelines for CLL.[10]

Pharmacokinetic analyses

Blood samples were collected in prechilled EDTA/K3 siliconized glass tubes on days 1 through 8 of cycle 1 and any subsequent cycle in which an intrapatient dose escalation had occurred. For the 1-hour infusion, samples were obtained predose and 0.5, 1 (immediately prior to the end of infusion), 1.25, 1.5, 2, 3, 5, 8, 24 (± 2 hours), 48 (± 2 hours), 72 (± 2 hours), 96 (± 2 hours), and 168 hours (± 2 hours) after the start of the obatoclax infusion. For the 3-hour infusion, samples were obtained predose and 0.5, 1, 2, 3 (immediately prior to the end of infusion), 3.5, 4, 5, 6, 10, 14, 24 (± 2 hours), 48 (± 2 hours), 72 (± 2 hours), 96 (± 2 hours), and 168 hours (± 2 hours) after the start of the obatoclax infusion. Pharmacokinetic (PK) parameters were determined for each dose level, and mean and standard deviation values were calculated.

Plasma concentrations of obatoclax were measured using a validated liquid chromatography with mass spectrometry (LC-MS) method. Curves of obatoclax concentration versus time in plasma were constructed for each patient and analyzed by a noncompartmental analysis technique. Maximum plasma concentration (C_{max}) and corresponding time (T_{max}), area under the concentration-versus-time curve through 24 hours ($AUC_{0\text{-}24\ hours}$), area under the concentration-versus-time curve through the last measurement (AUC_{last}), terminal half-life ($t_{1/2}$), plasma clearance (Cl_p), and volume of distribution (V_{ss}) were calculated.

Pharmacodynamic analyses

The release of Bax from the Bcl-2 family proteins leads to its activation through a conformational change that can be detected by specific antibodies. Activated Bax can form hetero-oligomers with Bax (Figure 1). Blood samples were drawn into BD CPT vacutainer tubes (BD, Franklin Lakes, NJ), followed by the isolation of peripheral blood mononuclear cells (PBMNCs) by centrifugation. For the 1-hour infusion, samples were obtained predose and 1, 2, 3, and 5 hours after the start of the obatoclax infusion. For the 3-hour infusion, samples were obtained predose and 3, 4, 5, and 8 hours after the start of the obatoclax infusion. Immunoprecipitation (IP) of activated Bak/Bax complexes were performed according to Cuconati et al[11] using, for the IP, the mouse monoclonal anti-Bak (Ab-1) IgG2a (TC-100; Calbiochem, San Diego, CA) and, for the Western blot analysis, the rabbit anti-Bax (Cell Signaling Technology, Beverly, MA). Control Western blot analysis with the anti-Bax antibody was performed to demonstrate that comparable amounts of total cellular protein extracts were used in all reactions.

Initially, additional plasma samples were obtained predose and at 24, 48, 72, and 96 hours (± 2 hours) after the start of the infusion to be used in enzyme-linked immunosorbent assays (ELISAs) for the determination of oligonucleosomal DNA/histone complexes. However, the protocol was later amended to obtain these samples from those leftover from PK sampling. These samples were processed using the Cell Death ELISA (Roche Applied Science, Indianapolis, IN) using control DNA obtained by mixing heterologous blood from healthy normal volunteers as recommended by the manufacturer.

Results

Patients

Twenty-six patients were enrolled between October 2004 and March 2006 (Table 1). Patients were typically of advanced stage and heavily pretreated with a median of 4 prior regimens. All had

Table 1. Patient characteristics

Characteristic	No.
Sex	
Men	20
Women	6
Age, y	
Median	60
Range	46-76
ECOG performance status	
0	7
1	18
ND	1
Rai stage	
I-II	7
III-IV	19
Prior regimens	
Median	4
Range	2-12
Fludarabine	26
Refractory to fludarabine	22
Alkylating agents	25
Rituximab	24
Alemtuzumab	11

ECOG indicates Eastern Cooperative Oncology Group; and ND, not documented.

been exposed to fludarabine, and 22 were refractory to it or had progressed within 6 months of their last fludarabine treatment. All but 1 patient had received alkylating agents, and all but 2 had received rituximab. Eleven patients had prior treatment with alemtuzumab.

Dose levels studied and toxicities observed

The dose levels evaluated, the number of cycles administered, and the dose escalations are summarized in Table 2. A total of 74 cycles were administered to 26 patients across all dose levels. The median number of cycles administered was 2, with 4 patients receiving 4 cycles, 1 patient each receiving 5 and 6 cycles, and 2 patients receiving 7 cycles. Overall, 21 of 26 patients (81%) received 2 cycles or more, and 24 of 26 patients (92%) were removed from study because of disease progression (1 for toxicity and 1 at the patient's request). Of note, after an accelerated titration from 3.5 to 7 mg/m^2, the initial patient treated at 3.5 mg/m^2 was reported to have a partial response (PR), and limited additional patients were enrolled at the lower dose levels, notwithstanding adequate tolerance to allow further dose escalations, to capture more PK data within what appeared to be a clinically active dose range.

The most common toxicity was infusion-related somnolence, often accompanied by euphoria (Table 3). Other neurologic symptoms included ataxia and confusion. These symptoms resolved promptly following the end of the infusion. To facilitate further dose escalations, the infusion was prolonged from 1 hour (used for doses from 3.5-14 mg/m^2) to 3 hours (used for doses of 20-40 mg/m^2). Neurologic symptoms including grade 3 somnolence, ataxia, and confusion were judged to be dose-limiting in 3 patients, 1 of 6 patients treated at 28 mg/m^2 and 2 of 4 patients treated at 40 mg/m^2. Thus, the recommended phase II dose was determined to be 28 mg/m^2 administered over 3 hours every 3 weeks. There were no obvious correlations between the intensity of central nervous system (CNS) symptoms and C_{max} or AUC at the 28 and 40 mg/m^2 dose levels. Of note, the patient experiencing DLT at 28 mg/m^2 experienced complete resolution of his infusion-associated symptoms by the 24-hour time point, but presented on day 10 with new left-sided weakness, ataxia, and confusion. Magnetic resonance imaging (MRI) of the brain revealed a mass in the right basal ganglia, a tentative diagnosis of leukoencephalopathy was made, and steroids were prescribed. A brain biopsy was considered, but the patient declined. This patient also had a prior squamous cell carcinoma of the nose, which had been resected and irradiated. He subsequently had a recurrence in the neck and had undergone a right neck dissection 3 weeks before entry into this study. At that time, the neurologic examination was normal, but baseline neurologic imaging was not obtained. Despite steroid therapy, the left-sided weakness progressed, and repeat MRI revealed interval progression of the lesion in the right ganglia with multiple additional brain abnormalities compatible with brain metastases. The patient was then referred for palliative radiotherapy.

Laboratory-based toxicities were also commonly reported (Table 4). With the exception of decreased O_2 saturation, an observation possibly related to somnolence, there was no clear dose response for these events.

Table 2. Obatoclax dose levels and cycles

Obatoclax dose, mg/m^2	No. of patients	Median cycles	Cycle range	Dose escalations
1-hour infusion				
3.5	2	3	3-6	1-10 mg/m^2
5	1	2	2	
7	2	2	2-3	1-10 mg/m^2
10	3	4	4-7	3-14 mg/m^2
14	4	2	1-4	
3-hour infusion				
20	3	2	2-5	
28	7	2	1-7	1-40 mg/m^2
40	4	2	1-2	
Overall	26	2	1-7	

Pharmacokinetics

Table 5 summarizes the results of the PK analyses for patients treated with 3-hour intravenous infusion only. The PK values in patients who received the drug in a 1-hour intravenous infusion schedule were not included in these analyses due to the very limited number of patients per dose group. Overall, the plasma C_{max} and AUC values of obatoclax were generally dose proportional. Peak plasma obatoclax concentrations were generally attained at the 1- to 2-hour time point for the nominal 3-hour infusions. The apparent $t_{1/2}$ ranged from 39.0 to 59.6; however, the majority of the drug was eliminated from plasma with a short $t_{1/2}$. The Cl_p and V_{ss} were relatively consistent, with mean values ranging from 71.6 to 162 L/h/m^2 and 866 to 1947 L/m^2, respectively.

Pharmacodynamics

The pharmacodynamic activity of obatoclax was monitored using 3 different end points: (1) the formation of the activated Bak/Bax complex in circulating peripheral mononuclear cells, indicative of their release from binding to the antiapoptotic Bcl-2 family proteins and the initiation of apoptosis (Figure 2); (2) the plasma concentration of oligonucleosomal DNA/histone complexes, indicative of endonuclease activation and the execution of apoptosis; and (3) relative decreases in circulating lymphocyte counts, indicative of the completion of apoptosis.

Figure 2A shows an example of the activation of Bax and Bak in the PBMNCs of a patient treated with 3.5 mg/m^2 obatoclax over 1 hour, who was later dose-escalated to 10 mg/m^2. There is a significant increase in the amount of Bax associated with the

Table 3. Number of patients with clinical adverse events occurring in 15% or more of patients

	Dose level, mg/m^2								
Adverse event	**3.5 N = 2**	**5 N = 1**	**7 N = 2**	**10 N = 5**	**14 N = 7**	**20 N = 3**	**28 N = 7**	**40 N = 5**	**Total N = 32**
Somnolence									
Grade 1	1/2		2/2	5/5	5/7	1/3			14/32
Grade 2						1/3	3/7	2/5	6/32
Grade 3							2/7	1/5	3/32
Euphoric mood									
Grade 1	2/2	1/1		4/5	4/7	3/3	1/7	1/5	16/32
Grade 2				1/5	1/7		1/7		3/32
Fatigue									
Grade 1	1/2	1/1			2/7	1/3	1/7	2/5	8/32
Grade 2			1/2						1/32
Grade 3							2/7		2/32
Cough									
Grade 1			1/2		3/7	1/3		2/5	7/32
Grade 2	1/2								1/32
Fever									
Grade 1		1/1			3/7		1/7	1/5	6/32
Grade 3								1/5	1/32
Diplopia									
Grade 1			1/2	1/5		1/3	2/7	2/5	7/32
Dizziness									
Grade 1	1/2			2/5		1/3	1/7		5/32
Grade 3								1/5	1/32
Headache									
Grade 1			1/2	1/5	1/7		1/7	1/5	5/32
Grade 3		1/1							1/32
Ataxia									
Grade 1	1/2			1/5					2/32
Grade 2							1/7		1/32
Grade 3							1/7	1/5	2/32
Confusional state									
Grade 1				1/5			1/7	1/5	3/32
Grade 2							1/7		1/32
Grade 3								1/5	1/32
Hangover									
Grade 1				2/5		1/3	1/7	1/5	5/32
Nausea									
Grade 1				1/5	2/7			1/5	4/32
Grade 2					1/7				1/32

activated form of Bak at the end of the infusion (1 hour), which was transient. When this patient received obatoclax at the higher dose, the pattern of activation of Bax and Bak was shifted to reach a peak at 3 hours and persisted until at least 4 hours after the start of the infusion. As patients were commonly lymphopenic from previous therapy (range $0.94\text{-}217 \times 10^3/\text{mm}^3$; 8 with $< 10 \times 10^3/\text{mm}^3$), only 16 had successfully processed serial samples containing sufficient protein to complete the IP assays. Of these, 12 of 16 across all dose levels showed activation of Bax and Bak persisting beyond the end of the 1- or 3-hour infusions with a trend toward a dose response (Table 6).

Low concentrations of oligonucleosomal DNA/histone complexes were commonly present at baseline, indicative of a low background rate of apoptosis in patients with advanced CLL. Following infusion of obatoclax and the activation of Bax and Bak, which was sustainable for hours, a substantial increase in the plasma concentrations of oligonucleosomal DNA/histone complexes could be observed to be sustained over days and reaching up to a 30-fold increase over baseline (data not shown).

The plasma concentrations of oligonucleosomal DNA/histone complexes are summarized for all patients in Figure 2B. Here, peak plasma concentration at any time is plotted against the AUC-24 hours of obatoclax. Since oligonucleosomal DNA/histone complex measurements were determined from the samples drawn for PK, precise timing of the early measurement varied between patients treated with 1- and 3-hour infusions. As a result, we show both the peak plasma concentrations obtained at any time, as well as those restricted to samples drawn at 24, 48, 72, and 96 hours after infusion, which are common time points for all patients. In both analyses, the peak plasma concentration of oligonucleosomal DNA/histone complexes increases with dose, with an apparent threshold of approximately 200 ng·h/mL.

Finally, reduction in lymphocyte count was observed in 18 of 26 patients, with a median reduction of 24% compared with baseline (Figure 2C). There was no relationship between C_{max} or $AUC_{(0\text{-}24\ hours)}$ and the percent decrease in lymphocyte count with any trends relating the hematologic responses to PK exposure.

Clinical efficacy

One patient with bulky lymphadenopathy experienced a PR following treatment with obatoclax at the 3.5 mg/m^2 dose level. This patient previously received fludarabine with rituximab, followed upon relapse by alemtuzumab, but was the only patient in this trial with no prior exposure to alkylating agents. Of note, an oligonucleosomal DNA response was not observed in this patient.

Table 4. Number of patients with laboratory abnormalities occurring in 15% or more of patients*

	Dose level (mg.m^2)								
Adverse event	**3.5 N = 2**	**5 N = 1**	**7 N = 2**	**10 N = 5**	**14 N = 7**	**20 N = 3**	**28 N = 7**	**40 N = 5**	**Total N = 32**
Anemia									
Grade 1	1/2		1/2		2/7		3/7		7/32
Grade 2					2/7	1/3	1/7		3/32
Grade 3									
Grade 4									
Thrombocytopenia									
Grade 1					1/7		5/7		6/32
Grade 2			1/2	1/5	1/7	1/3	1/7	2/5	7/32
Grade 3					1/7				1/32
Grade 4								1/5	1/32
Neutropenia									
Grade 2					1/7	1/3			2/32
Grade 3	1/2			1/5	1/7				3/32
Grade 4			1/2		1/7	1/3		2/5	5/32
SGOT increased									
Grade 1	1/2	1/1			2/7	1/3	1/7	1/5	7/32
Grade 2					2/7	1/3			3/32
SGPT increased									
Grade 1		1/1	1/2		1/7	1/3		2/5	6/32
Grade 2	1/2				1/7				2/32
Decreased O_2 saturation									
Grade 1								1/5	1/32
Grade 2					1/7				1/32
Grade 3				1/5	3/7			1/5	5/32

SGOT indicates serum glutamic oxaloacetic transaminase; and SGPT, serum glutamic pyruvic transaminase.
*NCI CLL Working Group Criteria for Hematological Toxicity.

Obatoclax did not cause myelosuppression in animal toxicology studies. Eleven patients had a baseline Hb less than 11 g/dL, and 14 patients had a baseline platelet count less than 100×10^9/L. Sustained improvements in Hb (3/11) and platelet counts (4/14) were seen at all dose levels, censoring patients receiving transfusions or growth factor support. Furthermore, 2 patients who had been red blood cell transfusion-dependent had Hb increases from 7.9 to 13.9 g/dL and 8.7 to 9.9 g/dL, respectively, and achieved sustained independence from transfusions, while another patient achieved an Hb increase from 8.7 to 10.6 g/dL. Sustained elevations in platelet counts were also observed in the same 3 patients from 65 to 106×10^3 platelets/mm^3, 47 to 105×10^3 platelets/mm^3, and 61 to 103×10^3 platelets/mm^3. Another patient with borderline anemia increased from 70 to 144×10^3 platelets/mm^3.

Discussion

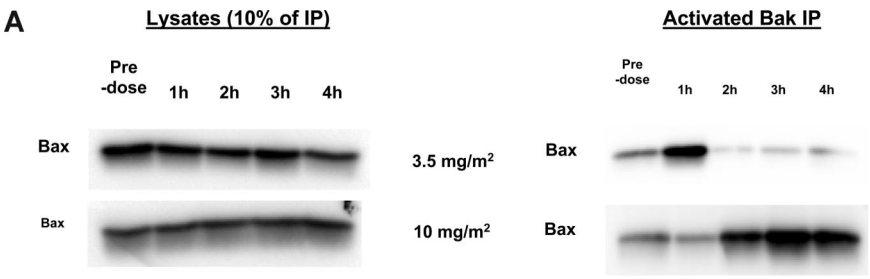
In this phase I trial in patients with refractory CLL, obatoclax infusion was followed by a dose-dependent activation of Bax and Bak in circulating mononuclear cells of the 12 of 26 patients for whom serial samples were successfully obtained. The activation of the mitochondrial pathway was followed by execution of apoptosis as evidenced by the exposure-dependent appearance of oligonucleosomal DNA fragments in the circulation and the subsequent decrease in circulating lymphocyte count in some patients. Of note, the plasma oligonucleosomal concentration may be affected by patient's characteristics other than the absolute number of apoptotic cells generated by the studied intervention. In particular, functional macrophages appear to be essential for the generation of plasma oligonucleosomal DNA, as in their absence, there is no release of DNA in the plasma.[12]

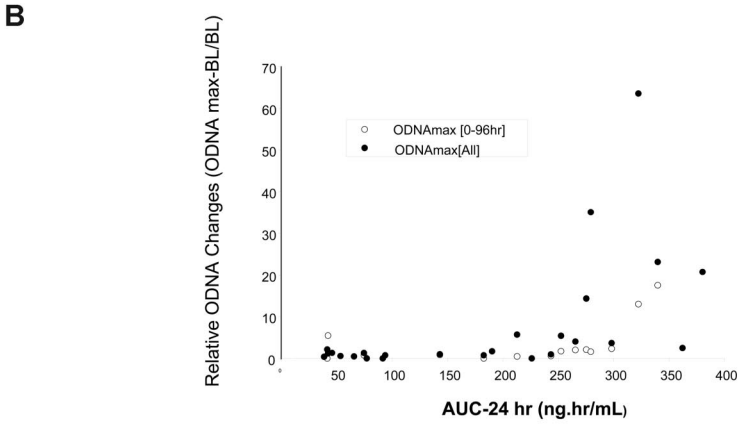
Further, the propensity to undergo apoptosis in response to a BH3-mimetic is dependent on the integration of proapoptotic signals inhibited by the integrated activity of all antiapoptotic Bcl-2 family of proteins. This varies across tumors and cell lines. This was demonstrated by engineering the KB cell line, which naturally expresses Mcl-1, but only traces of Bcl-2, to overexpress the Bcl-2 protein.[2] This model system then allowed exploration of how the cell line's sensitivity to the Bcl-2 (but not Mcl-1) inhibitor ABT-737 would be affected by small interfering RNA (siRNA)

Table 5. Summary of PK parameters across dose levels administered with a 3-hour infusion

			Mean (% CV)					
Dose, mg/m^2	**Infusion duration, h**	**n**	**T_{max}*, h**	**C_{max} (ng/mL)**	**$AUC_{0\text{-}24\ h}$ (ng·h/mL)**	**$t_{1/2,\ (h)}$**	**Clp (L/m^2/h)**	**V_{ss} (L/m^2)**
20	3	3	2	77.9 (24)	267 (18)	44.9 (NC)	71.6 (NC)	866 (NC)
28	3	6	1	72.9 (45)	245 (44)	39.0 (43)	162 (97)	1947 (74)
40	3	4	1	92.6 (40)	277 (47)	59.6 (39)	155 (57)	1878 (31)

CV indicates coefficient of variation; and NC, not calculated.
*Median presented (no % CV reported).





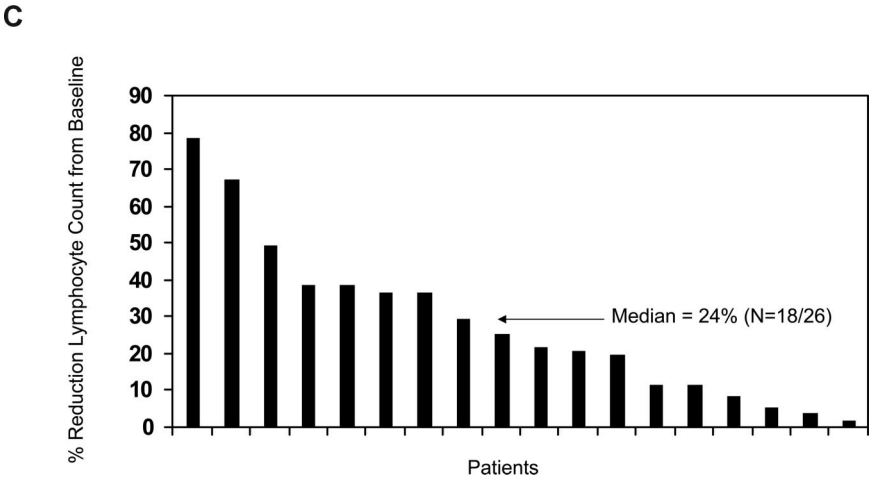


Figure 2. Pharmacodynamic activity of obatoclax monitored using 3 different end points. (A) Example of time course in PBMNCs following a 1-hour infusion of obatoclax in a patient initially receiving 3.5 mg/m^2 and later dose-escalated to 10 mg/m^2. Left panel, Western blot analysis of total Bax expression. Right panel, Western blot analysis of Bax coimmunoprecipitated with activated Bak. (B) Relationship between relative change in plasma concentration of oligonucleosomal DNA/histone complexes and obatoclax exposure in individual patients (N = 26). (C) Relative decrease in lymphocyte count from baseline in individual patients (N = 18).

knockdown of Mcl-1. Apoptosis in the presence of high concentrations of ABT-737 was dependent on the concomitant knockdown of Mcl-1, which itself was insufficient to trigger apoptosis in the absence of Bcl-2 inhibition.

A complementary experiment was conducted using low concentrations of obatoclax to block Mcl-1 function without completely inhibiting Bcl-2 function (higher levels of Bcl-2 protein expression than Mcl-1). Whereas neither obatoclax at low concentrations or ABT-737 at high concentrations could induce apoptosis in this model, a low concentration of obatoclax sensitized cells to ABT-737 in a dose-dependent manner.

From such observations, it is possible to extrapolate that the biologic effect of obatoclax is dependent not only on the dose (or

Table 6. Summary of BAX/BAK hetero-oligomer formation in PBMNCs in response to obatoclax

Infusion duration	Dose level, mg/m^2	Activated/informative	Time to peak, h	Time to resolution, h
1 hour	3.5	1/1*	1 h	2 h
	5	0/1	ND	ND
	7	0/0	ND	ND
	10	1/1*	3 h	> 4 h
	14	3/4	1-2 h	> 4 h
3 hour	20	1/2	2 h	5 h
	28	3/4	2-5 h	> 5-8 h
	40	3/3	3-4 h	5-> 8 h
Total		12/16		

ND indicates not documented.
*Patient was dose-escalated. See Figure 2A.

concentration) of this agent but also on the underlying biology ("priming") of the individual tumor. A low dose could have maximal activity for outlier tumors strongly primed to undergo apoptosis, whereas a maximally tolerated dose would be expected to be active against the broadest range of tumors.

The safety profile of obatoclax is distinct from that of classical cytotoxic agents. Its common toxicities are infusional in nature and composed of transient CNS events, most commonly somnolence and euphoria. The neurologic toxicity was expected based on preclinical toxicology experiments and was decreased by prolonging the rate of infusion. A recent report implicates Bcl-xl in synaptic function with disruption induced in vitro by the Bcl-2/Bcl-xl/Bcl-w antagonist ABT-737,[13] thus supporting the conclusion that the CNS effects of obatoclax, which is known to cross the blood brain barrier, represent an on-target effect. Monitoring of peripheral blood counts throughout the study revealed occasional episodes of grade 3 or 4 neutropenia and thrombocytopenia. Since obatoclax showed no evidence of myelosuppression in either animal toxicology studies or in another single-agent trial conducted in patients with refractory solid tumors or lymphomas with the more intensive weekly infusion schedule,[14] these hematologic events are likely to be related to the evolution of the underlying refractory CLL.

Our data also show clinical activity of single-agent obatoclax in heavily pretreated CLL patients. One of 26 patients (4%) achieved a PR, and it is notable that he was the least heavily pretreated patient in the trial. Furthermore, improvements in anemia and/or thrombocytopenia were reported across the entire dose range, irrespective of the status of the underlying CLL, with 2 patients achieving sustained red blood cell transfusion independence. Similar hematologic responses have been observed in poor prognosis myelodysplastic syndrome (MDS) patients receiving 24-hour infusions of obatoclax.[15]

In summary, single-agent obatoclax demonstrated biologic as well as modest clinical activity in heavily pretreated patients with CLL. Its safety profile makes it an attractive candidate for inclusion in combination trials for CLL and other hematologic and solid tumor malignancies.

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Authorship

Contribution: S.M.O. performed research, analyzed data, and wrote the manuscript; D.F.C, M.C., S.F., T.K., and M.J.K. performed research; J.V. designed the trial, analyzed data, and wrote the manuscript; and B.D.C. designed the trial and performed research. All authors reviewed the manuscript prior to submission.

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