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ORIGINAL REPORT

# Substantial Susceptibility of Chronic Lymphocytic Leukemia to BCL2 Inhibition: Results of a Phase I Study of Navitoclax in Patients With Relapsed or Refractory Disease

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## ABSTRACT

### Purpose
BCL2 overexpression is a hallmark of chronic lymphocytic leukemia (CLL). The novel BH3 mimetic navitoclax (ABT-263) specifically inhibits BCL2 and related proteins BCL-$x_L$ and BCL-w, potently inducing apoptosis of CLL cells in vitro. A phase I trial in patients with CLL was conducted to evaluate the safety, pharmacokinetics, and biologic activity of oral navitoclax.

### Patients and Methods
Twenty-nine patients with relapsed or refractory CLL received daily navitoclax for 14 days (10, 110, 200, or 250 mg/d; n = 15) or 21 days (125, 200, 250, or 300 mg/d; n = 14) of each 21-day cycle. Dose escalation decisions were informed by continual reassessment methodology.

### Results
Lymphocytosis was reduced by more than 50% in 19 of 21 patients with baseline lymphocytosis. Among 26 patients treated with navitoclax ≥ 110 mg/d, nine (35%) achieved a partial response and seven maintained stable disease for more than 6 months. Median treatment duration was 7 months (range, 1 to ≥ 29 months). Median progression-free survival was 25 months. Activity was observed in patients with fludarabine-refractory disease, bulky adenopathy, and del(17p) CLL. Thrombocytopenia due to BCL-$x_L$ inhibition was the major dose-limiting toxicity and was dose-related. Low MCL1 expression and high BIM:MCL1 or BIM:BCL2 ratios in leukemic cells correlated with response. We determined that the navitoclax dose of 250 mg/d in a continuous dosing schedule was optimal for phase II studies.

### Conclusion
BCL2 is a valid therapeutic target in CLL, and its inhibition by navitoclax warrants further evaluation as monotherapy and in combination in this disease.

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## INTRODUCTION

Chronic lymphocytic leukemia (CLL) is characterized by high-level expression of BCL2 in all patients, and the accumulation of mature leukemic lymphocytes is proposed to be a direct consequence of the antiapoptotic effect of BCL2. As such, BCL2 has long been considered a high priority therapeutic target in this disease, but previous attempts to target BCL2 clinically with oblimersen and obataclax have not demonstrated major antileukemic activity.[1,2]

The closely related compounds navitoclax and ABT-737 are BH3 mimetics, a new class of anticancer therapeutics that depend on inhibition of BCL2 and related antiapoptotic intracellular proteins for their anticancer activity.[3-5] Such targeted compounds were developed because of the critical role BCL2 is recognized to play in tumorogenesis[6] and chemotherapy resistance[7-10] when highly expressed. Both bind BCL2, BCL-$x_L$, and BCL-w, relieving repression of BAX and BAK, which then oligomerize on the mitochondrial outer membrane and trigger apoptosis.[3-5] Although much of the early preclinical data were generated by studying ABT-737, an analog with superior oral bioavailability was required for clinical application so navitoclax was developed. These drugs[3-5,11] display absolute dependence on the mitochondrial apoptotic pathway for cell killing, show significant single-agent activity against cell lines expressing high levels of BCL2 or BCL-$x_L$,[3,12,13]

and synergize with chemotherapeutic drugs in preclinical in vivo models of lymphoproliferative diseases.[5,14,15]

In vitro, primary CLL cells show marked sensitivity to ABT-737[3,16,17] and navitoclax,[18] including highly chemoresistant CLL cells and cells from patients with del(17p) and del(11q) CLL.[19] The modality of death is apoptosis, and this is observed within 4 hours of exposure.[17] Given this compelling biologic rationale, evaluation of navitoclax in patients with relapsed or refractory CLL was planned as one of three initial concurrent phase I studies.

Preclinical toxicology studies indicated that the anticipated human toxicities for navitoclax reflected the known essential nonredundant functions of the target proteins as defined by deficiency of BCL-$x_L$,[20] BCL2,[21] and BCL-w[22] in mice (thrombocytopenia, lymphopenia, and impaired spermatogenesis, respectively). Pharmacologic inhibition of BCL-$x_L$ in mice[20] or dogs[23] causes an acute, dose-proportional reduction in platelet count within 1 to 2 days via induction of apoptosis[24] and subsequent clearance of platelets from the circulation.[20] Megakaryocytes and reticulated platelets are less susceptible, and thus compensatory increased megakaryopoiesis and partial correction of the thrombocytopenia are observed in preclinical models during ongoing dosing.[23]

The objectives of this study were to define the safety profile, dose-limiting toxicity (DLT), maximum-tolerated dose (MTD), pharmacokinetics, and preliminary efficacy of navitoclax and to determine a recommended dose and schedule for phase II assessment in patients with relapsed or refractory CLL.

**Table 1.** Entry Criteria

| |
|---|
| Inclusion |
| Informed consent |
| Age > 18 years |
| ECOG performance status of 0 or 1 |
| Adequate hematology |
| Neutrophil count $\geq 1.0 \times 10^9$/L |
| Platelet count $\geq 75 \times 10^9$/L |
| Hemoglobin concentration $\geq$ 90 g/L |
| Serum creatinine $\leq$ 2.0 mg/dL or calculated creatinine clearance $\geq$ 50 mL/min |
| Adequate liver function |
| AST, ALT $\leq 3 \times$ ULN |
| Bilirubin $\leq 1.5 \times$ ULN |
| Normal coagulation profile |
| Exclusion |
| History of immune thrombocytopenia or refractoriness to platelet transfusion in the last 12 months |
| Ongoing use of an antiplatelet agent or anticoagulant therapy |
| Current bleeding or history of active peptic ulcer disease or erosive gastritis/esophagitis |
| History of significant cardiovascular disease (eg, myocardial infarction, thrombotic event, or thromboembolic event in the last 6 months) |
| Active infection |
| Current pregnancy or breastfeeding |
| Previous stem-cell transplantation |

NOTE. All patients had an original diagnosis of chronic lymphocytic leukemia, but lymphocytosis was not required at study entry.
Abbreviation: ECOG, Eastern Cooperative Oncology Group; ULN, upper limit of normal.

## PATIENTS AND METHODS

### Study Design

Study M06-873 (ClinicalTrials.gov Identifier: NCT00481091) was designed as an open-label, multicenter, dose-escalation phase I/IIA study. Phase I enrolled patients between July 2007 and June 2009 with data cutoff of July 16, 2010, and is the subject of this report.

The study was conducted according to the Declaration of Helsinki and relevant International Conference on Harmonization Good Clinical Practice guidelines and with approval from the local institutional review board, independent ethics committee, or research ethics board of all participating study sites. All participants provided written informed consent before participating in this study.

### Patient Eligibility

Patients with relapsed or refractory CLL requiring treatment were considered eligible if they met all of the inclusion criteria and none of the exclusion criteria (Table 1). Patients could not have received any anti-CLL therapy within 14 days (30 days for monoclonal antibody therapy) before the first dose of navitoclax.

### Treatment

Patients were assigned sequentially to dose-escalation cohorts of at least three. Initially, navitoclax was administered orally, once daily, for 14 days followed by 7 days off drug in each 21-day cycle. Subsequently, patients received a continuous, once daily, dosing schedule after a 7-day lead-in course of navitoclax 100 mg daily. Patients continued treatment in 21-day cycles as long as there was clinical benefit in the absence of significant toxicity. Dose reductions were allowed for toxicity. Patients received prophylaxis against tumor lysis syndrome and also received supportive care per local practice.

### Dose Escalation

A continual reassessment method (CRM) was used to efficiently obtain an estimate of the MTD (defined as the dose at which 30% of patients experience a DLT) and to determine the recommended phase II dose.[25,26] Once three patients in a dose cohort completed cycle 1, all available data were entered into a responder model of dose and probability of DLT generated by using preclinical and accumulating clinical data. The navitoclax dose for the next cohort was adjusted to the CRM-estimated MTD, provided that dose escalation did not exceed either an increase of more than 100 mg or a 40% increase from the just completed dose level. Dose escalation ceased when the model-estimated MTD was within 10% of the just-completed dose cohort. At this point, the model was considered to have converged and the trial-determined MTD was declared.

### Study Assessments

Safety assessments included history, physical examination, vital signs, ECG, 2D echocardiogram, blood chemistry, hematology, and urinalysis. Safety assessments were performed on screening, day 1 of lead-in, day 1 of cycle 1, weekly through cycle 2, day 1 of each subsequent cycle, and at the end of treatment. Platelet counts were intensively monitored at multiple time points throughout the study.

Adverse events (AEs) were graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0.[27] AEs that were judged possibly or probably related to navitoclax were considered to be DLTs if they satisfied any of the following criteria: grade 4 thrombocytopenia, grade $\geq$ 2 bleeding associated with thrombocytopenia, or all other grade $\geq$ 3 AEs with certain protocol-defined exceptions (grade 3 laboratory tumor lysis syndrome without clinical manifestations; grade 3 or 4 leukopenia, lymphopenia, or afebrile neutropenia; alopecia; and grade 3 nausea, vomiting, and/or diarrhea unless unresponsive to treatment). Each DLT required interruption and possible discontinuation of navitoclax therapy; navitoclax could be reintroduced at a reduced dose providing toxicity resolved to grade $\leq$ 1 or baseline if grade 2 toxicity was present at study entry. Following a DLT of thrombocytopenia, navitoclax could be reintroduced at a reduced dose if the platelet count returned to $\geq 50 \times 10^9$/L (grade $\leq$ 2 toxicity).

### Efficacy

Exploratory efficacy end points included progression-free survival (PFS), overall response rate (ORR), time to disease progression, overall survival, and duration of overall response. Objective response was evaluated by using the

**Table 2.** Patient Characteristics

| Characteristic | No./ Total | % |
|---|---|---|
| Age, years | | |
| Median | 67 | |
| Range | 50-79 | |
| Sex | | |
| Male | 19/29 | 66 |
| Female | 10/29 | 34 |
| Lymphocyte count $\times 10^9$/L | | |
| Median | 15.5 | |
| Range | 0.8-284.3 | |
| Splenomegaly | 13/29 | 45 |
| Bulky adenopathy | 12/29 | 41 |
| No. prior therapies | | |
| Median | 4.5 | |
| Range | 1-11 | |
| Fludarabine refractory | 9/29 | 31 |
| Unfavorable FISH* | | |
| 17p13.2 del | 11/25 | 44 |
| 11q22.3 del | 7/25 | 28 |

Abbreviation: FISH, fluorescent in situ hybridization.
*Reported per Döhner et al[30]; not all patients were evaluated.

1996 NCI Working Group response criteria for CLL[28] at the end of cycles 2 and 4, the end of every third cycle thereafter, and at the final visit. In addition, changes in lymph nodes and spleen were evaluated by serial computed tomography scanning,[29] and individual measurements were recorded. Information on pharmacokinetics and biologic studies is included in the Data Supplement.

### Statistical Analysis

Pharmacokinetic parameters were determined by using a noncompartmental approach. The method of Kaplan and Meier was used for time to event analyses. Comparisons and correlations between groups were performed by using GraphPad Prism (GraphPad Software, La Jolla, CA). The specific tests used are stated in the relevant figure legends.

## RESULTS

### Patient Characteristics

Twenty-nine patients with a median age of 67 years (range, 50 to 79 years) were enrolled. Their key clinical characteristics are summarized in Table 2. Twenty patients had at least one of the following adverse prognostic features: bulky lymphadenopathy, fludarabine resistance, or deletion of 17p13.2 or 11q22.3 by fluorescent in situ hybridization.

### Patient Flow

The first 15 patients were enrolled in the intermittent 14-/21-day dosing schedule (Fig 1A). Two patients remained on study and 13 discontinued because of disease progression (n = 5), AEs (n = 5), withdrawal of consent (n = 2), or death (n = 1). Five patients required dose reductions, predominantly because of severe thrombocytopenia in cycles 1 or 2. DLTs were observed in three patients in cycle 1: grade 3 tumor lysis syndrome (110 mg) and grade 4 thrombocytopenia (110 mg, 250 mg). All resolved with dose interruption (Data Supplement). By using the CRM methodology, the MTD was estimated to be 200 mg/d with intermittent dosing.

With the aim of avoiding dose-limiting thrombocytopenia, the protocol was amended to evaluate escalating doses on a continuous schedule following a 7-day 100 mg/d lead-in phase. Commencing at 125 mg/d (a dose level approximating the overall exposure of the 200 mg/d for the 14-/21-day regimen), 14 patients were enrolled in the 21-/21-day schedule (Fig 1B), and five remain on study. Reasons for study discontinuation included disease progression (n = 4), AEs (n = 3), withdrawal of consent (n = 1), and investigator discretion (n = 1). Nine patients required dose reductions. Three patients experienced DLTs in cycle 1: grade 4 thrombocytopenia (200 mg, 300 mg) and grade 2 nausea (250 mg). The MTD in the 21-/21-day schedule was estimated to be 250 mg/d, with thrombocytopenia being the DLT.

### Navitoclax-Induced Thrombocytopenia

On the 14-/21-day schedule, acute reductions in platelet counts were observed in all patients receiving navitoclax at more than 10 mg/d. Platelet nadirs were transient, typically occurring on days 2 to 5, followed by partial recovery during continued dosing and full recovery during the 7 days off drug. Re-exposure resulted in repetition of this cycle (Fig 2A). In cycle 1, the nadirs (mean $\pm$ standard deviation) were related to dose: 10 mg, $111 \pm 12 \times 10^9$/L; 110 mg, $67 \pm 51 \times 10^9$/L; 200 mg, $46 \pm 9 \times 10^9$/L; 250 mg, $26 \pm 5 \times 10^9$/L. Lead-in dosing of 100 mg/d for 7 days followed by dose escalation reduced the depth of the nadir and therefore the occurrence of grade 4 thrombocytopenia during the first cycle (Fig 2A), allowing escalation to higher doses than achievable on the 14-/21-day schedule. The mean respective nadirs for 125 mg, 200 mg, 250 mg, and 300 mg doses were 79 $\pm$ 24, 79 $\pm$ 65, 58 $\pm$ 5, and 40 $\pm$ 13 $\times 10^9$/L, respectively. Continuous dosing also minimized cyclic variability observed with intermittent dosing. Despite these adjustments, severe thrombocytopenia requiring dose reduction was also observed during later cycles in four of seven patients in the 300 mg and 250 mg continuous dosing cohorts. Grade 4 thrombocytopenia resolved to more than $50 \times 10^9$/L within 1 to 2 weeks of dose interruption, thus allowing resumption of navitoclax at a lower dose. Thrombocytopenia was not associated with any clinically significant bleeding during this study.

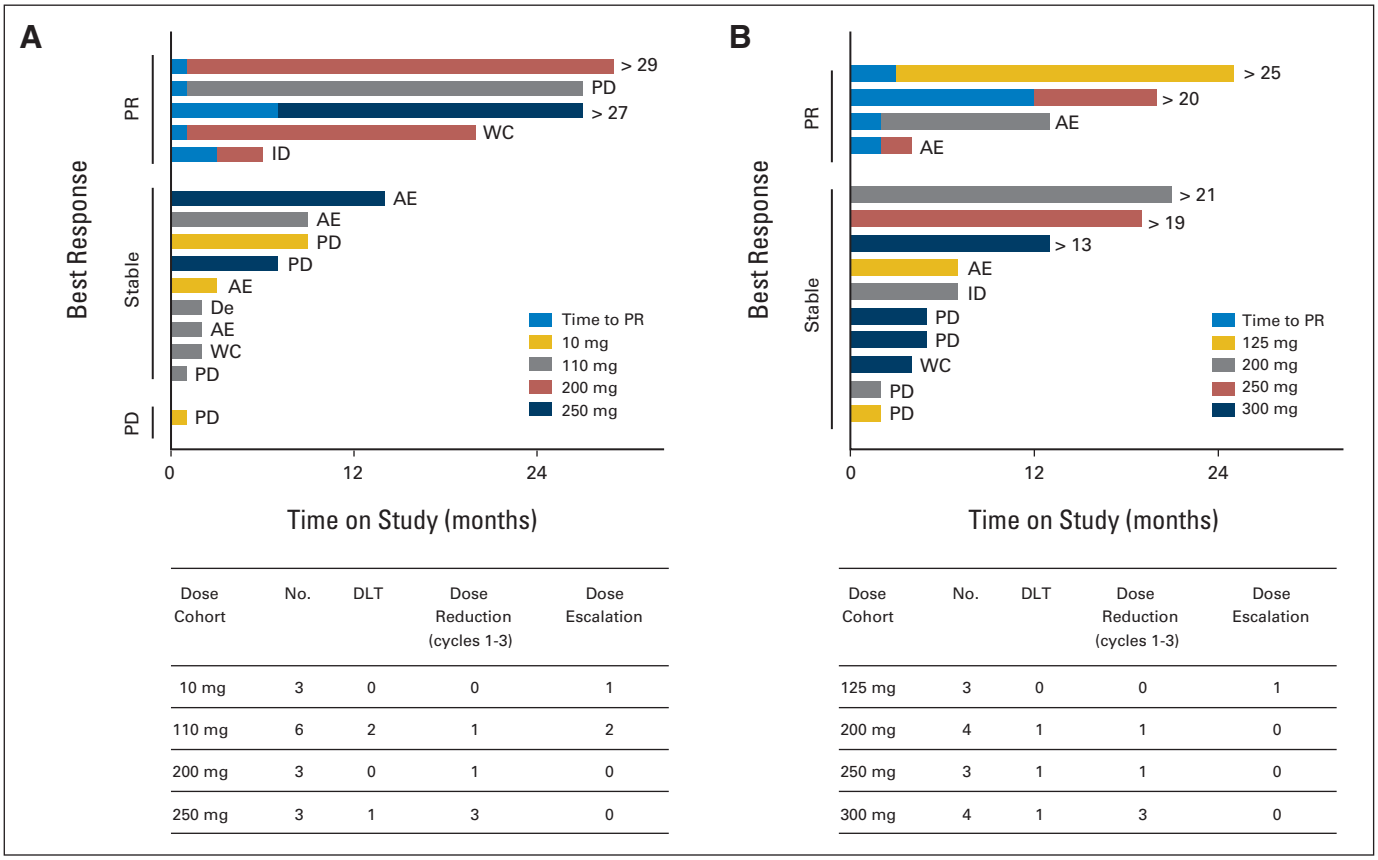
### Safety

Table 3 lists all AEs observed in 10% or more of the patients. The most common emergent AEs were diarrhea, nausea, vomiting, fatigue, thrombocytopenia, and neutropenia. The GI events were mild and were considered likely to be related to navitoclax. Serious AEs other than grade 4 thrombocytopenia considered potentially related to navitoclax included tumor lysis syndrome (n = 1; related), neutropenia (n = 8; related or probably related), polymerase chain reaction–confirmed progressive multifocal leukoencephalopathy (n = 1 heavily pretreated patient; possibly related), and myocardial infarction (n = 1; possibly related). Grade 4 neutropenia was observed both early (cycles 1 to 5) and late (cycles 7 to 14) and was reversible with either dose reduction or administration of granulocyte colony-stimulating factor. Three of four patients in the 300-mg cohort experienced grade 4 neutropenia within the first four cycles.

### Pharmacokinetics

Twenty-eight patients were included in the pharmacokinetic analysis (Data Supplement). Peak concentrations were observed 6 to 8 hours postdose in both the 14-/21-day (Fig 2B) and 21-/21-day schedules. The interpatient variability in area under the curve was 46%.




| Dose Cohort | No. | DLT | Dose Reduction (cycles 1-3) | Dose Escalation |
|---|---|---|---|---|
| 10 mg | 3 | 0 | 0 | 1 |
| 110 mg | 6 | 2 | 1 | 2 |
| 200 mg | 3 | 0 | 1 | 0 |
| 250 mg | 3 | 1 | 3 | 0 |

| Dose Cohort | No. | DLT | Dose Reduction (cycles 1-3) | Dose Escalation |
|---|---|---|---|---|
| 125 mg | 3 | 0 | 0 | 1 |
| 200 mg | 4 | 1 | 1 | 0 |
| 250 mg | 3 | 1 | 1 | 0 |
| 300 mg | 4 | 1 | 3 | 0 |

**Fig 1.** Patient status on study by dose and response. For the intermittent schedule cohort (A) and the continuous schedule cohort (B), the upper horizontal bar graphs indicate the time on study for each patient and the lower tables present a summary of dose-limiting toxicities (DLTs) and dose modifications. For the time on study graph, patients are grouped according to their observed best response, and the color of the bar reflects the navitoclax dose cohort they entered. For patients achieving a partial response (PR), the time on study to reach PR is represented by the lighter blue segment of the bar. When a patient discontinued the study, the reason is indicated on the right end of the colored bar, and for patients still receiving navitoclax, their duration on study in months is similarly indicated. AE, adverse event; De, death; ID, investigator discretion; PD, progressive disease; WC, withdrew consent.

Navitoclax exposure was dose-proportional between 10 mg and 250 mg in the 14-/21-day cohort. Pharmacokinetic parameters were stable over multiple cycles. No consistent trend was observed in dose-normalized steady-state trough concentrations over time. Navitoclax exposure did not demonstrate any apparent correlation with age, body weight, body surface area, renal function, or total bilirubin levels (data not shown).

### *Preliminary Efficacy*

*Peripheral blood lymphocytosis.* Nineteen (90%) of 21 patients with peripheral blood lymphocytosis at study entry achieved at least a 50% reduction from baseline at maximum response (Fig 2C). No apparent relationship was observed between increasing doses of navitoclax over 110 mg/d and reduction in circulating lymphocytes (Data Supplement). Significant reductions in lymphocytosis occurred within days of commencing navitoclax (Fig 2D). These were associated with morphologic (Data Supplement) and biochemical changes of apoptosis in circulating CLL cells (Fig 2E), confirming the mechanism of action of navitoclax. The median number of cycles to maximum reduction was three (range, one to 24).

*Splenomegaly and nodal disease.* In five of 13 patients with palpable splenomegaly at study entry, the splenomegaly resolved, and an additional five patients achieved partial reductions in splenic size; improvements were evident within three cycles in nine of these 10 responding patients. Nodal disease was reduced in 21 of 29 patients, with 11 achieving $\geq$ 50% reduction from baseline in tumor volume as assessed by computed tomography criteria[29]; 10 of these 11 patients had received $\geq$ 200 mg/d navitoclax (Fig 2F). The median time to nodal response was 50 days (range, 43 to 548 days).

*Overall response.* Nine patients achieved a partial response (PR). Bone marrow biopsies were performed when the patient was considered to have a potential complete response, and residual diffuse CLL involvement was observed in all patients so investigated. The ORR was 31% (nine of 29 patients). Among the 26 patients who received doses of navitoclax sufficient to achieve sustained exposure of biologically active concentrations ($\geq$ 110 mg/d; Fig 2B and Data Supplement), the ORR was 35%. Responses appeared durable, since seven patients had stable disease (SD) features for more than 12 months from commencement of therapy (Fig 1). SD was the best objective response in 18 patients and was sustained for at least 6 months in eight patients (44%). The median PFS and the median time to disease progression were both 25 months (Fig 3A; data not shown).

Among seven patients with fludarabine-refractory disease receiving $\geq$ 110 mg/d, one achieved a PR and five had overall SD while demonstrating some antitumor efficacy with either a more than 50% reduction in peripheral blood lymphocytosis and/or a substantial reduction in

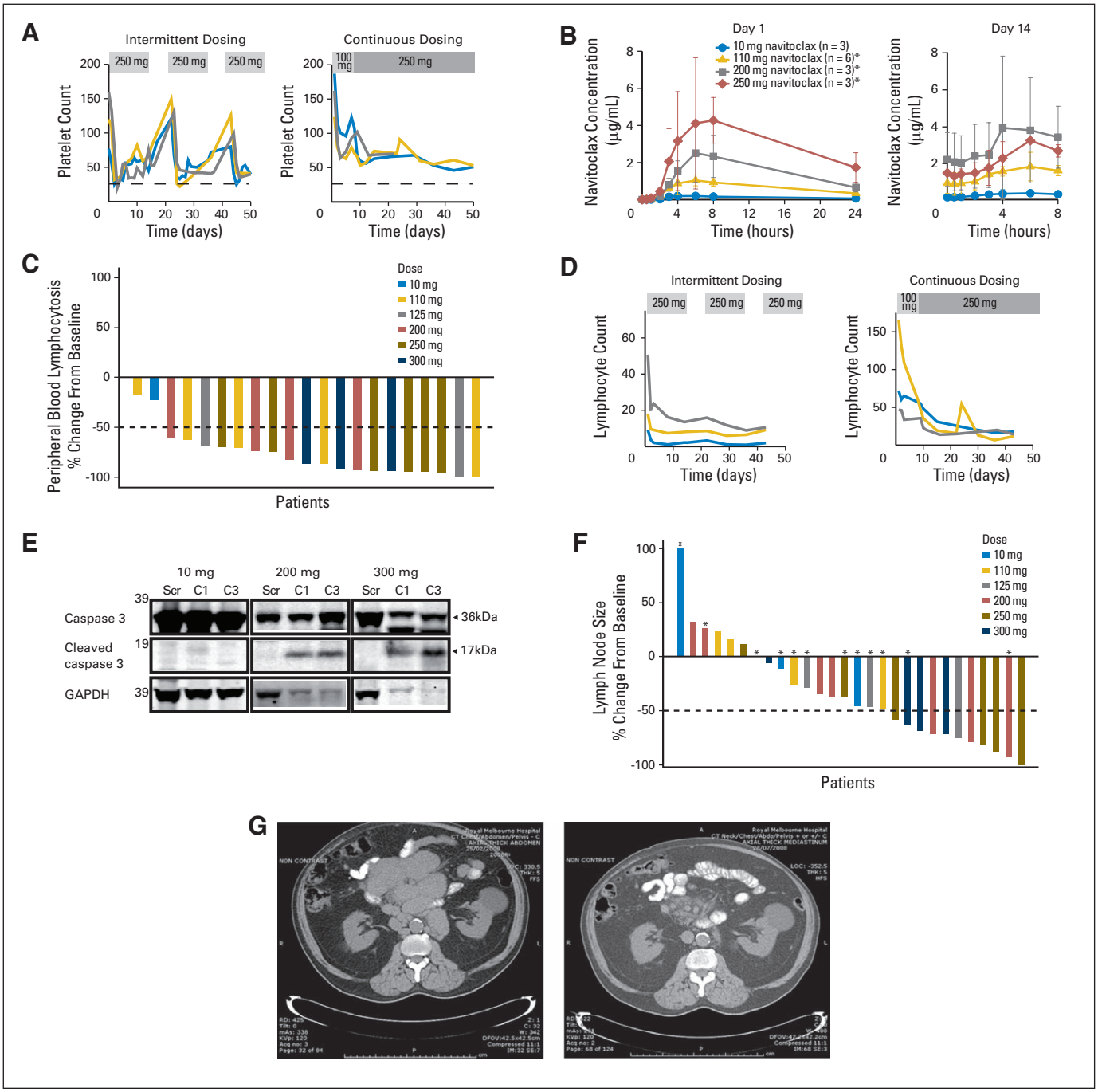



**Fig 2.** Navitoclax reduces platelet counts and chronic lymphocytic leukemia (CLL) burden in peripheral blood (PB) and lymph nodes. (A) Navitoclax scheduling modulates the severity of thrombocytopenia. The platelet counts of patients receiving 250 mg navitoclax daily in either the 14-days-on/7-days-off intermittent dosing cohort or the continuous dosing cohort incorporating a 7-day 100-mg lead-in phase are plotted for the first 50 days. The dotted line represents a platelet count of 25 $\times$ $10^9$/L, denoting the grade 4 toxicity level. One patient in the continuous cohort interrupted dosing prematurely for nonthrombocytopenic toxicity. (B) Navitoclax pharmacokinetics: The mean $\pm$ standard deviation plasma concentrations of navitoclax are plotted for the hours following initial dosing on day 1 and on day 14 for each dose cohort on the intermittent dosing schedule. (*) On day 14, n = 3 for 110 mg, n = 4 for 200 mg, and n = 2 for 250 mg. (C) Waterfall plot of most favorable changes in PB lymphocytosis. Only patients with lymphocytosis ($> 5 \times 10^9$/L) at study entry are included. (D) Rapid reduction in PB lymphocytosis. The PB lymphocyte counts ($\times 10^9$/L) during cycles 1 to 3 for individual patients enrolled in the 250-mg dose cohorts on either the intermittent or continuous schedules are presented. (E) Navitoclax induces apoptosis of CLL cells in vivo. Representative immunoblots of lysates of PB CLL cells from a patient from each of the 10-mg, 200-mg (intermittent), and 300-mg (continuous) dosing cohorts. For each patient, lysates from screening (Scr; pre-exposure), day 14 of cycle 1 (C1), and day 1 of cycle 3 (C3) were electrophoresed, before membranes were probed for caspase 3, cleaved caspase 3, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). In the 200-mg and 300-mg examples, but not the 10-mg example, the presence of cleaved caspase 3 in the cycle 1 and cycle 3 lanes reflects activation of apoptosis during exposure to navitoclax. GAPDH is reduced in these particular samples because it is known to be degraded during apoptosis. Cleaved caspase 3 was detected in six of nine patients analyzed after receiving $\geq$ 110 mg/d of navitoclax. (F) Waterfall plot of most favorable change in lymphadenopathy. Asterisks identify patients with bulky lymphadenopathy ($>$ 5 cm diameter masses) at study entry. (G) Representative images from abdominal computed tomography scans of a patient with fludarabine-refractory del(17p) CLL at study entry (left) and after seven cycles of navitoclax 200 mg/d (right). Bulky mesenteric adenopathy has almost completely resolved. A benign left renal cyst has not altered over time. This response has been maintained for more than 2 years.

**Table 3.** All Adverse Events Observed in ≥ 10% of Patients

| Adverse Event | No. | % | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|---|---|
| GI | | | | | | |
| Diarrhea | 22 | 76 | 15 | 7 | 0 | 0 |
| Nausea | 17 | 59 | 11 | 6 | 0 | 0 |
| Vomiting | 9 | 31 | 8 | 0 | 1 | 0 |
| Decreased appetite | 6 | 21 | 5 | 1 | 0 | 0 |
| Dyspepsia | 4 | 14 | 3 | 1 | 0 | 0 |
| Abdominal pain | 4 | 14 | 4 | 0 | 0 | 0 |
| General | | | | | | |
| Fatigue | 10 | 35 | 4 | 5 | 0 | 1 |
| Peripheral edema | 6 | 21 | 4 | 2 | 0 | 0 |
| Insomnia | 5 | 17 | 2 | 3 | 0 | 0 |
| Pyrexia | 4 | 14 | 2 | 2 | 0 | 0 |
| Dizziness | 4 | 14 | 2 | 2 | 0 | 0 |
| Headache | 4 | 14 | 4 | 0 | 0 | 0 |
| Hypertension | 3 | 10 | 1 | 2 | 0 | 0 |
| Blood | | | | | | |
| Thrombocytopenia | 8 | 28 | 0 | 0 | 3 | 5 |
| Neutropenia | 8 | 28 | 0 | 0 | 1 | 7 |
| Epistaxis | 5 | 17 | 4 | 1 | 0 | 0 |
| Contusion | 4 | 14 | 3 | 1 | 0 | 0 |
| Respiratory | | | | | | |
| Cough | 9 | 31 | 6 | 3 | 0 | 0 |
| Upper RTI | 7 | 24 | 2 | 5 | 0 | 0 |
| Sinusitis | 6 | 21 | 2 | 4 | 0 | 0 |
| Lower RTI | 4 | 14 | 0 | 1 | 3 | 0 |
| Pneumonia | 4 | 14 | 0 | 1 | 2 | 1 |
| Productive cough | 4 | 14 | 4 | 0 | 0 | 0 |
| Nasopharyngitis | 4 | 14 | 4 | 0 | 0 | 0 |
| Dyspnea | 3 | 13 | 2 | 1 | 0 | 0 |
| Pain | | | | | | |
| Arthralgia | 4 | 14 | 2 | 2 | 0 | 0 |
| Oropharyngeal | 3 | 10 | 2 | 1 | 0 | 0 |
| Extremity | 3 | 10 | 0 | 3 | 0 | 0 |
| Skin | | | | | | |
| Basal cell carcinoma | 4 | 14 | 0 | 3 | 1 | 0 |
| Skin lesion | 4 | 14 | 1 | 2 | 1 | 0 |
| Dry skin | 3 | 10 | 3 | 0 | 0 | 0 |
| Rash | 3 | 10 | 1 | 2 | 0 | 0 |
| Squamous cell carcinoma | 3 | 10 | 0 | 1 | 2 | 0 |
| Infections and other | | | | | | |
| Herpes simplex | 4 | 14 | 2 | 2 | 0 | 0 |
| Urinary tract | 4 | 14 | 0 | 4 | 0 | 0 |
| Viral | 4 | 14 | 4 | 0 | 0 | 0 |
| Influenza-like illness | 3 | 10 | 2 | 1 | 0 | 0 |
| Stomatitis | 3 | 10 | 1 | 2 | 0 | 0 |

Abbreviation: RTI, respiratory tract infection.

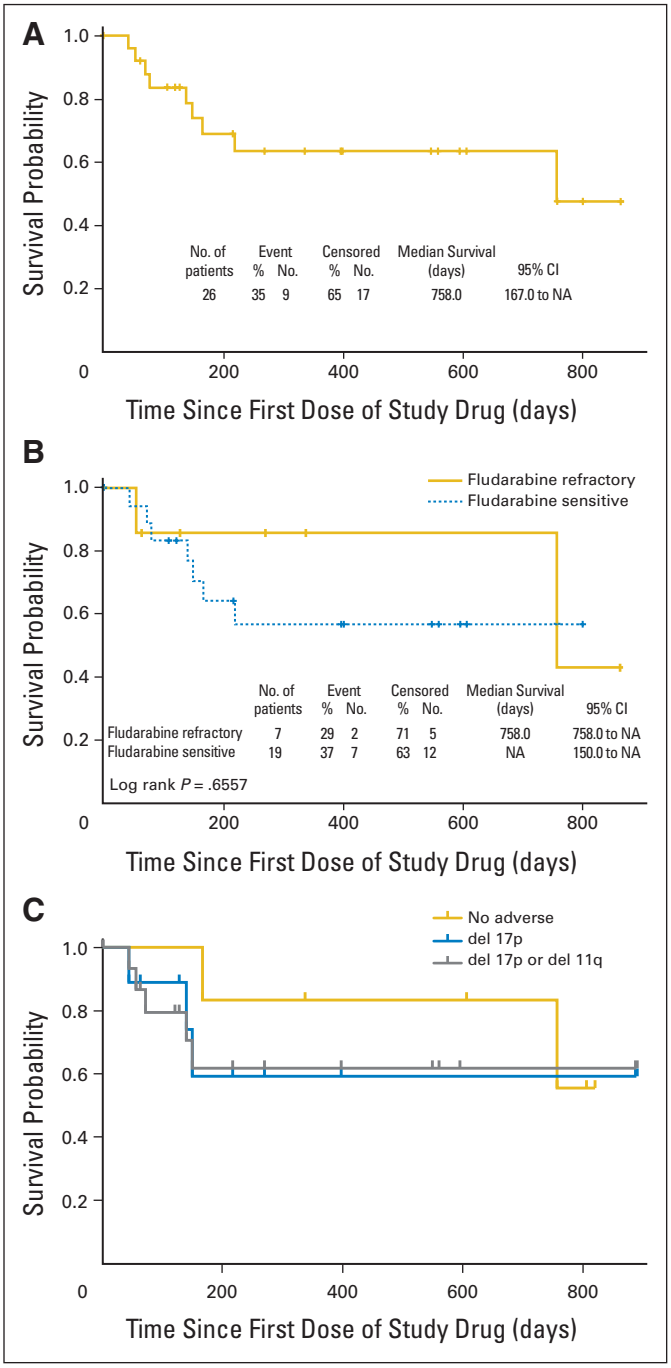



**Fig 3.** Durability of antileukemic activity of navitoclax. (A) The progression-free survival (PFS) for all patients receiving ≥ 110 mg/d navitoclax from study entry (n = 26) is displayed in a Kaplan-Meier plot. (B) PFS for a subset of these patients with fludarabine-refractory disease. Fluorescent in situ hybridization data were available for 22 of 26 patients receiving ≥ 110 mg/d navitoclax. (C) PFS for patients with del17p13.2 chronic lymphocytic leukemia (n = 9), either del17p13.2 or del11p22.3 (n = 16), or chronic lymphocytic leukemia with neither of these abnormalities (n = 6). NA, not applicable.

lymphadenopathy. The median PFS of fludarabine-refractory patients was 25 months (Fig 3B). Among nine patients with bulky lymphadenopathy receiving navitoclax at doses ≥ 110 mg/d, three achieved a PR and six had SD while demonstrating some antitumor efficacy. Similarly, three of nine patients with del(17p) CLL treated with navitoclax at ≥ 110 mg/d achieved a PR, and their median PFS has not been reached (Figs 2G and 3C).

### *BCL2 Family Proteins as Potential Biomarkers*

The levels of expression at baseline of the target proteins, as well as the related prosurvival protein MCL1 that is not inhibited by navitoclax and which can mediate navitoclax resistance in vitro,[4,5] and the BH3-only protein BIM, which antagonizes all BCL2-related prosurvival proteins,[31] were analyzed in a subset of patients treated at two centers to explore their potential as biomarkers. Neither BCL-w nor BCL-$x_L$ were detectable in high concentrations (Fig 4A; data not

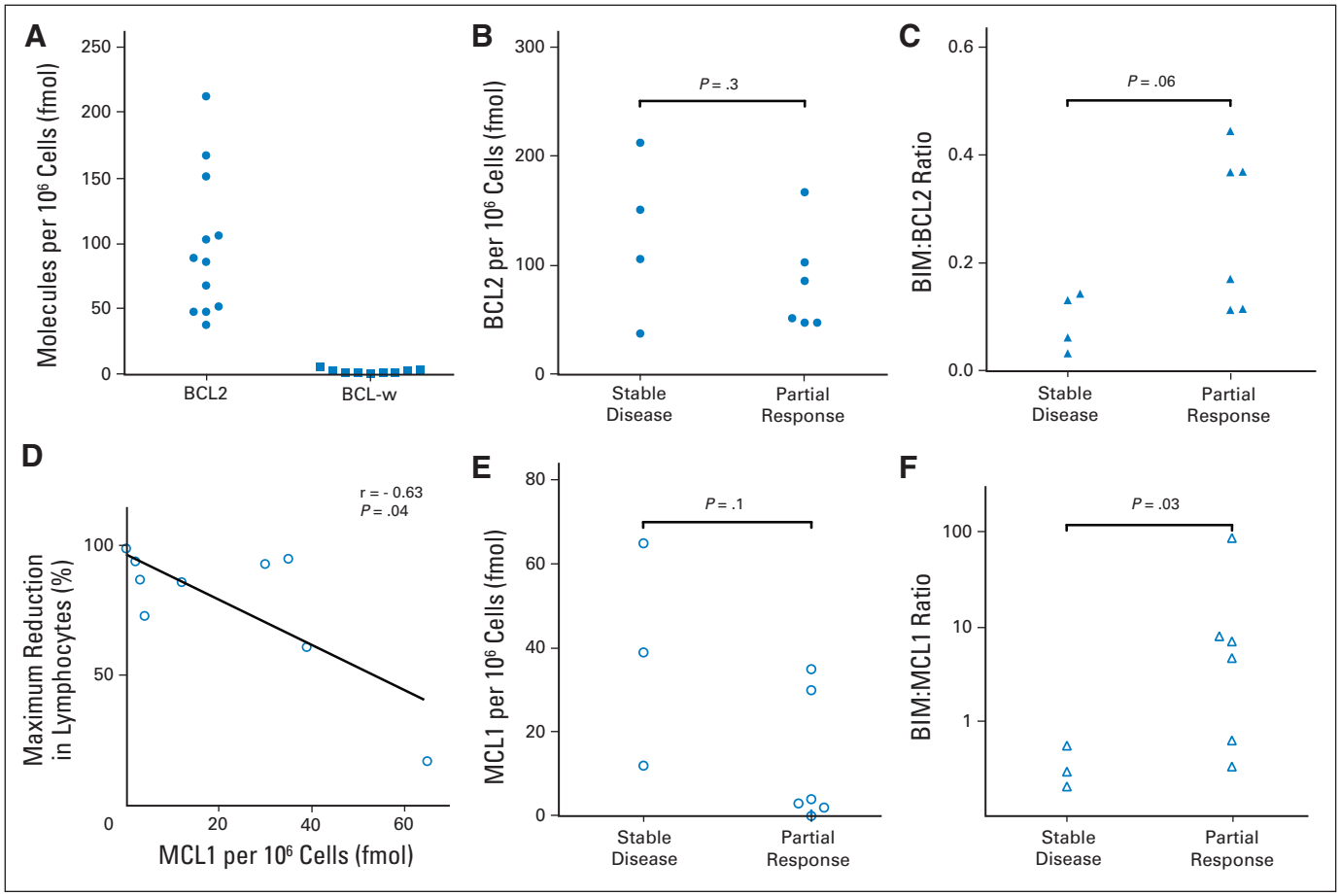



**Fig 4.** High-level BCL2 expression, but not BCL-w or BCL-$x_L$, in peripheral blood chronic lymphocytic leukemia cells and inverse correlation of MCL1 expression with response. The concentrations of BCL2, BCL-w, BCL-$x_L$, BIM, and MCL1 were measured as described in Patients and Methods by using immunoblots. BCL-$x_L$ was typically not detected (< 6 fmol/$10^6$ cells). (A) Concentrations of BCL2 and BCL-w for individual patients treated at two centers. (B and C) BCL2 concentrations and BIM:BCL2 ratios determined for 10 patients treated with ≥ 110 mg/d navitoclax *v* best observed clinical response ($P = .3$ and $P = .06$, respectively; unpaired two-tailed *t* test). (D) Shows significant inverse relationship between MCL1 expression in peripheral blood lymphocytes and the maximal reduction in peripheral blood lymphocytosis in the nine patients in whom it could be measured (Spearman coefficient $r = -0.63$; one-tailed $P = .04$), as hypothesized. (E and F) MCL1 concentrations and BIM:MCL1 ratios determined for the same patients *v* best observed clinical response ($P = .1$ and $P = .03$, respectively; unpaired two-tailed *t* test).

shown). BCL2 was highly but variably expressed, and MCL1 was expressed in intermediate levels in some samples (Fig 4). No correlation was observed between the level of BCL2 and in vivo response. MCL1 expression was inversely correlated with maximum reduction in lymphocytosis (Fig 4D). Navitoclax is thought to kill MCL1-expressing cells by displacing BIM from a stable complex with BCL2, thereby freeing BIM to antagonize MCL1.[16] Consistent with this, high BIM:MCL1 ratios and possibly high BIM:BCL2 ratios were associated with achieving PR (Fig 4).

## DISCUSSION

Navitoclax demonstrated significant single-agent activity against circulating, nodal, and splenic disease in patients with CLL. Antileukemic activity was evident within days, particularly in circulating lymphocyte counts, with maximum clinical responses typically observed within the first 4 months. Although no CRs were observed, durable PRs were seen in 35% and SD for more than 6 months in 27% of patients receiving continuing treatment with doses ≥ 110 mg/d. The depth of response was not clearly related to dose in these patients. Durable responses were observed in patients with fludarabine-refractory disease, bulky adenopathy, and del(17p) CLL.

This initial clinical experience with navitoclax, confirmed in the parallel phase I study in lymphoma,[32] provides the first convincing clinical validation of BCL2 as a useful therapeutic target in CLL. Previous attempts to target BCL2 have not resulted in the substantial antitumor activity seen in this study. Oblimersen, an antisense oligodeoxyribonucleotide designed to reduce BCL2 translation displayed minimal single-agent activity in a phase I/II study, with transient PRs achieved in only two of 26 patients.[2] Similarly, a phase I study of obataclax, a small molecule with inhibitory activity against BCL2 and related prosurvival proteins, reported only one PR in 26 patients with CLL.[1] Unlike with navitoclax, the mechanism of cell killing by obataclax in vitro is not exclusively via the BCL2 family–regulated mitochondrial apoptosis pathway,[33] and its lack of single-agent activity may be a consequence of inadequate inhibition of BCL2 family proteins.

In sharp contrast, prior preclinical evaluation has defined the mechanism of action of navitoclax as being dependent on binding and inhibition of BCL2, BCL-w, or BCL-$x_L$.[3,4] In the absence of significant BCL-$x_L$ or BCL-w expression in CLL, BCL2 is the key antiapoptotic

protein inhibited by navitoclax. The single-agent activity of navitoclax in vivo reflects the previously reported potent in vitro activity of ABT-737 and navitoclax[3,16,18,19] in this disease. Consistent with the rapid induction of apoptosis in vitro,[17] circulating CLL cells decreased rapidly in patients treated with navitoclax.

However, responses in patients were more heterogeneous than predicted by in vitro data, raising the possibility that insufficient BCL2 inhibition is being achieved in vivo. Peak concentrations of navitoclax observed were between 1 and 10 $\mu$mol/L in patients receiving doses $\geq$ 110 mg. Although these concentrations were within the predicted efficacy range estimated from preclinical animal studies[5] and whole blood assays for CLL cytoxicity,[18] it remains uncertain if they provide maximal antileukemic activity because the DLT of thrombocytopenia prevented exploration of higher doses. Development of more selective BH3 mimetics that inhibit BCL2 but not BCL-$x_L$ may be required to allow the full potential of BCL2 inhibitory therapy to be explored.

No significant correlation between level of expression of BCL2 and response was observed in this study. This suggests that BCL2-independent survival factors are operating, and these could provide an alternative explanation for the persistence of disease in vivo during ongoing therapy. MCL1 is a recognized resistance factor in vitro,[4,5] and this study provides the first clinical data supporting its role in determining response of tumor cells to navitoclax. Higher levels of MCL1 before therapy were associated with lesser reductions in lymphocytosis and best clinical response when adjusted for the level of BIM expression. The clinical utility of measuring MCL1 and BIM:MCL1 or BIM:BCL2 ratios as biomarkers requires formal assessment in future clinical trials. Resistance to BCL2 inhibition warrants exploration of combination therapy. In preclinical in vivo models, both ABT-737 and navitoclax display synergy with cytotoxic agents and targeted therapies against other lymphoproliferative diseases[5,14] and malignancies.[3,34] Along with the promising single-agent activity observed in this study of heavily pretreated patients, such data underpin the rationale for recently commenced clinical trials of combination therapies of navitoclax with current standard-of-care drugs in CLL.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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