A Phase Ib Dose-Escalation and Expansion Study of the BCL2 Inhibitor Venetoclax Combined with Tamoxifen in ER and BCL2–Positive Metastatic Breast Cancer

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Venetoclax, a potent and selective BCL2 inhibitor, synergizes with endocrine therapy in preclinical models of ER-positive breast cancer. Using a phase Ib 3 + 3 dose-escalation and expansion study design, 33 patients with ER and BCL2-positive metastatic disease (mean prior regimens, 2; range, 0–8) were treated with daily tamoxifen (20 mg) and venetoclax (200–800 mg). Apart from uncomplicated “on-target” lymphopenia, no dose-limiting toxicities or high-grade adverse events were observed in the escalation phase (15 patients), and 800 mg was selected as the recommended phase II dose (RP2D). In the expansion phase (18 patients), few high-grade treatment-related adverse events were observed. For 24 patients treated at the RP2D, the confirmed radiologic response rate was 54% and the clinical benefit rate was 75%. Treatment responses were preempted by metabolic responses (FDG-PET) at 4 weeks and correlated with serial changes in circulating tumor DNA. Radiologic responses (40%) and clinical benefit (70%) were observed in 10 patients with plasma-detected ESR1 mutations.

SIGNIFICANCE: In the first clinical study to evaluate venetoclax in a solid tumor, we demonstrate that combining venetoclax with endocrine therapy has a tolerable safety profile and elicits notable activity in ER and BCL2-positive metastatic breast cancer. These findings support further investigation of combination therapy for patients with BCL2-positive tumors.

See related commentary by Drago et al., p. 323.

INTRODUCTION

Luminal breast tumors, characterized by estrogen receptor (ER) expression, account for approximately 70% of all breast cancers and are responsible for the majority of breast cancer deaths (1, 2). Endocrine therapy is the mainstay of therapy for patients with metastatic disease. Options include selective ER modulators (such as tamoxifen), aromatase inhibitors, and selective ER degraders. Tumor response can be further enhanced with adjunctive therapy that includes mTOR inhibitors (3), isoform-specific PI3K inhibitors (4), and cyclin-dependent kinase (CDK) 4/6 inhibitors (5–7). The latter have transformed the treatment landscape with significant improvement in overall response and progression-free survival (PFS), as well as overall survival (OS) benefit for the subset of patients with endocrine-responsive disease (8). Although CDK4/6 inhibitors elicit potent antiproliferative effects, they do not induce tumor cell death (9). As a result, disease progression almost invariably occurs.

Inhibition of apoptosis is a hallmark of cancer. Upregulation of survival proteins has been implicated in tumor growth and reduced sensitivity to anticancer therapy (10, 11). BCL2, a key member of the BCL2 pro-survival family, is an estrogen-responsive gene (12) and is overexpressed in approximately 80% of primary ER-positive (ER+) breast cancers (13, 14). It is a well-recognized prognostic factor that can be readily assessed by IHC or in genomic assays such as Oncotype DX and PAM50/Prosigna (15–17). BCL2, however, is often expressed at high levels in poorer-prognosis luminal B tumors, as well as good-prognosis luminal A tumors (18). Indeed, the annualized mortality rate following an early breast cancer diagnosis is similar for ER or BCL2 positivity (17).

BH3 mimetics that target BCL2 or other antiapoptotic proteins have recently emerged as a promising new therapeutic class of drug. These compounds mimic the natural antagonists of BCL2 and related proteins (11, 19). Venetoclax (ABT-199/GDC-0199) is a potent and highly selective inhibitor of BCL2 (ref. 20; Supplementary Fig. S1). Recent clinical trials demonstrated remarkable activity as a single agent as well as in combination with monoclonal antibodies, including in patients with aggressive, treatment-refractory chronic lymphocytic leukemia (CLL), leading to its approval by the FDA (21, 22). Studies using combination therapy have also shown promising activity in several other types of hematologic malignancies (23–25). To date, venetoclax has not been evaluated in patients with solid tumors.

Preclinical data using patient-derived xenograft (PDX) models of ER+ breast cancer suggested that intermittent dosing with venetoclax synergized with tamoxifen to improve tumor response by increasing apoptosis (18). On the basis...
of these findings, we extended our preclinical work to model continuous venetoclax therapy, explore the effect of tamoxifen on BCL2 levels in a window-of-opportunity study, and undertook a phase Ib dose escalation and expansion study of venetoclax combined with tamoxifen in patients with metastatic ER$^+$ and BCL2$^+$ breast cancer. The primary aim was to determine the maximum tolerated dose (MTD), define dose-limiting toxicities (DLT), and identify the recommended phase II dose (RP2D).

RESULTS

Preclinical Modeling of Tamoxifen and Venetoclax in ER-Positive Breast Cancer

We previously showed that combining venetoclax with tamoxifen in short-term therapy (10 days per 21-day treatment cycle, for 2 cycles) was safe and effective in PDX models of ER$^+$ and BCL2$^+$ breast cancer (1B). To explore the impact of increasing the dose and duration of therapy, we treated mice bearing a luminal B PDX breast tumor with continuous (daily) venetoclax (Fig. 1A; Supplementary Fig. S2A). Although venetoclax alone was ineffective, continuous treatment with venetoclax at 25 mg/kg or 100 mg/kg daily augmented tumor response to tamoxifen, with superior responses elicited at the higher dose. Comparable doses in hematologic models predicted efficacy for venetoclax in patients with CLL (20–22). Together with previous findings, these data suggest that combination therapy would be required in the clinic to maximize benefit and that response is dose-dependent.

Window-of-Opportunity Tamoxifen Study

To investigate whether tamoxifen modulates BCL2 levels in tumors, we conducted a window-of-opportunity study in patients with newly diagnosed ER$^+$ breast cancer. Ten premenopausal women received tamoxifen 20 mg daily for 5 to 7 days following biopsy and prior to tumor resection (Supplementary Fig. S2B). As anticipated, a trend toward reduced proliferation was observed in paired samples, as determined by Ki67 immunostaining (Supplementary Fig. S2C). In the majority of cases, BCL2 protein levels were either unchanged or slightly increased (Supplementary Fig. S2D). RNA-sequencing analysis of paired treatment samples similarly indicated that the expression of BCL2 and other prosurvival genes including MCL1 and BCLXL did not change with tamoxifen therapy (Supplementary Fig. S2E). Thus, tamoxifen does not appear to overtly affect the expression of the therapeutic target BCL2. These findings provide a rationale for evaluating tamoxifen and venetoclax in the clinic.

Patient Selection and Demographics

A total of 96 patients with ER$^+$ HER2-nonamplified metastatic breast cancer were prescreened for the study (Fig. 1B and C). ER expression and HER2 expression were determined using American Society of Clinical Oncology/College of American Pathologists guidelines. BCL2 levels were scored by IHC for percent tumor cell positivity and intensity of staining, using a scale of 0 to 3 (Fig. 1D; ref. 17). Of 86 patients confirmed to have ER$^+$ and HER2-negative (nonamplified) disease on either a fresh or archival tissue sample, 62 (72%) tumors were BCL2$^+$ (defined as ≥50% positive cells and ≥2+ intensity on immunostaining; Fig. 1C; Supplementary Fig. S3). Thirty-three of these patients fulfilled the remaining eligibility criteria and were enrolled on the study.

Median age of the overall cohort was 65 years (range, 43–78), and 91% were postmenopausal (Table 1). All patients had ER$^+$, BCL2$^+$, and HER2-nonamplified metastatic breast cancer. Ten of 33 (30%) had negative or weak progesterone receptor (PgR) staining on their baseline or archival tissue sample, suggestive of luminal B biology (Table 1; Supplementary Table S1). Two patients had bone-only disease whereas all others had either nodal or visceral metastases with or without bone lesions.

Eleven patients (33%) were treated in the first-line setting. Twenty-two patients (67%) had received prior endocrine therapy or chemotherapy for metastatic disease, including 15 (45%) who received ≥2 lines of therapy. The mean number of lines of prior treatment received was 2 (median, 1; range, 0–8). Twelve patients (36%) had previous chemotherapy exposure for advanced disease. According to European School of Oncology-European Society for Medical Oncology consensus nomenclature (ABC 4), 3 patients had primary endocrine resistance, 24 had exhibited secondary endocrine resistance (26), and 6 patients had late relapse (endocrine-sensitive disease) or de novo metastatic disease. Notably, more than half the cohort had received tamoxifen either in the adjuvant (13 patients; 39%), metastatic (4 patients; 12%), or adjuvant and metastatic (4 patients; 12%) settings. Nine patients (27%) had previously developed progressive disease on tamoxifen (Table 1; Supplementary Table S2).

Dose Determination and Safety

For the dose-escalation component of the study, 3 patients were enrolled into each of the 4 predetermined dose levels consisting of daily 200, 400, 600, or 800 mg oral venetoclax in combination with oral tamoxifen 20 mg daily. Once the 800-mg dose cohort was completed, an additional 3 patients were recruited at that dose (Fig. 1B). No DLT (defined as ≥ grade 3 toxicity by Common Terminology Criteria for Adverse Events (CTCAE) v4.03; refer to Methods) was observed in any of the cohorts during the first 4 weeks of treatment, and as a result
The BCL2 Inhibitor Venetoclax in ER-Positive Breast Cancer

**A**

![Graph showing survival rates and dose response](image)

**B**

<table>
<thead>
<tr>
<th>Screening</th>
<th>Dose escalation</th>
<th>Dose expansion</th>
<th>Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+/BCL2+ MBC</td>
<td>Tamoxifen + venetoclax 800 mg (n=6)</td>
<td>Tamoxifen + venetoclax 800 mg (n=18)</td>
<td>Primary endpoint - Dose-limiting toxicity - Determine MTD and RP2D</td>
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<tr>
<td>ER+/BCL2+ MBC</td>
<td>Tamoxifen + venetoclax 600 mg (n=3)</td>
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<td>Secondary endpoints - Progression-free survival - Objective response rate</td>
</tr>
<tr>
<td>ER+/BCL2+ MBC</td>
<td>Tamoxifen + venetoclax 400 mg (n=3)</td>
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<td>Exploratory endpoints - Changes in PBMCs - Measure ctDNA - Metabolic response (FDG-PET)</td>
</tr>
<tr>
<td>ER+/BCL2+ MBC</td>
<td>Tamoxifen + venetoclax 200 mg (n=3)</td>
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<td></td>
</tr>
</tbody>
</table>

**Biomarker studies**

- ctDNA and PBMCs every 4 weeks
- Tissue biopsy every 12 weeks
- FDG-PET

**C**

- 96 Patients prescreening
- 6 Patients treated with chemotherapy
- 6 Patients no measurable disease
- 5 Patients declined study enrollment
- 3 Patients enrolled on alternative study
- 2 Patients poor performance status
- 2 Patients not eligible due to comorbidities
- 2 Patients ongoing response to prior therapy
- 1 Patient poor hepatic function
- 1 Patient concurrent renal cancer
- 1 Patient normal tissue on biopsy
- 3 Patients ER- 1 Patient HER2 amplified
- 1 Patient squamous cell carcinoma
- 24 Patients BCL2 low or negative (<50% staining and/or < moderate strength)
- 6 Patients BCL2 positive
- 1 Patient on tamoxifen
- 1 Patient concurrent renal cancer

**D**

![Images of BCL2 intensity](image)
**Table 1. Patient demographics and baseline characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose escalation n = 15 (%)</th>
<th>Dose expansion n = 18 (%)</th>
<th>Overall n = 33 (%)</th>
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<td><strong>Median age (range)</strong></td>
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<td>66 (43–75)</td>
<td>65 (43–78)</td>
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<td><strong>ECOG performance status</strong></td>
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<tr>
<td>0</td>
<td>7 (47%)</td>
<td>14 (78%)</td>
<td>21 (64%)</td>
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<td>1</td>
<td>8 (53%)</td>
<td>4 (22%)</td>
<td>12 (36%)</td>
</tr>
<tr>
<td><strong>Menopausal status, n (%)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal or perimenopausal</td>
<td>2 (13%)</td>
<td>1 (6%)</td>
<td>3 (9%)</td>
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<tr>
<td>Postmenopausal</td>
<td>13 (87%)</td>
<td>17 (94%)</td>
<td>30 (91%)</td>
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<td><strong>Histology</strong></td>
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<td></td>
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<td>Invasive ductal carcinoma/no special type</td>
<td>10 (67%)</td>
<td>15 (83%)</td>
<td>25 (76%)</td>
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<td>Invasive lobular carcinoma</td>
<td>4 (27%)</td>
<td>1 (6%)</td>
<td>5 (15%)</td>
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<td>Others or not specified</td>
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<td>2 (11%)</td>
<td>3 (9%)</td>
</tr>
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<td><strong>Receptor status</strong></td>
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<tr>
<td><strong>ER</strong></td>
<td></td>
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<tr>
<td>Strong</td>
<td>12 (80%)</td>
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<td>1 (6%)</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>Weak</td>
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<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>PgR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>11 (73%)</td>
<td>8 (44%)</td>
<td>19 (58%)</td>
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<td>1 (7%)</td>
<td>3 (17%)</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>Weak</td>
<td>2 (13%)</td>
<td>3 (17%)</td>
<td>5 (15%)</td>
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<tr>
<td>Negative</td>
<td>1 (7%)</td>
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<td>5 (15%)</td>
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<tr>
<td><strong>BCL2</strong></td>
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<tr>
<td>Strong</td>
<td>11 (73%)</td>
<td>17 (94%)</td>
<td>28 (85%)</td>
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<td>Moderate</td>
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<td>1 (6%)</td>
<td>5 (15%)</td>
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<tr>
<td>Weak</td>
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<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Median Ki67 % (range)</strong></td>
<td>10% (1–30)</td>
<td>30% (5–60)</td>
<td>15% (1–60)</td>
</tr>
<tr>
<td><strong>Sites of disease</strong></td>
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<tr>
<td>Bone</td>
<td>14 (93%)</td>
<td>12 (67%)</td>
<td>26 (79%)</td>
</tr>
<tr>
<td>Visceral metastases</td>
<td>9 (60%)</td>
<td>11 (61%)</td>
<td>20 (61%)</td>
</tr>
<tr>
<td>Liver</td>
<td>3 (20%)</td>
<td>8 (44%)</td>
<td>11 (33%)</td>
</tr>
<tr>
<td>Lung</td>
<td>7 (47%)</td>
<td>5 (28%)</td>
<td>12 (36%)</td>
</tr>
<tr>
<td>Nodal</td>
<td>7 (47%)</td>
<td>9 (50%)</td>
<td>16 (48%)</td>
</tr>
<tr>
<td><strong>Adjuvant endocrine therapy (%)</strong></td>
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</tr>
<tr>
<td>Tamoxifen only</td>
<td>6 (40%)</td>
<td>5 (28%)</td>
<td>11 (33%)</td>
</tr>
<tr>
<td>Aromatase inhibitor only</td>
<td>2 (13%)</td>
<td>4 (22%)</td>
<td>6 (18%)</td>
</tr>
<tr>
<td>Tamoxifen and aromatase inhibitor</td>
<td>1 (7%)</td>
<td>5 (28%)</td>
<td>6 (18%)</td>
</tr>
<tr>
<td>Other (toremefi ne)</td>
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<td>1 (3%)</td>
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<td>5 (33%)</td>
<td>4 (22%)</td>
<td>9 (27%)</td>
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<tr>
<td><strong>Prior lines of metastatic therapy, n (%)</strong></td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td>3 (20%)</td>
<td>8 (44%)</td>
<td>11 (33%)</td>
</tr>
<tr>
<td>1</td>
<td>3 (20%)</td>
<td>4 (22%)</td>
<td>7 (21%)</td>
</tr>
<tr>
<td>≥2</td>
<td>9 (60%)</td>
<td>6 (33%)</td>
<td>15 (45%)</td>
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<td>2.7 (0–6)</td>
<td>1.5 (0–8)</td>
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<td><strong>Prior tamoxifen exposure (%)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>5 (33%)</td>
<td>7 (39%)</td>
<td>12 (36%)</td>
</tr>
<tr>
<td>Adjuvant setting only</td>
<td>4 (27%)</td>
<td>9 (50%)</td>
<td>13 (39%)</td>
</tr>
<tr>
<td>Metastatic setting only</td>
<td>3 (20%)</td>
<td>1 (6%)</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>Adjuvant and metastatic</td>
<td>3 (20%)</td>
<td>1 (6%)</td>
<td>4 (12%)</td>
</tr>
<tr>
<td><strong>Prior disease progression on tamoxifen</strong></td>
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<tr>
<td>Yes</td>
<td>6 (40%)</td>
<td>3 (17%)</td>
<td>9 (27%)</td>
</tr>
<tr>
<td>No</td>
<td>9 (60%)</td>
<td>15 (83%)</td>
<td>24 (73%)</td>
</tr>
<tr>
<td><strong>Prior chemotherapy exposure (%)</strong></td>
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<td>5 (33%)</td>
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<td>12 (36%)</td>
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<td>4 (27%)</td>
<td>5 (28%)</td>
<td>9 (27%)</td>
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<tr>
<td>Metastatic setting only</td>
<td>3 (20%)</td>
<td>2 (11%)</td>
<td>5 (15%)</td>
</tr>
<tr>
<td>Adjuvant and metastatic</td>
<td>3 (20%)</td>
<td>4 (22%)</td>
<td>7 (21%)</td>
</tr>
</tbody>
</table>

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

*Sixteen patients were enrolled based on results of archival tissue.*
MTD was not reached. There were only two incidences of low-grade lymphopenia (≤ grade 2; 500–800 × 10^6/L) during the DLT reporting time that were not considered DLTs per the protocol.

Because the highest predetermined dose level was reached and due to the potential “pill burden” of taking more than 8 × 100 mg venetoclax tablets with tamoxifen, venetoclax 800 mg/day was selected as the RP2D, and no higher doses were explored. This dose, which is higher than the FDA-approved dose of 400 mg/day for CLL, produces approximately double the exposure at steady state (21). A further 18 patients were enrolled at the RP2D as part of the dose-expansion phase of the study (totaling 24 patients enrolled at 800 mg), with ongoing reporting of adverse events (AE).

Investigator-assessed, treatment-related AEs occurring in ≥ 10% of patients are summarized in Table 2 and Supplementary Table S3. The most common AEs were leukopenia and lymphopenia, the latter an expected side effect of venetoclax, both observed in 29 of 33 (88%) patients. Grade ≥3 lymphopenia occurred in 10 (30%) patients. Other hematologic AEs included neutropenia in 24 patients (73%; 67% grade 1–2, 6% grade 3), anemia in 13 patients (39%; 33% grade 1–2, 6% grade 3), and thrombocytopenia in 11 patients (33%; all ≤ grade 2). There were no episodes of febrile neutropenia.

The most common nonhematologic AE was nausea in 22 patients (67%; none > grade 2). Nausea was generally mild, short-lived, and typically occurred within 2 hours of ingesting the study medication. In cases requiring pharmacologic management, antiemetic therapy with metoclopramide was highly effective. Other common AEs included vomiting, diarrhea, infection, fatigue, lethargy, pruritus, and rash. There were no incidences of tumor lysis syndrome, which was a DLT in the phase I study in CLL (21).

Three possible treatment-related serious AEs (SAE) were observed on study in relation to hospital admissions for a dermatomal herpes zoster infection, right upper lobe pneumonia, and cellulitis. All SAEs occurred in the context of grade 2 to 4 lymphopenia and normal neutrophil count. The patient who experienced herpes zoster infection had received concurrent high-dose dexamethasone while undergoing palliative radiotherapy and had transient grade 4 lymphopenia. There were no cases of study drug discontinuation due to AEs. Two patients required dose reduction of venetoclax (from 800 to 400 mg, as per protocol) for prolonged grade 2 nausea and grade 3 lymphopenia, respectively. These AEs resolved at the reduced dose.

In preclinical studies, endometrial thinning had been observed when tamoxifen was combined with ABT-737, a preclinical lead that targets both BCL2 and BCL-XL (18, 27). Three patients underwent transvaginal ultrasound, revealing mild endometrial hyperplasia normally associated with tamoxifen.

### Antitumor Activity

Tumor response as per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria, matching circulating tumor DNA mutations, and PFS are shown in Table 2 and Supplementary Table S3. Thirty-one of 33 patients had measurable disease. One patient (from the 800-mg cohort) achieved complete response (CR) within 12 weeks of treatment, having received 3 prior lines of therapy (anastrozole, capecitabine, and letrozole/palbociclib) for metastatic disease. Partial response (PR) was observed in 14 of 33 (42%) patients. This was observed in 4 of 15 (27%) patients from the dose-escalation cohort and 10 of 18 (56%) patients from the dose-expansion cohort. Partial response (PR) was observed in 14 of 33 (42%) patients. This was observed in 4 of 15 (27%) patients from the dose-escalation cohort and 10 of 18 (56%) patients from the dose-expansion cohort. Therefore, an objective response rate (ORR), defined as CR plus PR, was observed in 15 of 33 (45%) patients (48% for the 31 patients with measurable disease). Eight (24%) patients had stable disease (SD) lasting more than 24 weeks. Taken together, the clinical benefit rate (CBR), defined as PR + CR + SD, was observed in 23 of 33 (70%) patients for the overall cohort. Median time...
to objective response was 12 weeks (the first staging time point), with a median duration of response of 42 weeks at the time of data analysis (mean 46; range, 8–100+ weeks).

All 24 patients who received the RP2D of 800 mg venetoclax had measurable disease. For this group, the ORR was 54% (1 CR and 12 PR), SD was 21% (5 patients), with a CBR of 75%. Median PFS was not reached at the time of data analysis (>51 weeks). Although the study was not powered to detect differences between subgroups, these responses were higher than for patients who received <800 mg venetoclax, where ORR was 22% (0 CR and 2 PR) and CBR was 56%. Additionally, patients in the 800-mg cohort demonstrated prolonged PFS compared with patients receiving <800 mg (median PFS 23 vs. 51 weeks at the time of data analysis, P = 0.03; Supplementary Fig. S4).

Patients who received treatment in de novo or first-line relapsed metastatic disease experienced a higher ORR (9 of 11 patients; 82%) and CBR (10 of 11; 91%) compared with patients treated in later-line relapse (Supplementary Table S4A). For the 9 patients from the 800-mg cohort treated in first-line relapse, ORR was 78% (7 patients) and CBR was 89% (8 patients). However, tumor responses or prolonged SD were also observed in 8 of 12 (67%) patients who had received more than 3 prior lines of therapy for metastatic disease. Notably, tumor responses or prolonged SD were also observed in 5 of 9 (56%) patients who had previously received the combination of an aromatase inhibitor and CDK4/6 inhibitor for metastatic disease (Supplementary Table S4B).

We further evaluated response according to guidelines for endocrine-resistant disease (26). For the entire cohort, clinical benefit was seen in 1 of 3 patients with primary endocrine resistance (33%; 1 SD), 16 of 24 patients with secondary endocrine resistance (67%; 1 CR, 9 PR, 6 SD, 8 PD), and all 6 patients with late relapse (endocrine-sensitive disease) or de novo metastatic breast cancer (100%; 6 PR). Together, these findings suggest that venetoclax may augment tumor response in patients either with endocrine-sensitive disease or who develop secondary endocrine resistance.

### Pharmacodynamics and Biomarker Analyses

Fresh tumor biopsies were collected before treatment and after 28 days of treatment in 8 patients. Seven paired biopsies were conducted on patients from the RP2D (800 mg) cohort, 3 of whom subsequently achieved a radiologic response. Samples were analyzed by IHC for changes in hormone receptor expression, proliferation (Ki67), BCL2, and measures of apoptosis [cleaved caspase-3 (CC3)]. No notable changes were observed in ER, PgR, or BCL2 expression. A trend toward decreased Ki67 was observed (Fig. 3A). Few CC3-positive cells were noted in tumors (likely reflecting the late timing of the treatment biopsy), except in the tumor biopsy from patient 01-033 who subsequently demonstrated a complete radiologic response (Fig. 3B).

Tumor biopsies at the time of progressive disease were obtained on a small number of patients (n = 9) to investigate changes in biomarker expression compared with archival samples. ER expression was reduced in 3 tumor biopsies. Although BCL2 levels appeared unchanged in 3 samples, reduced immunostaining for BCL2 was observed in 2 samples and BCL2 expression was absent in 4 samples (Supplementary Fig. S5). Because BCL2 expression was either unchanged or reduced at the time of progressive disease compared with archival samples, diverse mechanisms (such as loss of dependence on ER signaling, upregulation of other prosurvival BCL2 family members, or mutations in BCL2 or effector proteins BAX or BAK) may account for tumor resistance and progression.

Sixteen patients who received the RP2D of 800-mg venetoclax underwent paired FDG-PET scans at baseline and after 4 weeks of therapy as an exploratory endpoint for tumor response (Fig. 3C and D). Of the 11 patients (68%) who achieved partial metabolic response (PMR; based on changes in maximum standardized uptake value [SUVmax] in target lesions), 8 patients had an objective partial response on CT as per RECIST v1.1 (7 at 12 weeks, 1 at 24 weeks). Conversely, metabolic progression...
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Figure 2. Efficacy assessment by subject. **A**, Waterfall plot of the best radiologic response for 33 evaluable patients treated. Best response was assessed per RECIST v1.1. **B**, Matching ctDNA for evaluable patients. Blue squares identify detection of mutation in ctDNA at study enrollment. **C**, Swimmer plot of time on treatment for 33 evaluable patients. Individual patients represented as lines.
activity in the remaining patient (01-021) would be consistent with a “flare” response (28), because a partial response by RECIST was observed at 12 weeks and metabolic activity was reduced at 16 weeks (not shown). Overall, patients with PMR at 4 weeks demonstrated prolonged PFS compared with those with PMD or SMD (PFS 25 vs. 65 weeks, $P = 0.004$; Supplementary Fig. S6A). Together, these findings suggest a possible role for FDG-PET as an early marker of therapeutic response in this treatment setting.

Plasma was collected for circulating tumor DNA (ctDNA) studies at baseline, Cycle 1 Day 15, and Day 1 of each subsequent treatment cycle. ctDNA was screened for 39 genes known to be recurrently mutated in breast cancer (Supplementary Tables S5–S8). ctDNA mutations were identified at baseline in 28 of 33 (85%) patients (Fig. 3E; Supplementary Table S8). The most common mutations were present in PIK3CA (14 of 33; 42%) and ESR1 (10 of 33; 30%). Other mutations detected at lower frequency included GATA3 (15%), MAP3K1 (12%), CDH1 (12%), and PTEN (9%). ESR1 and MAP3K1 mutations were mutually exclusive, as recently reported (29). No obvious association between mutation status and response was observed (Supplementary Fig. S6B). Within 28 days of treatment, a significant reduction in ctDNA levels was observed in both ESR1 (median difference $-441.9$ copies/mL, $P = 0.008$) and PIK3CA (median difference $-91.94$ copies/mL, $P = 0.02$) mutations, respectively. Notably, PR or SD was observed in 4 (40%) and 3 (30%) of 10 patients with ctDNA-detected ESR1 mutations, respectively. Three of 4 (75%) with PR and 2 of 3 (66%) with SD had D538G mutations (Supplementary Tables S8 and S9). Six of the 7 patients experiencing PR or SD had previously received tamoxifen in the adjuvant and/or metastatic setting (Supplementary Table S9). In patients who experienced a partial response, a significant reduction in ctDNA ESR1 was observed within 28 days of treatment, $P = 0.008$ (Supplementary Fig. S6C). Subsequent rises in ctDNA appeared to preempt radiologic progression (Fig. 3F). These findings are consistent with emerging evidence on the utility of ctDNA in monitoring disease (30).

**Effect of Venetoclax on the Innate and Adaptive Immune System**

Prior clinical trials have evaluated venetoclax in heavily pretreated patients with hematologic malignancies, where extensive bone marrow infiltration and immune defects are common. We therefore complemented clinical hematology findings with serial analysis of peripheral blood lineages by flow cytometry to describe the effects of venetoclax on the innate and adaptive immune system (Fig. 4; Supplementary Figs. S7A–S7D and S8). Compared with baseline, treatment with venetoclax and tamoxifen resulted in a significant reduction in hemoglobin, neutrophil, and platelet counts, although this was not clinically significant or actionable (Fig. 4A). An early and sustained decrease in eosinophils and B cells was observed within 4 weeks of treatment (Fig. 4B). Consistent with the reduction in B-cell numbers, a reduction in IgG and IgM was observed, although IgG and overall gamma globulin levels were unaffected (Fig 4C; Supplementary Fig. S8A–S8B). Although a modest reduction in total T-cell count was seen, no significant changes in the actual numbers or percentages of T-cell subsets [including CD4+, CD8+, and regulatory T cells (Treg)] was observed (Fig. 4D; Supplementary Fig. S8C). Similarly, circulating natural killer (NK) cells, monocyte and dendritic cell subsets were unaffected (Fig. 4E; Supplementary Fig. S8D). Collectively, these data reveal that venetoclax-associated peripheral blood lymphopenia is largely attributable to a reduction in B cells, with modest changes in other lineages that do not appear to be associated with increased risk of opportunistic infections.

**DISCUSSION**

Here, we report results from a phase Ib study evaluating the safety and preliminary efficacy of combining venetoclax with tamoxifen in 33 patients with metastatic ER+ and BCL2+ breast cancer. The MTD of venetoclax was not reached, and the RP2D was determined to be 800 mg daily. No DLTs were reported in the dose-escalation phase. Combination therapy was well tolerated overall, with the most common AEs being nonclinically significant cytopenia and nausea similar to that reported for patients with CLL treated with venetoclax (21). Dose modifications were infrequent, and no patients required multiple dose reductions or cessation of treatment due to safety concerns. Despite previous signal in hematologic trials, no tumor lysis was observed in this cohort. Allopurinol was prescribed for patients in the dose-escalation phase, but was removed from the study protocol for the dose-expansion cohort. Overall, the venetoclax combination appears to have a favorable toxicity profile when compared with other adjunctive therapies used with endocrine therapy, such as the mTOR, PIK3CA, and CDK4/6 inhibitors.

Promising antitumor activity was observed with tamoxifen and venetoclax, including in heavily pretreated patients. Tumor response or prolonged SD was observed in 8 of 12 patients who were treated in ≥ fourth-line therapy. It is noteworthy that a proportion of responding patients had previously been treated with tamoxifen, with clinical benefit seen in 5 of 9 patients who had previously progressed on single-agent tamoxifen. Clinical benefit was also observed in the 4 patients who had previously received a CDK4/6
Figure 4. Effect of venetoclax on the innate and adaptive immune system. A, Clinical hematology values for patients treated in the dose-expansion cohort (800 mg venetoclax), WCC, white cell count. B–E, Characterization of representative mononuclear cell subsets and immunoglobulin. B, B cells; C, immunoglobulin levels; D, T cells; E, monocytes. Paired t test values are shown; *, P < 0.05; **, P < 0.005; ***, P < 0.001; ****, P < 0.0001.
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inhibitor. Most tumor responses were generally observed by the first RECIST measurement at 12 weeks, perhaps indicative of a rapid response achieved by combining endocrine therapy with a proapoptotic agent.

As this was a small phase I study, direct comparison with randomized phase III studies evaluating single-agent tamoxifen is speculative. Nevertheless, it is noteworthy that the ORR (54%) and CBR (75%) observed here for the 800-mg cohort compares favorably with historical studies of patients treated with tamoxifen in first-line relapse, where reported ORR ranged between 17% and 33% and CBR between 38% and 56% (31–34). Objective response rates seem comparable to those reported with “modern-day” therapies comprising an aromatase inhibitor and a CDK4/6 inhibitor. The ORR (78%) and CBR (89%) for the small number of patients treated in the first-line setting at 800 mg are similar to those reported for letrozole and palbociclib (55% and 85%, respectively) in PALOMA-2, where approximately 31% of patients had de novo metastatic disease (5). Although tamoxifen and venetoclax appeared to produce rapid responses, median PFS and duration of response for the RP2D cohort remains an open question, because the data were not sufficiently mature at the time of analysis. Larger, randomized studies where PFS is a primary or secondary endpoint will be required to properly address these issues.

We elected to combine venetoclax with tamoxifen based on our preclinical data, which raised the possibility that tamoxifen therapy could induce “mitochondrial priming” through elevation of BCL2 (18), thereby rendering tumor cells more susceptible to BH3 mimetic therapy (35). The small window-of-opportunity study suggested that BCL2 protein expression remains high (and in some cases may be increased) following short-term treatment with tamoxifen. Similarly, BCL2 levels remained high in the 8 patients who underwent paired tumor biopsy after 4 weeks of tamoxifen and venetoclax therapy (data not shown). Although the day 28 biopsy established that BCL2 expression was sustained, an earlier on-treatment biopsy may have been required to reliably detect apoptosis. Presumably, a reliance on the preexisting high levels of BCL2 present in most ER+ breast tumors facilitated apoptosis following combination therapy. We speculate that venetoclax will similarly augment tumor responses in conjunction with other commonly used endocrine therapies such as aromatase inhibitors or fulvestrant (32–34, 36, 37). These questions should in part be addressed in VERONICA (W040181), an ongoing randomized phase II study of fulvestrant with or without venetoclax in patients with ER+ HER2- breast cancer who have progressed on a CDK4/6 inhibitor (NCT03584009).

Biopsies of a small number of tumors at progression suggested that pleiotropic mechanisms are likely to account for the development of resistance to venetoclax. BCL2 levels were reduced or absent in a subset of tumors, raising the possibility that other prosurvival factors (such as BCL-XL or MCL1) could be involved. Such functional redundancy among prosurvival BCL2 family members seems plausible, given that they are commonly coexpressed in ER+ tumors (18, 38). Pertinently, MCL1 appears to be amplified in a subset of triple-negative tumors that fail to respond to neoadjuvant therapy (39). In other tumor progression biopsies, BCL2 expression did not change. It will be interesting to determine whether mutations in BCL2 or downstream effectors BAK and BAX contribute to resistance in this subset of tumors. Importantly, tumor heterogeneity or sampling issues are also likely to have contributed to discordant findings on ER and BCL2 expression at progression.

Plasma ctDNA analysis revealed ESR1 mutations in 30% of patients at study entry, consistent with heavy pretreatment with aromatase inhibitors. Mutations mirrored those that have been previously described, principally affecting the ligand-binding domain (LBD). In several patients, more than 1 mutation was identified, consistent with clonal heterogeneity developing in response to the selective pressure of endocrine therapy. Although mutations in the LBD have been reported to confer relative resistance to tamoxifen and fulvestrant (40), it is noteworthy that a tumor response or prolonged SD (accompanied by a significant reduction in mutant ESR1 ctDNA) was observed in 7 of 10 patients harboring ESR1 mutations, notably in those with a D538G mutation. These findings are consistent with responses observed with tamoxifen in vitro in ER+ tumor cells expressing this LBD mutation (41, 42). Although tumor responses could have been due to tamoxifen alone, it seems likely that the addition of venetoclax to tamoxifen (or fulvestrant) therapy could amplify tumor response in vitro.

A role for FDG-PET/CT in predicting response to endocrine therapy in metastatic breast cancer is currently unclear. A pilot study suggested that PMR to FDG-PET at ~10 weeks was predictive of improved PFS (43). Consistent with these data, we observed semiquantitative metabolic responses at 4 weeks compared with baseline that broadly correlated with radiologic response by RECIST at 12 weeks as well as improved PFS, compared with those with PMD or SMD. Our findings suggest that investigating a role for FDG-PET/CT at 4 weeks in patients with ER+ metastatic breast cancer may be worthwhile.

Serial analysis of peripheral blood leukocyte subsets revealed asymptomatic lymphopenia that was largely confined to B cells, with minimal changes in T-cell subsets (including CD4, CD8, and Tregs). A mild reduction in neutrophil count was observed, while NK cells, monocyte and dendritic cell subsets were unaffected. These findings do not preclude the possibility that venetoclax differentially modulates immune subsets within the intratumoral environment.

In this first clinical study to evaluate venetoclax in a solid tumor, we demonstrate that combining venetoclax with tamoxifen is highly tolerable and elicits encouraging activity in ER+ and BCL2+ metastatic breast cancer. Because BCL2 was expressed at high levels in approximately 70% of metastatic biopsy samples from patients with ER+ tumors (Supplementary Fig. S2), a large proportion of patients could potentially be affected by these findings. The ready availability of BCL2 as a possible predictive biomarker should facilitate further investigation of combination therapy for patients with BCL2-positive breast cancer as well as other types of cancers.

METHODS

Study Design and Objectives

This was a phase I, multicenter, open-label study of venetoclax in combination with tamoxifen in patients with metastatic ER-positive and BCL2-positive breast cancer (“mBEP,” BCL2 inhibition
in ER-positive metastatic breast cancer). Patients were recruited from two tertiary centers in Melbourne, Australia, from July 28, 2015, to April 16, 2018. The cutoff date for data analysis for this publication was October 19, 2018. As of this date, 8 of 33 patients enrolled remained on active study treatment. A further 8 patients were recruited before study closure on July 31, 2018, and will be included in a later report once sufficient follow-up data are available. All potential patients had BCL2 IHC performed on either fresh or archival tissue during the prescreening process to ensure eligibility. The primary objective of the study was to define the safety and tolerability of venetoclax in combination with tamoxifen by determining the DLTs in the first 4 weeks of treatment, as well as the MTD and the RP2D. Secondary objectives included overall response rates of the combination treatment as defined by CR or partial response, the CBR as defined by CR, PR, or SD for >24 weeks, and to determine the PFS and OS. Exploratory objectives included evaluation of changes in ER, PR, and BCL2 gene-expression profiles, changes in plasma ctDNA mutations and alterations in peripheral blood leukocyte subsets. All patients gave written informed consent per Declaration of Helsinki recommendations, and the protocol was reviewed and approved by the Melbourne Health Institutional Review Board prior to study commencement. The study was registered on ISRCTN (ISRCTN98335443) and ACTRN (ACTRN1261500072516). Study data were collected and managed using REDCap electronic data capture tools hosted at The Walter and Eliza Hall Institute of Medical Research (44).

Study Population

Patients ages >18 years with histologically confirmed ER-positive (defined as >1% positive stained carcinoma cells) and BCL2-positive metastatic breast cancer were enrolled. BCL2 status was determined by IHC on archival or fresh tumor biopsies and defined as positive if >10% cells stained positive with at least moderate cytoplasmic staining (intensity 2-3 on 0-3 scale). Of note, tumors from all patients recruited to mBEP exhibited BCL2-positive immunostaining in >50% of cells, with 2+ or 3+ intensity. Eligibility criteria included ECOG performance status of 0 to 1, evaluable disease as defined by RECIST v1.1, life expectancy of >6 months, and adequate end-organ function. Patients in the dose-escalation phase must not have received prior therapy in the metastatic setting and required measurable disease. Exclusion criteria included tamoxifen use within the last 6 months, and adequate end-organ function. All patients gave written informed consent per Declaration of Helsinki recommendations, and the protocol was reviewed and approved by the Melbourne Health Institutional Review Board prior to study commencement. The study was registered on ISRCTN (ISRCTN98335443) and ACTRN (ACTRN1261500072516). Study data were collected and managed using REDCap electronic data capture tools hosted at The Walter and Eliza Hall Institute of Medical Research (44).

Study Treatment

Patients received tamoxifen 20 mg daily with dose escalation of venetoclax using a standard 3 + 3 design. The venetoclax dose was escalated from 200 mg daily (dose level 1), 400 mg daily (dose level 2), 600 mg daily (dose level 3) to the maximum planned dose of 800 mg daily (dose level 4). The MTD was defined as the highest dose at which <33% of patients experienced a DLT during the DLT-evaluable period. Once dose level 4 was reached, 3 additional patients were recruited and 800 mg determined as the RP2D in discussion with the study Safety Monitoring Committee, taking into account clinical and ACTRN (ACTRN1261500072516). Study data were collected and managed using REDCap electronic data capture tools hosted at The Walter and Eliza Hall Institute of Medical Research (44).

Safety

Patients in the dose-escalation phase were observed for the presence of DLTs during the first 4 weeks of treatment. A DLT was defined as a venetoclax-related toxicity that was at least grade 3 in severity as defined by CTCAE v4.03 with the exception of grade 3 nausea, vomiting, and fatigue that improves with appropriate therapy; grade 3 thrombocytopenia without evidence of bleeding and transient grade 3 hyperuricemia, hypocalcemia, or hyperkalemia lasting <48 hours. Grade 1 clinical or laboratory tumor lysis syndrome (TLS) according to the Cairo-Bishop definition (45) was also considered a DLT. Safety assessments were conducted at baseline, weekly during the DLT evaluation period, and every 4 weeks thereafter. All AEs were collected until 30 days following the last treatment regardless of attribution to study drug. AE severity was graded according to NCI CTCAE v4.03. There were no prespecified special AEs of interest in the study.

Due to reports of TLS in early-phase studies of venetoclax in hematopoietic malignancies (ClinicalTrials.gov Identifier: NCT01328626), tumor lysis prophylaxis was implemented for all patients in this study, despite the low risk in this study population. Patients received prophylactic allopurinol 300 mg daily commencing at least 72 hours prior to and continuing for up to a week after starting study treatment. Laboratory investigations in the form of serum uric acid, phosphate, calcium, creatinine, and lactate dehydrogenase measurements were performed 24 hours after the first dose of venetoclax and tamoxifen. Patients were also advised to remain well hydrated during the first week of taking the study treatment. Due to the absence of TLS, the requirement for TLS prophylaxis was removed from the study protocol for the dose-expansion cohort.

Efficacy Assessment

Tumor response was evaluated locally based on RECIST v1.1 by means of CT scan with intravenous contrast of chest, abdomen, and pelvis, which were performed at screening and every 12 weeks after starting study treatment until progression. The best overall response was defined as the best response recorded from the start of treatment until disease progression or relapse. Objective response was considered to be confirmed if the response was maintained at a subsequent scheduled CT assessment, at least 4 weeks after the criteria for response were first met. Bone scans were performed every 24 weeks if bone metastases were identified at baseline.

Pharmacodynamic Assessment

Paired tumor biopsies in consenting patients were conducted at baseline and following 28 days of treatment for PD assessment. Tumor samples were assessed for decreased proliferation (as measured by Ki67), BCL2 pathway proteins, as well as measures of downstream activation of apoptosis, including cleaved caspase-3.

As an exploratory marker of response to therapy, FDG-PET scans were obtained at baseline and at 28 days after commencing study treatment for a subset of patients in the dose-expansion phase. For FDG-PET response evaluation, up to 5 target lesions with a target to background uptake level of >2 were selected at the screening scan. An FDG-PET PMR was evaluated locally and defined as a decrease of >15% in the percentage change in the maximum standardized uptake value (SUVmax) of the target lesion.

Statistical Methods

The sample size for this study was obtained based on the dose-escalation rules described in the study design. Patient characteristics and AEs are summarized using descriptive statistics. Safety analyses included all enrolled patients who fulfilled eligibility criteria, received at least one dose of the study treatment, and are DLT-evaluable. Efficacy analyses included all enrolled patients who fulfilled eligibility criteria, received at least one dose of the study treatment, are
DLT-evaluable and had at least one post-baseline efficacy assessment. The response rate and CBR are estimated with 95% confidence interval calculated using exact methods based on binomial distribution. Time-to-event endpoints (PFS) are described using Kaplan–Meier methods with 95% confidence intervals. Patients who continue on study treatment were censored at the time of reporting.

**Role of the Funding Source**

This was an investigator-initiated study sponsored by Melbourne Health. AbbVie and Roche/Genentech provided venetoclax and funds for the study, which were supplemented by grant support from the National Health and Medical Research Council (Australia), Victorian Cancer Agency and National Breast Cancer Foundation (Australia) to conduct the clinical and translational research. Protocol development, conduct of the study, and reporting were carried out independently of the funding agencies. AbbVie and Roche/Genentech provided comments on the protocol and manuscript but played no role in its preparation or reporting.

**“Pretreat” Window-of-Opportunity Study**

An exploratory window study (Pretreat; ACTRN12614000695606) was initiated prior to the main study to evaluate changes in mRNA expression in patients with ER+ breast cancer in response to tamoxifen following diagnostic biopsy and prior to definitive surgery. A total of 10 premenopausal women were enrolled. Tissue samples were collected at baseline from the diagnostic core biopsy and at the time of surgery for paired analyses. The primary endpoint was global changes in mRNA expression, including BCL2 family members by gene-expression profiling and RT-PCR on paired tumor samples following short-term tamoxifen treatment in ER-positive breast cancer. Secondary endpoints were changes in ER, PR, Ki67, and BCL2 family expression, as determined by IHC. All patients gave written informed consent as per Declaration of Helsinki recommendations, and the protocol was reviewed and approved by the Melbourne Health Institutional Review Board prior to study commencement. The study was supported by funds from a National Health and Medical Research Council (Australia) grant.

**Disclosure of Potential Conflicts of Interest**

S.W. Lok, J.R. Whittle, F. Vaillant, C.E. Teh, J. Desai, L.C. Gandolfo, D.H.D. Gray, H.K. Liu, B. Pal, A.N. Policheni, A.W. Roberts, K. Shackleton, G.K. Smyth, J.E. Visvader, and G.J. Lindeman are employees of the Walter and Eliza Hall Institute, which receives milestone and royalty payments from AbbVie and Genentech in relation to venetoclax (ABT-199). Employees may be eligible for benefits related to these payments. AbbVie and Roche/Genentech provided funds that contributed to the conduct of this study. S.W. Lok is an employee of the Walter and Eliza Hall Institute, which receives milestone royalty payments from AbbVie and Genentech in relation to venetoclax (ABT-199). C.E. Teh reports receiving commercial research support from Genentech. J. Desai reports receiving commercial research grants from Roche, Bristol-Myers Squibb, and Novartis and is a consultant/advisory board member for Amgen, Novartis, Lilly, Biomornics, and Beigene. A.W. Roberts is employed at Walter and Eliza Hall Institute of Medical Research, which receives milestones and royalties related to venetoclax, and has received other remuneration from Walter and Eliza Hall Institute of Medical Research. B. Yeo has served on Advisory Boards for Amgen and Genentech and received honoraria payments from Genentech. D.H.D. Gray reports receiving a commercial research grant from Servier, reports receiving AbbVie Investigator-Initiated Study support and Amgen Investigator-Initiated Study support, has received honoraria from the speakers bureaus of Amgen and Genentech, and is a consultant/advisory board member for AbbVie. No potential conflicts of interest were disclosed by the other authors.

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A Phase Ib Dose-Escalation and Expansion Study of the BCL2 Inhibitor Venetoclax Combined with Tamoxifen in ER and BCL2–Positive Metastatic Breast Cancer

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