A phase 1b dose-escalation and expansion study of the BCL-2 inhibitor venetoclax combined with tamoxifen in ER and BCL-2–positive metastatic breast cancer

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ABSTRACT

Venetoclax, a potent and selective BCL-2 inhibitor, synergizes with endocrine therapy in pre-clinical models of ER-positive breast cancer. Using a phase 1b 3+3 dose escalation and expansion study design, 33 patients with ER and BCL-2-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 2 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 clinical benefit rate 75%. Treatment responses were pre-empted 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 BCL-2-positive metastatic breast cancer. These findings support further investigation of combination therapy for patients with BCL-2-positive tumors.
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, as well as overall survival benefit for the subset of patients with endocrine responsive disease (8). While CDK4/6 inhibitors elicit potent anti-proliferative 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 anti-cancer therapy (10,11). BCL-2, a key member of the BCL-2 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 immunohistochemistry or in genomic assays such as Oncotype DX® and PAM50/Prosigna® (15-17). BCL-2, 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 BCL-2 positivity (17).

BH3 mimetics that target BCL-2 or other anti-apoptotic proteins have recently emerged as a promising new therapeutic class of drug. These compounds mimic the natural antagonists of BCL-2 and related proteins (11,19). Venetoclax (ABT-199/GDC-0199) is a potent and highly selective inhibitor of BCL-2 (20) (Supplementary Fig. 1). Recent clinical trials demonstrated remarkable activity as single agent as well as 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 hematological malignancies (23-25). To date, venetoclax has not been evaluated in patients with solid tumors.

Pre-clinical 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). Based on these findings, we extended our pre-clinical work to model continuous venetoclax therapy, explore the effect of tamoxifen on BCL-2 levels in a window of opportunity study, and undertook a Phase 1b dose escalation and expansion study of venetoclax combined with tamoxifen in patients with metastatic ER$^+$ and BCL-2$^+$ breast cancer. The primary aim was to determine the maximum tolerated dose (MTD), define dose-limiting toxicities (DLTs) and identify the recommended phase 2 dose (RP2D).

RESULTS

Pre-clinical 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 BCL-2$^+$ breast cancer (18). 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. 2A). While 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 hematological 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 BCL-2 levels in tumors, we conducted a window of opportunity study in patients with newly diagnosed ER$^+$ breast cancer. Ten pre-menopausal women received tamoxifen 20 mg daily for 5-7 days following biopsy and prior to tumor resection (Supplementary Fig. 2B). As anticipated, a trend towards reduced proliferation was observed in paired samples, as determined by Ki67 immunostaining (Supplementary Fig. 2C). In
the majority of cases BCL-2 protein levels were either unchanged or slightly increased (Supplementary Fig. 2D). RNA-seq analysis of paired treatment samples similarly indicated that the expression of BCL-2 and other pro-survival genes including MCL-1, BCL-XL did not change with tamoxifen therapy (Supplementary Fig. 2E). Thus, tamoxifen does not appear to overtly impact on the expression of the therapeutic target BCL-2. 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-non-amplified metastatic breast cancer were pre-screened for the study (Fig. 1B, 1C). ER and HER2 expression were determined using ASCO/CAP guidelines. BCL-2 levels were scored by immunohistochemistry for per cent tumor cell positivity and intensity of staining, using scale of 0 - 3 (Fig. 1D) (17). Of 86 patients confirmed to have ER+ and HER2-negative (non-amplified) disease on either a fresh or archival tissue sample, 62 (72%) tumors were BCL-2 positive (defined as ≥ 50% positive cells and ≥ 2+ intensity on immunostaining) (Fig. 1C, Supplementary Fig. 3). 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 post-menopausal (Table 1). All patients had ER+, BCL-2+ and HER2 non-amplified metastatic breast cancer. Ten of 33 (30%) had negative or weak progesterone receptor (PR) staining on their baseline or archival tissue sample, suggestive of luminal B biology (Table 1, Supplementary Table 1). Two patients had bone only disease whilst 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 or chemotherapy for metastatic disease, including 15 (45%) who received ≥ 2 lines of therapy. The mean number of lines of prior treatment received was 2.0 (median 1, range 0 - 8). Twelve patients (36%) had previous chemotherapy exposure for advanced disease. According to ESO-ESMO 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 and Supplementary Table 2).

**Dose determination and safety**

For the dose escalation component of the study, 3 patients were enrolled to each of the 4 pre-determined dose levels consisting of daily 200, 400, 600 and 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 dose-limiting toxicity (DLT, defined as ≥ Grade 3 toxicity by CTCAE v4.03; refer Methods) was observed in any of the cohorts during the first 4 weeks of treatment and as a result maximum tolerated dose (MTD) was not reached. There were only 2 incidences of low-grade lymphopenia (≤ Grade 2; 500 - 800 x 10⁶/L) during the DLT reporting time that were not considered as DLTs per the protocol.

Since the highest pre-determined dose level was reached and due to the potential ‘pill burden’ of taking more than 8 x 100 mg venetoclax tablets with tamoxifen, venetoclax 800 mg/day was selected as the recommended phase 2 dose (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 (totalling 24 patients enrolled at 800 mg), with ongoing reporting of adverse events (AEs).

Investigator assessed, treatment related AEs occurring in ≥ 10% of patients are summarised in Table 2 and Supplementary Table 3. The most common adverse events 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 hematological adverse events 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 non-hematological adverse event 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 pharmacological management, anti-emetic therapy with metoclopramide was highly effective. Other common adverse events included vomiting, diarrhea, infection, fatigue, lethargy, pruritus and rash. There were no incidences of tumor lysis syndrome, which was a dose limiting toxicity in the Phase 1 study in CLL (21).

Three possible treatment related serious adverse events (SAEs) 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 adverse events. 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 pre-clinical studies, endometrial thinning had been observed when tamoxifen was combined with ABT-737, a pre-clinical lead that targets both BCL-2 and BCL-XL (18,27). Three patients underwent transvaginal ultrasound, revealing mild endometrial hyperplasia normally associated with tamoxifen.

**Anti-tumor activity**
Tumor response as per RECIST v1.1 criteria, matching circulating tumor DNA mutations, and progression free survival are shown in Fig. 2A-C and Table 3. 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. Therefore, an objective response (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, clinical benefit (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 progression-free survival 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 progression free survival (PFS) compared to patients receiving <800 mg (median PFS 23 vs 51 weeks at the time of data analysis, p = 0.03) (Supplementary Fig. 4).

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 to patients treated in later line relapse (Supplementary Table 4A). 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 stable disease 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 stable disease were also observed in 5 of 9 (56%) patients who had previously experienced disease progression on tamoxifen. Clinical benefit (1 CR, 1 PR and 2 SD) was seen in all four patients who had previously received the combination of an aromatase inhibitor and CDK4/6 inhibitor for metastatic disease (Supplementary Table 4B).

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 with either 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 radiological response. Samples were analyzed by immunohistochemistry for changes in hormone receptor expression, proliferation (Ki67), BCL-2 and measures of apoptosis (cleaved caspase 3). No notable changes were observed in ER, PR or BCL-2 expression. A trend towards decreased Ki67 was observed (Fig. 3A). Few cleaved caspase 3 (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 radiological 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 to archival samples. ER expression was reduced in 3 tumor biopsies. While BCL-2 levels appeared unchanged in 3 samples, reduced immunostaining for BCL-2 was observed in 2 samples and BCL-2 expression was absent in 4 samples (Supplementary Fig. 5). Since BCL-2 expression was either unchanged or reduced at the time of progressive disease compared to archival samples, diverse mechanisms (such as loss of dependence on ER signaling, upregulation of other pro-survival BCL-2 family members or mutations in BCL-2 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, D). Of the 11 patients (68%) who achieved partial metabolic response (PMR) (based on changes in SUV max 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 (PMD) or stable metabolic disease (SMD) observed on FDG-PET in 5 patients was associated with subsequent RECIST progression in 4 patients (Fig. 3D). The increased metabolic activity in the remaining
patient (01-021) would be consistent with a ‘flare’ response (28), since 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 to those with metabolic progression (PMD) or stable metabolic disease (SMD) (PFS 25 vs 65 weeks, p = 0.004) (Supplementary Fig. 6A). 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 studies at baseline, C1D15 and D1 of each subsequent treatment cycle. ctDNA was screened for 39 genes known to be recurrently mutated in breast cancer (Supplementary Tables 5-8). ctDNA mutations were identified at baseline in 28 of 33 (85%) patients (Fig. 3E, Supplementary Table 8). 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. 6B). Within 28 days of treatment, a significant reduction in ctDNA levels were 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 8, 9). Six of the 7 patients experiencing PR or SD had previously received tamoxifen in the adjuvant and/or metastatic setting (Supplementary Table 9). 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. 6C). Subsequent rises in ctDNA appeared to pre-empt radiological 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 pre-treated patients with hematological malignancies, where extensive bone marrow infiltration and immune defects are common. We therefore complemented clinical haematology 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 and Supplementary Fig. 7A-D and 8). Compared to baseline, treatment with venetoclax and tamoxifen resulted in a significant reduction in haemoglobin, 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 IgA and IgM was observed, although IgG and overall gamma globulin levels were unaffected (Fig 4C, Supplementary Fig. 8A, B). 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 (Treg) cells) was observed (Fig. 4D, Supplementary Fig. 8C). Similarly, circulating natural killer (NK) cells, monocyte and dendritic cell (DC) subsets were unaffected (Fig. 4E, Supplementary Fig. 8D). 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 1b study evaluating the safety and preliminary efficacy of combining venetoclax with tamoxifen in 33 patients with metastatic ER+ and BCL-2+ 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 adverse events being non-clinically significant cytopenia and nausea similar to that reported for patients treated with CLL (21). Dose modifications were infrequent and no patients required multiple dose reductions or cessation of treatment due to safety concerns. Despite previous signal in hematological 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 to other adjunctive therapies used with endocrine therapy such as the mTOR, PIK3CA and CDK4/6 inhibitors.

Promising anti-tumor activity was observed with tamoxifen and venetoclax, including in heavily pre-treated patients. Tumor response or prolonged stable disease was observed in 8 of 12 patients
who were treated in ≥ 4th 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 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 pro-apoptotic agent.

As this was a small phase 1 study, direct comparison with randomised phase 3 studies evaluating single agent tamoxifen is at best speculative. Nevertheless, it is noteworthy that the ORR (54%) and CBR (75%) observed here for the 800 mg cohort, compares favorably to historical studies of patients treated with tamoxifen in first line relapse, where reported ORR ranged between 17-33% and CBR between 38-56% (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 progression free survival and duration of response for the RP2D cohort remains an open question, since 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 pre-clinical data, which raised the possibility that tamoxifen therapy could induce ‘mitochondrial priming’ through elevation of BCL-2 (18), thereby rendering tumor cells more susceptible to BH3 mimetic therapy (35). The small window of opportunity study suggested that BCL-2 protein expression remains high (and in some cases may be increased) following short-term treatment with tamoxifen. Similarly, BCL-2 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 BCL-2 expression was sustained, an earlier on treatment biopsy may have been required to reliably detect apoptosis. Presumably a reliance on the pre-existing high levels of BCL-2 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 (WO40181), an ongoing randomized phase 2 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. BCL-2 levels were reduced or absent in a subset of tumors, raising the possibility that other pro-survival factors (such as BCL-XL or MCL-1) could be involved. Such functional redundancy among pre-survival BCL-2 family members seems plausible, given that they are commonly co-expressed in ER\(^+\) tumors (18,38). Pertinently, MCL-1 appears to be amplified in a subset of triple negative tumors that fail to respond to neoadjuvant therapy (39). In other tumor progression biopsies, BCL-2 expression did not change. It will be interesting to determine whether mutations in BCL-2 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 BCL-2 expression at progression.

Plasma ctDNA analysis revealed *ESR1* mutations in 30% of patients at study entry, consistent with heavy pre-treatment 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 stable disease (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 vivo.*
A role for FDG PET/CT in predicting response to endocrine therapy in metastatic breast cancer is currently unclear. A pilot study suggested that partial metabolic response to FDG-PET at ~10 weeks was predictive of improved progression free survival (43). Consistent with these data, we observed semiquantitative metabolic responses at 4 weeks compared to baseline that broadly correlated with radiological response by RECIST at 12 weeks as well as improved PFS, compared to those with metabolic progression (PMD) or stable metabolic disease (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 Treg cells). A mild reduction in neutrophil count was observed, while natural killer (NK) cells, monocyte and dendritic cell (DC) 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 BCL-2\(^+\) metastatic breast cancer. Since BCL-2 was expressed at high levels in approximately 70% of metastatic biopsy samples from patients with ER\(^+\) tumors (Supplementary Fig. 2), a large proportion of patients could potentially be impacted by these findings. The ready availability of BCL-2 as a possible predictive biomarker should facilitate further investigation of combination therapy for patients with BCL-2-positive breast cancer as well as other types of cancer.

**METHODS**

**Study Design and Objectives**

This was a phase 1, multi-center, open-label study of venetoclax in combination with tamoxifen in patients with metastatic ER positive and BCL-2 positive breast cancer (‘mBEP’, BCL-2 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 cut-off 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 is available. All potential patients had BCL-2 immunohistochemistry performed on either fresh or archival tissue during the pre-screening 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 dose limiting toxicities (DLTs) in the first 4 weeks of treatment, as well as the maximum tolerated dose (MTD) and the recommended phase two dose (RP2D). Secondary objectives included overall response rates of the combination treatment as defined by complete response (CR) or partial response (PR), the clinical benefit rate as defined by CR, PR or Stable Disease (SD) for >24 weeks; and to determine the progression free and overall survival. Exploratory objectives included evaluation of changes in ER, PR and BCL-2 gene expression profiles, changes in plasma circulating tumor DNA 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 (ACTRN12615000702516). 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 aged >18 years with histologically confirmed ER positive (defined as >1% positive stained carcinoma cells) and BCL-2 positive metastatic breast cancer were enrolled. BCL-2 status was determined by immunohistochemistry 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 to 3 scale). Of note, tumor from all patients recruited to mBEP exhibited BCL-2 positive immunostaining in >50% of cells, with 2+ or 3+ intensity. Eligibility criteria included ECOG performance status of 0-1, evaluable disease as defined by RECIST v1.1, life expectancy of >6 months and adequate end-organ function. Patients in the dose expansion phase must not have received ≥3 lines of prior therapy in the metastatic setting and required measurable disease.
Exclusion criteria included tamoxifen use within the last 3 months, absolute contraindication to tamoxifen and uncontrolled brain metastases.

**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 synthesis of all available toxicity information. Additional patients were then recruited to receive the RP2D dose in the dose expansion phase of the study.

Patients received oral venetoclax, available as 100 mg tablets and taken together with tamoxifen 20 mg daily in continuous 28 day cycles. The allocated treatment was continued until disease progression according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, unacceptable toxicity, death or withdrawal of informed consent. Dose modifications according to the study protocol were permitted in the event of drug related toxicity.

**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 4 weekly thereafter. All adverse events (AEs) were collected until 30 days following the last treatment regardless of attribution to study drug. AE severity was graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03. There were no pre-specified special adverse events 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 LDH 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), BCL-2 pathway proteins as well as measures of downstream activation of apoptosis including cleaved caspase 3 (CC3).

As an exploratory marker of response to therapy, 2-deoxy-2-[fluorine-18]fluoro-D-glucose (\(^{18}\text{F-FDG}\)) positron emission tomography (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 partial metabolic response (PMR) was
evaluated locally and defined as a decrease of >15% in the average percentage change in the maximum standardized uptake value (SUV$_{\text{max}}$) of the target lesions.

**Statistical Methods**

The sample size for this study was obtained based on the dose escalation rules described in the study design. Patient characteristics and adverse events are summarised 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 clinical benefit rate is 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.

**‘Pre-Treat’ Window of Opportunity Study**

An exploratory window study (Pre-Treat; 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 pre-menopausal 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 BCL-2 family members by gene expression
profiling and RT-PCR on paired tumour samples following short-term tamoxifen treatment in ER positive breast cancer. Secondary endpoints were changes in ER, PR, Ki67 and BCL-2 family expression, as determined by immunohistochemistry. 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 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 royalty payments from AbbVie and Genentech in relation to venetoclax (ABT-199). AbbVie and Roche/Genentech provided funds that contributed to the conduct of this study. G.J. Lindeman has served on Advisory Boards for AbbVie, Amgen and Genentech, received honoraria payments from AbbVie, Amgen and Genentech and research funding from Servier. A.W. Roberts has received research funding support from AbbVie. B. Yeo has served on Advisory Boards for Amgen and Genentech and received honoraria payments from Genentech. J. Visvader has received research funding from Servier.

Authors’ Contributions


Writing, review and/or revision of the manuscript: S. W. Lok, J.R. Whittle, F. Vaillant, L.L. Lo, A.W. Roberts, S.J. Dawson, D.H.D. Gray, J. E. Visvader, G.J. Lindeman.
Administrative, technical or material support: G.B. Mann, L. Taylor, K. Shackleton.

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REFERENCES


FIGURE LEGENDS

**Figure 1.** A, Kaplan-Meier survival curve for ER$^+$BCL-2$^+$ PDX 315 (n = 6-10 mice per arm). Mice were treated with vehicle, tamoxifen, venetoclax, or the combination at indicated doses. Venetoclax oral gavage, 5 days per week. Tamoxifen was injected subcutaneously daily. Mice, which remained otherwise healthy, were sacrificed when tumor size reached the experimental ethical endpoint (> 600 mm$^3$). Log rank (Mantel-Cox) p value for combination therapy with ABT-199 25 mg/kg vs combination with ABT-199 100 mg/kg: p = 0.01. Tumor growth curves for individual mice from PDX model 315 are shown in Fig. S2. B, Study design and consort flow diagram. Patients with metastatic ER$^+$BCL-2$^+$ breast cancer were treated with tamoxifen and venetoclax (in escalating doses) in a 3+3 Phase 1 design continuously until progression, unacceptable toxicity or death. Once the RP2D was determined, an additional 18 patients were enrolled as of the reporting date. Pharmacodynamic studies were performed on plasma and tumor tissue as well as metabolic studies using FDG-PET. C, Clinical trial consort diagram shows the number of subjects entering the study from enrollment. D, Immunohistochemical evaluation of BCL-2 staining intensity. 0 = negative, 1 = weak, 2 = moderate, 3 = strong. Scale bar, 50 µM.

**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 circulating tumor DNA at study enrolment. C, Swimmer plot of time on treatment for 33 evaluable patients. Individual patients represented as lines.

**Figure 3.** Biomarkers of response. A, Ki67 of individual patients at baseline and following 28 days of treatment. B, Post-treatment biopsy of patient 01-033 (28 days). Immunostaining of ER, PR, BCL-2, Ki67 and cleaved caspase 3 (CC3). Scale bars, 50 µm. Black arrows indicate CC3 foci. C, Representative images of FDG-PET scans at baseline and following 28 days of treatment. White arrows for patient 01-019 indicate skull metastases. D, Waterfall plot of change in SUV$_{\text{max}}$ and best radiographic response for 16 patients treated in dose expansion cohort. For patient 01-024 the lateral component of the soft tissue iliac mass seen was chosen as the target lesion. E, Numbers of mutations detected in ctDNA at baseline for 33 patients by targeted...
amplicon sequencing. F, Dynamic changes in ctDNA in response to treatment for patients 01-011 and 01-014 by droplet digital PCR.

**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). 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.
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>
</tr>
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<tbody>
<tr>
<td>Median age (range)</td>
<td>65 (45-78)</td>
<td>66 (43-75)</td>
<td>65 (43-78)</td>
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<td>ECOG performance status</td>
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<td>0</td>
<td>7 (47%)</td>
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<td>21 (64%)</td>
</tr>
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<td>8 (53%)</td>
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<td>Premenopausal or peri menopausal</td>
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<td>5 (15%)</td>
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<td>Receptor status*</td>
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<tr>
<td>ER</td>
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<td>4 (12%)</td>
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<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>PR</td>
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<tr>
<td>Strong</td>
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<td>8 (44%)</td>
<td>19 (58%)</td>
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<tr>
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<td>3 (17%)</td>
<td>5 (15%)</td>
</tr>
<tr>
<td>Negative</td>
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<tr>
<td>BCL-2</td>
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<td>28 (85%)</td>
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<td>5 (15%)</td>
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<tr>
<td>Weak</td>
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<td>0 (0%)</td>
<td>0 (0%)</td>
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<td>Median Ki-67 % (range)</td>
<td>10% (1-30)</td>
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<td>15% (1-60)</td>
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<td>Sites of disease</td>
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<tr>
<td>Bone</td>
<td>14 (93%)</td>
<td>12 (67%)</td>
<td>26 (79%)</td>
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<td>Visceral metastases</td>
<td>9 (60%)</td>
<td>11 (61%)</td>
<td>20 (61%)</td>
</tr>
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<td>Liver</td>
<td>3 (20%)</td>
<td>8 (44%)</td>
<td>11 (33%)</td>
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<tr>
<td>Lung</td>
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<td>5 (28%)</td>
<td>12 (36%)</td>
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<tr>
<td>Nodal</td>
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<td>Adjuvant endocrine therapy (%)</td>
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<tr>
<td>Tamoxifen only</td>
<td>6 (40%)</td>
<td>5 (28%)</td>
<td>11 (33%)</td>
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<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 (toremefine)</td>
<td>1 (7%)</td>
<td>0 (0%)</td>
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<td>None</td>
<td>5 (33%)</td>
<td>4 (22%)</td>
<td>9 (27%)</td>
</tr>
<tr>
<td>Prior lines of metastatic therapy, n (%)</td>
<td></td>
<td></td>
<td></td>
</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>
</tr>
<tr>
<td>Mean prior lines of treatment (range)</td>
<td>2.7 (0-6)</td>
<td>1.5 (0-8)</td>
<td>2.0 (0-8)</td>
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<td>Prior tamoxifen exposure (%)</td>
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</tr>
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<td>None</td>
<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>Prior disease progression on tamoxifen</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>Prior chemotherapy exposure (%)</td>
<td></td>
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</tr>
<tr>
<td>None</td>
<td>5 (33%)</td>
<td>7 (39%)</td>
<td>12 (36%)</td>
</tr>
<tr>
<td>Adjuvant setting only</td>
<td>4 (27%)</td>
<td>5 (28%)</td>
<td>9 (27%)</td>
</tr>
<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>

*16 patients were enrolled based on results of archival tissue
Table 2. Any grade treatment related adverse events reported in at least 10% of patients

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>&lt; 800 mg n = 9(n (%))</th>
<th>800 mg n = 24(n (%))</th>
<th>Total n = 33(n (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased WCC</td>
<td>8 (89)</td>
<td>21 (88)</td>
<td>29 (88)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>9 (100)</td>
<td>20 (83)</td>
<td>29 (88)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>5 (56)</td>
<td>19 (79)</td>
<td>24 (73)</td>
</tr>
<tr>
<td>Nausea</td>
<td>6 (67)</td>
<td>16 (67)</td>
<td>22 (67)</td>
</tr>
<tr>
<td>Anemia</td>
<td>5 (56)</td>
<td>8 (33)</td>
<td>13 (39)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>4 (44)</td>
<td>7 (29)</td>
<td>11 (33)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3 (33)</td>
<td>8 (33)</td>
<td>11 (33)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 (22)</td>
<td>7 (29)</td>
<td>9 (27)</td>
</tr>
<tr>
<td>Infection (any)</td>
<td>0 (0)</td>
<td>9 (38)</td>
<td>9 (27)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1 (33)</td>
<td>5 (21)</td>
<td>6 (18)</td>
</tr>
<tr>
<td>Lethargy</td>
<td>0 (0)</td>
<td>4 (17)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>Pruritis</td>
<td>0 (0)</td>
<td>4 (17)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>Rash</td>
<td>1 (33)</td>
<td>3 (13)</td>
<td>4 (12)</td>
</tr>
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</table>
### Table 3. Summary of efficacy data

<table>
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<tr>
<th>Variable</th>
<th>Dose escalation n = 15</th>
<th>Dose expansion n = 18</th>
<th>All patients n = 33</th>
<th>800 mg cohort n = 24</th>
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</thead>
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<tr>
<td><strong>Best overall response, n (%)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete (CR)</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>1 (3)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Partial response (PR)</td>
<td>4 (27)(^#)</td>
<td>10 (56)</td>
<td>14 (42)(^\wedge)</td>
<td>12 (50)</td>
</tr>
<tr>
<td>Stable disease (SD)</td>
<td>6 (40)</td>
<td>2 (11)</td>
<td>8 (24)</td>
<td>5 (21)</td>
</tr>
<tr>
<td>Progressive disease (PD)</td>
<td>5 (33)</td>
<td>5 (28)</td>
<td>10 (30)</td>
<td>6 (25)</td>
</tr>
<tr>
<td>Overall response rate (ORR)</td>
<td>4 (27)(^#)</td>
<td>11 (61)</td>
<td>15 (45)(^\wedge)</td>
<td>13 (54)</td>
</tr>
<tr>
<td>Clinical benefit rate (CBR)</td>
<td>10 (67)</td>
<td>13 (72)</td>
<td>23 (70)</td>
<td>18 (75)</td>
</tr>
<tr>
<td><strong>Median PFS (weeks)</strong></td>
<td>36</td>
<td>48</td>
<td>36</td>
<td>51</td>
</tr>
<tr>
<td>Median duration of response (Range)</td>
<td>61 (11-100+)</td>
<td>39 (8 - 83+)</td>
<td>42 (8 - 100+)</td>
<td>42 (8 - 100+)</td>
</tr>
</tbody>
</table>

\(^\#\) PR and ORR was 31% for the 13 patients with measurable disease

\(^\wedge\) PR and ORR were 45% and 48%, respectively, for the 31 patients with measurable disease

* Duration of objective response shown for the cut-off date, with 8 patients from the 800 mg cohort remaining on study treatment.

**Abbreviations:** PFS, Progression Free Survival
Figure 1

A

**Percent survival**

**Vehicle**
- Tamoxifen
- Venetoclax 100 mg/kg
- Tamoxifen / Venetoclax 25 mg/kg
- Tamoxifen / Venetoclax 100 mg/kg

**Time (days)**
- 0
- 20
- 40
- 60
- 80
- 100

B

**Screening**
- ER +ve
- BCL-2 +ve MBC

**Dose Escalation**
- Tamoxifen + Venetoclax 200 mg
  - n = 3
- Tamoxifen + Venetoclax 400 mg
  - n = 3
- Tamoxifen + Venetoclax 600 mg
  - n = 3
- Tamoxifen + Venetoclax 800 mg
  - n = 6

**Dose Expansion**
- Tamoxifen + Venetoclax 800 mg
  - n = 18

**Endpoints**
- Primary endpoint
  - Dose limiting toxicity
  - Determine MTD and RP2D
- Secondary endpoints
  - Progression free survival
  - Objective response rate
- Exploratory endpoints
  - Changes in PBMCs
  - Measure ctDNA
  - Metabolic response (FDG-PET)

**Biomarker studies**
- ctDNA and PBMCs every 4 weeks
- Response assessment every 12 weeks
- Disease Progression

**Cycle**
- Screening
- Cycle 1
- Cycle 2
- Cycle 3
- Cycle n

**Optional**
- Tissue Biopsy
- FDG-PET

C

**96 patients pre-screening**
- 1 patient no biopsy
- 1 patient no tissue available
- 1 patient developed bowel obstruction
- 1 unknown
- 1 patient normal tissue on biopsy
- 3 patients ER –
- 1 patient HER2 amplified
- 1 patient squamous cell carcinoma
- 24 patients BCL-2 low or negative
  (≤50% staining and/or < moderate strength)
- 6 patients treated with chemotherapy
- 6 patients no measurable disease
- 5 patients declined study enrolment
- 3 patients enrolled on alternative study
- 2 patients poor PS
- 2 patients not eligible due to comorbidities
- 2 patients ongoing response to prior therapy
- 1 patient poor hepatic function
- 1 patient on tamoxifen
- 1 patient concurrent renal cancer

**92 patients tissue assessed**
- 86 patients ER +/HER2 –
- 62 patients BCL-2 positive

**33 patients enrolled**

D

**BCL-2 intensity**

0
1
2
3

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Figure 2

A

Author Manuscript Published OnlineFirst on December 5, 2018; DOI: 10.1158/2159-8290.CD-18-1151

* Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited:

![Graph showing change from baseline (%)]

**RESPONSE**

- **Progressive Disease**
- **Stable Disease (≥24wks)**
- **Partial response**
- **Complete response**

**B**

- **PIK3CA**
- **ESR1**
- **GATA3**
- **MAP3K1**
- **CDH1**
- **PTEN**

**ctDNA Mutation**

**C**

- **Patient ID**

![Graph showing patient ID and response status]

**Legend:**

- **200 mg**
- **400 mg**
- **600 mg**
- **800 mg**
- **Non-measurable**

- **Complete response**
- **Progressive disease**
- **Partial response**
- **Stable disease**
- **Ongoing response**

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Figure 3

A) Ki67

B) ER, PR, BCL2, Ki67, CC3

C) Baseline and 4 weeks images

D) SUV max and RECIST v1.1

E) ctDNA mutations

F) ctDNA level (copies/ml)
A phase 1b dose-escalation and expansion study of the BCL-2 inhibitor venetoclax combined with tamoxifen in ER and BCL-2-positive metastatic breast cancer

Geoffrey J Lindeman, Sheau Wen Lok, James R Whittle, et al.

Cancer Discov  Published OnlineFirst December 5, 2018.

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Supplementary Material  Access the most recent supplemental material at: http://cancerdiscovery.aacrjournals.org/content/suppl/2018/11/27/2159-8290.CD-18-1151.DC1

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