VERONICA: Randomized Phase II Study of Fulvestrant and Venetoclax in ER-Positive Metastatic Breast Cancer Post-CDK4/6 Inhibitors – Efficacy, Safety, and Biomarker Results

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ABSTRACT

Purpose: Despite promising activity in hematopoietic malignancies, efficacy of the B-cell lymphoma 2 (BCL2) inhibitor venetoclax in solid tumors is unknown. We report the prespecified VERONICA primary results, a randomized phase II clinical trial evaluating venetoclax and fulvestrant in estrogen receptor (ER)-positive, HER2-negative metastatic breast cancer, post–cyclin-dependent kinase (CDK) 4/6 inhibitor progression.

Patients and Methods: Pre-/postmenopausal females ≥18 years were randomized 1:1 to venetoclax (800 mg orally daily) plus fulvestrant (500 mg intramuscular; cycle 1: days 1 and 15; subsequent 28-day cycles: day 1) or fulvestrant alone. The primary endpoint was clinical benefit rate (CBR); secondary endpoints were progression-free survival (PFS), overall survival, and safety. Exploratory biomarker analyses included BCL2 and BCL extra-large (BCLXL) tumor expression, and *PIK3CA* circulating tumor DNA mutational status.

Results: At primary analysis (cutoff: August 5, 2020; $n = 103$), venetoclax did not significantly improve CBR [venetoclax plus fulvestrant: 11.8% ($n = 6/51$; 95% confidence interval (CI), 4.44–23.87); fulvestrant: 13.7% (7/51; 5.70–26.26); risk difference −1.96% (95% CI, −16.86 to 12.94)]. Median PFS was 2.69 months (95% CI, 1.94–3.71) with venetoclax plus fulvestrant versus 1.94 months (1.84–3.55) with fulvestrant (stratified HR, 0.94; 95% CI, 0.61–1.45; $P = 0.7853$). Overall survival data were not mature. A nonsignificant improvement of CBR and PFS was observed in patients whose tumors had strong BCL2 expression (IHC 3+), a BCL2/BCLXL Histoscore ratio ≥1, or *PIK3CA*-wild-type status.

Conclusions: Our findings do not indicate clinical utility for venetoclax plus fulvestrant in endocrine therapy–resistant, CDK4/6 inhibitor–refractory metastatic breast tumors, but suggest possible increased dependence on BCLXL in this setting.

Introduction

Estrogen receptor (ER)-positive, HER2-negative tumors, which account for approximately 70% of all breast cancers, are responsible for most breast cancer–related deaths (1). Standard first- and second-line treatments for ER-positive, HER2-negative metastatic breast cancer includes various endocrine therapies, such as ER modulators (e.g., tamoxifen), steroidal/nonsteroidal aromatase inhibitors (e.g., exemestane/anastrozole and letrozole), and selective ER degraders (e.g., fulvestrant; ref. 2). Combination therapy with a

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Prior presentations: These analyses have been presented in part at the (1) Oral presentation at the American Society of Clinical Oncology (ASCO) Virtual Congress 2021, June 4–8, 2021 (results from VERONICA: a randomized, phase II study of second-/third-line venetoclax (VEN) + fulvestrant (F) vs. F alone in estrogen receptor (ER)-positive, HER2-negative, locally advanced, or metastatic breast cancer (LA/MBC); and (2) Poster presentation at the San Antonio Breast Cancer Symposium (SABCS) 2021, December 7–10, 2021 (exploratory biomarker analysis in VERONICA, a phase II study of fulvestrant + venetoclax, vs. fulvestrant alone, in patients with ER-positive, HER2-negative mBC following progression on CDK4/6 inhibitor therapy).

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Clin Cancer Res 2022;28:3256–67

doi: 10.1158/1078-0432.CCR-21-3811

Translational Relevance

Targeting the B-cell lymphoma 2 (BCL2) prosurvival protein has shown considerable promise in hematopoietic malignancies but has not been properly explored in solid tumors. VERONICA was the first randomized phase II study of BCL2 inhibition in hormone receptor–positive metastatic breast cancer with venetoclax and endocrine therapy. Venetoclax plus fulvestrant in patients progressing on endocrine and cyclin-dependent kinase (CDK) 4/6 inhibitor therapy did not improve clinical benefit rate (CBR) or progression-free survival (PFS). Exploratory analyses revealed a slightly improved CBR and PFS with venetoclax where tumors exhibited strong BCL2 expression (IHC 3+), a BCL2/BCLXL Histoscore ratio ≥1, or *PIK3CA*-wild-type status (particularly those with high BCL2 expression). These data suggest an important dependence of tumor survival on BCLXL in the post-CDK4/6 inhibitor setting, and on BCL2 in *PIK3CA*-wild-type tumors. The role for targeting BCL2 in an endocrine therapy–responsive, CDK4/6 inhibitor–naïve setting remains unknown.

cyclin-dependent kinase (CDK) 4/6 inhibitor (e.g., palbociclib, ribociclib, or abemaciclib) has rapidly become standard of care in the first-/second-line metastatic setting, due to its profound impact on progression-free survival (PFS) and overall survival (OS; refs. 3–7). Nevertheless, at least one-third of patients experience disease progression on first-line therapy within 2 years, with the majority of tumors becoming refractory to therapy by 3 years (8).

Targeted induction of apoptosis represents a novel therapeutic strategy for targeting ER-positive breast tumors. B-cell lymphoma 2 (BCL2) is a key member of the BCL2 prosurvival family, which includes BCL2, BCL extra-large (BCLXL), and myeloid cell leukemia 1 (MCL1). These proteins play a central role as guardians against apoptosis by keeping proapoptotic "sensor" and/or "effector" proteins in check (9). BCL2 inhibition frees the effectors, resulting in mitochondrial outer membrane permeabilization, cytochrome c release, caspase activation, and, finally, cell death (9). BCL2 is overexpressed in approximately 80% and 70% of ER-positive primary and metastatic breast cancers, respectively, whereas BCLXL expression is ubiquitous (albeit at differing levels; refs. 10–12). In the primary breast cancer setting, BCL2 overexpression is associated with a favorable prognosis, presumably reflecting its relationship with ER expression (as an estrogen-responsive gene; refs. 10, 13). Nevertheless, poorer-prognosis luminal B tumors also express high levels (14), and a substantial proportion of patients with BCL2-positive tumors develop incurable metastatic disease (10).

Targeting BCLXL and BCL2 with agents such as navitoclax has proved challenging for drug development, due to the induction of thrombocytopenia (15). This effect is "on-target", as a result of the dependence of platelets on BCLXL for survival (16, 17). Selective targeting of BCL2 to mitigate BCLXL-associated thrombocytopenia has proved to be more manageable. Venetoclax is a potent, selective inhibitor of BCL2 and has assumed an important place in the treatment of several hematopoietic malignancies [including a subset of chronic lymphocytic leukemia (CLL), acute myeloid leukemia, and small lymphocytic lymphoma; ref. 18]. While venetoclax has single-agent activity in CLL, combination therapy appears to be required to trigger apoptosis in breast cancer cells (12). Dual inhibition of BCL2 and ER with venetoclax and tamoxifen has been shown to promote apoptosis and enhance tumor response in endocrine therapy–naïve, BCL2-positive, patient-derived xenograft models (14). In a small phase Ib dose-escalation and -expansion study ("mBEP"; ISRCTN98335443), venetoclax (800 mg) plus tamoxifen was well tolerated and had encouraging clinical benefit rates (CBR) in patients with ER-positive, BCL2-positive metastatic breast cancer, most of whom had never received CDK4/6 inhibitor therapy (11).

Based on these findings, we conducted the second study of venetoclax in a solid tumor. The VERONICA trial (NCT03584009) was a phase II study of venetoclax plus fulvestrant, versus fulvestrant alone, in patients with ER-positive, HER2-negative locally advanced or metastatic breast cancer, following disease progression on endocrine therapy and a CDK4/6 inhibitor. Here we report its prespecified primary and updated PFS and OS analyses.

Patients and Methods

Patients and trial design

VERONICA was a phase II, multicenter, open-label, randomized trial (Supplementary Fig. S1) across 40 sites in five countries. Patients were pre-/postmenopausal females ≥18 years with histologically/cytologically confirmed ER-positive, HER2-negative, locally advanced or metastatic breast cancer [as per the American Society of Clinical Oncology (ASCO)/College of American Pathology (CAP) guidelines; refs. 19, 20], and at least one measurable lesion [per Response Evaluation Criteria In Solid Tumors (RECIST) v1.1] and inoperable disease. Patients had adequate organ and bone marrow function, had an Eastern Cooperative Oncology Group Performance Status of 0 or 1, had previously received ≤2 prior lines of endocrine therapy with no prior cytotoxic chemotherapy in the locally advanced or metastatic breast cancer settings, had experienced disease recurrence or progression during/after CDK4/6 inhibitor therapy (must have been administered for ≥8 weeks prior to progression), and were eligible for endocrine therapy at study enrollment. Key exclusion criteria included: untreated or active central nervous system metastasis; current severe, uncontrolled, systemic disease; prior treatment with fulvestrant, other selective ER degraders, or BCL2 inhibitors; anticancer therapy within 21 days of cycle 1; and radiotherapy within 21 days of cycle 1, or previous radiotherapy to the target lesion sites.

Patients were randomized 1:1 to venetoclax (800 mg oral daily) plus fulvestrant (500 mg intramuscular; days 1 and 15 of cycle 1; day 1 of subsequent 28-day cycles) or fulvestrant alone, and were treated until disease progression, unacceptable toxicity, withdrawal of consent, death, or predefined study end, whichever occurred first (Supplementary Fig. S1). Patients were stratified by prior lines of therapy in the locally advanced or metastatic breast cancer settings (one versus two) and BCL2 clinical status (high versus low).

Trial oversight

The study was conducted according to the International Council for Harmonisation E6 Guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research was conducted, whichever were the more stringent. The protocol and amendments were approved by independent review boards/ethics committees. All patients provided written informed consent; tumor specimens for BCL2, BCLXL, and other biomarker analyses; and blood for circulating tumor DNA (ctDNA) studies.

Endpoints and assessments

The primary endpoint was CBR (complete response, partial response, or stable disease for ≥24 weeks). Secondary endpoints

included PFS, OS, and safety and tolerability. Incidence and severity of adverse events (AE) were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (21). An exploratory biomarker subgroup analysis of PFS and CBR, according to tumor protein expression levels of BCL2 and BCLXL, and *PIK3CA* mutational status (determined from ctDNA), was also conducted.

Tumor assessments occurred by documenting all known sites of disease at screening (within 28 days prior to cycle 1, day 1) and reassessing at each subsequent tumor evaluation (every 8 weeks ± 5 days from the date of randomization until week 24, and every 12 weeks thereafter). Tumor assessments were conducted regardless of dose delays or early discontinuation, until investigator-assessed disease progression, predefined study end, or until death, whichever occurred first. Response assessments were made by the investigator using physical examinations, CT scans, or MRI and/or bone scans per RECIST v1.1. The same radiographic procedure used to assess disease sites at screening was used throughout the study.

BCL2 and BCLXL protein expression and clinical status were determined centrally by IHC on formalin-fixed paraffin-embedded primary or metastatic tissue samples from prestudy biopsies or archival specimens using the CONFIRM anti-bcl-2 (clone 124) mouse monoclonal primary antibody assay (Ventana Medical Systems, Inc., Oro Valley, AZ; catalog number 790–4464; ref. 22) and the anti-BCLXL rabbit monoclonal antibody (clone 54H6; Cell Signaling Technology; catalog number 2764S), respectively. BCL2-high or BCL2-low/-negative clinical status was prespecified and determined as "high" if ≥50% of tumor cells had BCL2 expression at 2+ or 3+ staining intensity by IHC, based on the criteria used in the phase 1 mBEP clinical trial (ISRCTN98335443; ref. 11). Additional BCL2 scoring for subgroup analyses was not predefined and used IHC clinical scores of 0/1+/2+/3+, as described for diffuse large B-cell lymphoma (23). Exploratory biomarker analyses of BCLXL protein expression did not have predefined cutoffs. For this, we derived a BCLXL Histoscore (H-score) and changes between quartile expressions were analyzed, as previously reported (24). The ratio of BCL2 to BCLXL H-score protein expression was also assessed at a cutoff of ≥1, as previously described for multiple myeloma (25, 26). ER status was centrally confirmed by IHC using the CONFIRM anti-ER (SP1) rabbit monoclonal primary antibody (Ventana Medical Systems, Inc.; catalog number 790–4,325) in accordance with ASCO/CAP guidelines (19). Baseline plasma-derived ctDNA was evaluated using the FoundationOne Liquid assay (CF3 baitset, Foundation Medicine, Inc.) to examine short variants (SV) and copy-number alterations (CNA) of known or likely pathogenicity, as described previously (27). We focused on *PIK3CA* mutations, given their utility as a predictive biomarker for targeted therapy (27).

Statistical analysis

The intention-to-treat (ITT) population comprised all randomized or enrolled patients regardless of study treatment administration; the safety-evaluable population included all patients who received any study drug.

The planned sample size was approximately 100 patients (50 with BCL2-high tumors). The study was not designed with defined statistical power for hypothesis testing for primary or secondary endpoints; thus, all *P* values shown are intended to be for descriptive rather than hypothesis-testing purposes. The planned sample size provided a 15% precision for an 80% confidence interval (CI) when calculating the absolute difference in CBR between the two treatment arms. This assumed that the expected observed CBR was 40% based on Cristofanilli and colleagues (28) with fulvestrant and 60% with venetoclax plus fulvestrant (i.e., 80% CI, 5%–35%).

The primary analysis was conducted when all patients had discontinued study treatment, or 6 months after the last patient was enrolled, whichever occurred first.

An estimate of CBR for measurable disease at baseline and its 95% CI was calculated. The CIs for the difference in CBR were determined using the normal approximation to the binomial distribution.

Kaplan–Meier methodology was used to estimate median PFS and OS, with stratified log-rank tests used to compare treatment arms. HRs and their 95% CIs were determined by the stratified Cox proportional hazards model. Stratification factors were prior lines of therapy in the locally advanced or metastatic breast cancer settings (one versus two) and BCL2 clinical status (high versus low).

Safety analyses were descriptive in the safety-evaluable population, and predefined methodology was used for toxicity reporting. All the AEs were summarized by treatment arm, with number of patients and percentages.

Subgroup analyses were exploratory without multiple testing adjustments and statistical power was not predefined. For the purposes of our analyses, we followed the protocol plan by assessing the association between candidate biomarkers and measures of efficacy with the study drug venetoclax plus fulvestrant, and standard treatment fulvestrant. Unstratified/stratified Cox proportional hazard models were used for time-to-event outcomes. The normal approximation was used to determine the 95% CI for CBR.

Data availability

For eligible studies, qualified researchers may request access to individual patient level clinical data through a data request platform. At the time of writing, this request platform is Vivli: https://vivli.org/ourmember/roche/. For updated details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see https://go.roche.com/data_sharing.

Results

Patients

Between September 6, 2018 and February 5, 2020, 103 patients were randomized to receive venetoclax plus fulvestrant ($n = 51$) or fulvestrant alone ($n = 52$). At the primary analysis clinical cutoff (August 5, 2020), 10 patients (9.7%) remained on treatment and median follow-up was 9.9 months (Supplementary Tables S1 and S2).

Baseline demographics were balanced between treatment arms (**Table 1**). Median patient ages were 58.0 years and 59.5 years in the venetoclax plus fulvestrant and fulvestrant arms, respectively, and 72 of 103 (69.9%) patients were <65 years. Median duration of CDK4/6 inhibitor therapy in the venetoclax plus fulvestrant versus the fulvestrant arm was 15.0 months (range: 1.8–51.2) versus 16.5 months (range: 1.9–63.0). Central testing of tumor ER expression revealed concordance with the local results. Only one tumor exhibited low (<10%) ER expression, where ER expression was observed in 1% of tumor nuclei. Approximately 65% ($n = 67/103$) of patients had BCL2-high tumors. Across both treatment arms, there was a high percentage of patients with visceral [venetoclax plus fulvestrant: $n = 47/51$ (92.2%); fulvestrant: $n = 43/52$ (82.7%)] and liver metastases [$n = 35/51$ (68.6%); $n = 28/52$ (53.8%); only one patient in each arm had bone-only metastasis]. A high prevalence of *ESR1* and *TP53* mutations was also observed [$n = 40/94$ (42.6%) and $n = 39/94$

Table 1. Baseline demographics, prior cancer therapies, and biomarker status.

	Venetoclax plus fulvestrant $n = 51$	Fulvestrant $n = 52$
Median age, years	58.0	59.5
<65	38 (74.5)	34 (65.4)
ECOG performance status		
0	28 (54.9)	31 (59.6)
Race,[a] *n* (%)		
White	40 (78.4)	46 (88.5)
Asian	6 (11.8)	3 (5.8)
Black or African American	3 (5.9)	2 (3.8)
Breast cancer histologic subtype[b]		
Ductal	40 (78.4)	34 (65.4)
Lobular	10 (19.6)	16 (30.8)
Other	5 (9.8)	8 (15.4)
Site of metastatic disease (≥1 lesion)		
Visceral[c]	47 (92.2)	43 (82.7)
Liver	35 (68.6)	28 (53.8)
Lung	19 (37.3)	18 (34.6)
Bone[d]	36 (70.6)	39 (75.0)
Line of prior metastatic breast cancer ET		
1	41 (80.4)	43 (82.7)
2	10 (19.6)	9 (17.3)
Prior cancer therapy		
Neoadjuvant	12 (23.5)	7 (13.5)
Adjuvant	30 (58.8)	27 (51.9)
Metastatic (ET)	51 (100)	52 (100)
CDK4/6 inhibitor (metastatic)		
Palbociclib	29 (56.9)	39 (75.0)
Ribociclib	22 (43.1)	13 (25.0)
Median duration of therapy, months (range)	15.0 (1.8–51.2)	16.5 (1.9–63.0)
BCL2 status[e]		
High	33 (64.7)	34 (65.4)
Low	18 (35.3)	18 (34.6)
Gene mutations[f]	$n = 48$	$n = 46$
PIK3CA SV	19 (39.6)	14 (30.4)
ESR1 SV	21 (43.8)	19 (41.3)
TP53 SV	23 (47.9)	16 (34.8)
RB1 SV	9 (18.8)	4 (8.7)
CDH1 SV	9 (18.8)	5 (10.8)
PTEN SV	5 (10.4)	4 (8.7)
BRCA2 SV	4 (8.3)	2 (4.3)
ERBB2 SV	4 (8.3)	1 (2.2)
AKT1 SV	2 (4.2)	4 (8.7)
ATM SV	2 (4.2)	3 (6.5)
FGFR1 CNA	4 (8.3)	7 (15.2)

Note: Data are *n* (%) unless otherwise specified.

Abbreviations: BCL2, B-cell lymphoma 2; CDK4/6, cyclin-dependent kinase 4/6; CNA, copy-number alteration; ECOG, Eastern Cooperative Oncology Group; ET, endocrine therapy; SV, short variant.

[a]Venetoclax plus fulvestrant arm: one patient - multiple categories, one patient - unknown (2.0%); fulvestrant arm: one patient - unknown (1.9%).

[b]Missing at time of snapshot: venetoclax plus fulvestrant: one patient (1.9%); fulvestrant: four patients (7.7%).

[c]Visceral: lung, liver, adrenal gland, central nervous system, pleural cavity, or peritoneal cavity.

[d]There were two patients with bone-only metastatic lesions, one in each arm.

[e]BCL2 clinical status was determined as "high" if ≥50% of tumor cells expressed BCL2 at 2+ or 3+ staining intensity by immunohistochemistry. Note: 62% of tissue specimens were archival, collected >1 year prior to study start; 39% of tissue specimens were from metastatic sites compared with 59% from primary sites (unknown in two patients). There were no patients who had tumor specimens collected from both primary and metastatic sites.

[f]Gene mutation analysis is based only on known or likely pathogenic SVs and CNAs detected with a prevalence of >5% in the total population of samples evaluable for plasma circulating tumor DNA.

(41.5%), respectively]. Some numerical, nonsignificant imbalances between treatment arms were seen in the frequency of *PIK3CA*, *TP53*, *CDH1*, and *RB1* mutations, all of which were more prevalent in the venetoclax plus fulvestrant arm (**Table 1**).

Efficacy

CBR in the ITT population with baseline measurable disease was low across both treatment arms, with no complete responses and few partial responses (**Fig. 1A**). CBR for venetoclax plus fulvestrant was 11.8% (n = 6/51; 95% CI, 4.44–23.87), compared with 13.7% for fulvestrant alone [n = 7/51; 95% CI, 5.70–26.26; risk difference –1.96% (95% CI, –16.86 to 12.94)]. Median PFS was 2.69 months (95% CI, 1.94–3.71) in the venetoclax plus fulvestrant arm versus 1.94 months (95% CI, 1.84–3.55) in the fulvestrant arm [stratified HR: 0.94; 95% CI, 0.61–1.45; P = 0.7853; **Fig. 1B**]. OS, based on the observed median, HR, and Kaplan–Meier plot, favored fulvestrant; however, data maturity was limited by short follow-up at the time of the primary analysis [venetoclax plus fulvestrant: 16.76 months (95% CI, 10.12–not evaluable (NE)); fulvestrant: NE (95% CI, 16.00–NE); stratified HR: 2.56 (95% CI, 1.11–5.89); P = 0.0218; **Fig. 1C**]. At the updated analysis (June 23, 2021), PFS (stratified HR, 0.96; 95% CI, 0.63–1.47; P = 0.87; Supplementary Fig. S2) and OS results were consistent with the primary analysis (stratified HR, 1.87; 95% CI, 1.02–3.43; P = 0.0403; **Fig. 1D**).

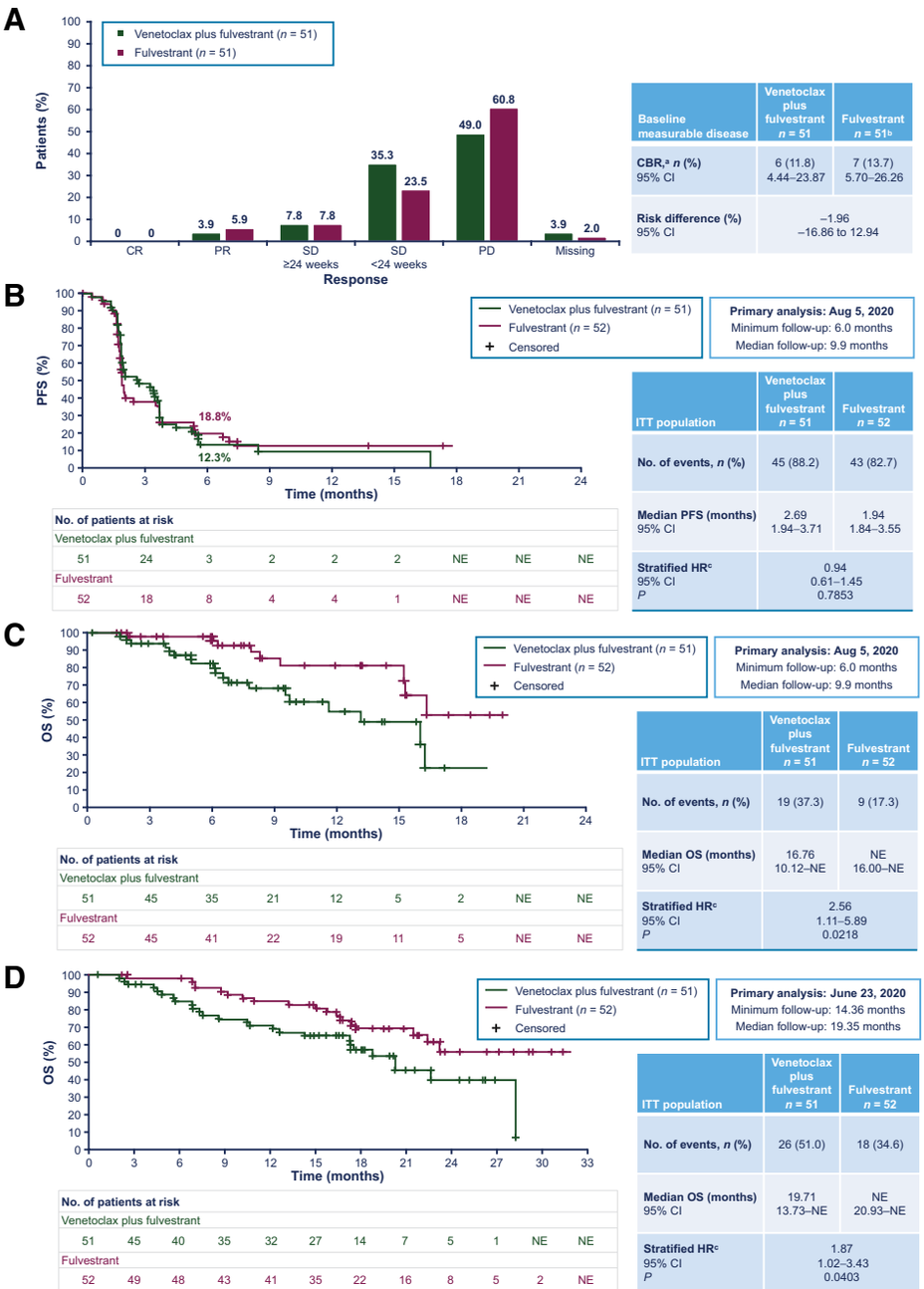


Figure 1.
Primary and secondary endpoints in the ITT population. CBR (**A**), PFS (**B**), OS (**C**; August 5, 2020 cutoff date), and OS (**D**; June 23, 2021 cutoff date). BCL2, B-cell lymphoma 2; CBR, clinical benefit rate; CI, confidence interval; CR, complete response; ITT, intention-to-treat; NE, not evaluable; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease. [a]CBR included CR, PR, and SD ≥24 weeks. [b]One patient did not report measurable disease at baseline. [c]Stratified by prior line of therapy in the locally advanced or metastatic breast cancer setting (one versus two) and BCL2 status (high versus low).

Safety

The safety summary is presented in **Table 2**. The majority of treatment-related AEs with venetoclax were grade 1–2 and manageable. There was one serious AE that led to patient death (urosepsis) in the venetoclax plus fulvestrant arm; this was considered unrelated to study treatment by the investigator. A numerical imbalance of deaths in the venetoclax plus fulvestrant arm, versus the fulvestrant arm [n = 19/50 (38.0%) versus n = 9/51 (17.6%)], was mainly due to progressive disease >28 days following the last dose of treatment (**Table 2**).

The most common all-grade AEs with the biggest difference between treatment arms included nausea [venetoclax plus fulvestrant: n = 32/50 (64.0%) versus fulvestrant: n = 9/51 (17.6%)], diarrhea [n = 27/50 (54.0%) versus n = 5/51 (9.8%)], and vomiting [n = 15/50 (30.0%) versus n = 1/51 (2.0%); Supplementary Table S3]. Tumor lysis syndrome (which can occur in CLL; ref. 29) was not observed. Any-grade neutropenia occurred in 8 of 50 [16.0%; grade 3–4: n = 6/50 (12.0%)] patients in the venetoclax and fulvestrant arm versus 0 in the fulvestrant arm.

Exploratory biomarker analysis of BCL2 and BCLXL tumor expression and *PIK3CA* subgroups

BCL2 tumor protein expression to determine predefined high and low/negative clinical status was performed prospectively (n = 103), using samples from primary tumors (59%) or metastatic sites (39%; **Table 1**). CBR was similar between the venetoclax plus fulvestrant and fulvestrant arms in the BCL2-high subgroup [n = 6/33 (18.2%) and n = 6/33 (18.2%); risk difference

Table 2. Overall safety summary and subsequent cancer therapies.

	Venetoclax plus fulvestrant n = 50	**Fulvestrant n = 51**
≥1 AE	47 (94.0)	39 (76.5)
Total AEs	399	218
Grade 3–4 AEs[a]	13 (26.0)	6 (11.8)
SAEs	4 (8.0)	1 (2.0)
SAEs leading to death	1 (2.0)	0
Urosepsis (unrelated to study drug)	1 (2.0)	NA
AEs related to study drug[b]	43 (86.0)	26 (51.0)
Venetoclax	43 (86.0)	NA
Grade 1–2	32 (64.0)	NA
Grade 3–4	11 (22.0)	NA
Fulvestrant	27 (54.0)	26 (51.0)
Related AEs leading to drug withdrawal[c]	4 (8.0)	0
Venetoclax	4 (8.0)	NA
Fulvestrant	1 (2.0)	0
AEs leading to dose modification/interruption	22 (44.0)	1 (2.0)
Deaths	19 (38.0)	9 (17.6)
≤28 days following last dose	2 (4.0)	0
AE	1 (2.0)[d]	0
PD	1 (2.0)	0
>28 days following last dose	17 (34.0)	9 (17.6)
PD	16 (32.0)	8 (15.7)
Other	1 (2.0)	1 (2.0)
Pneumonia	0	1 (2.0)
Post-study reporting death	1 (2.0)	0
Subsequent cancer therapies[e]	n = 51	n = 52
Radiotherapy	7 (13.7)	8 (15.4)
Bone	3 (5.9)	4 (7.7)
Brain	3 (5.9)	3 (5.8)
Antineoplastic agents[f]	35 (68.6)	41 (78.8)
Capecitabine	19 (37.3)	24 (46.2)
Paclitaxel	9 (17.6)	9 (17.3)
Everolimus	2 (3.9)	10 (19.2)
ET[f]	5 (9.8)	13 (25.0)
Exemestane	4 (7.8)	10 (19.2)

Note: Safety-evaluable population (one patient in each arm did not receive study drug). Data are n (%).
Abbreviations: AE, adverse event; ET, endocrine therapy; ITT, intention-to-treat; NA, not applicable; PD, progressive disease; SAE, serious adverse event.
[a]Difference in incidence of grade 3–4 AEs between the venetoclax plus fulvestrant arm versus the fulvestrant arm was driven by neutropenia [six patients (12.0%) versus zero patients, respectively].
[b]AEs were mainly grade 1–2 and manageable.
[c]All study drugs.
[d]Fatal urosepsis SAE unrelated to study drug.
[e]ITT population.
[f]Antineoplastic agents and ET reported are those used by >10% of patients in either treatment arm.

Table 3. CBR in BCL2-high, BCL2-low, *PIK3CA*-wild-type, and *PIK3CA*-mutant subgroups.

	BCL2-high		BCL2-low		*PIK3CA*-wild-type		*PIK3CA*-mutant	
	Venetoclax plus fulvestrant $n = 33$	Fulvestrant $n = 33$	Venetoclax plus fulvestrant $n = 18$	Fulvestrant $n = 18$	Venetoclax plus fulvestrant $n = 29$	Fulvestrant $n = 31$	Venetoclax plus fulvestrant $n = 19$	Fulvestrant $n = 14$
CBR, *n* (%)	6 (18.2)	6 (18.2)	0	1 (5.6)	6 (20.7)	3 (9.7)	0	2 (14.3)
Risk difference (%)	0		−5.56		11.01		−14.29	
95% CI	−21.64 to 21.64		−21.69 to 10.58		−10.37 to 32.40		−38.82 to 10.25	

Note: ITT population with baseline measurable disease.
Abbreviations: BCL2, B-cell lymphoma 2; CBR, clinical benefit rate; CI, confidence interval; ITT, intention-to-treat.

0% (95% CI, −21.64 to 21.64); **Table 3**]. No clinical improvement was observed in the venetoclax plus fulvestrant and fulvestrant arms in the BCL2-low subgroup [$n = 0/18$ and $n = 1/33$ (5.6%); risk difference −5.56% (95% CI, −21.69 to 10.58)]. Median PFS was similar between the venetoclax plus fulvestrant and fulvestrant arms in the BCL2-low subgroup [2.04 months (95% CI, 1.81–3.71) versus 2.00 months (95% CI, 1.87–3.75); HR, 0.96], although a slight, nonsignificant difference (not reflected in HR) between treatment arms was observed in the BCL2-high subgroup [3.45 months (95% CI, 1.87–3.71) versus 1.94 months (95% CI, 1.80–3.71); HR, 0.92; **Fig. 2A** and **B**].

Additional exploratory biomarker analyses were performed to understand the unexpectedly low CBR in VERONICA, with the caveats that subgroup sizes were small and that treatment groups may not have been balanced for other key disease characteristics. Exploratory analyses of BCL2 subgroups by IHC score suggested that patients with tumors that exhibited the strongest BCL2 protein expression (IHC 3+) had a slight, nonsignificant improvement in CBR and median PFS in the venetoclax plus fulvestrant arm [3.88 months (95% CI, 1.71–NE) versus 1.74 months (95% CI, 1.02–3.65); HR, 0.38], albeit with small patient numbers ($n = 13$; Supplementary Fig. S3). Protein expression of the BCL2 family member BCLXL was successfully evaluated in tumor specimens from 96 patients (93% of the ITT). Interestingly, patients' tumors that had the lowest BCLXL protein expression also had the greatest (albeit nonsignificant) improvement in median PFS with venetoclax plus fulvestrant versus fulvestrant alone (Supplementary Fig. S4). Indeed, median PFS was higher with venetoclax plus fulvestrant versus fulvestrant alone in patients with a BCL2/BCLXL H-score ratio ≥1; no difference in median PFS was observed between the treatment arms in patients with a BCL2/BCLXL ratio <1 (**Fig. 2C**).

Plasma ctDNA was evaluated for SVs and CNAs of known or likely pathogenicity in 94 patients (91% of the ITT). In the *PIK3CA*-wild-type subgroup, CBR was 20.7% ($n = 6/29$) in the venetoclax plus fulvestrant arm and 9.7% ($n = 3/31$) in the fulvestrant arm (risk difference 11.01%; 95% CI, −10.37 to 34.40; **Table 3**). In contrast, CBR in *PIK3CA*-mutant tumors was 0% and 14.3% ($n = 2/14$), respectively (risk difference −14.29%; 95% CI, −38.82 to 10.25). Although median PFS was similar between treatment arms in the *PIK3CA*-mutant subgroup, a nonsignificant improvement in PFS was observed in the venetoclax plus fulvestrant arm for *PIK3CA*-wild-type tumors [venetoclax plus fulvestrant: 3.71 months (95% CI, 1.94–4.53) versus fulvestrant: 1.87 months (95% CI, 1.74–3.55); HR, 0.66; 95% CI, 0.38–1.17; $P = 0.1549$; **Fig. 3A** and **B**]. Notably, further evaluation of *PIK3CA* mutational status and BCL2 protein expression revealed that patients with *PIK3CA*-wild-type and BCL2-high tumors had the greatest, albeit nonsignificant, improvement in median PFS and CBR with venetoclax plus fulvestrant, versus fulvestrant alone [median PFS, 3.7 months versus 1.9 months (HR, 0.58; 95% CI, 0.28–1.19); CBR: 31.6% ($n = 6$) versus 13.6% ($n = 3$), respectively; Supplementary Fig. S5].

Discussion

The VERONICA study was conducted in patients with ER-positive, HER2-negative metastatic breast cancer progressing on CDK4/6 inhibitor therapy. The overall study population exhibited largely endocrine therapy–unresponsive disease, with a high proportion of patients displaying adverse prognostic features, reflective of highly advanced disease. In this setting, VERONICA did not meet its primary endpoint; venetoclax plus fulvestrant did not significantly improve CBR or PFS, when compared with fulvestrant alone. Exploratory biomarker analyses, however, revealed a slightly, although nonsignificantly, improved CBR and PFS in patients receiving venetoclax where tumors exhibited strong BCL2 protein expression (IHC 3+) or where the BCL2/BCLXL H-score ratio was ≥1, suggesting that the balance between BCL2 and BCLXL proteins may predict sensitivity to venetoclax. A modest, nonsignificant improvement in CBR and PFS was also observed when tumors were *PIK3CA*-wild-type, most notably when BCL2 expression was high. The small sample sizes and the exploratory nature of this subgroup analysis, which was not predefined or statistically powered, mean that confirmation of these results is required.

The reason for the lack of clinical benefit in VERONICA is unclear. Of note, patients entering VERONICA had a much lower median PFS on prior endocrine and CDK4/6 inhibitor therapy (median of 15.0 and 16.5 months, respectively), compared with that reported in the PALOMA-2 (27.6 months) and MONALEESA-2 (25.3 months) trials (3, 30), suggesting that patients in VERONICA had more aggressive disease upon study entry. The short median PFS (<2.0 months) and low CBR in the fulvestrant monotherapy arm of VERONICA underscores the endocrine therapy–resistant status of the overall population and the need for more effective therapeutic options in this setting. A high proportion had visceral, including liver, metastases, which have been associated with a much poorer response to endocrine therapy (31). Only one patient in each treatment arm had bone-only metastatic disease. The rate of visceral metastases in VERONICA (87.4%) is higher than that seen previously in the SOLAR-1 (56.6%) phase III study of fulvestrant plus alpelisib or placebo for patients with *PIK3CA*-mutated, hormone receptor–positive, HER2-negative advanced breast cancer progressing on aromatase inhibitor therapy, although the breakdown of visceral metastases for the small number of patients who had previously received a CDK4/6 inhibitor was not reported (32). The rate of visceral metastases was also higher than that in the BYLieve (66.9%) trial of alpelisib in patients with *PIK3CA*-mutated, hormone receptor–positive, HER2-negative advanced breast cancer

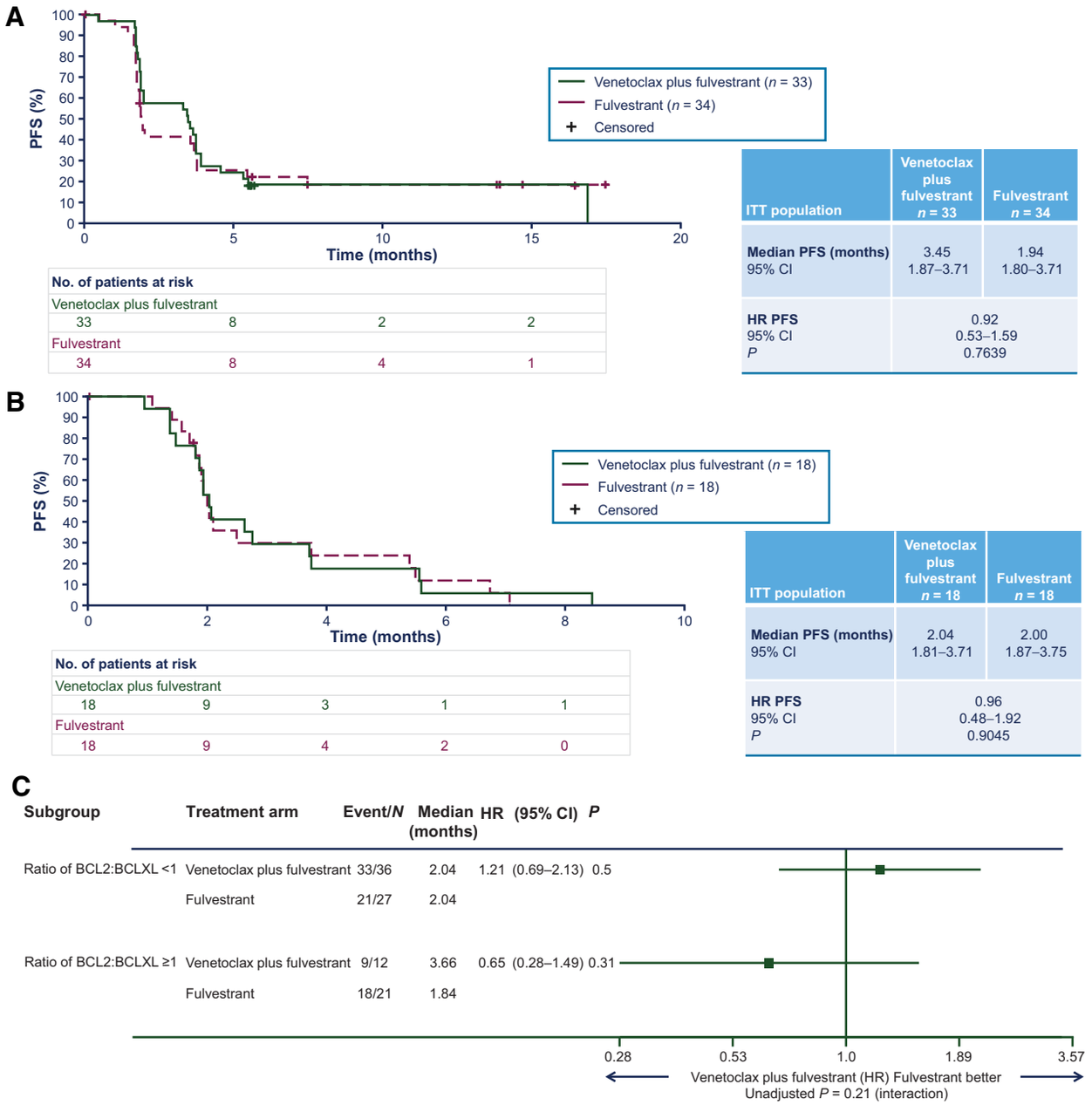
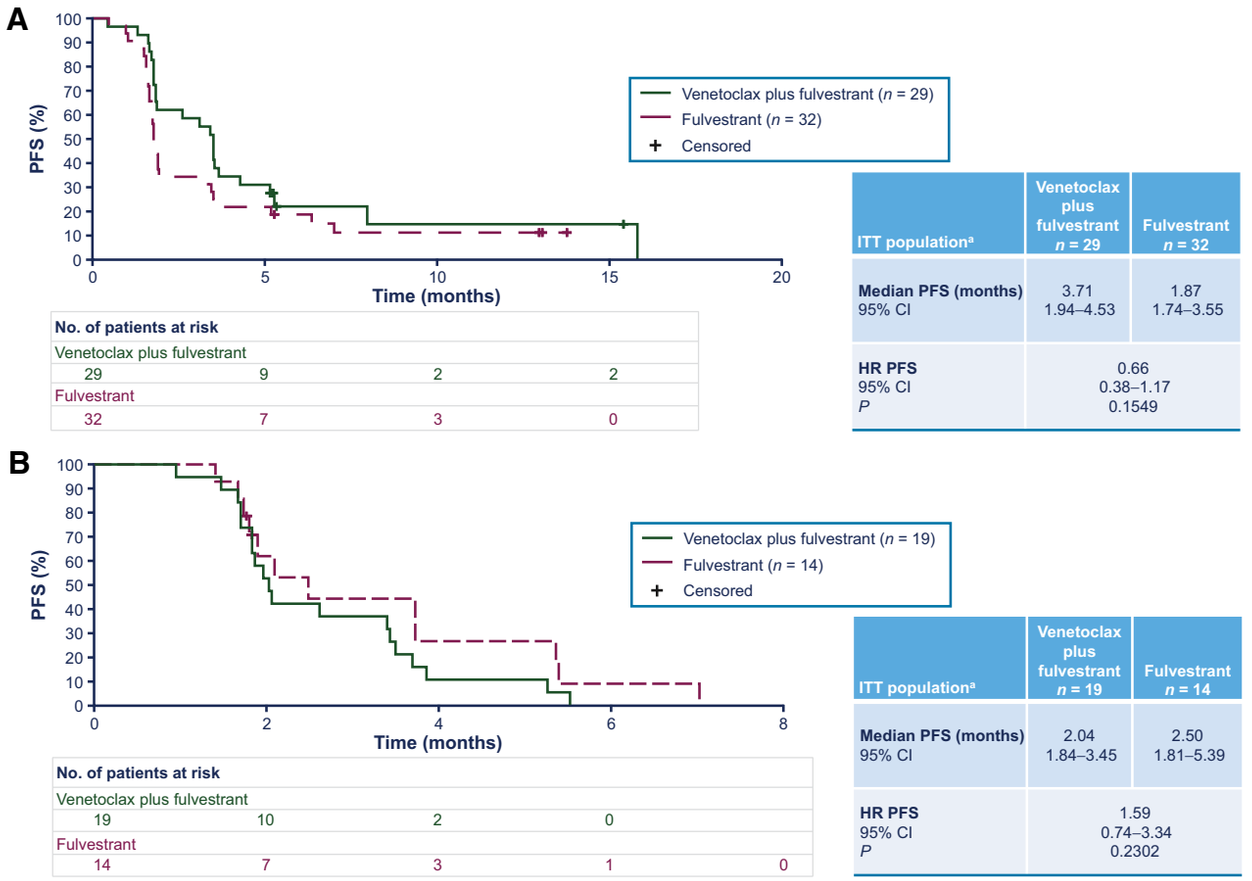


Figure 2.
PFS in the BCL2-high subgroup (**A**), the BCL2-low subgroup (**B**), and tumors with a BCL2/BCLXL ratio ≥1 and <1 (**C**). BCL2, B-cell lymphoma 2; BCLXL, B-cell lymphoma extra-large; CI, confidence interval; PFS, progression-free survival.

progressing on a CDK4/6 inhibitor (33). While the precise definition of visceral disease differed slightly between VERONICA and BYLieve, the difference is largely driven by the presence of liver metastases, that is, 46.5% in BYLieve versus 61.2% in VERONICA (33). Different selection criteria between BYLieve and VERONICA and thus a differing patient population may have contributed to these differences. For example, 15 (11.8%) patients in BYLieve received alpelisib as first-line therapy for metastatic breast cancer (no patients received treatment in the first-line setting in VERONICA), 24 (18.9%) had bone-only disease versus 2 (1.9%) in VERONICA, and patients with either *PIK3CA*-mutated or -wild-type tumors were recruited to VERONICA (33).

Further highlighting the endocrine therapy–resistant status of the overall population, the VERONICA cohort also exhibited a high rate of *ESR1* and *TP53* mutations, although variations in the incidence of these mutations were not significantly different between treatment arms by univariate analysis. Approximately 20–40% of patients who have received an aromatase inhibitor for metastatic breast cancer have *ESR1* mutations, with prevalence varying by sites of metastatic disease (34). For example, in the PALOMA-3 trial, 21.8% and 15.7% of



ITT population[a]	Venetoclax plus fulvestrant *n* = 29	Fulvestrant *n* = 32
Median PFS (months) 95% CI	3.71 1.94–4.53	1.87 1.74–3.55
HR PFS 95% CI *P*	0.66 0.38–1.17 0.1549	

ITT population[a]	Venetoclax plus fulvestrant *n* = 19	Fulvestrant *n* = 14
Median PFS (months) 95% CI	2.04 1.84–3.45	2.50 1.81–5.39
HR PFS 95% CI *P*	1.59 0.74–3.34 0.2302	

Figure 3.

PFS in the *PIK3CA*-wild-type subgroup (**A**) and *PIK3CA*-mutant subgroup (**B**). CI, confidence interval; ITT, intention-to-treat; PFS, progression-free survival; SV, short variant. [a]Analysis is based on known or likely pathogenic *PIK3CA* SVs detected from samples evaluable for plasma ctDNA only.

patients had baseline *ESR1* and *TP53* mutations, respectively (35). A similarly high *ESR1* mutation rate (40.1%) to that of VERONICA was observed in the PADA-1 trial (36), although direct comparisons between VERONICA and PADA-1 are challenging due to the different patient populations, study designs, and research questions being addressed. Both *ESR1* and *TP53* mutations have been linked to endocrine-therapy resistance and worse patient outcomes in ER-positive metastatic breast cancer (35, 37–39). *RB1* mutations were also common, consistent with CDK4/6 inhibitor resistance and escape from cell-cycle arrest (40). Thus, the VERONICA patient population exhibited a range of adverse clinical features.

Preclinical modeling of ER- and BCL2-positive, primary (treatment-naïve) breast cancer has suggested that combining endocrine therapy with venetoclax elicits a more profound apoptotic response than endocrine therapy alone (14). In contrast, single-agent venetoclax was not effective, suggesting that triggering of apoptosis requires an endocrine stressor (14). Consistent with this theory, combining venetoclax with tamoxifen in patients with endocrine-responsive disease or who displayed secondary resistance elicited a high CBR and prolonged PFS in the preceding mBEP study (11), providing a rationale for further investigation of venetoclax and endocrine therapy. At the time that the VERONICA study was initiated, no studies had been conducted with venetoclax in the post-CDK4/6 inhibitor setting. There were limited preclinical data and very few [$n = 4/33$ (12.1%)] patients had received prior CDK4/6 inhibitor therapy in mBEP (11).

Strong BCL2 (IHC 3+) expression may be necessary to elicit a tumor response to venetoclax in ER-responsive breast cancer. In mBEP, all patients were required to have BCL2-high tumors (with the same criteria used in VERONICA); however, almost all patients [$n = 28/33$ (84.8%)] had BCL2 3+ tumors (11). A CBR of 72% was observed in mBEP in the dose-expansion cohort, in which 17 of 18 (94%) patients had tumors with strong BCL2 expression (11). Only 2 of those 18 patients had received prior CDK4/6 inhibitor therapy. Similar to what has been reported in previous literature (10–12), 65% of patients in VERONICA had tumors that were BCL2-high. However, of these patients, only 13 (19%) had strong (IHC 3+) BCL2 tumor expression.

Functional redundancy between BCL2 family members may have also contributed to the poor efficacy observed with venetoclax in VERONICA. The improved, albeit nonsignificant, CBR and PFS observed in patients receiving venetoclax plus fulvestrant for tumors with low BCLXL expression or BCL2:BCLXL H-score ratio ≥1 are similar to findings in the hematopoietic setting for multiple myeloma (26, 41). MCL1 levels could also be relevant, given that high expression is associated with tumor progression and poor prognosis (42). Importantly,

the CDK4/6 inhibitors palbociclib and abemaciclib were recently shown to increase BCLXL (and MCL1 for abemaciclib) expression in breast cancer cell lines (43, 44), suggesting that apoptosis evasion may become less dependent on BCL2 and more reliant on BCLXL. Taken together, it seems likely that lower BCL2 levels coupled with increased dependence on BCLXL for tumor cell survival in the post-CDK4/6 inhibitor setting contributed to the poor clinical responses to venetoclax plus fulvestrant in VERONICA. These findings suggest that targeting BCLXL or both BCL2 and BCLXL with a dual inhibitor such as navitoclax may be worth exploring in the post-CDK4/6 inhibitor setting.

The safety profile of venetoclax plus fulvestrant was consistent with the known safety profiles of each drug; no new safety signals indicating novel adverse drug reactions were identified. The most common side effects included low-grade toxicity, such as nausea and diarrhea (usually treatable with simple intermittent therapy). Leukopenia was also common, but typically low grade, with uncomplicated Grade 3–4 neutropenia and lymphopenia observed in 12% and 4%, respectively, of patients treated with venetoclax plus fulvestrant.

The reason for the less-favorable OS in the venetoclax plus fulvestrant arm, compared with the fulvestrant arm, is unclear. The single serious AE that led to patient death was due to urosepsis and was deemed unrelated to the study drug. Deaths were primarily due to progressive disease occurring >28 days following the last dose of study treatment. It seems unlikely that the relatively short duration of venetoclax treatment in this study could have impacted bone marrow reserve and compromised delivery of subsequent chemotherapy, especially considering that hematologic toxicity was largely low grade. There was a small numerical imbalance in several poor prognostic variables between treatment arms; for example, patients in the venetoclax plus fulvestrant arm, versus the fulvestrant alone arm, were more likely to have visceral disease, including liver metastases, and had a higher incidence of *PIK3CA*, *TP53*, *RB1*, and *CDH1* mutations. Furthermore, patients in the venetoclax plus fulvestrant arm were more likely to have received prior chemotherapy in the adjuvant/neoadjuvant setting and less likely to receive subsequent cancer therapies, including antineoplastic agents and endocrine therapy. In the mBEP study, the expected OS far exceeds the 16.76 months observed in VERONICA (11). While it is possible that anti-BCL2 treatment may have had a detrimental effect on OS, perhaps through upregulation/selection of other pro-survival pathways in tumor cells, there is currently insufficient data to investigate this hypothesis.

In an exploratory analysis, a slightly increased CBR and PFS was also observed in patients with *PIK3CA*-wild-type, compared with *PIK3CA*-mutant, tumors, although the difference was nonsignificant. This may reflect differing mechanisms of resistance in *PIK3CA*-mutant tumors where PI3K/AKT signaling may be a dominant contributor to oncogenic signaling and tumor survival, reducing dependence on BCL2. Activated AKT can block apoptosis by phosphorylating transcription factors that regulate proapoptotic genes (such as FoxO; ref. 45) or prosurvival genes (such as IKKα and CREB; refs. 46–48), or directly inhibit the caspase-mediated apoptotic cascade via phosphorylation of the BCL2-associated agonist of cell death protein (BAD; ref. 49). Furthermore, *PIK3CA*-mutant breast cancer cell lines have been shown to exhibit increased dependence on BCLXL and MCL1, with dual inhibition resulting in strong antitumor activity (50). It is possible that increased BCL2 dependence occurs in *PIK3CA*-wild-type tumors. Indeed, we observed increased CBR in *PIK3CA*-wild-type, BCL2-high tumors in the venetoclax plus fulvestrant versus fulvestrant arms (no such clinical improvement was observed in *PIK3CA*-wild-type, BCL2-low tumors). These data highlight potential differential dependence of the BCL2 family members between *PIK3CA*-wild-type and -mutant tumors that could merit further investigation.

Conclusion

The VERONICA primary analysis found that addition of venetoclax to fulvestrant did not improve CBR or PFS in patients with ER-positive, HER2-negative, locally advanced or metastatic breast cancer, post-CDK4/6 inhibitor therapy. Overall, these results do not suggest clinical utility of venetoclax plus fulvestrant in an endocrine therapy–resistant, CDK4/6 inhibitor–refractory setting. Our exploratory biomarker data may point to a dependence of tumors on BCLXL for survival in the post-CDK4/6 inhibitor setting and on BCL2 in *PIK3CA*-wild-type tumors. Selection of patients based on strong BCL2 (IHC 3+) tumor expression and/or profiling of BCL2 family member expression could be important for identifying subgroups who may be sensitive to BCL2 inhibitor therapy.

Our exploratory results, together with other preclinical data, suggest that BCLXL levels and the BCL2:BCLXL H-score ratio may need to be considered in the design of future clinical trials. Future studies could investigate targeting of BCLXL or dual targeting of BCL2 and BCLXL in the post–CDK4/6 inhibitor setting. This may prove feasible in the future as BCLXL inhibitors that do not induce thrombocytopenia are developed. The possibility that poorer OS might be observed with venetoclax would also need to be considered in future clinical trial design. Finally, the role for BCL2 inhibition in a CDK4/6 inhibitor–naive setting (where promising activity was observed with tamoxifen; ref. 11), or in combination with chemotherapy (12), remains unknown. Because CDK4/6 inhibitor therapy is standard-of-care first-line therapy for ER-positive metastatic breast cancer, the safety and efficacy of adding venetoclax in this endocrine therapy–responsive setting also remains an open question, currently being explored in the PALVEN study (NCT03900884; ref. 43).

Authors' Disclosures

G.J. Lindeman reports grants and non-financial support from F. Hoffmann-La Roche Ltd during the conduct of the study. G.J. Lindeman also reports personal fees from AbbVie and Pfizer, as well as grants from AbbVie, Amgen, F. Hoffmann-La Roche Ltd, and Pfizer outside the submitted work. In addition, G.J. Lindeman is an employee of the Walter and Eliza Hall Institute, which receives milestone and royalty payments related to venetoclax (all employees may be eligible for benefits related to these payments and G.J. Lindeman reports receiving benefits); G.J. Lindeman is also supported by a National Health and Medical Research Council (Australia) Leadership Fellowship and the Breast Cancer Research Foundation. T.M. Fernando reports other support from Genentech, Inc. and F. Hoffmann-La Roche Ltd during the conduct of the study, as well as other support from Genentech, Inc. and F. Hoffmann-La Roche Ltd outside the submitted work. R. Bowen reports personal consulting or advisory fees from GSK, AstraZeneca, and Clovis. K.J. Jerzak reports other support from F. Hoffmann-La Roche Ltd during the conduct of the study. K.J. Jerzak also reports personal fees from Amgen, Apo Biologix, Eisai, Genomic Health/Exact Sciences, Knight Therapeutics, Merck, Myriad Genetics, Novartis, Purdue Pharma, Pfizer, and F. Hoffmann-La Roche Ltd, as well as grants and personal fees from AstraZeneca, Eli Lilly, and Seagen outside the submitted work; in addition, K.J. Jerzak has a patent regarding the use of dronedarone and related derivatives as anticancer agents (patent publication #WO/2017/106974) issued. X. Song reports other support from BMS, Merck, Novartis, and Pfizer outside the submitted work. T. Decker reports personal fees from Novartis and IOMEDICO outside the submitted work. F. Boyle reports other support from F. Hoffman-La Roche Ltd during the conduct of the study. F. Boyle also reports other support from Eii Lilly, Eisai, Pfizer, Novartis, and The Friends of the Master Foundation, as well as personal fees and other support from Novartis outside the submitted work. S. McCune reports other support from F. Hoffmann-La Roche Ltd/Genentech, Inc. during the conduct of the study. A. Armstrong reports other support from AstraZeneca, personal fees from Gilead and MSD, grants from

AstraZeneca, and non-financial support from Gilead and MSD outside the submitted work. C. Shannon reports personal fees from Gilead, Novartis, AstraZeneca, F. Hoffmann-La Roche Ltd, and GSK outside the submitted work. C.-W. Chang reports personal fees from Genentech, Inc. outside the submitted work. T.R. Wilson reports other support from Genentech, Inc. and F. Hoffmann-La Roche Ltd during the conduct of the study, as well as other support from Genentech, Inc. and F. Hoffmann-La Roche Ltd outside the submitted work. A. Flechais reports other support from F. Hoffmann-La Roche Ltd during the conduct of the study, as well as other support from F. Hoffmann-La Roche Ltd outside the submitted work. A. Bardia reports grants and personal fees from Genentech, Inc., Novartis, Pfizer, Merck, Sanofi, Radius Health/Menarini, Immunomedics/Gilead, Daiichi Pharma/AstraZeneca, and Eli Lilly during the conduct of the study, as well as grants and personal fees from Genentech, Inc., Novartis, Pfizer, Merck, Sanofi, Radius Health/Menarini, Immunomedics/Gilead, Daiichi Pharma/AstraZeneca, and Eli Lilly outside the submitted work. No disclosures were reported by the other authors.

Disclaimer

Anonymized records for individual patients across more than one data source external to F. Hoffmann-La Roche Ltd cannot, and should not, be linked due to a potential increase in risk of patient reidentification.

Authors' Contributions

G.J. Lindeman: Conceptualization, resources, supervision, investigation, writing–original draft, writing–review and editing. **T.M. Fernando:** Conceptualization, data curation, software, funding acquisition, validation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **R. Bowen:** Conceptualization, resources, supervision, investigation, writing–review and editing. **K.J. Jerzak:** Conceptualization, resources, investigation, writing–review and editing. **X. Song:** Conceptualization, resources, investigation, writing–review and editing. **T. Decker:** Conceptualization, resources, investigation, writing–review and editing. **F. Boyle:** Conceptualization, resources, investigation, writing–review and editing. **S. McCune:** Conceptualization, resources, investigation, writing–review and editing. **A. Armstrong:** Conceptualization, resources, investigation, writing–review and editing. **C. Shannon:** Conceptualization, resources, investigation, writing–review and editing. **G. Bertelli:** Conceptualization, resources, investigation, writing–review and editing. **C.-W. Chang:** Conceptualization, data curation, funding acquisition, validation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **R. Desai:** Conceptualization, data curation, funding acquisition, validation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **K. Gupta:** Conceptualization, data curation, software, formal analysis, funding acquisition, validation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **T.R. Wilson:** Conceptualization, data curation, funding acquisition, validation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **A. Flechais:** Conceptualization, data curation, supervision, funding acquisition, validation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **A. Bardia:** Conceptualization, resources, supervision, investigation, writing–review and editing.

Acknowledgments

The VERONICA trial was supported by F. Hoffmann-La Roche Ltd (Basel, Switzerland), the trial sponsor. The authors would like to thank all the patients, investigators, and site staff who contributed to the study. We would also like to thank Jerry Hsu, MD, PhD (formerly of Genentech, Inc.), Mehrdad Mobasher, MD, MPH (formerly of Genentech, Inc.), and Saeed Rafii, MD, PhD, FRCP (F. Hoffmann-La Roche Ltd), for their contributions during the study. Research support for third-party writing assistance for this manuscript, provided by Stephen Salem, BSc, of Health Interactions, was funded by F. Hoffmann-La Roche Ltd.

The following investigators participated in the VERONICA trial: Australia – Frances Boyle, Geoffrey J. Lindeman, Catherine Shannon; Canada – Susan Dent, Andrea Eisen, Katarzyna Jerzak, Farrah Kassam, Michel Pavic, Xinni Song, Ellen Warner; Germany – Thomas Decker, Claus Hanusch, Susanna Hegewisch-Becker, Volker Möbus, Toralf Reimer, Joachim Rom, Manfred Welslau; United Kingdom – Gianfilippo Bertelli, Gargi Patel, Sarah Khan, Peter Schmid, Anne Armstrong, Rebecca Bowen; United States – Aditya Bardia, Joseph Beck, Ursa Brown-Glaberman, Barbara Haley, Venu Konala, Ajay Kundra, Steve McCune, Madhu Midathada, Alberto Montero, Halle Moore, Donald Northfelt, Paula Rosenblatt, Rebecca Shatsky, Thomas Stanton, Frances Valdes-Albini, Manasa Vulchi, Robyn Young.

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Received November 5, 2021; revised December 16, 2021; accepted May 16, 2022; published first May 18, 2022.

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