Role of ctDNA in Predicting the Outcome of Patients with Hormone Receptor–Positive, HER2-Negative Advanced Breast Cancer Treated with First-line Ribociclib and Letrozole: BioItaLEE Trial

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ABSTRACT

Purpose: This phase IIIb study prospectively evaluated the prognostic and predictive value of baseline and dynamic circulating tumor DNA (ctDNA) in postmenopausal patients with hormone receptor–positive (HR+), human epidermal growth factor receptor 2–negative (HER2−) advanced breast cancer (ABC) treated with first-line ribociclib/letrozole.

Experimental Design: A total of 287 patients were enrolled, with ctDNA analyzed at baseline ($n = 263$), day 15 of cycle 1 (C1D15; $n = 238$), C2D1 ($n = 241$), and first imaging ($n = 206$). The primary objective was to identify ctDNA alterations, characterize their evolution across treatment time points, and assess their association with progression-free survival (PFS).

Results: Median PFS was 23.4 months (95% confidence interval, 20.8 to not estimable). At baseline, the most frequently altered genes were *PIK3CA* (22.1%) and *TP53* (15.5%). Alterations in *TP53*, *MYC*, and HER− and cyclin-dependent kinase 4/6− pathway genes were linked to early progression. Absence of a detectable mutation at baseline ($n = 150$, 57%) was associated with a better prognosis [hazard ratio (HR) = 0.41]. Among patients with a detectable mutation at baseline ($n = 104$), early clearance (mutation undetectability) was observed in 47.1% at C1D15 and 52.4% at C2D1 and was associated with improved PFS (C1D15, HR = 0.51; C2D1, HR = 0.44). In patients without a detectable mutation at baseline, 22.7% ($n = 34$) developed new mutations at C1D15, C2D1, or first imaging. Patients without new mutations had a lower risk of progression (HR = 0.45).

Conclusions: Pretreatment and early dynamics of ctDNA represent promising prognostic and predictive biomarkers in patients with HR+/HER2− ABC treated with ribociclib/letrozole. Early ctDNA dynamics seem to be a promising surrogate biomarker for treatment. Further studies are warranted to validate their clinical utility.

Introduction

The current mainstay of treatment for hormone receptor–positive (HR+), human epidermal growth factor receptor 2–negative (HER2−) advanced breast cancer (ABC) is the globally approved cyclin-dependent kinase 4/6 (CDK4/6) inhibitors, in combination with endocrine therapy (ET; refs. 1, 2). In the phase III MONALEESA program, ribociclib combined with ET versus placebo with ET demonstrated significant progression-free survival (PFS) and overall survival benefits in HR+/HER2− ABC, regardless of the ET partner, lines of therapy, or menopausal status (3–6). Despite the survival benefit, up to 30% of patients have disease progression during the first 6 months of CDK4/6 inhibitor therapy (7, 8). However, robust and clinically validated predictive biomarkers to evaluate the benefit of or resistance to CDK4/6 inhibitors are limited. Liquid biopsy represents a highly versatile diagnostic tool that allows for the molecular characterization of the disease and provides more information in the context of tumor heterogeneity and its

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Translational Relevance

Safety and efficacy of first-line ribociclib/letrozole in the phase IIIb BioItaLEE trial were consistent with those of MONALEESA-2. *FGFR* alterations were frequently found in aggressive disease, and aberrations in *TP53*, *MYC*, *HER*, and cyclin-dependent kinase 4/6 were associated with early progression. The presence of any detectable mutation, defined as somatic single-nucleotide variants or insertions/deletions identified in baseline ctDNA, was associated with an unfavorable prognosis. Approximately 50% of patients with baseline mutations experienced early variant allele frequency clearance at cycle 1 day 15, which was predictive of a better outcome. Additionally, the lack of appearance of detectable mutations at any later time points was linked to a lower risk of progression.

noninvasive dynamic monitoring (9, 10). Several circulating biomarkers, including circulating tumor DNA (ctDNA), miRNA, exosomes, and proteins, as well as metabolites and circulating tumor cells, are being investigated for their potential to identify targets for targeted therapies, predict resistance, be used in the early monitoring of the impact of treatments, and guide therapeutic decisions at progression (8, 10). ctDNA represents the most promising and mature biomarker among others to evaluate the response to CDK4/6 inhibitors (11).

BioItaLEE is a phase IIIb study, with a primary objective of identifying baseline ctDNA genomic alterations, characterizing their evolution during first-line ribociclib plus letrozole treatment in postmenopausal patients with HR+/HER2− ABC, and evaluating their association with PFS. Herein, we report the clinical relevance of baseline and dynamic ctDNA changes and the safety and efficacy outcomes of the treatment, as well as the baseline ctDNA mutational landscape.

Materials and Methods

Study design and treatment

BioItaLEE is a phase IIIb, multicenter, open-label, single-arm study (NCT03439046) conducted in Italy. Patients were treated with ribociclib 600 mg orally daily for 3 weeks on (days 1–21 of each 28-day cycle) and 1 week off (days 22–28) in combination with letrozole 2.5 mg orally once daily (Supplementary Fig. S1; core phase). Whole blood samples were prospectively collected at baseline (day 0), cycle 1 day 15 (C1D15), C2D1, the first imaging time point (at 12 weeks ± 7 days, C2D15–C6D1), and upon disease progression or at the end of treatment if interrupted for reasons other than progressive disease (PD). At progression, patients with *PIK3CA* mutations were offered a second-line treatment with alpelisib and fulvestrant at the conventional registered doses within an extension phase of the study.

Patients

The core phase included postmenopausal patients with HR+/HER2− ABC (locoregionally recurrent, not amenable to surgery, or metastatic) in the first-line setting. Patients had a histologically confirmed diagnosis of estrogen receptor (ER)–positive (≥1%) and/or progesterone receptor (PgR)–positive (≥1%), HER2− breast cancer as defined by a local laboratory according to the current American Society of Clinical Oncology/College of American Pathologists definition (12). Patients had an Eastern Cooperative Oncology Group performance status of ≤2 and adequate bone marrow and organ function. Patients with endocrine-sensitive tumors were allowed to participate, including those with *de novo* metastatic disease and those who received (neo)adjuvant ET with a disease-free interval of ≥12 months from the completion of treatment. Patients must have had no previous treatment for ABC or received ≤28 days of letrozole or anastrozole prior to enrollment. Patients with measurable and evaluable disease (including bone-only disease with at least one nonirradiated lytic lesion) were eligible for the study.

Study objectives

The primary objective was to identify ctDNA genomic alterations at baseline, characterize their evolution during first-line ribociclib plus letrozole treatment in postmenopausal patients with HR+/HER2− ABC, and evaluate their association with PFS. At baseline, single-nucleotide variants (SNV), insertion/deletion, and copy-number alterations (CNA) were assessed, and only SNVs were assessed at the following time points. The secondary objectives included the assessment of the safety and efficacy parameters [PFS, clinical benefit rate (CBR), and overall response rate (ORR)].

Sample collection and processing

Peripheral blood samples for ctDNA analysis were collected in 10 mL tubes at predefined time points (day 0, C1D15, C2D1, first imaging time point, and end of treatment) and were processed according to standardized procedures outlined in the laboratory manual. All samples were shipped to a central laboratory for analysis.

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Genetic alterations, variant analyses, assessments, and schedule

ctDNA from plasma was extracted using the QIAamp Circulating Nucleic Acid Kit (QIAGEN). The extracted ctDNA was used for library preparation using the ThermoFisher Ion AmpliSeq Library Kit HD according to the manufacturer's procedure. Molecular barcodes were linked to each initial DNA molecule to increase the confidence in mutation calling with respect to amplification errors.

After library preparation and enrichment, sequencing was performed on a ThermoFisher S5 Sequencer. Binary Alignment Map (BAM) files generated from the sequencing were automatically uploaded to the ThermoFisher Ion Reporter server version 5.12 for data analysis.

Genomic alterations in ctDNA were assessed using a 533-amplicon Custom AmpliSeqHD Panel with amplicons covering the selected hotspot areas of the coding exons of 39 breast cancer–related genes (Supplementary Table S1). Variants were analyzed as both individual genes and as predefined pathways (Supplementary Table S1). The CNA status of 12 genes was analyzed by the Oncomine Pan-Cancer Cell-Free Assay (Thermo Fisher Scientific) panel only on baseline samples. For the purpose of assessing ctDNA dynamic changes, mutations were defined as SNV or insertion/deletion detected at baseline. When multiple mutations were detected, the one with the highest variant allele frequency (VAF) was considered. VAF clearance was defined as a 100% decrease in a mutation (mutation not detectable at the following time point).

Tumor assessments were carried out every 12 weeks by local investigators according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria. Investigators were blinded to all biomarker assessments until after the completion of the analysis to minimize potential bias.

Statistical analysis

The intent-to-treat (ITT) population included all eligible patients who received ≥1 dose of either ribociclib or letrozole, and the biomarker population included only patients who had ≥1 valid baseline ctDNA sample (Supplementary Fig. S2). All the analyses performed in this study were carried out without adjustment for multiple testing due to the exploratory objective of the trial, which is descriptive in nature.

The efficacy evaluation (PFS, ORR) was performed in the ITT population. The association between baseline predefined genomic groups (genes altered in ≥3% of patients, groups including patients with ≥1 genetic alteration vs. the presence of ≥2 genetic alterations, pathway alterations) and several clinicopathologic variables was assessed using the type III sum of squares of the Wald χ^2 test in the biomarker population. The association between ctDNA assessments and PFS was evaluated using multivariate Cox models adjusted for the type of metastatic occurrence (recurrent vs. *de novo*), tumor type (luminal B–like vs. luminal A–like), absence or presence of visceral metastases, and the number of organs involved by metastases (≥3 vs. <3). Luminal A–like and luminal B–like were defined using locally assessed IHC variables (luminal A–like: Ki67 <20%, ER-positive, PgR ≥20%, HER2− or Ki67 <20%, ER-negative, PgR ≥20%, HER2−; Luminal B–like: Ki67 ≥20% or PgR <20%).

Ethics

The study was conducted in accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Harmonized Tripartite Guidelines, the Good Clinical Practice guidelines, the principles of the Declaration of Helsinki, and applicable local regulations (including the European Directive 2001/20/European Commission and the United States Code of Federal Regulations Title 21). The study-related protocol was approved by the Independent Ethics Committee. Written informed consent was obtained from all patients before conducting any study-specific procedures.

Results

Patient characteristics

From February to December 2018, 287 postmenopausal women were enrolled across 47 study centers in Italy to represent the ITT population (**Table 1**). The biomarker population included 263 patients (91.6%); seven patients were excluded due to protocol deviations that affected the analysis population, and 17 patients did not have a sample collected at baseline (**Table 1**; Supplementary Fig. S2). The median follow-up was 26.9 months (data cutoff: October 15, 2020). No significant differences were observed between the ITT and biomarker populations. In the ITT population, the median age was 65 years, and luminal B–like was the most represented molecular subtype (64.5%). Patients with *de novo* metastatic disease represented 39.7% of the ITT population, and visceral metastases were present in 44.3% of patients, with 18.8% having ≥3 metastatic sites.

Genomic alterations in ctDNA at baseline

At least one CNA or SNV alteration was detected in 51% of patients, and more than one alteration was observed in 28% of patients. The co-occurrence and types of genomic alterations found in the baseline ctDNA of patients who had at least one alteration are represented in the oncoplot (**Fig. 1A**). The following genetic alterations (either CNA or SNV) were found in >3% of patients: *PIK3CA* (22.1%), *TP53* (15.5%), *FGFR1* (6.6%), *CCND1*, and *CCND2* (4.8% each), *KMT2C* (4.4%), *MYC* (4.1%), *CDK4* (3.7%), and *AKT1* and *PTEN* (3.3% each; **Fig. 1A**). No genomic alterations were found in the following genes: *CCNE1*, *ERBB3*, *HRAS*, *MLH1*, *MSH2*, *NF1*, *NOTCH1*, *RET*, *RUNX1*, and *SRC*. The functional clusters or pathways of the genes most frequently altered in baseline samples (more than 10% of patients with genomic alterations) were *PIK3CA* (26.9%), *TP53*, DNA repair, cell cycle–related, receptor tyrosine kinases, CDK4/6, and ER nuclear function (ERnf) pathways (**Fig. 1B**).

Correlation between genomic alterations at baseline and clinicopathologic variables

Mutations in *KMT2C* or alterations in genes belonging to the ERnf pathway (*KMT2C*, *ESR1*, *GATA3*, and *MYC*) were more frequently altered in patients with recurrent versus *de novo* disease. Mutations in *KMT2C* were also more frequent in patients with PgR <20% versus those with PgR ≥20% (**Tables 2** and **3**). Additional details are provided in Supplementary Data S1.

Copy-number gain of *FGFR* genes (*FGFR1*, *FGFR2*, and *FGFR3*) was more frequent in patients with visceral metastases ($P = 0.0144$) and in patients with ≥3 metastatic sites ($P = 0.0052$). *MYC* copy-number gains or alterations in the *ERnf* genes were more frequent in ER+/PgR− tumors versus ER+/PgR+ tumors, and alterations in *ERnf* genes were also more frequent in patients with Ki67 ≥14%.

Alterations in the genes of the CDK4/6− (*CCND1*, *RB1*, *CDK4* and *CDK6*, *CDKN2A*) and HER− family (*EGFR*, *ERBB2*, and *ERBB4*) pathways were associated with a significantly higher frequency of PD at the first tumor evaluation. With regard to single-

Table 1. Patient disposition, demographics, and disease characteristics.

Variables	Biomarker population (*N* = 263)	ITT population (*N* = 287)
Age (median), years (range)	66 (47–86)	65 (47–86)
Age category, years, *n* (%)		
<70	170 (64.6)	189 (65.9)
ECOG performance status		
0	191 (72.6)	205 (71.4)
1	68 (25.9)	77 (26.8)
2	4 (1.5)	5 (1.7)
Tumor type,[a] *n* (%)		
Luminal A–like	74 (28.1)	83 (28.9)
Luminal B–like	173 (65.8)	185 (64.5)
Unknown	16 (6.1)	19 (6.6)
De novo disease,[b] *n* (%)	105 (39.9)	114 (39.7)
Metastatic sites, *n* (%)		
Bone	193 (73.4)	206 (71.8)
Bone only	62 (23.6)	64 (22.3)
Visceral	114 (43.3)	127 (44.3)
Liver	36 (13.7)	41 (14.3)
Lung	87 (33.1)	96 (33.5)
Liver and lung	105 (39.9)	117 (40.8)
Other visceral	17 (6.5)	18 (6.3)
Number of organs affected by metastases, *n* (%)		
1	99 (37.6)	107 (37.3)
2	113 (43)	124 (43.2)
≥3	50 (19)	54 (18.8)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; eCRF, electronic case report form.
[a]Luminal A–like: Ki67 <20%, ER-positive, PgR ≥20%, HER2-negative, or Ki67 <20%, ER-negative, PgR ≥20%, HER2-negative. Luminal B–like: Ki67 ≥20% or PgR <20%.
[b]*De novo* patients are defined as patients with the "date of first recurrence/progression" information blank in the "Diagnosis and extent of cancer" eCRF page.

gene alterations, both *MYC* gains and *TP53* mutations were significantly associated with PD at first imaging.

ctDNA levels across study time points and the association with clinical outcomes

In the biomarker population, we evaluated serial ctDNA at C1D15 ($n = 238$, 90.5%), C2D1 ($n = 241$, 91.6%), and first imaging ($n = 201$, 76.4%; Supplementary Fig. S2). A graphical representation of the distribution of VAF in patients with ≥1 detectable mutation at baseline across early time points (first cycle) is displayed in **Fig. 2A**. The mean pretreatment VAF at baseline was 11.3%. A significant VAF reduction compared with baseline was observed at C1D15 [mean (SD) −64.3% (55.9), $P < 0.0001$] and at C2D1 [−68.6% (52.2), $P < 0.0001$; **Fig. 2A**]. Early VAF clearance of mutations was observed in 47.1% ($n = 49/104$) and 52.4% ($n = 55/105$) of patients at C1D15 and C2D1, respectively (**Fig. 2A**). Overall, the average VAF was similar at C1D15 and C2D1 although variance at the individual patient level was observed, with a significant decrease in the paired comparison ($P < 0.001$).

At baseline, mutations were detected in 113 patients (43%), whereas in 150 patients (57%), there were no detectable mutations (**Fig. 2B**). Patients with no detectable mutations at baseline had a significantly lower risk of progression versus patients with detectable mutations [hazard ratio (HR) = 0.41; 95% confidence interval (CI), 0.27–0.61; $P = 0.0001$; **Fig. 2B**]. The median PFS (mPFS) was not estimable (NE) and was 16.59 months in patients without or with detectable mutations, respectively (**Fig. 2B**).

Among 104 patients with evaluable ctDNA at baseline and C1D15, VAF clearance was observed in 49 (47.1%), whereas 55 patients (52.9%) retained detectable mutations. Patients with VAF clearance at C1D15 had a significantly lower risk of progression versus those without VAF clearance (HR = 0.51; 95% CI, 0.28–0.91; $P = 0.0228$), corresponding to a PFS of 21.85 versus 12.09 months (**Fig. 2C**).

In a similar analysis of patients with evaluable ctDNA at baseline and C2D1 ($n = 105$), VAF clearance ($n = 55$, 52.4%) was associated with better PFS (HR = 0.44; 95% CI, 0.25–0.78; $P = 0.0052$). Considering groups with VAF clearance versus those without at C2D1, the mPFS was 22.11 and 12.32 months, respectively (**Fig. 2D**).

About 34 patients (22.7%) had detection of ≥1 alteration at one of the early time points: C1D15, C2D1, and first imaging. The absence of detectable mutations at all post-baseline time points was associated with a significantly lower risk of progression (HR = 0.45; 95% CI, 0.24–0.85; $P = 0.0143$; **Fig. 3A**).

We assessed whether ctDNA at C1D15 alone may provide predictive information, avoiding repeated ctDNA assessments. Patients with available samples at C1D15 and valid ctDNA results ($n = 238$) were included, and three groups were defined as follows: (i) no detectable mutations at C1D15 ($n = 159$, 66.8%), (ii) mutated with low VAF at C1D15 (below median value; $n = 39$, 16.4%), and (iii) mutated with high VAF at C1D15 (above median value; $n = 40$, 16.8%; **Fig. 3B**). These three groups displayed significantly different clinical outcomes ($P < 0.001$). Compared with patients with a

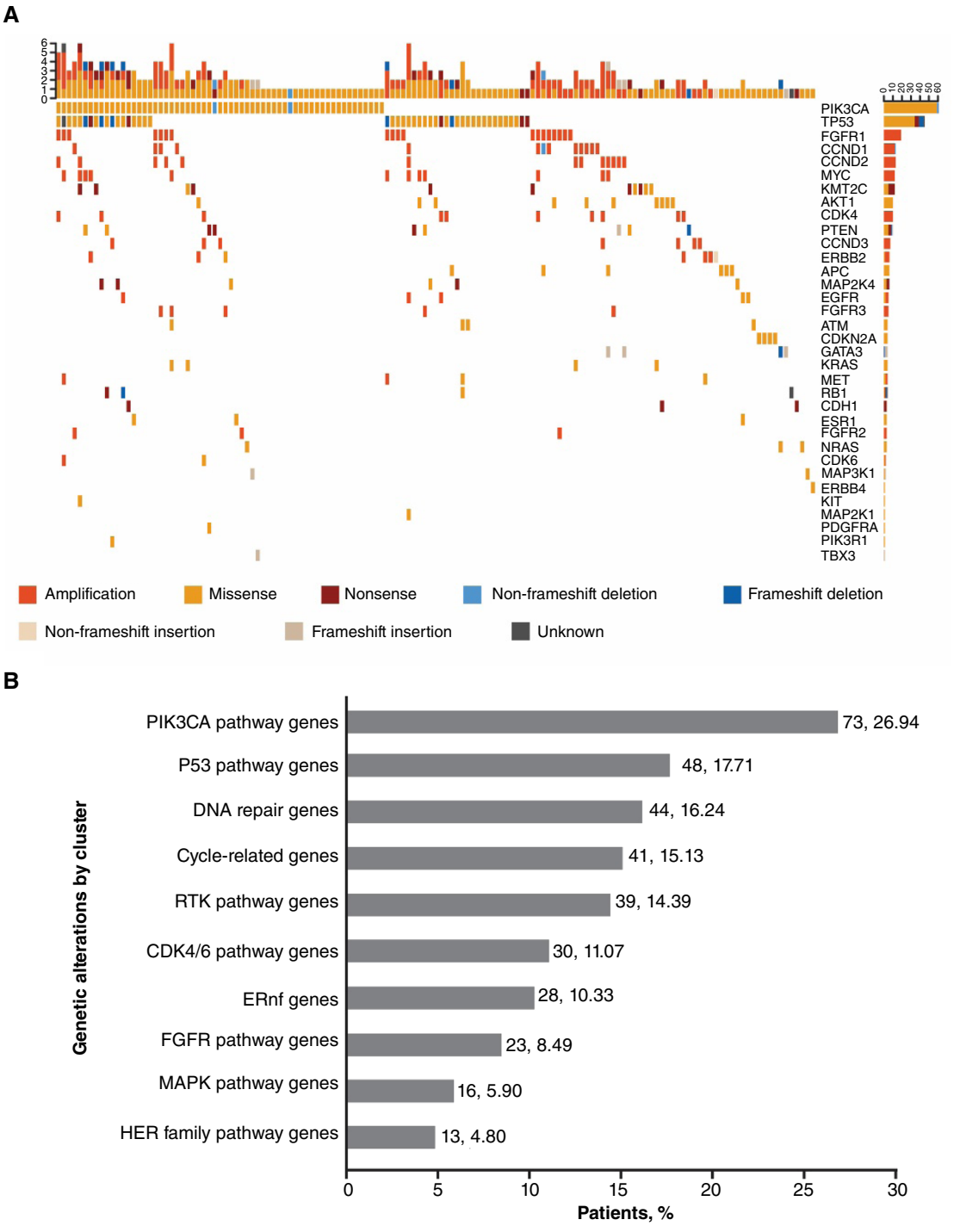

Figure 1.

Genomic landscape at baseline considering single gene and frequency of gene clusters/pathways alteration using blood samples. **A,** Oncoplot: pattern and type of genomic alterations at baseline, including only patients with at least one genomic alteration. **B,** Frequency of genomic alterations at baseline by functional cluster or pathways of genes. RTK, receptor tyrosine kinase.

detectable mutation and high VAF (mPFS = 11.07 months), patients without a detectable mutation at C1D15 showed a favorable outcome (mPFS = NE; HR = 0.32; 95% CI, 0.20–0.51; $P < 0.0001$), and patients with a detectable mutation and low VAF showed an intermediate outcome (mPFS = 16.53 months; HR = 0.56; 95% CI, 0.30–1.04; $P = 0.065$; **Fig. 3B**).

Table 2. Significant correlations between detection of at least one genomic alteration in a gene/pathway at baseline and clinicopathologic variables.

Clinicopathologic variables	≥1 Genomic alterations *n* (%)	No genomic alterations *n* (%)	*P* value[a]
ERnf genes in			
De novo	6 (5.5)	103 (94.5)	
Recurrent	22 (13.6)	140 (86.4)	0.0381
ERnf genes in			
Ki67 <14%	2 (2.9)	68 (97.1)	
Ki67 ≥14%	22 (12.2)	159 (87.9)	0.0396
ERnf genes in			
ER+ and PgR−	9 (22.5)	31 (77.5)	
ER+ and PgR+	18 (8)	208 (92)	0.0073
KMT2C in			
De novo	1 (0.9)	108 (99.1)	
Recurrent	11 (6.8)	151 (93.2)	0.0499
KMT2C in			
PgR <20%	7 (8.9)	72 (91.1)	
PgR ≥20%	5 (2.7)	178 (97.3)	0.0392
MYC in			
ER+ and PgR−	4 (10)	36 (90)	
ER+ and PgR+	6 (2.7)	220 (97.4)	0.0361
FGFR pathway genes in			
1 metastatic organ	3 (2.9)	99 (97.1)	
2 metastatic organs	9 (7.8)	107 (92.2)	
≥3 metastatic organs	10 (19.6)	41 (80.4)	0.0052
FGFR pathway genes in			
Bone only	2 (3.1)	62 (96.9)	
Visceral excluded	4 (4.4)	86 (95.6)	
Visceral included	17 (14.5)	100 (85.5)	0.0144

All genes and clinicopathologic characteristics were analyzed, and the variables presented in this table were selected based on their potential relevance for hypothesis generation. Univariate logistic models were performed to evaluate the correlation between pathway/gene and clinicopathologic variables/early progression, and no multivariate analysis was performed.
[a]Only results with an unadjusted *P* value < 0.05 (without correction for multiple testing) are reported in this table in the *post hoc* analysis.

Efficacy and safety

In the ITT population, the mPFS was 23.4 months (95% CI, 20.8 to NE; Supplementary Fig. S3A). For the first imaging time point, we considered the 225 patients who underwent the first imaging evaluation at the recommended time (12 weeks); PD did not preclude patients from the biomarker population. At first

Table 3. Significant correlations between detection of at least one genomic alteration in a gene/pathway at baseline and early progression (first imaging).

Clinicopathologic variables	≥1 Genomic alterations *n* (%)	No genomic alterations *n* (%)	*P* value[a]
Early progression with			
HER family pathway genes in			
PD	3 (30)	19 (8.9)	0.0426
CR + PR + stable disease	7 (70)	195 (91.1)	
MYC in			
PD	3 (33.3)	19 (8.8)	
CR + PR + stable disease	6 (66.7)	196 (91.2)	0.028
TP53 in			
PD	8 (23.5)	14 (7.4)	0.0058
CR + PR + stable disease	26 (76.5)	176 (92.6)	
CDK4/6 pathway genes in			
PD	6 (22.2)	16 (8.1)	0.0272
CR + PR + stable disease	21 (77.8)	181 (91.9)	

All genes were analyzed, and the variables presented in this table were selected based on their potential relevance for hypothesis generation. Univariate logistic models were performed to evaluate the correlation between pathway/gene and clinicopathologic variables/early progression, and no multivariate analysis was performed.
[a]Only results with an unadjusted *P* value < 0.05 (without correction for multiple testing) are reported in this table.

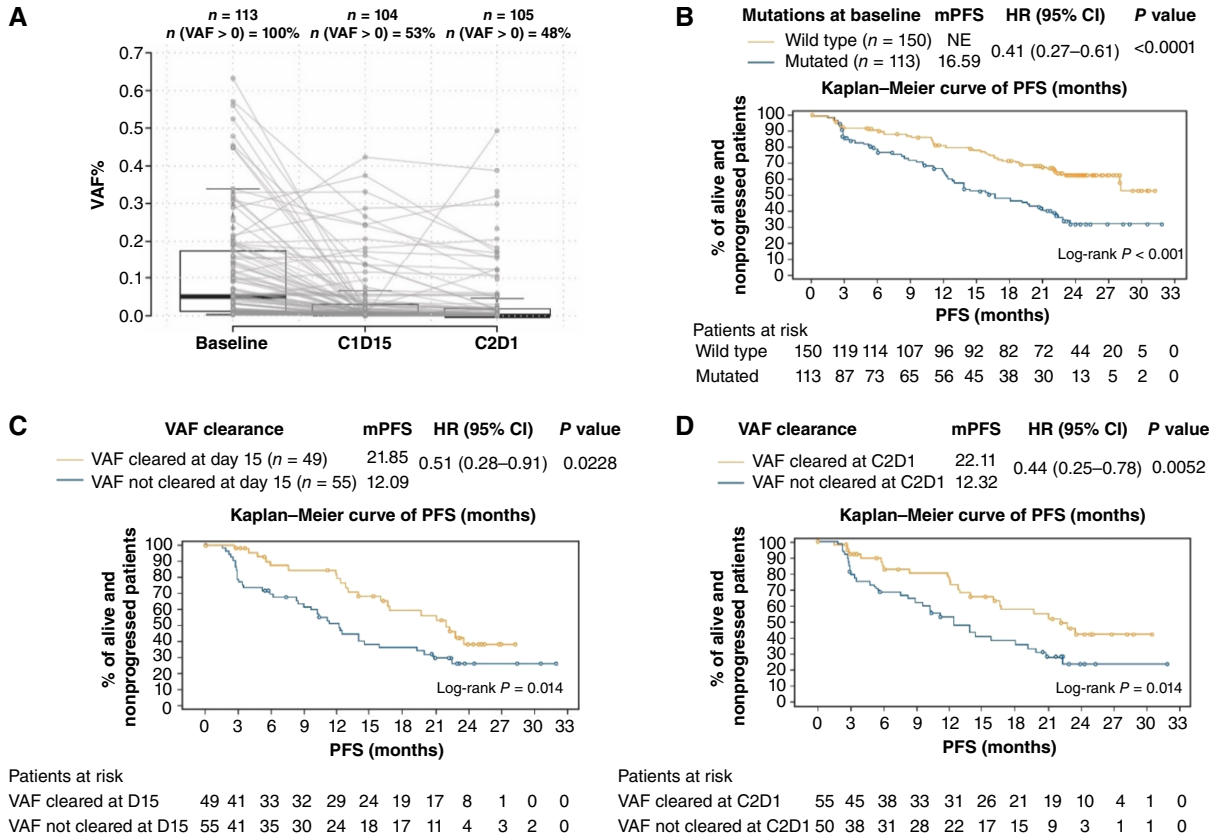

Figure 2.

VAF levels across study time points and correlation between PFS and ctDNA levels assessed at different time points. **A,** Box plots showing VAF of mutations detected at baseline and the paired VAF dynamics at C1D15 and C2D1 time points. **B,** Kaplan–Meier estimates of PFS for patients with or without mutations at baseline. **C,** Kaplan–Meier estimates of PFS for patients with or without VAF cleared at C1D15; analysis performed on patients with matched baseline and C1D15. **D,** Kaplan–Meier estimates of PFS for patients with or without VAF cleared at C2D1; analysis performed on patients with matched baseline and C2D1 samples. The mutation at baseline refers to the hotspot mutation detected at screening with the highest molecular frequency, specifically those with a frequency greater than 1%. This definition excludes SNPs and ensures consistency across analyses. **C,** includes patients with at least one hotspot mutation detected at baseline and valid ctDNA results at C1D15—total $N = 104$; (**D**) patients with at least one hotspot mutation detected at baseline and valid ctDNA results at C2D1—total $N = 105$.

imaging, the CBR, defined as complete response (CR) + partial response (PR) + stable disease, was 89.8% ($n = 202$), with only 10.2% ($n = 23$) of patients showing early PD and 16.4% demonstrating a response at this early time of assessment (37/225; 5 CR and 32 PR). ORR and CBR at 24 weeks were achieved in 35.9% and 72.3%, respectively (Supplementary Table S2), with a median duration of response that was not reached (95% CI, 22.8 to not reached). A total of 80.65% ($n = 125/155$) of patients with measurable disease had tumor shrinkage (Supplementary Fig. S3B).

Overall, 15.7% of patients discontinued the study drug (ribociclib) due to adverse events (AE); 6.3% of patients had severe AEs (SAE). Of the 10 fatal SAEs, ABC was identified as the cause of death in four cases. In the remaining six cases, three (1%) were indicated as being possibly related to ribociclib [respiratory failure ($n = 1$), pneumonia ($n = 1$), and undetermined death ($n = 1$)], and three were reported as not related to ribociclib (Supplementary Table S3). The most common (≥10% of patients) treatment-emergent AEs are reported in Supplementary Table S4.

Discussion

ctDNA analysis is emerging as a promising noninvasive approach to characterize tumor biology, track its evolution over time, predict treatment benefit, and guide therapeutic strategies (13–15). Although validated ctDNA assays for detecting actionable alterations such as *PIK3CA* or *ESR1* mutations are now part of standard care in ABC, the use of ctDNA for treatment monitoring is not yet endorsed by international guidelines due to insufficient evidence supporting its clinical validity (14, 16). In this context, prospective trials are essential to define the role of ctDNA in guiding treatment decisions.

The phase IIIb BioItaLEE trial was designed to prospectively evaluate how genomic alterations detected by ctDNA evolve during treatment and how these changes correlate with clinical outcomes. Successful ctDNA analysis in more than 90% of patients at three early time points demonstrated the feasibility of implementing dynamic ctDNA monitoring in large-scale clinical trials. Consistent with pooled analyses from the MONALEESA program (17), baseline ctDNA alterations were associated with poor prognosis, independent

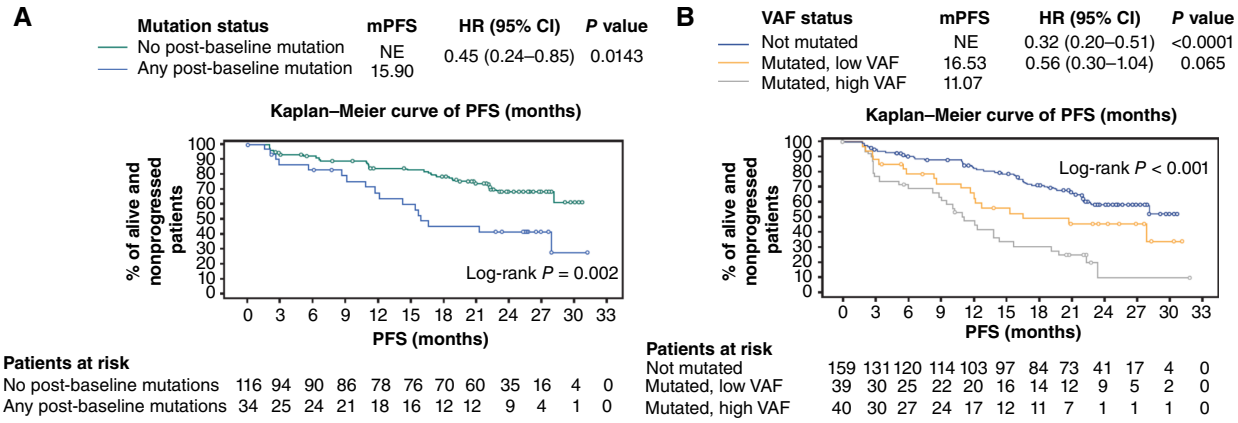

Figure 3.

Correlation between PFS and ctDNA levels assessed at different time points. **A,** Kaplan–Meier estimates of PFS in patients with no vs. any post-baseline mutation status. **B,** Kaplan–Meier estimates of PFS in patients without detectable mutations at C1D15, detection of at least one mutation at C1D15 with low VAF, and detection of at least one mutation at C1D15 with high VAF.

of clinicopathologic features. This association has also been described in other metastatic solid tumors (18, 19). Although surrogate luminal subtypes were included in multivariate models, further studies are needed to assess the independent prognostic value of baseline ctDNA within PAM50-defined subtypes, particularly luminal and HER2-enriched, which seem to derive the most benefit from ribociclib (20). Although prognostic factors in metastatic disease do not have clear clinical utility, the strong prognostic value of baseline ctDNA could be considered an additional stratification factor in ABC trials.

Patients with detectable mutations at baseline exhibited marked ctDNA dynamics as early as 2 weeks into treatment and at C2D1. At these time points, approximately half of these patients achieved ctDNA clearance, which was associated with a significantly lower risk of progression. The results also showed that early ctDNA dynamic changes may indeed help further prognostic stratification within the group of patients with detectable mutations at baseline. These findings align with results from the PALOMA-3 trial, in which early reduction in *PIK3CA* VAF predicted better outcomes (21). Our data further suggest that both ctDNA clearance and the degree of VAF reduction at C1D15 provide prognostic information, supporting a continuous rather than binary interpretation of ctDNA response. This is consistent with emerging frameworks such as the liquid biopsy RECIST criteria 3 and findings from other malignancies (15).

Although the absence of a placebo arm limits definitive conclusions about predictive value, the observed associations support the potential of early ctDNA dynamics as surrogate biomarkers of treatment benefit (22). This is further supported by data from the SERENA-2 trial, in which early ctDNA changes predicted the superiority of camizestrant over fulvestrant (23). Similar predictive utility has been proposed in non–small cell lung cancer (18). Our findings reinforce the rationale for incorporating ctDNA dynamics into early-phase drug development, in line with recent FDA guidance (24). The absence of detectable mutations in nearly half of patients at baseline likely reflects the low abundance of ctDNA in the BioItaLEE study setting (patients with metastatic luminal breast cancer undergoing first-line treatment) and the intrinsic sensitivity of the methodology used. Nonetheless, our findings align with prior studies (6, 17). More sensitive sequencing approaches may be needed to fully capture the mutational landscape in this patient population.

The use of next-generation sequencing enabled broad mutation profiling without restricting analysis to specific alterations. This approach expands the applicability of ctDNA monitoring although the clinical relevance of dynamic changes in certain mutations—such as *ESR1*—may be limited (21). Evaluation at three early time points enhanced the robustness of the analysis. Although C1D15 was strongly predictive, interpatient variability between C1D15 and C2D1 suggests that both time points may offer complementary insights, warranting further investigation in larger cohorts.

The emergence of new mutations by first imaging in patients without baseline mutations was associated with worse outcomes, highlighting the potential of ctDNA for ongoing tumor monitoring. Future strategies may include personalized gene panels that track baseline and resistance-associated mutations, such as *ESR1*, to inform adaptive treatment strategies (25). The most frequent baseline alterations—*PIK3CA*, *KMT2C*, and *ERnf*—were consistent with prior studies (26, 27). We acknowledge the broad biological role of KMT2C, which transcends the scope of ERnf (28). Alterations in TP53, MYC, and HER−/CDK4/6− pathway genes were enriched in patients with early progression, underscoring their known roles in resistance to ET and CDK4/6 inhibition (29–31). The safety and efficacy of ribociclib plus letrozole in BioItaLEE were consistent with findings from MONALEESA-2 and CompLEEment-1 (4, 32). A key strength of BioItaLEE was its high rate of blood sample collection across multiple time points, enabling robust ctDNA analysis. However, the absence of a monotherapy control arm limits the interpretation of prognostic versus predictive effects.

Conclusions

Overall, the BioItaLEE study illustrates different types of information that can be obtained from ctDNA assessment in a prospective ABC trial. Specifically, the trial describes the molecular landscape of tumors at study entry, the potential correlation of genomic alterations in individual genes or pathways

with prognosis and treatment benefit, and the early assessment of ctDNA dynamic changes as a predictive biomarker. Moreover, by monitoring the appearance of target alterations before the established clinical progression, the BioItaLEE trial is a proof-of-concept study suggesting a role for molecular-based monitoring of tumor progression with the potential to inform treatment decisions at times of clinical progression. These findings not only identify potential prognostic and predictive biomarkers for the ribociclib/letrozole combination but also pave the way for further trials designed to validate the clinical utility of ctDNA as a surrogate marker to enhance and expedite drug development for advanced-stage solid tumors.

Data Availability

The datasets generated or analyzed during this study are not publicly available. Novartis is committed to sharing with qualified external researchers access to patient-level data and supporting clinical documents from eligible studies. These requests are reviewed and approved on the basis of scientific merit. All data that may be provided will be anonymized to respect the privacy of patients who have participated in the trial, in line with applicable laws and regulations. The data may be requested from the corresponding author of the article.

Authors' Disclosures

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Note

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

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