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Genomic Profiling of Premenopausal HR+ and HER2– Metastatic Breast Cancer by Circulating Tumor DNA and Association of Genetic Alterations With Therapeutic Response to Endocrine Therapy and Ribociclib

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abstract

PURPOSE This analysis evaluated the genomic landscape of premenopausal patients with hormone receptor–positive and human epidermal growth factor receptor 2–negative advanced breast cancer and the association of genetic alterations with response to ribociclib in the phase III MONALEESA-7 trial.

METHODS Premenopausal patients were randomly assigned 1:1 to receive endocrine therapy plus ribociclib or placebo. Plasma collected at baseline was sequenced using targeted next-generation sequencing for approximately 600 relevant cancer genes. The association of circulating tumor DNA alterations with progression-free survival (PFS) was evaluated to identify biomarkers of response and resistance to ribociclib.

RESULTS Baseline circulating tumor DNA was sequenced in 565 patients; 489 had evidence of ≥ 1 alteration. The most frequent alterations included *PIK3CA* (28%), *TP53* (19%), *CCND1* (10%), *MYC* (8%), *GATA3* (8%), receptor tyrosine kinases (17%), and the Chr8p11.23 locus (12%). A treatment benefit of ribociclib was seen with wild-type (hazard ratio [HR] 0.45 [95% CI, 0.33 to 0.62]) and altered (HR 0.57 [95% CI, 0.36 to 0.9]) *PIK3CA*. Overall, patients with altered *CCND1* had shorter PFS regardless of treatment, suggesting *CCND1* as a potential prognostic biomarker. Benefit with ribociclib was seen in patients with altered (HR 0.21 [95% CI, 0.08 to 0.54]) or wild-type (HR 0.52 [95% CI, 0.39 to 0.68]) *CCND1*, but greater benefit was observed with altered, suggesting predictive potential of *CCND1*. Alterations in *TP53*, *MYC*, Chr8p11.23 locus, and receptor tyrosine kinases were associated with worse PFS, but ribociclib benefit was independent of alteration status.

CONCLUSION In this study—to our knowledge, the first large study of premenopausal patients with hormone receptor–positive and human epidermal growth factor receptor 2–negative advanced breast cancer—multiple genomic alterations were associated with poor outcome. A PFS benefit of ribociclib was observed regardless of gene alteration status, although in this exploratory analysis, a magnitude of benefits varied by alteration.

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ASSOCIATED CONTENT

Data Supplement

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INTRODUCTION

Breast cancer is the leading cause of cancer-related death in women worldwide.[1] Approximately 20% of breast cancer cases in the United States are diagnosed in women under 50 years old.[2] Globally, breast cancer comprises almost half of all cancer diagnoses in women under 50 years and the incidence of advanced breast cancer (ABC) in premenopausal women is increasing.[3]

Approximately 65% of breast cancer cases in women under 50 years old are hormone receptor-positive (HR+) and human epidermal growth factor receptor 2–negative (HER2–).[4] Premenopausal women with HR+ tumors, especially younger women, typically have a worse prognosis and are under-represented in clinical trials compared with postmenopausal women.[3,5,6] Consequently, treatment approaches for premenopausal women with HR+ and HER2– ABC are usually extrapolated from data for postmenopausal patients.

To date, there is a lack of biomarker profiling, especially genomic profiling of premenopausal ABC, and the limited biomarker data available have been specific to the early disease setting.[7] Previous gene

CONTEXT

Key Objective

Premenopausal patients with hormone receptor–positive (HR+) and human epidermal growth factor receptor 2–negative (HER2–) advanced breast cancer (ABC) typically have a worse prognosis compared with postmenopausal women. Here, we sought to characterize genomic alterations detectable in plasma circulating tumor DNA and to determine their relationship with ribociclib treatment benefit in premenopausal patients with HR+ and HER2– ABC in the MONALEESA-7 trial.

Knowledge Generated

A progression-free survival benefit was observed with ribociclib treatment regardless of genomic alteration status, although a magnitude of benefits varied on the basis of specific alterations. Several were associated with a worse outcome in premenopausal patients with HR+ and HER2– ABC including alterations in *TP53*, *MYC*, Chr8p11.23 locus, and receptor tyrosine kinases.

Relevance

Understanding the impact of genomic alterations on prognosis or sensitivity to therapies could potentially inform treatment decisions in premenopausal patients with HR+ and HER2– ABC, and further confirmatory studies are warranted for clinical utility.

expression, whole-exome, and transcriptome studies of early breast cancer from pre- and postmenopausal HR+ patients have shown that the molecular characteristics of premenopausal tumors are distinct from those of postmenopausal tumors.[7] Understanding the genomic landscape of HR+ and HER2– ABC in premenopausal patients could inform treatment strategies in this group.

Ribociclib, a cyclin-dependent kinase (CDK) 4/6 inhibitor, is established as an effective treatment for many patients with HR+ and HER2– ABC; however, there are patients who exhibit de novo and/or acquired resistance to CDK4/6 inhibitors or endocrine therapy (ET), which may result from several mechanisms.[8,9] ET resistance can be driven by dysregulation of the estrogen receptor (ER) pathway via modulation of receptor tyrosine kinase (RTK) signaling, which affects ER activity; alteration of *GATA3*, which can drive aberrant ER-mediated transcriptional activities; or dysregulation of FGFR1 signaling.[10-17] Additionally, a study profiling the genomic landscape of endocrine-resistant ABCs indicated that genes involved in ER transcriptional machinery, including *MYC*, were enriched in endocrine-resistant tumors.[9] Because various mechanisms can cause resistance to ET or CDK4/6 inhibition,[8] identifying biomarkers predictive of sensitivity to these therapies can inform treatment decisions and future research. Thus, a key objective has been to identify biomarkers of resistance or response to ribociclib.

To our knowledge, the phase III MONALEESA-7 trial was the first trial of a targeted therapy performed exclusively in premenopausal patients with HR+ and HER2– ABC. The results of this trial demonstrated that treatment with ribociclib plus ET resulted in significantly longer median progression-free survival (PFS) and overall survival versus ET alone.[18,19] Here, we report the results from the first (to our knowledge) analysis dedicated to characterizing the genomic landscape of premenopausal patients with HR+ and HER2– ABC. In this analysis, we evaluated molecular alterations detected in circulating tumor DNA (ctDNA) collected at baseline and their impact on PFS in MONALEESA-7. This is the largest data set of premenopausal patients with ABC receiving first-line endocrine-based therapy in the metastatic setting.

METHODS

Trial and Patients

The study population consisted of premenopausal patients enrolled in MONALEESA-7, the details of which have been previously published.[18,19] In brief, MONALEESA-7 is a randomized, placebo-controlled, international, double-blind, phase III study evaluating ribociclib plus ET and goserelin versus placebo plus ET with goserelin in pre- or perimenopausal women with HR+ and HER2– ABC. The primary end point was investigator-assessed PFS, and the key secondary end point was overall survival; the results for these analyses were previously reported.[18,19] In this analysis, PFS was defined as the time from random assignment to the first documented progression per local radiology assessment (RECIST version 1.1) or death from any cause. Genomic profiling by next-generation sequencing was an exploratory end point to characterize molecular alterations in ctDNA and correlate these alterations with outcomes in MONALEESA-7.

Biomarker Sample Collection and Assessment of Genetic Alterations

Baseline (before study treatment initiation) plasma samples were collected from 632 patients, and cell-free DNA was extracted. Total extracted cell-free DNA was used to generate next-generation sequencing libraries, which were enriched for a specific 2.9-Mb region of the human genome designed to contain approximately 600 genes relevant to cancer. Single-nucleotide variants were identified using

MuTect.[20] Copy number variants were called using PureCN, and indels were called using PINDEL.[21,22] Germline mutations and artifacts were filtered out using publicly available databases dbSNP and ExAC and an internal database (Novartis Institutes for Biomedical Research) of normal circulating free DNA samples from healthy individuals without cancer.

Statistical Analysis

Correlative analyses with PFS were performed for genes altered in ≥ 8% of patients (leading to approximately 20 patients/arm), RTK genes, and the Chr8p11.23 amplicon (*ZNF703*, *WHSC1L1*, and *FGFR1*). Alterations in RTKs (*FGFR1*, *EGFR*, *ERBB2*, *ERBB3*, *ERBB4*, *IGF1R*, *IGF1*, *KDR*, *KIT*, *PDGFRA*, *PDGFRB*, and *VEGFA*) were grouped to evaluate infrequently altered genes with similar downstream signaling. The Chr8p11.23 amplicon was analyzed after a strong overlap of *ZNF703*, *WHSC1L1*, and *FGFR1*, which are known to be on the same amplicon, was observed.

For each gene or group in ctDNA, patients were classified as altered if ≥ 1 alteration, defined as the presence of a copy number alteration, short insertion or deletion, or mutation, was detected and as wild-type (WT) if no alterations were detected (excluding patients with zero alterations detected per assay limitation). Kaplan-Meier curves were generated, and median PFS (95% CI) was estimated by treatment and biomarker status. A Cox proportional hazards (PH) model was used to estimate the hazard ratios (HRs) of treatment benefit (ribociclib *v* placebo) for PFS by biomarker status. To test for a difference in treatment benefit by biomarker status, a gene-treatment interaction term was included in a Cox PH model. Cox PH models were stratified by the presence of liver

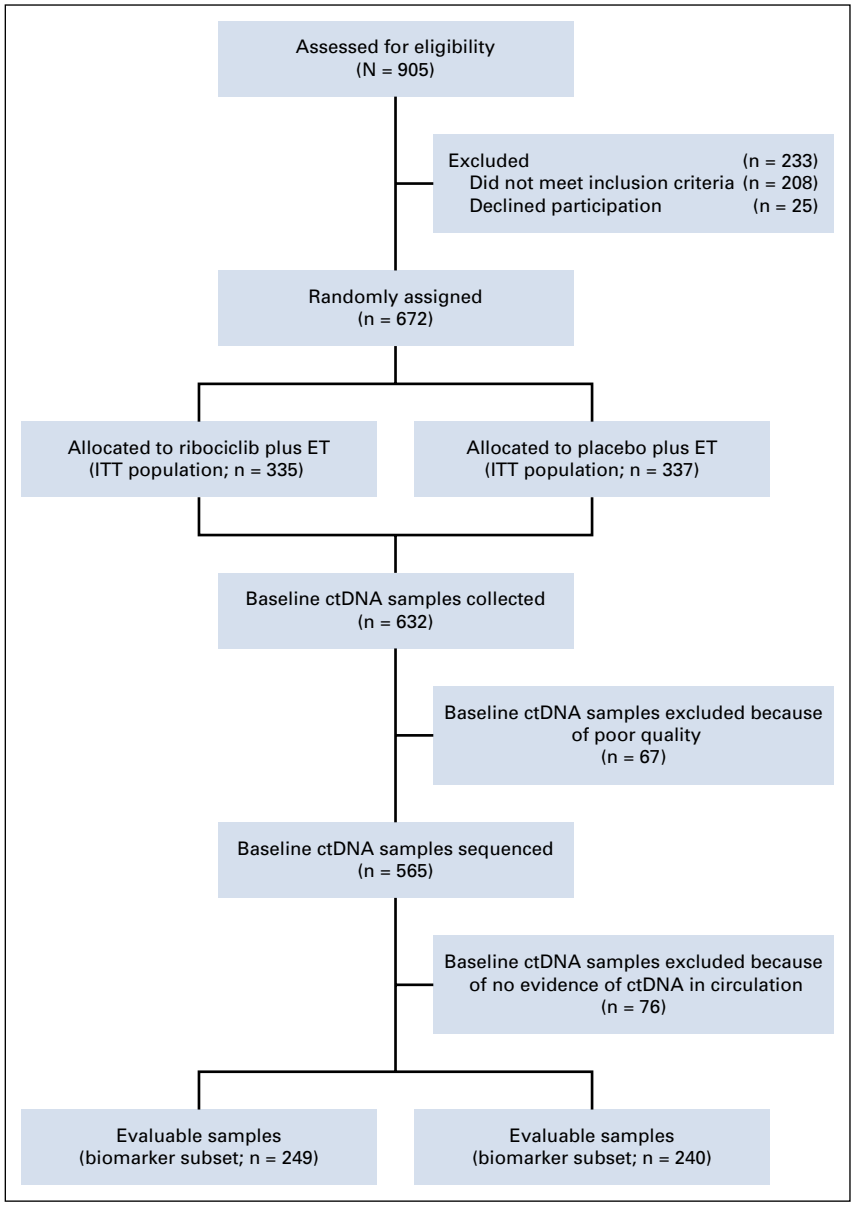


FIG 1. CONSORT diagram of ctDNA sample collection and analysis. Baseline ctDNA was sequenced from 565 patients. All analyses focused on patients with evidence of tumor DNA in circulation with the presence of ≥ 1 genetic alteration. In all, 489 of 565 patients met all criteria and were subject to downstream analysis. ctDNA, circulating tumor DNA; ET, endocrine therapy; ITT, intention to treat.

TABLE 1. Patient's Baseline Characteristics: Biomarker Population (n = 489) Versus ITT Population (N = 672)

	Biomarker Population (ctDNA)				ITT Population			
	RIB		PBO		RIB		PBO	
Category	**No.**	**Percent**	**No.**	**Percent**	**No.**	**Percent**	**No.**	**Percent**
Race								
Asian	84	34	77	32	99	30	99	29
Black	7	3	6	2	10	3	9	3
White	126	51	138	57	187	56	201	60
Others or unknown	32	13	19	8	39	12	28	8
ECOG PS								
Missing	1	0	3	1	3	1	3	1
0	178	71	176	73	245	73	255	76
1	70	28	60	25	87	26	78	23
2	0	0	1	0	0	0	1	0
Disease-free interval								
Existing > 12 months	134	54	137	57	176	53	190	56
Existing ≤ 12 months	14	6	9	4	23	7	13	4
Newly diagnosed	101	41	94	39	136	41	134	40
Previous neoadjuvant or adjuvant ET								
Missing	2	1	NA	NA	2	1	1	0
None	151	61	140	58	208	62	196	58
Yes; progression > 12 months after the end of ET	16	6	22	9	25	7	35	10
Yes; progression on or within 12 months of the end of ET	80	32	78	32	100	30	105	31
Previous CT								
For advanced disease	33	13	32	13	47	14	47	14
Neoadjuvant or adjuvant only	105	42	101	42	138	41	138	41
None	111	45	107	45	150	45	152	45
No. of metastatic sites								
< 3	156	63	153	64	219	65	216	64
≥ 3	93	37	87	36	116	35	121	36
Site of metastases								
Soft tissue								
Yes	22	9	14	6	25	7	21	6
Bone								
Yes	188	76	185	77	251	75	247	73
Bone only								
Yes	60	24	60	25	81	24	78	23
Visceral								
Yes	142	57	126	52	193	58	188	56
Lymph nodes								
Yes	108	43	115	48	142	42	158	47
Skin								
Yes	8	3	5	2	8	2	8	2
Age, median (range), years	43 (25-58)		45 (29-58)		43 (25-58)		45 (29-58)	

Abbreviations: CT, chemotherapy; ctDNA, circulating tumor DNA; ECOG PS, Eastern Cooperative Oncology Group performance status; ET, endocrine therapy; ITT, intent-to-treat; NA, not achieved; PBO, placebo; RIB, ribociclib.

or lung metastases, previous chemotherapy for advanced disease, and endocrine combination partner (tamoxifen or nonsteroidal aromatase inhibitor).[18,19] No adjustments for multiple testing were made, as these analyses were hypothesis generating. Statistical analyses were performed using the R package.[23]

RESULTS

Patient Characteristics and Sample Collection and Analysis

Between December 17, 2014, and August 1, 2016, 672 premenopausal patients underwent 1:1 random assignment to receive ribociclib (n = 335) or placebo (n = 337).[18,19] Baseline characteristics were balanced among treatment groups.[18,19]

Baseline ctDNA was successfully sequenced in 565 patients (Fig 1); 489 patients with HR+ and HER2– ABC had ≥ 1 genetic alteration and were included in this analysis. The demographic and baseline characteristics of the biomarker population were balanced and generally representative of the intent-to-treat population (Table 1).

Genomic Landscape of Premenopausal Metastatic HR+ and HER2– Breast Cancer in ctDNA

In these 489 patients, 32 genes were altered in ≥ 5% of patients (Fig 2). *PIK3CA* was the most frequently altered gene (28%), followed by *TP53* (19%). Other frequently altered genes of interest, including *CCND1*, *MYC*, and *GATA3*, were altered in 10%, 8%, and 8% of patients, respectively (Fig 2). *CCND1*, *FGF4*, *FGF3*, and *FGF19* were localized on Chr11q13.3 and were coamplified in 48 of 489 patients (10%; data not shown). *FGFR1*, *ZNF703*, and *WHSC1L1* were localized on Chr8p11.23 and were altered in 12% of patients and coamplified in 28 of 489 patients (6%). Alterations in other genes of interest, including *NF1* (6%), *PTEN* (4%), *AKT1* (3%), *ESR1* (3%), *ERBB2* (3%), *CDKN2A* (3%), and *RB1* (2%), were also identified (Fig 2).

Association of PFS With Genes Involved in Cell Cycle Regulation

Median PFS was determined for each genetic subgroup, and a corresponding HR was calculated (Fig 3).

CCND1. Patients with altered *CCND1* had a shorter PFS regardless of treatment (the median PFS in patients with WT and altered *CCDN1* for ribociclib *v* placebo was 22.1 *v* 12.9 months and 11.3 *v* 5.5 months, respectively). Benefit with ribociclib was observed in both altered *CCND1* and WT groups; although the magnitude varied, benefit with ribociclib appeared greater in patients with altered *CCND1* (HR, 0.21 [95% CI, 0.08–0.54]) than in those with WT *CCND1* (HR 0.52 [95% CI, 0.39 to 0.68]; *P* value of gene-treatment interaction = .047; Fig 4A).

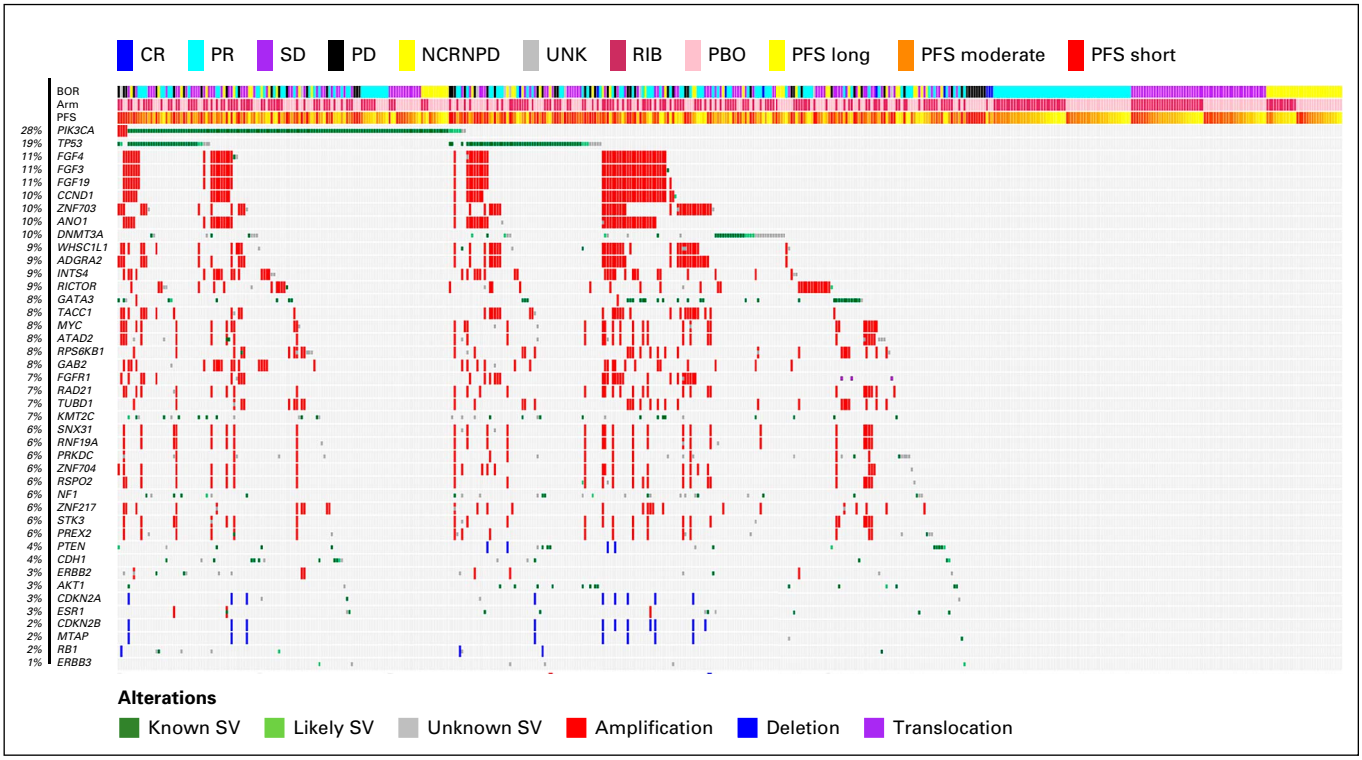
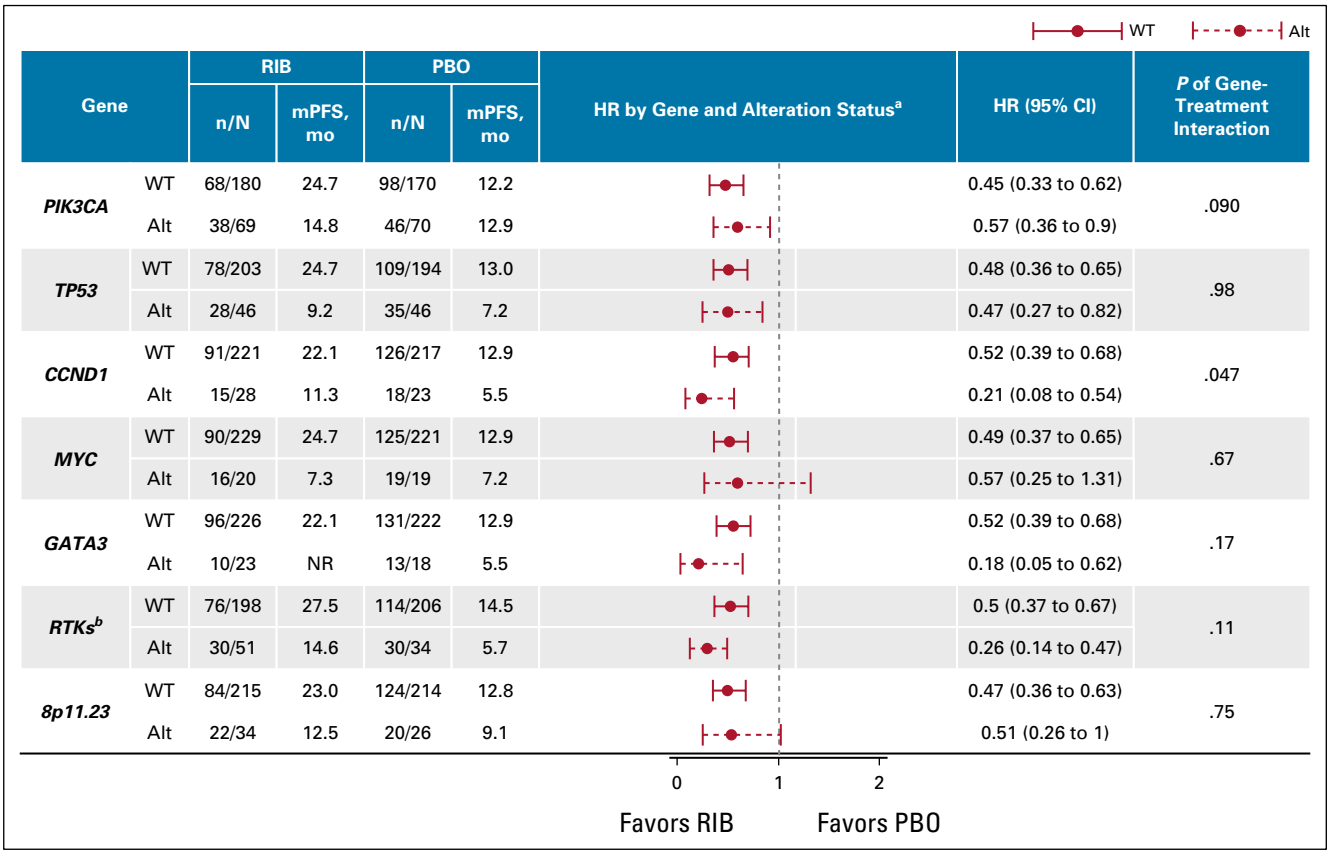


FIG 2. Genomic landscape of advanced breast cancer in premenopausal women. Oncoprint depicting the results of patient's ctDNA NGS data. MONALEESA-7 cfDNA at screening: Genes with frequency > 5% or genes of interest are included. BOR, best overall response; cfDNA, circulating free DNA; CR, complete response; ctDNA, circulating tumor DNA; NCRNPD, neither complete response nor progressive disease; NGS, next-generation sequencing; PBO, placebo; PD, progressive disease; PFS, progression-free survival; PR, partial response; RIB, ribociclib; SD, stable disease; SV, structural variations; UNK, unknown.



Gene		RIB n/N	RIB mPFS, mo	PBO n/N	PBO mPFS, mo	HR by Gene and Alteration Status[a]	HR (95% CI)	*P* of Gene-Treatment Interaction
PIK3CA	WT	68/180	24.7	98/170	12.2		0.45 (0.33 to 0.62)	.090
	Alt	38/69	14.8	46/70	12.9		0.57 (0.36 to 0.9)	
TP53	WT	78/203	24.7	109/194	13.0		0.48 (0.36 to 0.65)	.98
	Alt	28/46	9.2	35/46	7.2		0.47 (0.27 to 0.82)	
CCND1	WT	91/221	22.1	126/217	12.9		0.52 (0.39 to 0.68)	.047
	Alt	15/28	11.3	18/23	5.5		0.21 (0.08 to 0.54)	
MYC	WT	90/229	24.7	125/221	12.9		0.49 (0.37 to 0.65)	.67
	Alt	16/20	7.3	19/19	7.2		0.57 (0.25 to 1.31)	
GATA3	WT	96/226	22.1	131/222	12.9		0.52 (0.39 to 0.68)	.17
	Alt	10/23	NR	13/18	5.5		0.18 (0.05 to 0.62)	
RTKs[b]	WT	76/198	27.5	114/206	14.5		0.5 (0.37 to 0.67)	.11
	Alt	30/51	14.6	30/34	5.7		0.26 (0.14 to 0.47)	
8p11.23	WT	84/215	23.0	124/214	12.8		0.47 (0.36 to 0.63)	.75
	Alt	22/34	12.5	20/26	9.1		0.51 (0.26 to 1)	

FIG 3. PFS by genetic subgroup. Forest plot analysis of PFS benefit from treatment with ribociclib. [a]Stratified by the presence of lung or liver metastases, previous CT, and combination partner (NSAI/tamoxifen). [b]Receptor tyrosine kinase genes include *EGFR*, *ERBB2*, *ERBB3*, *ERBB4*, *FGFR1*, *IGF1*, *IGF1R*, *KDR*, *KIT*, *PDGFRA*, *PDGFRB*, and *VEGFA*. Alt, altered; CT, chemotherapy; HR, hazard ratio; mPFS, median PFS; n/N, events/total; NR, no response; NSAI, nonsteroidal aromatase inhibitor; PBO, placebo; PFS, progression-free survival; RIB, ribociclib; WT, wild-type.

TP53. A similar PFS benefit of ribociclib was observed in patients with WT *TP53* (HR 0.48 [95% CI, 0.36 to 0.65]) and altered *TP53* (HR 0.47 [95% CI, 0.27 to 0.82]; *P* = .98; Fig 4B). Although *TP53* was not predictive of response to ribociclib, median PFS in patients with altered *TP53* (ribociclib *v* placebo, 9.2 *v* 7.2 months) was numerically shorter than that in patients with WT *TP53* (24.7 *v* 13.0 months, respectively) in both arms.

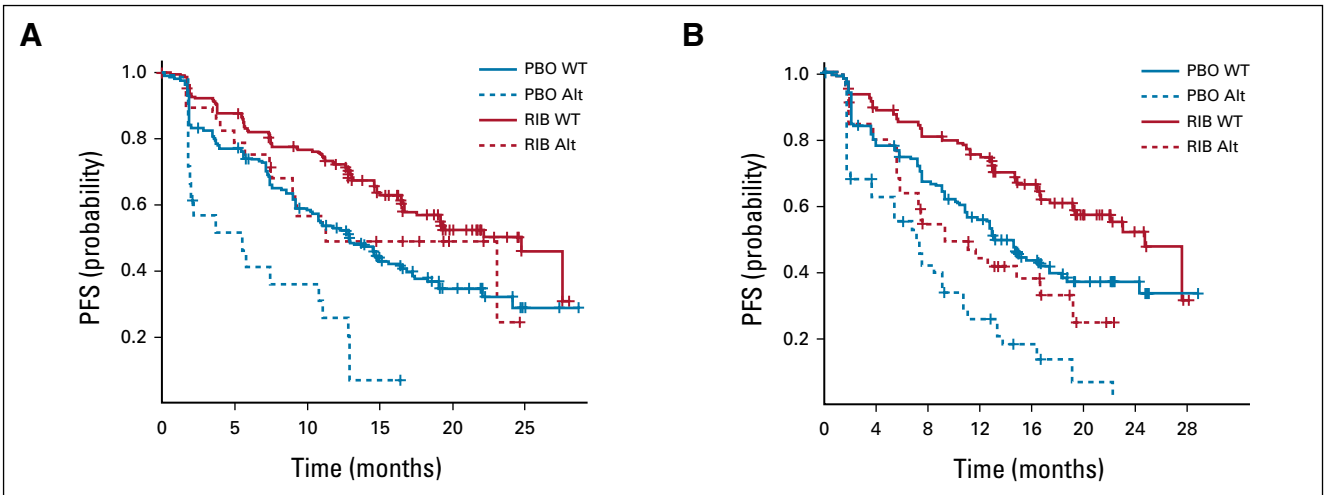
Association of PFS With Genes Relevant to the ER Pathway

GATA3. Alterations in *GATA3* were identified in 41 of 489 patients (8%) in this analysis.[24] Alterations in *GATA3* were associated with a shorter PFS in patients receiving placebo plus ET than in patients with WT *GATA3* (the median PFS for WT *v* altered with placebo was 12.9 *v* 5.52 months). These findings suggest that patients with alteration of *GATA3* might be resistant to ET and have a worse outcome than patients with WT *GATA3*. However, a PFS benefit of ribociclib was observed among patients with both WT (HR 0.52 [95% CI, 0.39 to 0.68]) and altered *GATA3* (HR 0.18 [95% CI, 0.05 to 0.62]; *P* = .17; Fig 5A).

MYC. Alterations in *MYC* were observed in 39 of 489 patients (8%).[25,26] Patients with altered *MYC* had shorter PFS regardless of treatment, suggesting that these patients have a worse outcome (the median PFS in patients with WT and altered *MYC* for ribociclib *v* placebo was 24.7 *v* 12.9 months and 7.3 *v* 7.2 months, respectively; Fig 5B); these results were consistent with other studies in ABC demonstrating *MYC* as a prognostic marker.[27] A similar PFS benefit of ribociclib was observed in patients with WT (HR 0.49 [95% CI, 0.37 to 0.65]) and altered *MYC* (HR 0.57 [95% CI, 0.25 to 1.31]; Fig 5B) on the basis of HRs (*P* = .67).

Association of PFS With Genes in the RTK Pathway

PIK3CA. *PIK3CA* was altered in 139 of 489 patients (28%). The median PFS in patients with WT *PIK3CA* receiving ribociclib versus placebo was 24.7 (95% CI, 22.1 to not achieved) versus 12.2 months (95% CI, 9.2 to 14.6); in patients with altered *PIK3CA*, it was 14.8 (95% CI, 11.0 to 19.4) versus 12.9 months (95% CI, 7.4 to 15.0), respectively. A PFS benefit of ribociclib was observed in patients with WT (HR 0.45 [95% CI, 0.33 to 0.62]) and



	No.	Events	PFS, Median (95% CI), months	HR (95% CI)
PBO WT	217	126	12.88 (10.78 to 15.01)	0.52 (0.39 to 0.68)
RIB WT	221	91	22.11 (16.62 to NA)	
PBO Alt	23	18	5.52 (1.87 to 11.04)	0.21 (0.08 to 0.54)
RIB Alt	28	15	11.27 (7.56 to NA)	

	No.	Events	PFS, Median (95% CI), months	HR (95% CI)
PBO WT	194	109	12.98 (11.04 to 16.53)	0.48 (0.36 to 0.65)
RIB WT	203	78	24.67 (19.35 to NA)	
PBO Alt	46	35	7.16 (3.68 to 9.13)	0.47 (0.27 to 0.82)
RIB Alt	46	28	9.23 (5.82 to 16.49)	

FIG 4. Association of PFS with genes involved in cell cycle regulation: (A) PFS by *CCND1* alteration status and (B) PFS by *TP53* alteration status. Kaplan-Meier curves of PFS in patients who exhibited alterations in the indicated genes in circulating tumor DNA. PFS in patients in the ribociclib treatment arm is shown in red; in patients in the placebo treatment arm, it is shown in blue. The WT subgroup is indicated by a solid line, and the altered subgroup is indicated by a dashed line. HR (95% CI) estimates and median PFS (95% CI) values are shown in the corresponding tables. Alt, altered; HR, hazard ratio; NA, not achieved; PBO, placebo; PFS, progression-free survival; RIB, ribociclib; WT, wild-type.

altered *PIK3CA* (HR 0.57 [95% CI, 0.36 to 0.90]; $P = .09$; Fig 6A).

Receptor tyrosine kinases. RTK gene alterations (Fig 6B) were identified in 17% of patients. A PFS benefit was observed with ribociclib among patients with WT (HR 0.50 [95% CI, 0.37 to 0.67]) and altered RTKs (HR 0.26 [95% CI, 0.14 to 0.47]; $P = .11$; Fig 6C). Median PFS generally favored patients with WT versus altered RTKs (the median PFS in patients with WT and altered RTKs for ribociclib *v* placebo was 27.5 *v* 14.5 months and 14.6 *v* 5.7 months, respectively).

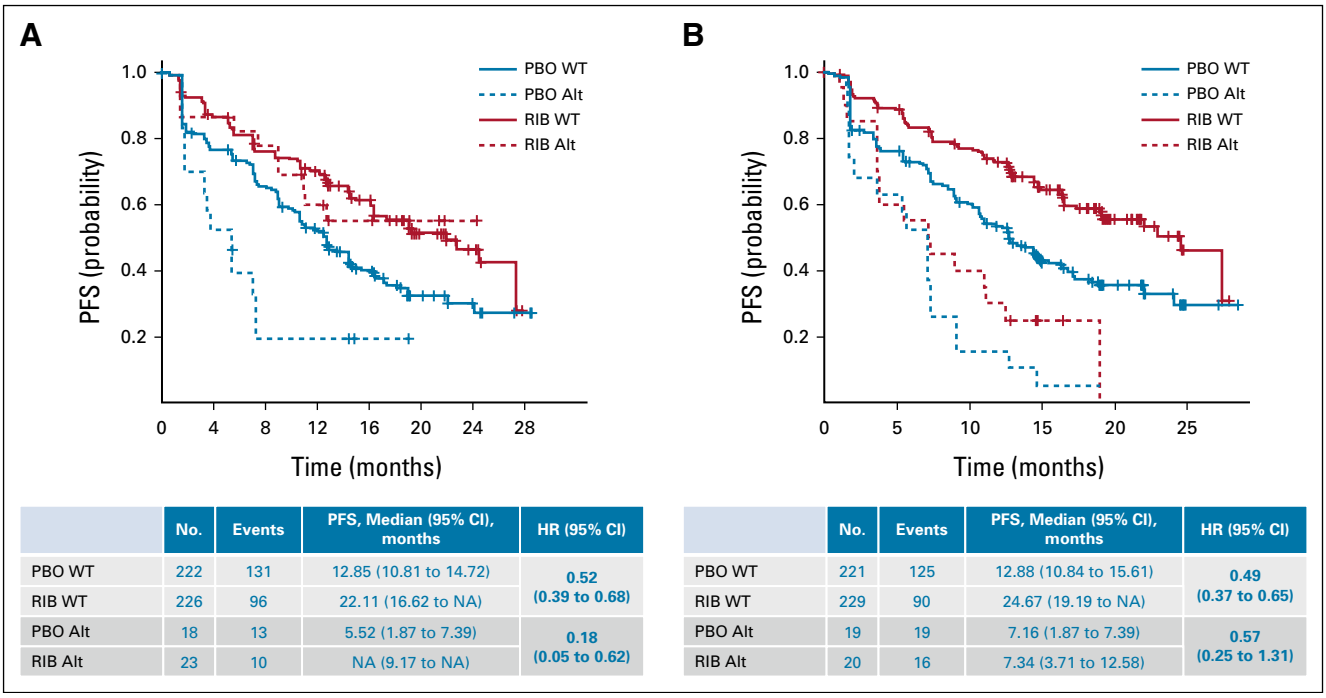
Chr8p11.23. Previous studies demonstrated frequent amplification of the Chr8p11.23 locus driven by the *FGFR1*, *ZNF703*, and *WHSC1L1* genes in patients with breast cancer.[10] Alterations (mainly amplification) of *FGFR1*, *ZNF703*, and *WHSC1L1* were identified in 60 of 489 patients (12%; Fig 6D). Alterations in Chr8p11.23 were prognostic of shorter PFS overall, irrespective of treatment (the median PFS for WT and altered Chr8p11.23 for ribociclib *v* placebo was 23.0 *v* 12.8 months and 12.5 *v* 9.1 months, respectively); these findings were in accordance with other studies demonstrating their prognostic relevance in HR+ ABC.[28,29] However, on the basis of HRs, a similar PFS benefit of ribociclib was observed in patients with WT (HR 0.47 [95% CI, 0.36 to 0.63]) or altered Chr8p11.23 locus (HR 0.51 [95% CI, 0.26 to 1.0]; $P = .75$; Fig 6E).

Prognosis and Treatment Benefit in Patients Without Detectable Alterations

Compared with the biomarker population (patients with ≥ 1 alteration; n = 489), patients with no detected alterations (n = 76) had better Eastern Cooperative Oncology Group performance status and were less likely to progress on or within 12 months of completing ET (Data Supplement). Additionally, patients without detectable alterations exhibited a trend toward improved PFS with ribociclib and placebo compared with the biomarker population (Data Supplement). These findings suggest a better prognosis in patients without detectable alterations.[30]

DISCUSSION

Before this study, there were limited clinical trials with a focus on premenopausal patients with breast cancer and analyses of the association of genomic profiles with clinical outcomes in this patient population; thus, there was little information regarding biomarkers that were prognostic and/or predictive of sensitivity or resistance to therapies in premenopausal women with HR+ and HER2– ABC. To our knowledge, MONALEESA-7 was the first trial conducted exclusively in premenopausal patients with HR+ and HER2– ABC, and therefore, this is the first large-scale genomic biomarker profiling study to date. Frequent alterations were identified in genes that regulate the cell cycle, the endocrine pathway, and RTK pathways. These



	No.	Events	PFS, Median (95% CI), months	HR (95% CI)
PBO WT	222	131	12.85 (10.81 to 14.72)	0.52 (0.39 to 0.68)
RIB WT	226	96	22.11 (16.62 to NA)	
PBO Alt	18	13	5.52 (1.87 to 7.39)	0.18 (0.05 to 0.62)
RIB Alt	23	10	NA (9.17 to NA)	

	No.	Events	PFS, Median (95% CI), months	HR (95% CI)
PBO WT	221	125	12.88 (10.84 to 15.61)	0.49 (0.37 to 0.65)
RIB WT	229	90	24.67 (19.19 to NA)	
PBO Alt	19	19	7.16 (1.87 to 7.39)	0.57 (0.25 to 1.31)
RIB Alt	20	16	7.34 (3.71 to 12.58)	

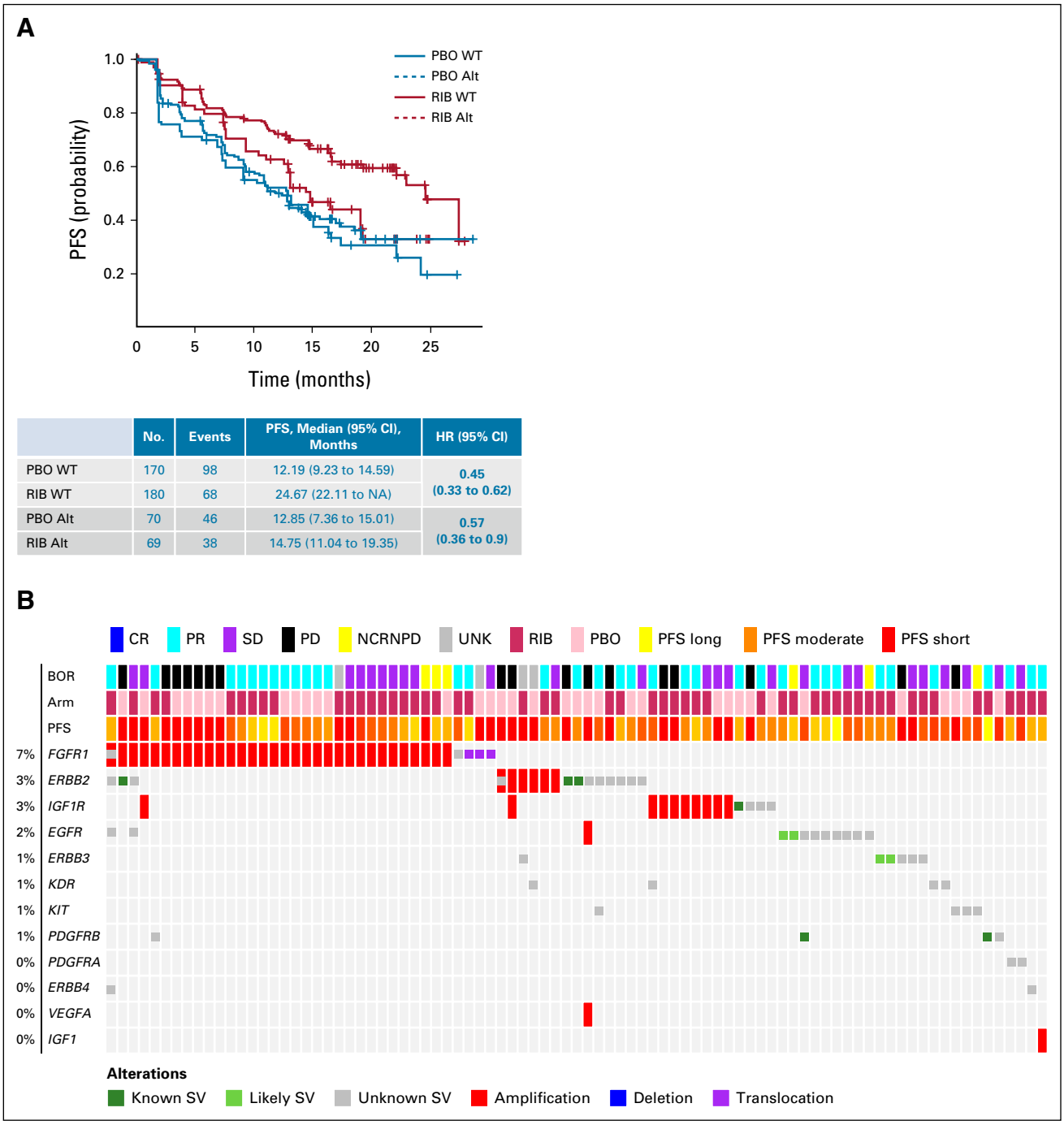
FIG 5. Association of PFS with genes relevant to the estrogen receptor pathway: (A) PFS by *GATA3* alteration status and (B) PFS by *MYC* alteration status. Kaplan-Meier curves of PFS in patients who exhibited alterations in the indicated genes in circulating tumor DNA. PFS in patients in the ribociclib treatment arm is shown in red; in patients in the placebo treatment arm, it is shown in blue. The WT subgroup is indicated by a solid line, and the alteration subgroup is indicated by a dashed line. HR (95% CI) estimates and median PFS (95% CI) values are shown in the corresponding tables. Alt, altered; HR, hazard ratio; NA, not achieved; PBO, placebo; PFS, progression-free survival; RIB, ribociclib; WT, wild-type.

results indicate that an increase in PFS with ribociclib plus ET versus ET alone was observed regardless of biomarker alteration status. Particularly, the PFS benefit of ribociclib treatment is independent of alteration status of *TP53*, *MYC*, and genes on the Chr8p11.23 locus. Additionally, patients with WT and altered *PIK3CA* exhibited a PFS benefit with ribociclib. The ctDNA biomarker data presented here provide insight into the genomic landscape of premenopausal HR+ and HER2– ABC and impact on clinical outcomes.

CCND1 and *TP53* are regulators of the cell cycle, and in this analysis, alterations in *CCND1* and *TP53* were frequent (10% and 19%, respectively) and associated with a worse outcome. *CCND1* amplification and/or overexpression has been associated with ER+ breast cancer and was reported to render ER+ cancer cell lines more sensitive to CDK4/6 inhibition.[31-33] Although data from PALOMA-1 and PALOMA-2 indicate that expression levels of cyclin D1 or *CCND1* amplification were not associated with benefit from CDK4/6 inhibition by palbociclib in these studies, which comprised postmenopausal patients,[34,35] our results suggest that premenopausal patients with an alteration of *CCND1* experience a more pronounced PFS benefit with ribociclib, with a 79% difference in relative risk of progression with ribociclib versus placebo with altered *CCND1* (HR 0.21 [95% CI, 0.08 to 0.54]) and only a 48% difference in relative risk of progression with ribociclib versus placebo with WT *CCND1* (HR 0.52 [95% CI, 0.39 to 0.68]). Thus, it is possible that there may be a greater treatment interaction with *CCND1* in premenopausal versus postmenopausal patients, but this requires confirmation.

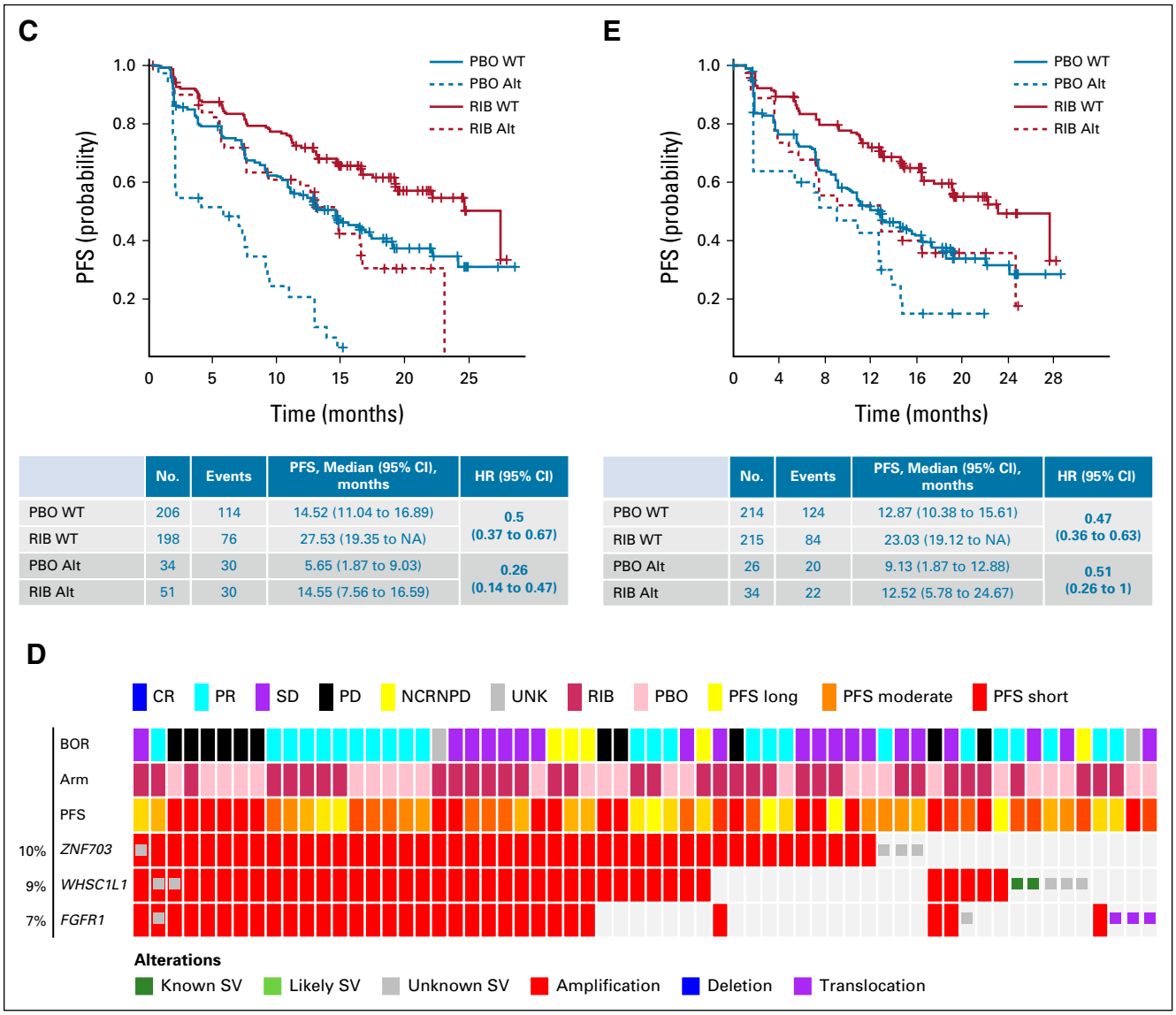
TP53 has been established as a prognostic biomarker in breast cancer.[36] In this analysis, there was a PFS benefit of ribociclib regardless of *TP53* alteration status; however, patients with altered *TP53* (19% of patients) versus WT *TP53* had a shorter PFS regardless of treatment. In postmenopausal patients in MONARCH 3, there was a PFS benefit with abemaciclib plus nonsteroidal aromatase inhibitors in patients with WT and altered *TP53* (26% of patients), but a greater benefit was observed in patients with WT *TP53*.[37] In postmenopausal patients in MONALEESA-2, a PFS benefit with ribociclib was observed in patients with WT or altered *TP53* (12% of patients), although a numerically shorter PFS was observed in patients with altered versus WT *TP53* irrespective of treatment.[38] Thus, *TP53* represents a prognostic biomarker for ABC in both pre- and postmenopausal HR+ patient populations.

ESR1 mutations were only observed in 13 of 489 patients, and thus, a correlation between *ESR1* alteration status and PFS could not be evaluated. *GATA3* has been implicated in breast cancer development and ET resistance by driving ER-mediated transcriptional regulation of downstream gene expression.[12] Our findings suggest that patients with altered *GATA3* derived a more pronounced PFS benefit



	No.	Events	PFS, Median (95% CI), Months	HR (95% CI)
PBO WT	170	98	12.19 (9.23 to 14.59)	0.45 (0.33 to 0.62)
RIB WT	180	68	24.67 (22.11 to NA)	
PBO Alt	70	46	12.85 (7.36 to 15.01)	0.57 (0.36 to 0.9)
RIB Alt	69	38	14.75 (11.04 to 19.35)	

FIG 6. Association of PFS with genes or pathways in parallel to estrogen receptor and cyclin-dependent kinase 4/6 pathways: (A) PFS by *PIK3CA* alteration status, (B) oncoprint displaying altered RTKs (MONALEESA-7 cfDNA at screening: RTKs [87 of 489 samples]) identified in NGS analysis of patient's circulating tumor DNA, (C) PFS by RTK alteration status, (D) oncoprint displaying alterations (MONALEESA-7 cfDNA at screening: Chr8p11.23 [62 of 489 samples]) in the Chr8p11.23 region, and (E) PFS by Chr8p11.23 alteration status. (A, C, and E) Kaplan-Meier curves of PFS in patients who exhibited alterations in the indicated genes in ctDNA. PFS in patients in the ribociclib treatment arm is shown in red; in patients in the placebo treatment arm, it is shown in blue. The WT subgroup is indicated by a solid line; the Alt subgroup is indicated by a dashed line. HR (95% CI) estimates and median PFS (95% CI) values are shown in the corresponding tables. (B and D) Oncoprint depicting a subset of altered genes identified in NGS analysis of patient's ctDNA. Alt, altered; BOR, best overall response; cfDNA, circulating free DNA; CR, complete response; ctDNA, ctDNA, circulating tumor DNA; HR, hazard ratio; NA, not achieved; NCRNPD, neither complete response nor progressive disease; NGS, next-generation sequencing; PBO, placebo; PD, progressive disease; PFS, progression-free survival; PR, partial response; RIB, ribociclib; RTK, receptor tyrosine kinase; SD, stable disease; SV, structural variations; UNK, unknown; WT, wild-type.



	No.	Events	PFS, Median (95% CI), months	HR (95% CI)
PBO WT	206	114	14.52 (11.04 to 16.89)	0.5 (0.37 to 0.67)
RIB WT	198	76	27.53 (19.35 to NA)	
PBO Alt	34	30	5.65 (1.87 to 9.03)	0.26 (0.14 to 0.47)
RIB Alt	51	30	14.55 (7.56 to 16.59)	

	No.	Events	PFS, Median (95% CI), months	HR (95% CI)
PBO WT	214	124	12.87 (10.38 to 15.61)	0.47 (0.36 to 0.63)
RIB WT	215	84	23.03 (19.12 to NA)	
PBO Alt	26	20	9.13 (1.87 to 12.88)	0.51 (0.26 to 1)
RIB Alt	34	22	12.52 (5.78 to 24.67)	

FIG 6. (Continued).

with ribociclib than patients with WT *GATA3*, although this difference was not statistically significant. Thus, *GATA3* status may be predictive of response to ribociclib in premenopausal patients.

Previous studies have shown that patients with altered RTKs have poor outcomes with ET alone, as RTK pathway dysregulation can drive resistance to ET.[11,14,15,39] The HRs from this analysis indicate that patients with altered RTKs derived a PFS benefit from ribociclib, suggesting that ribociclib was able to overcome ET resistance in patients harboring RTK gene alterations. Further investigation is needed to identify the specific RTK genes that are driving this effect. Additionally, because *FGFR1*, *WHSC1L1*, and *ZNF703*, located on Chr8p11.23 locus and mostly coamplified in this cohort, have been reported separately to play a role in mechanisms of resistance to ET,[14,16,17] we investigated the association between treatment benefit and alterations of this Chr8p11.23 locus. Our results indicate that patients with WT and altered genes at the Chr8p11.23 locus had a similar PFS benefit with ribociclib. However, patients with gene alterations at the Chr8p11.23 locus appeared to have a poorer outcome overall, as these patients had a shorter PFS irrespective of treatment, highlighting the prognostic potential of this amplicon in HR+ ABC. Since the genes at the Chr8p11.23 locus are mostly coamplified, further research is needed to identify which individual genes are driving this effect.

In this study, *PIK3CA* was altered in 28% of patients, whereas a higher incidence of *PIK3CA* mutations (30%-40%) was reported in studies of CDK4/6 inhibitors in postmenopausal patients.[37,40-42] The improvement in median PFS for ribociclib versus placebo was more

pronounced in patients with WT versus altered *PIK3CA* (HR 0.45 [95% CI, 0.33 to 0.62] *v* 0.57 [95% CI, 0.36 to 0.9]; interaction *P* value = .09), although this difference was not statistically significant. However, studies in postmenopausal patients with ribociclib, palbociclib, and abemaciclib have shown increased benefit of study treatment versus ET alone regardless of *PIK3CA* mutation status, with the exception of MONARCH 3, which showed a more pronounced benefit in patients with WT *PIK3CA*.

This study has several limitations. First, this is a retrospective exploratory analysis. Second, some subgroup analyses included very small sample sizes, and the results have not been adjusted for multiple testing. Third, these analyses are hypothesis generating and should be confirmed in additional studies. Fourth, the sensitivity to detect DNA alterations is higher in samples with more tumor DNA in circulation, which has itself been shown to be prognostic.[30] Finally, this study does not address acquired resistance and the role of other biomarkers, including epigenetic alterations and RNA and protein expression. Thus, further biomarker analyses are ongoing.

This analysis provides insight into the genomic landscape of premenopausal women with HR+ ABC and potential differences in the genetic landscape of HR+ and HER2– ABC in pre- and postmenopausal women. This study uncovered biomarkers associated with worse outcomes overall, but ribociclib treatment resulted in increased PFS benefit regardless of alteration status of these biomarker genes, although the magnitude of this benefit varied across subsets. Altogether, these findings highlight the potential role of genomic alterations in modulating clinical outcomes in premenopausal patients with ABC although these results are hypothesis generating and require confirmation in larger data sets.

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