ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

ORIGINAL ARTICLE

Pooled ctDNA analysis of MONALEESA phase III advanced breast cancer trials

F. André[1]*, F. Su[2], N. Solovieff[3], G. Hortobagyi[4], S. Chia[5], P. Neven[6], A. Bardia[7], D. Tripathy[4], Y.-S. Lu[8], A. Lteif[2], T. Taran[9], N. Babbar[2], D. Slamon[10] & C. L. Arteaga[11]

[1]Department of Medical Oncology and INSERM U981, Institut Gustave Roussy, Université Paris Saclay, Villejuif, France; [2]Novartis Pharmaceuticals, East Hanover; [3]Novartis Institutes for BioMedical Research, Cambridge; [4]The University of Texas MD Anderson Cancer Center, Houston, USA; [5]British Columbia Cancer Agency, Vancouver, Canada; [6]Multidisciplinary Breast Centre, Universitair Ziekenhuis Leuven, Leuven, Belgium; [7]Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, USA; [8]National Taiwan University Hospital, Taipei, Taiwan; [9]Novartis Pharma AG, Basel, Switzerland; [10]David Geffen School of Medicine at UCLA, Los Angeles; [11]UT Southwestern Simmons Comprehensive Cancer Center, Dallas, USA

Available online 5 September 2023

Background: The phase III MONALEESA trials tested the efficacy and safety of the cyclin-dependent kinase (CDK)4/6 inhibitor ribociclib with different endocrine therapy partners as first- or second-line treatment of hormone receptor−positive/human epidermal growth factor receptor 2−negative advanced breast cancer (ABC). Using the largest pooled biomarker dataset of the CDK4/6 inhibitor ribociclib in ABC to date, we identified potential biomarkers of response to ribociclib.

Patients and methods: Baseline circulating tumour DNA from patients in the MONALEESA trials was assessed using next-generation sequencing. An analysis of correlation between gene alteration status and progression-free survival (PFS) was carried out to identify potential biomarkers of response to ribociclib.

Results: Multiple frequently altered genes were identified. Alterations in *ERBB2*, *FAT3*, *FRS2*, *MDM2*, *SFRP1*, and *ZNF217* were associated with a greater PFS benefit with ribociclib versus placebo. Patients with high tumour mutational burden (TMB) and with *ANO1*, *CDKN2A/2B/2C*, and *RB1* alterations exhibited decreased sensitivity to ribociclib versus placebo.

Conclusions: Although exploratory, these results provide insight into alterations associated with the improved response to ribociclib treatment and may inform treatment sequencing in patients with actionable alterations following progression on CDK4/6 inhibitors. Validation of potential biomarkers identified here and development of prospective trials testing their clinical utility are warranted.

ClinicalTrials.gov identifiers: NCT01958021, NCT02422615, NCT02278120.

Key words: CDK4/6 inhibitor, advanced breast cancer, progression-free survival, ribociclib, biomarkers

INTRODUCTION

Cell cycle regulatory mechanisms are proven therapeutic targets in breast cancer.[1] Cyclin-dependent kinase 4 (CDK4) is dysregulated in most hormone receptor−positive (HR+) breast cancers.[2] CDK4 phosphorylates and inactivates retinoblastoma protein (encoded by the *RB1* gene), promoting G_1 to S phase transition.[3] CDK4 is activated upon coupling to cyclin D1—a transcriptional target of the oestrogen receptor (ER) and mitogenic signalling pathways.[4] CDK4 activation also results from p16 (encoded by the *CDKN2A* gene) inactivation.[4] Given the reported role of CDK4 in endocrine therapy (ET) resistance mechanisms and that inhibitors of CDK4 have decreased proliferation of HR+ breast cancer cells, CDK4/6 inhibitors (CDK4/6i) were developed for treating HR+ advanced breast cancer (ABC).[1,5]

CDK4/6i improve progression-free survival (PFS) in patients with HR+/human epidermal growth factor receptor 2−negative (HER2−) ABC; significant overall survival benefits have been demonstrated by abemaciclib plus ET in MONARCH-2 and by ribociclib plus ET in the MONALEESA-2, MONALEESA-3, and MONALEESA-7 trials.[6-10] Minimal clinical biomarker data are available to support identification of patients who might exhibit enhanced or reduced benefit from CDK4/6i. *CCNE1* overexpression was associated with decreased sensitivity to palbociclib plus fulvestrant in analyses of patients with HR+/HER2− ABC in PALOMA 3 (n = 302) and PEARL (n = 219).[11,12] While these preliminary data

*Correspondence to: Prof. Fabrice André, Department of Medical Oncology and INSERM Unit U981, Gustave Roussy Cancer Campus, 114 Rue Edouard Vaillant, Villejuif, 94800, France

E-mail: FABRICE.ANDRE@gustaveroussy.fr (F. André).

suggest that expression of some genes could predict efficacy of CDK4/6i, there is no large amount of clinical evidence that DNA alterations predict response to CDK4/6i. Genomic alterations, including *FGFR1* amplification, *PTEN* loss, *CDK6* amplification, *RB1* loss, and *FAT1* loss, were reported to mediate CDK4/6i resistance in preclinical models and limited clinical samples but have not translated into validated predictive clinical biomarkers.[13-26] Using a large pooled biomarker dataset from three phase III trials, we assessed the predictive value of genomic alterations for efficacy of ribociclib, a CDK4/6i that preferentially inhibits CDK4 versus CDK6, in patients with HR+/HER2− ABC.[27,28]

PATIENTS AND METHODS

Trials and patients

The analysis population consisted of patients enrolled in the MONALEESA-2, MONALEESA-3, and MONALEESA-7 trials, the details of which have been previously published (MONALEESA-2, NCT01958021; MONALEESA-3, NCT02422615; MONALEESA-7, NCT02278120).[9,29-31] In brief, the MONALEESA trials were randomized, placebo-controlled, international, double-blind, phase III studies that evaluated ribociclib plus ET versus placebo plus ET in patients with HR+/HER2− ABC. The ET partners used in MONALEESA-2, MONALEESA-3, and MONALEESA-7 were letrozole, fulvestrant, and a nonsteroidal aromatase inhibitor (NSAI) or tamoxifen, respectively. The MONALEESA-2 trial assessed the efficacy of ribociclib plus letrozole versus placebo plus letrozole for postmenopausal patients who received no prior therapy in the ABC setting.[30] The MONALEESA-3 trial tested ribociclib plus fulvestrant versus placebo plus fulvestrant for patients who received ≤1 prior line of ET in the ABC setting.[31] The MONALEESA-7 trial evaluated ribociclib plus goserelin and either an NSAI or tamoxifen versus placebo plus goserelin and NSAI or tamoxifen for premenopausal or perimenopausal patients who received no prior ET for ABC and ≤1 line of chemotherapy in the ABC setting.[29] The primary endpoint of each MONALEESA trial was investigator-assessed PFS. Results of these analyses were previously reported.[29-31] In the analysis presented here, PFS was defined as the time from the date of randomization to the date of 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 endpoint to characterize molecular alterations in circulating tumour DNA (ctDNA) and correlate these alterations with efficacy outcomes in the MONALEESA trials.

All patients in the MONALEESA trials provided written informed consent before enrolment. The study protocols, including any modifications, were approved by an independent ethics committee or institutional review board at each site. These trials were conducted in accordance with the Good Clinical Practice guidelines and Declaration of Helsinki. A steering committee made up of Novartis representatives and participating international investigators oversaw each study. Safety data were assessed by an independent data-monitoring committee. This manuscript is compliant with REMARK reporting guidelines.

Biomarker sample collection and assessment of genetic alterations and tumour mutational burden

The analysis of samples from MONALEESA trials was carried out retrospectively. The investigators who carried out the biological experiments were blinded for clinical data. The strategy for biomarker analysis was developed before data were analysed. Additional details on the analysis of gene alterations are outlined in the 'Statistical analysis' section.

The sample size in the analysis was based on sample and data availability. The total sample size for each trial was determined for the primary endpoint of each trial. We then attempted to sequence all available baseline plasma ctDNA samples with adequate volume and then included all samples for which next-generation sequencing was successful (i.e. passed quality control measures). Given this, the biomarker analysis was not designed to detect a specified effect size at a target power.

Blood samples were collected in K2-EDTA blood collection tubes and processed within 30 min of the collection by centrifugation at 1600 *g* (±150 *g*) for 10 min; the clear supernatant (plasma) was transferred to one fresh centrifuge tube and then centrifuged for another 10 min at 3000 *g* (±150 *g*). The plasma aliquots were then stored in a −70°C or colder freezer and shipped in batches to the central laboratory on dry ice and stored in −70°C or colder freezers. There was no freeze-thaw before DNA extraction. Cell-free DNA was extracted from frozen plasma samples collected at baseline (before the initiation of study treatment) using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Germantown, MD) and constructed into sequencing libraries with end repair, A-tailing, and PCR amplification (TruSeq Nano Library Preparation Kit, Illumina, San Diego, CA). Cell-free DNA was then sequenced on an Illumina HiSeq instrument targeted to a depth of ×1000 coverage using a targeted panel of 558 genes including all exonic regions, select introns for translocation detection, and tiled regions for improved copy number calling. The limit of detection was determined to be 1% (Supplementary Figures S1 and S2, available at https://doi.org/10.1016/j.annonc.2023.08.011). Samples collected after first exposure of study treatment were removed. A total of 766 patients had a sample collected the same day as the first exposure to study treatment, and 411 patients had a plasma sample collected before the first exposure to study treatment (1/411 patients had samples collected between day −29 and day −44; 278/411 patients had samples collected between day −1 and day −28).

Single-nucleotide variants were identified using MuTect (Broad Institute, Cambridge, MA), and indels were identified using Pindel.[32,33] PureCN was used to call copy number alterations while accounting for the ploidy and ctDNA fraction of the sample.[34] Amplifications were defined as ≥6 copies and ≥7 copies for focal and nonfocal events, respectively. Deletions were defined as 0 copies. Non-

frameshift mutations were defined as an insertion or deletion that does not cause a frameshift. Germline mutations and artefacts were filtered out using the publicly available dbSNP and ExAC databases. To remove artefacts, an internal database (Novartis Institutes for BioMedical Research) of normal circulating free DNA samples from 50 healthy individuals without cancer was used to estimate position-specific error rates and mutations were removed if they did not surpass the position-specific error rate. Variants with mutant allele fraction <1% were filtered out. Mutations/indels with an allelic fraction >40% and unknown functional significance were also removed because matched normal DNA was not sequenced. Synonymous mutations/indels were removed. To remove somatic mutations due to clonal haematopoiesis of indeterminate potential (CHIP), a classification approach was used to determine the CH status of each mutation/indel. Variants classified as CH positive were removed unless the variant was present at least 5 times in the breast cancer cohort of COSMIC.[35] To account for variability in levels of ctDNA shedding, patients with ctDNA fraction <1% were excluded from all analyses and ctDNA fraction was included as a covariate in all models.

Tumour mutational burden (TMB) was estimated as the number of somatic mutations per megabase using an established workflow.[36] The estimation includes synonymous and non-synonymous mutations in coding regions.

Statistical analysis

For statistical analyses, each gene in the ctDNA was classified in two ways. For the first approach, each gene was classified as altered if ≥1 alteration—defined as the presence of a copy number alteration, short insertion/deletion, or mutation—was detected and as wild-type (WT) if no alterations were detected. For the second approach, the classification focused on mutations/indels with known or likely functional significance and each gene was classified as altered if ≥1 alteration was detected and as WT if no alterations were detected. Correlative analyses were carried out for genes altered in ≥3% of patients (68 genes from the first approach; 9 genes from the second approach) and genes of interest (*RB1*, *CDKN2A/B/C*, *BRCA1/2*, excluding amplifications) with respect to clinical outcome (PFS; Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2023.08.011).

For key genes of interest (top predictive signals from predefined analysis or resistance genes reported in the literature), additional *ad hoc* analyses were carried out that focused on specific amino acid changes or types of alterations such as *PTEN* nonsense mutations and deletions, *NF1* nonsense and frameshift mutations, *FRS2*/*MDM2* amplifications, *BRCA1/2* alterations excluding amplifications and variants of unknown functional significance, CDKN2A/B/C loss-of-function variants (deletions, frameshift, nonsense).

TMB was divided into quartiles (Q1, 0-0.7; Q2, 0.7-1.3; Q3, 1.3-3.3; Q4, >3.3 mutations/Mb) and a cut-off of 10 mutations/Mb.

To identify potential biomarkers of response and resistance, the relationship between each biomarker and PFS was assessed. PFS was evaluated instead of overall survival because PFS was the primary endpoint of the MONALEESA trials and, at the time of this analysis, overall survival data were available only for MONALEESA-3 and MONALEESA-7. Kaplan—Meier curves were generated, and median PFS [95% confidence interval (CI)] was estimated by treatment arm and biomarker status. A Cox proportional hazards model was used to estimate the HRs of treatment benefit (ribociclib versus placebo) for PFS by biomarker status. Differential treatment benefit of ribociclib versus placebo between alteration and WT genes was assessed by including an interaction term between treatment and biomarker in the Cox proportional hazards model. All Cox proportional hazards models were stratified by study and adjusted for ctDNA fraction as a covariate. A false discovery rate (FDR) adjustment was applied to the gene—treatment interaction *P* value. Results for biomarkers with a nominal gene—treatment interaction *P* value < 0.10 are discussed in the 'Results' section. This is an exploratory analysis; thus, results are hypothesis generating. Additional research is needed to confirm the signals.

To compare patients with ctDNA fraction <1% versus ≥1%, a Fisher's exact test was used to test the association with demographic and clinical variables. A Cox proportional hazards model stratified by study was used to assess the relationship between ctDNA fraction categories and PFS.

All statistical analyses were carried out using the R package.[37]

RESULTS

Patient characteristics and genomic landscape per circulating tumour DNA analysis

Next-generation sequencing data were generated for 1674 baseline plasma ctDNA samples; among these, 1045 patients with ctDNA fraction ≥1% were analysed. Patient characteristics and treatment efficacy were similar in the biomarker ($n = 1045$) and overall ($N = 2066$) populations (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2023.08.011). In the biomarker population, 68 genes were altered in ≥3% of patients (Figure 1). Some of the most frequently altered genes were *PIK3CA* (42%), *TP53* (21%), *FGF4* (15%), *FGF3* (15%), *ANO1* (14%), *FGF19* (14%), *CCND1* (13%), *ZNF703* (12%), *ADGRA2* (11%), *CDH1* (11%), and *WHSC1L1* (11%). Median PFS with corresponding hazard ratios (HRs) for treatment benefit was determined for each genetic subgroup (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2023.08.011).

Gene alterations associated with increased sensitivity to ribociclib treatment

Genes with a nominal gene—treatment interaction <0.10 are reported in Figure 2 and discussed. Note that none of the genes survived a multiple testing correction (see FDR-

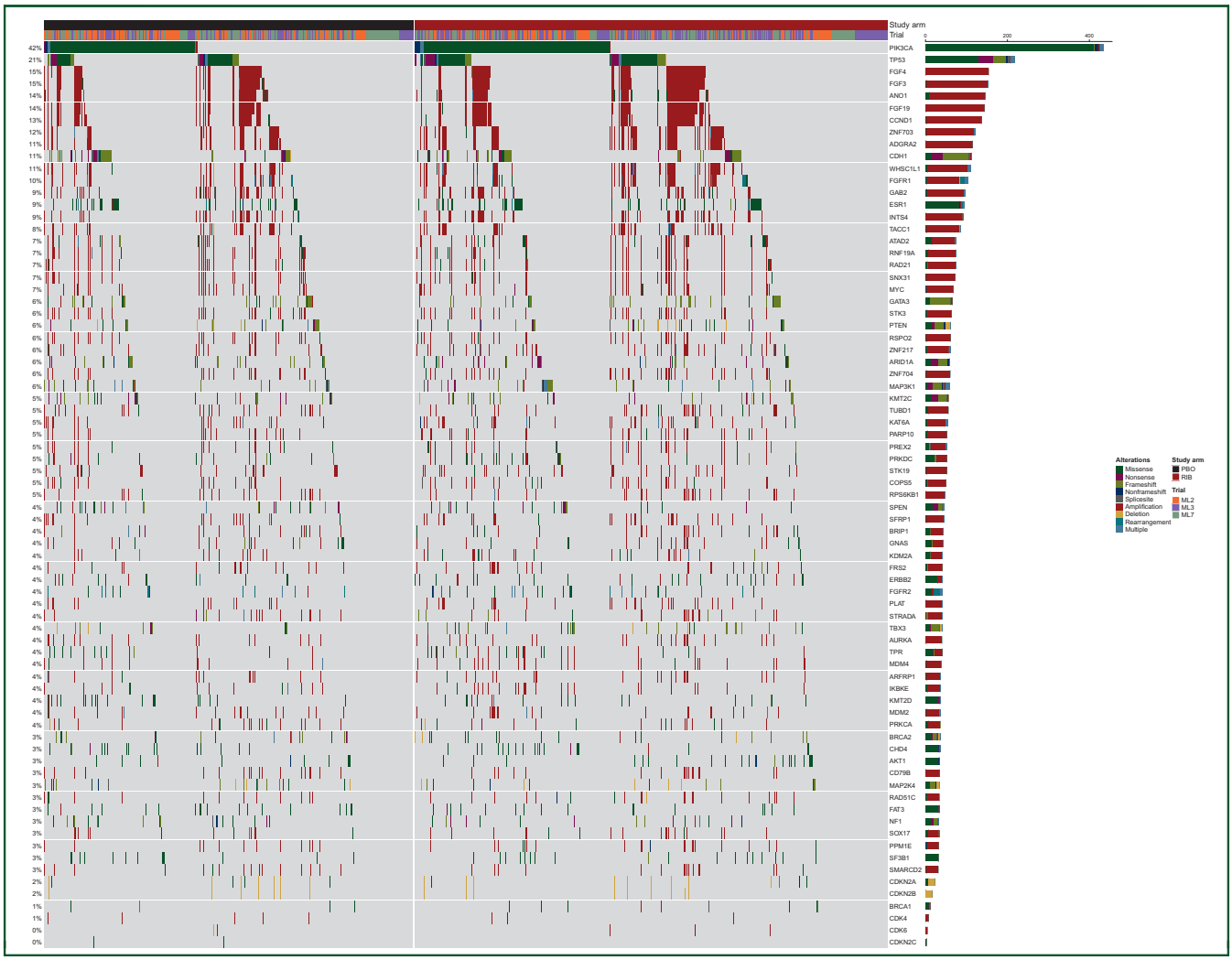


Figure 1. Oncoprint of genes altered in ≥3% of patients and genes of interest.
ML, MONALEESA; PBO, placebo; RIB, ribociclib.

adjusted *P* values in Figure 2). Ribociclib sensitivity was defined by PFS; genes with an alteration frequency of ≥3% were assessed, as were several genes of interest, including *RB1*, *BRCA1/2* (excluding amplifications), and *CDKN2A/B/C*. Gene alterations associated with higher sensitivity to ribociclib (nominal *P* value for nominal gene—treatment interaction <0.10) are reported in Figure 2A and Supplementary Figure S3A, available at https://doi.org/10.1016/j.annonc.2023.08.011. These include *ERBB2*, *FAT3*, *FRS2*, *MDM2*, *SFRP1*, and *ZNF217*. Alterations in these genes were generally associated with a poor response to placebo and an added benefit of ribociclib treatment (Figure 2B-E). *FRS2* and *MDM2* alterations were individually associated with high sensitivity ($P = 0.0714$ and $P = 0.0159$, respectively) (Figure 2F and G). *FRS2* and *MDM2*, located on the same amplicon (12q15), were co-amplified in 34/1045 patients (3%) (Supplementary Figure S3B, available at https://doi.org/10.1016/j.annonc.2023.08.011); no additional genes in this region were included in the panel; thus, amplification here may be a surrogate for amplifications somewhere within this region. Patients with co-amplified *FRS2*/*MDM2* exhibited a greater reduction in disease progression with ribociclib versus placebo [HR = 0.23 (95% CI 0.11-0.51), $P = 0.0255$] (Figure 2H).

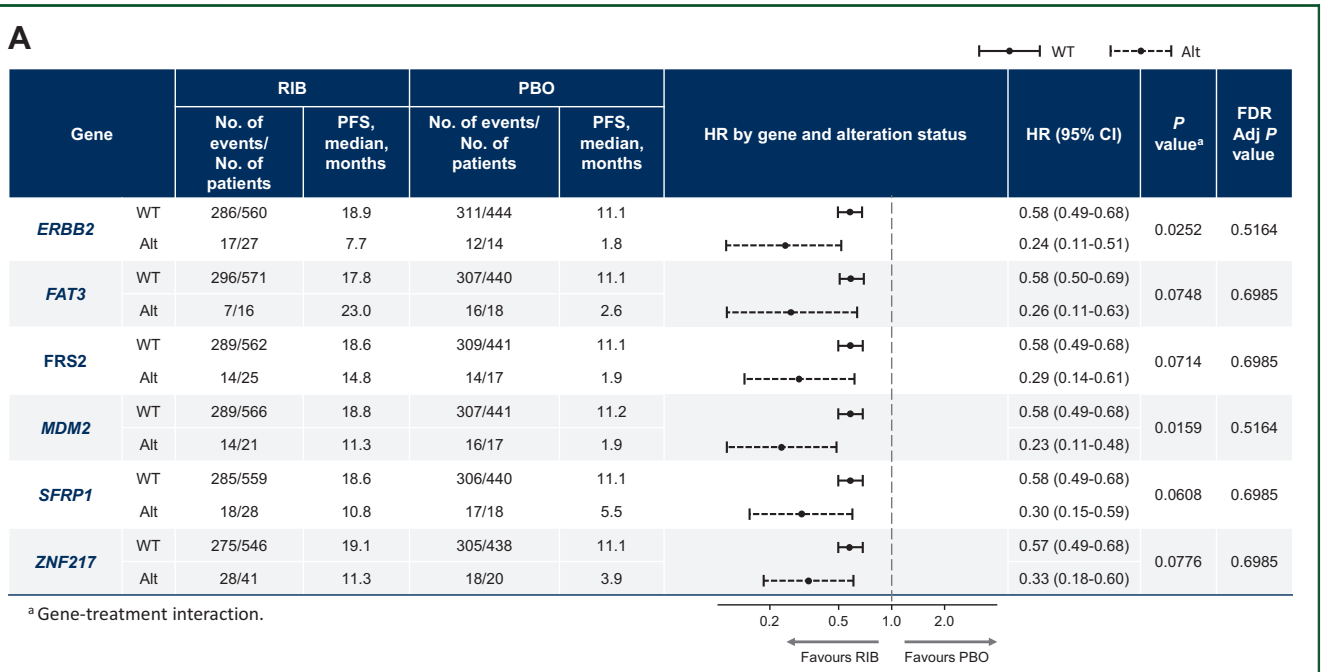
Gene alterations associated with lower sensitivity/relative resistance to ribociclib treatment

Gene alterations associated with lower sensitivity/relative resistance to ribociclib (*P* value for gene—treatment interaction <0.10) are reported in Figure 3A and Supplementary Figure S3A, available at https://doi.org/10.1016/j.annonc.2023.08.011. These included *ANO1*, *CDKN2A/B/C*, and *RB1*. In patients with alterations in *ANO1* (147/1045; 14%), there was limited benefit of ribociclib versus placebo, with median PFS values of 10.5 versus 7.4 months [HR = 0.78 (95% CI 0.53-1.16); interaction $P = 0.0749$] (Figure 3B); however, the benefit of ribociclib over placebo was evident in patients with WT *ANO1* [HR = 0.53 (95% CI 0.44-0.63)]. For genes of interest though with lower alteration rate, *CDKN2A/B/C* alterations occurred in 27/1045 samples (3%). Patients with altered *CDKN2A/B/C* did not experience a benefit with ribociclib versus placebo [median PFS 6.5

A

Gene		RIB No. of events/ No. of patients	RIB PFS, median, months	PBO No. of events/ No. of patients	PBO PFS, median, months	HR by gene and alteration status	HR (95% CI)	*P* value[a]	FDR Adj *P* value
ERBB2	WT	286/560	18.9	311/444	11.1		0.58 (0.49-0.68)	0.0252	0.5164
	Alt	17/27	7.7	12/14	1.8		0.24 (0.11-0.51)		
FAT3	WT	296/571	17.8	307/440	11.1		0.58 (0.50-0.69)	0.0748	0.6985
	Alt	7/16	23.0	16/18	2.6		0.26 (0.11-0.63)		
FRS2	WT	289/562	18.6	309/441	11.1		0.58 (0.49-0.68)	0.0714	0.6985
	Alt	14/25	14.8	14/17	1.9		0.29 (0.14-0.61)		
MDM2	WT	289/566	18.8	307/441	11.2		0.58 (0.49-0.68)	0.0159	0.5164
	Alt	14/21	11.3	16/17	1.9		0.23 (0.11-0.48)		
SFRP1	WT	285/559	18.6	306/440	11.1		0.58 (0.49-0.68)	0.0608	0.6985
	Alt	18/28	10.8	17/18	5.5		0.30 (0.15-0.59)		
ZNF217	WT	275/546	19.1	305/438	11.1		0.57 (0.49-0.68)	0.0776	0.6985
	Alt	28/41	11.3	18/20	3.9		0.33 (0.18-0.60)		

[a] Gene-treatment interaction.



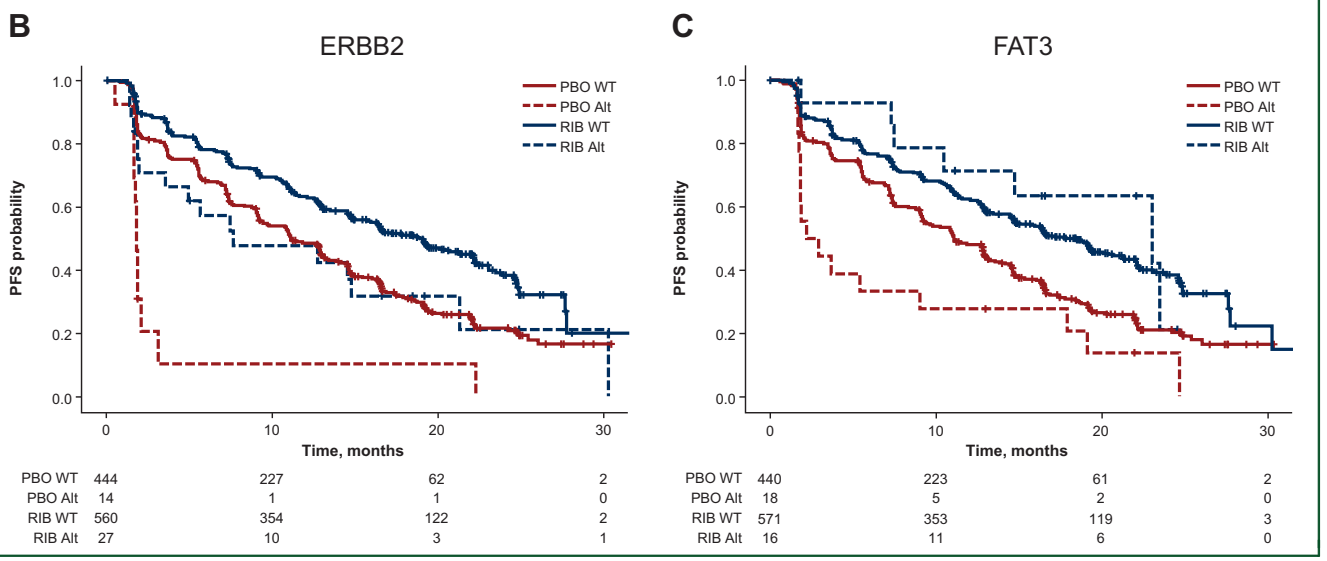


Figure 2. PFS in patients with gene alterations associated with improved outcomes with RIB.
(A) Forest plot analysis of potential biomarkers of RIB response. (B) PFS by *ERBB2* Alt status. (C) PFS by *FAT3* Alt status. (D) PFS by *SFRP1* Alt status. (E) PFS by *ZNF217* Alt status. (F) PFS by *FRS2* amplification status. (G) PFS by *MDM2* Alt status. (H) PFS by *FRS2/MDM2* Alt status. (B-H) Kaplan—Meier curves for PFS in patients who exhibited alterations in the indicated genes in circulating tumour DNA. PFS in patients in the RIB treatment arm is shown in blue; PFS in patients in the PBO treatment arm is shown in red. The WT subgroup is indicated by a solid line, and the Alt subgroup is indicated by a dashed line. Number of patients at risk are displayed in the tables below each Kaplan—Meier curve.
Adj, adjusted; Alt, alteration; CI, confidence interval; FDR, false discovery rate; HR, hazard ratio; PBO, placebo; PFS, progression-free survival; RIB, ribociclib; WT, wild-type.

versus 9.2 months; HR = 1.20 (95% CI 0.50-2.88); interaction *P* = 0.0874] (Figure 3C). Patients with *RB1* alterations (29/1045; 3%) experienced a decreased benefit with ribociclib versus placebo (Figure 3D), with a median PFS of 3.8 versus 9.2 months [HR = 1.48 (95% CI 0.65-3.38)] compared with that of 18.9 versus 11.1 months for those with WT *RB1* [HR = 0.56 (95% CI 0.47-0.66)].

Additional genes of interest

ESR1 alterations occurred in 92/1045 samples [9% overall, 5.7% in MONALEESA-2, 15.5% in MONALEESA-3 (3.6%-7.0% in the first-line setting, 17.8%-46.7% in the second-line setting), and 3.8% in MONALEESA-7] (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2023.08.011). While patients with *ESR1* alterations versus WT treated with placebo had poor PFS outcomes, a similar treatment benefit with ribociclib was observed with altered *ESR1* [HR = 0.49 (95% CI 0.31-0.78); median PFS for ribociclib versus placebo, 8.6 versus 3.7 months] or WT *ESR1* [HR = 0.57 (95% CI 0.48-0.68); median PFS for ribociclib versus placebo, 19.2 versus 11.4 months]. *FGFR1* alterations occurred in 92/1045 samples (9%) (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2023.08.011). Patients benefited from ribociclib regardless of *FGFR1*

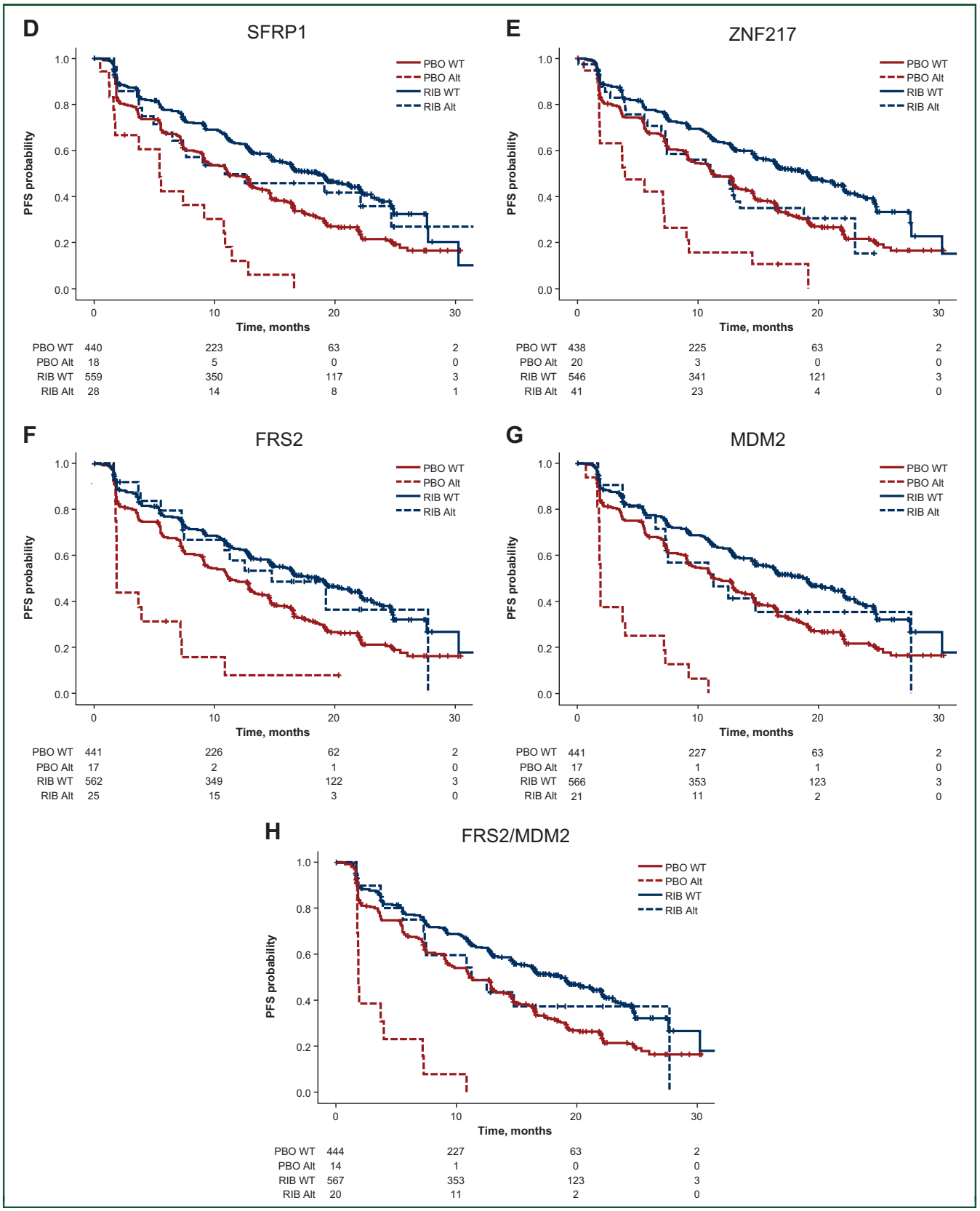


Figure 2. **Continued.**

A

Gene		RIB No. of events/ No. of patients	RIB PFS, median, months	PBO No. of events/ No. of patients	PBO PFS, median, months	HR by gene and alteration status	HR (95% CI)	*P* value[a]	FDR Adj *P* value
ANO1	WT	238/494	19.3	281/404	11.1		0.53 (0.44-0.63)	0.0749	0.6984
	Alt	65/93	10.5	42/54	7.4		0.78 (0.53-1.16)		
CDKN2A/ 2B/2C	WT	291/573	18.9	314/445	11.1		0.55 (0.47-0.65)	0.0874	0.7080
	Alt	12/14	6.5	9/13	9.2		1.2 (0.50-2.88)		
RB1	WT	292/573	18.9	311/443	11.1		0.56 (0.47-0.66)	0.0227	0.5164
	Alt	11/14	3.8	12/15	9.2		1.48 (0.65-3.38)		

WT Alt

0.5 1.0 2.0

Favours RIB Favours PBO

[a] Gene-treatment interaction.

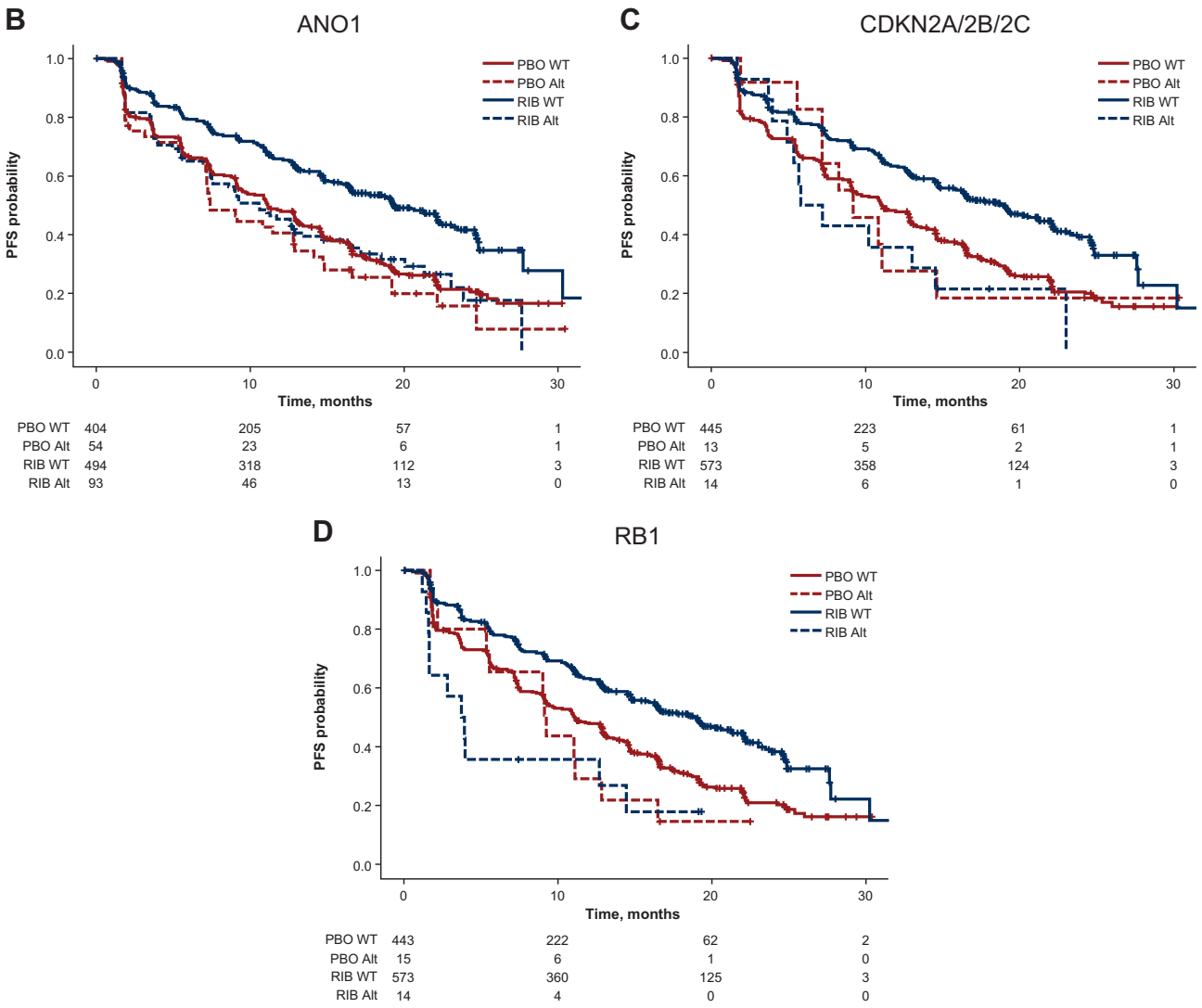


Figure 3. PFS in patients with gene alterations associated with decreased RIB activity.
(A) Forest plot analysis of potential biomarkers of RIB resistance. (B) PFS by *ANO1* Alt status. (C) PFS by *CDKN2A/2B/2C* Alt status. (D) PFS by *RB1* Alt status. (B-D) Kaplan—Meier curves for PFS in patients who exhibited alterations in the indicated genes in circulating tumour DNA. PFS in patients in the RIB treatment arm is shown in blue; PFS in patients in the PBO treatment arm is shown in red. The WT subgroup is indicated by a solid line, and the Alt subgroup is indicated by a dashed line. Number of patients at risk are displayed in the tables below each Kaplan—Meier curve.
Adj, adjusted; Alt, alteration; CI, confidence interval; FDR, false discovery rate; HR, hazard ratio; PBO, placebo; PFS, progression-free survival; RIB, ribociclib; WT, wild-type.

https://doi.org/10.1016/j.annonc.2023.08.011

alterations [altered: HR = 0.44 (95% CI 0.28-0.71); WT: HR = 0.57 (95% CI 0.48-0.68)]. With ribociclib versus placebo, median PFS was 18.9 versus 11.1 months in patients with WT *FGFR1* and 11.0 versus 6.1 months in patients with altered *FGFR1*. *BRCA1/2* alterations (excluding amplifications) occurred in 43/1045 samples (4%) (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2023.08.011). Median PFS in patients with altered *BRCA1/2* was 14.6 months with ribociclib and 8.1 months with placebo [HR = 0.38 (95% CI 0.19-0.76)]; in patients with WT *BRCA1/2*, it was 18.8 months with ribociclib and 11.1 months with placebo [HR = 0.58 (95% CI 0.49-0.68)]. *BRCA2* alterations occurred in 36/1045 samples (3%). Regardless of *BRCA2* alteration status, the benefit of ribociclib was observed over placebo, similar to that observed in patients with alterations in *BRCA1/2* (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2023.08.011). *AURKA* alterations were identified in 40/1045 samples (4%) (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2023.08.011). Benefit with ribociclib was observed regardless of alteration status [altered: HR = 0.34 (95% CI 0.17-0.69); WT: HR = 0.57 (95% CI 0.49-0.68)]. *PTEN* loss (nonsense mutation or gene deletion) was observed in 19/1045 patients (2%) and was associated with worse prognosis, regardless of treatment (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2023.08.011). In these patients, median PFS was 8.9 months with ribociclib and 10.9 months with placebo (HR, not estimable). *NF1* alterations were observed in 34/1045 samples (3%), of which 16/34 were nonsense/frameshift alterations (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2023.08.011). Overall, patients with *NF1* alterations responded poorly to placebo versus ribociclib [median PFS 1.8 versus 13 months; HR = 0.32 (95% CI 0.15-0.68)], and this was particularly evident in those with nonsense/frameshift *NF1* alterations (median PFS 1.8 versus 23.5 months; HR, not estimable).

ctDNA fraction analysis

An analysis of patients with <1% versus ≥1% ctDNA fraction was undertaken. There were no significant differences between treatment arms in ctDNA fraction representativeness (Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2023.08.011). Patients with <1% ctDNA fraction generally had a more favourable best overall response, lower rate of prior chemotherapy and prior hormone therapy, higher frequency of *de novo* disease, better Eastern Cooperative Oncology Group (ECOG) performance status, and were composed of a slightly higher percentage of whites and a lower percentage of Asians. Total target lesion diameter at baseline was lower among patients with a ctDNA fraction <1% ($P < 0.0001$) (Supplementary Figure S4A, available at https://doi.org/10.1016/j.annonc.2023.08.011) and higher ctDNA fraction was prognostic of poorer PFS regardless of treatment (Supplementary Figure S4B, available at https://doi.org/10.1016/j.annonc.2023.08.011). The PFS benefit of ribociclib over placebo was observed regardless of ctDNA fraction.

Tumour mutational burden and efficacy of ribociclib

TMB was estimated in 1045 patients and the predictive relationship with PFS was assessed (Figure 4). There was no significant difference in TMB between arms, but TMB was lower in MONALEESA-7 than in MONALEESA-2 and MONALEESA-3. Higher TMB has been associated with increased age; thus, this observation could be explained by the enrolment of premenopausal patients in MONALEESA-7 (Supplementary Figure S5, available at https://doi.org/10.1016/j.annonc.2023.08.011).[38] The *P* value for interaction between TMB quartile and treatment effect was 0.0498. The benefit of ribociclib was observed across all quartiles, but the magnitude of benefit was weakest in the top quartile (HR = 0.74). A relative reduction in the efficacy of ribociclib versus placebo was observed with TMB >10 mutations/Mb [$n = 34$; median PFS 7.5 versus 5.1 months; HR = 0.59 (95% CI 0.22-1.57)]; this was consistent with the results in the top quartile in which the median PFS for ribociclib versus placebo was 9.2 versus 7.4 months [HR = 0.74 (95% CI 0.56-0.99)]. Higher TMB was prognostic of worse PFS regardless of treatment (Supplementary Figure S6, available at https://doi.org/10.1016/j.annonc.2023.08.011).

DISCUSSION

This is the largest pooled biomarker analysis characterizing the correlation between genomic alterations in baseline ctDNA and clinical efficacy of a CDK4/6i.

This analysis has implications for further clinical research and practice, including identification of patients with CDK4/6i-resistant tumours. Consistent with preclinical studies showing that *RB1* mutations confer CDK4/6i resistance, patients with *RB1* mutations demonstrated little PFS benefit with ribociclib [HR = 1.48 (95% CI 0.65-3.38)], although alterations in *RB1* occurred in 3% of patients.[39] Patients with loss-of-function alterations of CDKN2A/B/C had numerically shorter PFS treated with ribociclib compared with placebo; this is in contrast with reported preclinical observations.[40,41] Compared with WT, alterations in *ANO1* were observed to be associated with lower ribociclib sensitivity. Among patients with alterations associated with decreased sensitivity to ribociclib, PFS with ET was in the same range as that observed in patients without alteration, suggesting that these genes could be involved in CDK4/6i but not ET resistance. Additionally, when the *PTEN* analysis was restricted to nonsense mutations or gene deletions ($n = 19$), median PFS was shorter with ribociclib than ET alone in patients with altered *PTEN*, implicating *PTEN* loss-of-function mutations as potential biomarkers of ribociclib resistance. These data are consistent with prior studies and could provide rationale to combine PI3K pathway inhibitors, CDK4/6i, and ET in patients with *PTEN* loss-of-function mutations.[14] As previously reported, *PIK3CA* mutations were not predictive of CDK4/6i efficacy.[42,43]

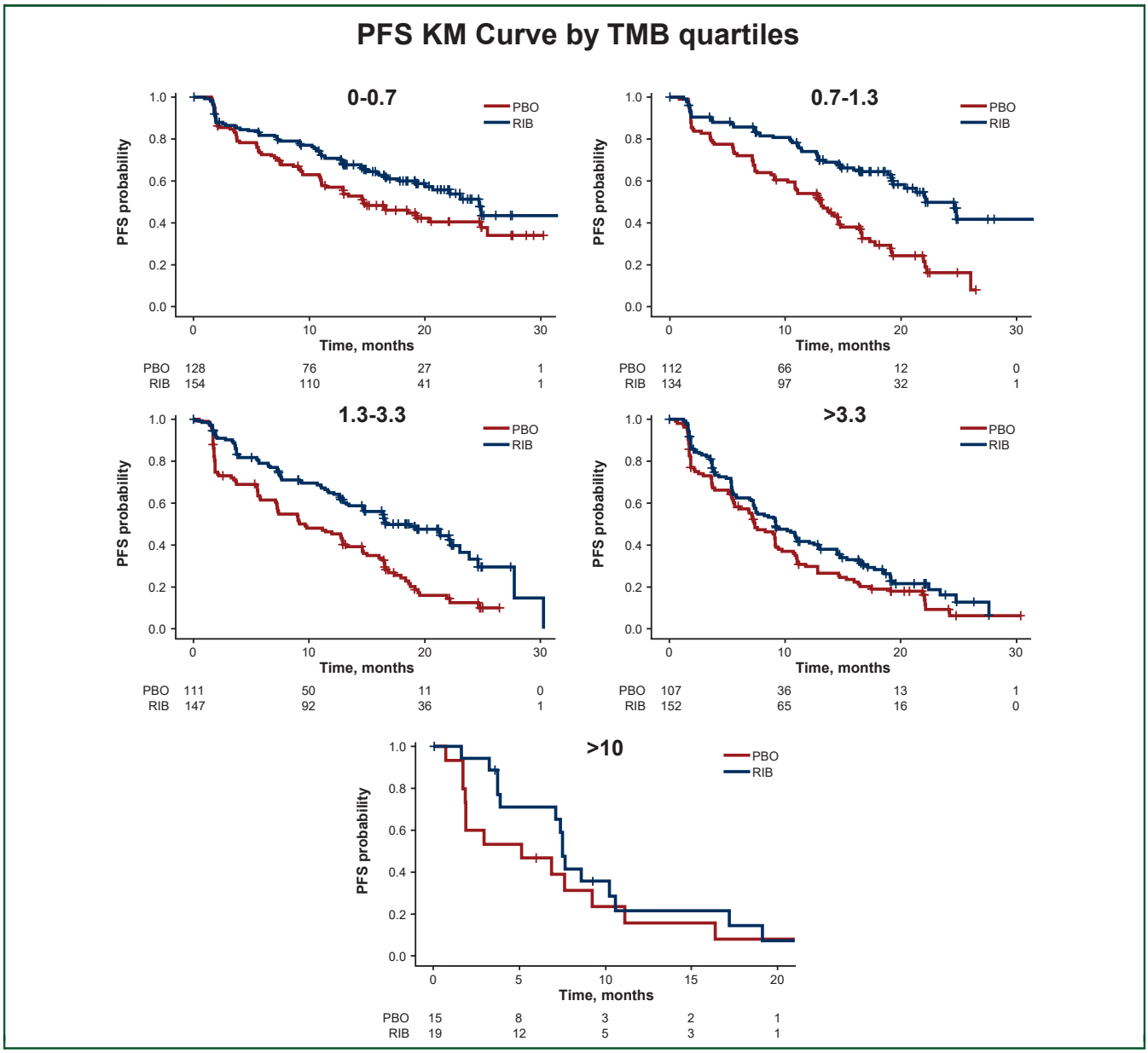


Figure 4. Prognostic and predictive relationship between PFS and TMB.
PFS by TMB. PFS in patients in the RIB treatment arm is shown in blue; PFS in patients in the PBO treatment arm is shown in red. HR (95% CI) estimates and median PFS (95% CI) values are shown in the corresponding table.
CI, confidence interval; HR, hazard ratio; KM, Kaplan—Meier; NA, not available; PBO, placebo; PFS, progression-free survival; Q, quartile; RIB, ribociclib; TMB, tumour mutational burden.

ERBB2, *FAT3*, *FRS2*, *MDM2*, *SFRP1*, and *ZNF217* alterations were associated with sensitivity to ribociclib treatment. Patients treated with ET alone with alterations in these genes had a shorter PFS than those with WT genes, suggesting that these genes are associated with ET resistance. Although these alterations are associated with ET resistance, the addition of a CDK4/6i appears to reverse the resistance leading to a large relative increase in PFS. An interesting observation is that, while *MDM2* alterations were associated with sensitivity to ribociclib and met the interaction $P < 0.10$ threshold, *TP53* alterations did not meet this threshold. The results of *MDM2* may be related to the fact that *MDM2* and *FRS2* were co-amplified, with *FRS2* being the potential driver of the ribociclib benefit observed. With respect to additional genes of interest, in the case of *ESR1* and *FGFR1*, while a benefit was observed for ribociclib over ET alone regardless of whether an alteration was present, those with alterations in these genes had a shorter PFS, regardless of treatment. The identification of genomic alterations associated with sensitivity to CDK4/6i could help identify which patients derive benefit from CDK4/6i in the adjuvant setting.

Higher baseline purity/ctDNA levels have been shown to be prognostic of poor response and to correlate with prognostic clinical factors.[44,45] In this analysis, we found that a ctDNA fraction of <1% was associated with a more favourable best overall response, lower rates of prior chemotherapy and prior hormone therapy, higher

https://doi.org/10.1016/j.annonc.2023.08.011

TMB quartile	Treatment	n/N	PFS, median, months	PFS 95% CI	HR (95% CI)
Q1 (0-0.7)	PBO	71/128	14.69	11.01-20.30	0.63 (0.45-0.88)
	RIB	63/154	24.67	19.19-NA	
Q2 (0.7-1.3)	PBO	79/112	13.11	10.25-14.59	0.43 (0.30-0.60)
	RIB	53/134	22.14	19.35-NA	
Q3 (1.3-3.3)	PBO	86/111	9.17	7.20-12.91	0.47 (0.35-0.64)
	RIB	78/147	16.59	13.47-22.21	
Q4 (>3.3)	PBO	87/107	7.43	5.65-9.17	0.74 (0.56-0.99)
	RIB	109/152	9.23	7.36-11.07	
TMB >10	PBO	14/15	5.1	1.7-9.2	0.59 (0.22-1.57)
	RIB	15/19	7.5	3.7-10.5	

Figure 4. Continued.

frequency of *de novo* disease, and better ECOG performance status. We also observed that ctDNA fraction <1% was associated with a lower total target lesion diameter at baseline and was prognostic of better PFS; however, the benefit of ribociclib was observed over ET alone regardless of ctDNA fraction.

While ribociclib generally demonstrated benefit in patients with TMB in lower quartiles (Q1-Q4), it was weakest in the top quartile, and patients with TMB >10 did not experience a clinically relevant benefit with ribociclib. These patients therefore represent a large unmet need, and there remains a necessity to develop prospective clinical trials testing new drugs in this population. A basket study recently showed that pembrolizumab is effective in patients with high TMB, irrespective of tumour origin.[46] Prior reports suggested that patients with HR+, TMB-high metastatic cancers could be responsive to anti–programmed cell death protein 1 antibodies.[47] These findings challenge the conventional sequence of therapy in HR+ ABC, for which consensus groups suggest that patients should start with ET.[48] Thus, there is rationale to test cytotoxic agents and immunotherapies in this selected population because studies suggest that TMB-high cancers could be sensitive to chemotherapy.[49]

In addition to its large size, this analysis had several strengths (Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2023.08.011). Use of ctDNA as a surrogate pool of all metastases in a patient is likely more informative than an analysis of a single metastatic tumour biopsy specimen. Analysis of genomic status at study entry is another strength, given that many published reports used archival material from primary tumours, which is unlikely to reflect the genomic profile of the metastatic tumour. Nevertheless, this analysis had several limitations. Data generation was initiated when unique molecular identifiers were not routinely used, limiting the sensitivity of the assay. Given the relative insensitivity of ctDNA detection, the requirement of ctDNA fraction >1% for inclusion, and the previous findings that patients with detectable ctDNA have a worse outcome overall, the selection of the population included in this analysis could have been biased towards patients with poorer prognosis.[50] The ability to detect copy number is more limited in ctDNA, and the frequency of amplified genes is likely underestimated here. Subclonal variants may be missing in patients with low levels of shedding or very low mutant allele fractions. Patients presented a large variability of ctDNA that could potentially affect the mutant allele fraction of the genes. This could be relevant for genes associated with resistance, as high ctDNA levels could be associated with more aggressive disease. To mitigate this limitation, we limited the analyses to patients who had ctDNA levels ≥1%. TMB was estimated using all non-synonymous and synonymous alterations but was not adjusted for CHIP, which is another limitation of this analysis; however, patients with CHIP generally have few CHIP mutations (generally 1 or fewer), and thus the impact on TMB should be limited.[35,51-53] Germline sequencing was not carried out, but germline mutations were filtered out bioinformatically (Methods). This analysis was exploratory, and if an adjustment was carried out, none of the alterations would have remained significant with a *P* value of 0.05. This dataset did not have the power to test the predictive value of alterations occurring at low frequency, thus splitting the current population into training and test sets would have limited the power of the analysis. Small sample sizes and wide 95% CIs in some cases should also be noted. While adjusted for each trial, the analyses cannot test whether the predictive value of an alteration is specific to an ET backbone. This analysis tested the association between gene alteration and drug resistance or sensitivity, but only mechanistic investigation will provide evidence of causality.

This analysis identified biomarkers that may be associated with response of or resistance to ribociclib that could affect future drug development for patients with ABC. These data are hypothesis generating, and while they do not have immediate clinical implications, they do provide direction for future research.

FUNDING

This analysis was sponsored by Novartis, which also provided financial support for medical editorial assistance.

ACKNOWLEDGEMENTS

We thank the patients who participated in these trials, their families and caregivers, datamonitoring committee members, study steering committee members, and the staff who assisted with the trial at each site. We thank Casey Nielsen, PhD, of MediTech Media, for medical editorial assistance with this manuscript. Ribociclib was discovered by Novartis

Institutes for BioMedical Research in collaboration with Astex Pharmaceuticals.

DISCLOSURE

FA's institution has received research funding from AstraZeneca, Lilly, Novartis, Pfizer, and Roche. FS, NS, AL, TT, and NB report employment and stock ownership with Novartis. GH reports receiving consulting or advisory role fees from Agendia, Lilly, Merck, Novartis, Peregrine Pharmaceuticals, and Roche and travel expenses from Novartis, and his institution has received research funding from Novartis. SC reports advisory board fees, and his institution has received funds for participation in clinical trials by Novartis, Pfizer, F. Hoffmann-La Roche Ltd., and Eli Lilly. AB's institution has received research grants from Genentech, Novartis, Pfizer, Merck, Sanofi, Radius Health, Immunomedics, Mersana, and Innocrin, and he has received advisory board and consulting fees from Biotheranostics Inc.; advisory board, consulting fees, steering committee, and travel support from Pfizer, Novartis, Genentech, Merck, Radius Health, and Immunomedics; advisory board, consulting fees, and travel support from Spectrum Pharma, Taiho, and Daiichi Sankyo; and advisory board and consulting fees from Puma Biotechnology. DT has received consulting fees and clinical research support paid to his institution from Novartis and reports consulting fees from Pfizer, Genomic Health, GSK, Polyphor, and Sellas Life Sciences Group and scientific advisory board (unpaid) for Puma Biotechnology. Y-SL has received clinical trial study fees, grants for clinical study of *ESR1* mutation detected by cell-free DNA, advisory board consultation fee, and speaker fee from Novartis; consultation fee from Pfizer; contracted research grant from Roche, Merck Sharp & Dohme, and Pfizer; and invited speaker fees from Eisai and AstraZeneca. DS has received board of directors (stock) and travel expenses from BioMarin; stock ownership, research funding, and travel expenses from Pfizer; advisory board, consulting, research funding, and travel expenses from Novartis; consulting fees from Eli Lilly; and stock ownership with Amgen and Seagen Inc. CLA has received research funding grant from Pfizer, Lilly, Takeda, Radius Health, and Puma Biotechnology and advisory board fees from Novartis, Lilly, Merck, Sanofi, Symphogen, Taiho Oncology, Puma Biotechnology, Radius, H3 Biomedicine, OrigiMed, and Daiichi Sankyo. PN has declared no conflicts of interest.

REFERENCES

1. Finn RS, Aleshin A, Slamon DJ. Targeting the cyclin-dependent kinases (CDK) 4/6 in estrogen receptor-positive breast cancers. *Breast Cancer Res*. 2016;18:17.
2. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490:61-70.
3. Ingham M, Schwartz GK. Cell-cycle therapeutics come of age. *J Clin Oncol*. 2017;35:2949-2959.
4. Peurala E, Koivunen P, Haapasaari KM, Bloigu R, Jukkola-Vuorinen A. The prognostic significance and value of cyclin D1, CDK4 and p16 in human breast cancer. *Breast Cancer Res*. 2013;15:R5.
5. Bosco EE, Wang Y, Xu H, et al. The retinoblastoma tumor suppressor modifies the therapeutic response of breast cancer. *J Clin Invest*. 2007;117:218-228.
6. Spring LM, Wander SA, Andre F, Moy B, Turner NC, Bardia A. Cyclin-dependent kinase 4 and 6 inhibitors for hormone receptor-positive breast cancer: past, present, and future. *Lancet*. 2020;395:817-827.
7. Sledge GW Jr, Toi M, Neven P, et al. The effect of abemaciclib plus fulvestrant on overall survival in hormone receptor-positive, ERBB2-negative breast cancer that progressed on endocrine therapy-MONARCH 2: a randomized clinical trial. *JAMA Oncol*. 2020;6:116-124.
8. Slamon DJ, Neven P, Chia S, et al. Overall survival with ribociclib plus fulvestrant in advanced breast cancer. *N Engl J Med*. 2020;382:514-524.
9. Im SA, Lu YS, Bardia A, et al. Overall survival with ribociclib plus endocrine therapy in breast cancer. *N Engl J Med*. 2019;381:307-316.
10. Hortobagyi GN, Stemmer SM, Burris HA, et al. Overall survival with ribociclib plus letrozole in advanced breast cancer. *N Engl J Med*. 2022;386:942-950.
11. Turner NC, Liu Y, Zhu Z, et al. Cyclin E1 expression and palbociclib efficacy in previously treated hormone receptor-positive metastatic breast cancer. *J Clin Oncol*. 2019;37:1169-1178.
12. Pascual J, Gil-Gil M, Zielinski C, et al. CCNE1 mRNA and cyclin E1 protein expression as predictive biomarkers for efficacy of palbociclib plus fulvestrant versus capecitabine in the phase III PEARL study. *J Clin Oncol*. 2021;39(suppl 15):1014.
13. Formisano L, Lu Y, Servetto A, et al. Aberrant FGFR signaling mediates resistance to CDK4/6 inhibitors in ER+ breast cancer. *Nat Commun*. 2019;10:1373.
14. Costa C, Wang Y, Ly A, et al. PTEN loss mediates clinical cross-resistance to CDK4/6 and PI3Kα inhibitors in breast cancer. *Cancer Discov*. 2020;10:72-85.
15. Yang C, Li Z, Bhatt T, et al. Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. *Oncogene*. 2017;36:2255-2264.
16. Li Z, Razavi P, Li Q, et al. Loss of the FAT1 tumor suppressor promotes resistance to CDK4/6 inhibitors via the hippo pathway. *Cancer Cell*. 2018;34:893-905.e8.
17. O'Leary B, Cutts RJ, Huang X, et al. Circulating tumor DNA markers for early progression on fulvestrant with or without palbociclib in ER+ advanced breast cancer. *J Natl Cancer Inst*. 2021;113:309-317.
18. Piezzo M, Cocco S, Caputo R, et al. Targeting cell cycle in breast cancer: CDK4/6 inhibitors. *Int J Mol Sci*. 2020;21:6479.
19. Condorelli R, Spring L, O'Shaughnessy J, et al. Polyclonal RB1 mutations and acquired resistance to CDK 4/6 inhibitors in patients with metastatic breast cancer. *Ann Oncol*. 2018;29:640-645.
20. O'Leary B, Cutts RJ, Liu Y, et al. The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. *Cancer Discov*. 2018;8:1390-1403.
21. Nayar U, Cohen O, Kapstad C, et al. Acquired HER2 mutations in ER(+) metastatic breast cancer confer resistance to estrogen receptor-directed therapies. *Nat Genet*. 2019;51:207-216.
22. Drago JZ, Formisano L, Juric D, et al. FGFR1 amplification mediates endocrine resistance but retains TORC sensitivity in metastatic hormone receptor-positive (HR+) breast cancer. *Clin Cancer Res*. 2019;25:6443-6451.
23. Wander SA, Cohen O, Gong X, et al. The genomic landscape of intrinsic and acquired resistance to cyclin-dependent kinase 4/6 inhibitors in patients with hormone receptor-positive metastatic breast cancer. *Cancer Discov*. 2020;10:1174-1193.
24. Mao P, Cohen O, Kowalski KJ, et al. Acquired FGFR and FGF alterations confer resistance to estrogen receptor (ER) targeted therapy in ER(+) metastatic breast cancer. *Clin Cancer Res*. 2020;26:5974-5989.
25. Xu G, Chhangawala S, Cocco E, et al. ARID1A determines luminal identity and therapeutic response in estrogen-receptor-positive breast cancer. *Nat Genet*. 2020;52:198-207.
26. Bertucci F, Ng CKY, Patsouris A, et al. Genomic characterization of metastatic breast cancers. *Nature*. 2019;569:560-564.

27. Chen P, Lee NV, Hu W, et al. Spectrum and degree of CDK drug interactions predicts clinical performance. *Mol Cancer Ther*. 2016;15:2273-2281.
28. Gelbert LM, Cai S, Lin X, et al. Preclinical characterization of the CDK4/6 inhibitor LY2835219: in-vivo cell cycle-dependent/independent antitumor activities alone/in combination with gemcitabine. *Invest New Drugs*. 2014;32:825-837.
29. Tripathy D, Im SA, Colleoni M, et al. Ribociclib plus endocrine therapy for premenopausal women with hormone-receptor-positive, advanced breast cancer (MONALEESA-7): a randomised phase 3 trial. *Lancet Oncol*. 2018;19:904-915.
30. Hortobagyi GN, Stemmer SM, Burris HA, et al. Updated results from MONALEESA-2, a phase III trial of first-line ribociclib plus letrozole versus placebo plus letrozole in hormone receptor-positive, HER2-negative advanced breast cancer. *Ann Oncol*. 2018;29:1541-1547.
31. Slamon DJ, Neven P, Chia S, et al. Phase III randomized study of ribociclib and fulvestrant in hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer: MONALEESA-3. *J Clin Oncol*. 2018;36:2465-2472.
32. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol*. 2013;31:213-219.
33. Ye K, Schulz MH, Long Q, Apweiler R, Ning Z. Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. *Bioinformatics*. 2009;25:2865-2871.
34. Riester M, Singh AP, Brannon AR, et al. PureCN: copy number calling and SNV classification using targeted short read sequencing. *Source Code Biol Med*. 2016;11:13.
35. Fairchild L, Whalen J, D'Aco K, et al. Clonal hematopoiesis detection in patients with cancer using cell-free DNA sequencing. *Sci Transl Med*. 2023;15:eabm8729.
36. Oh S, Geistlinger L, Ramos M, et al. Reliable analysis of clinical tumor-only whole-exome sequencing data. *JCO Clin Cancer Inform*. 2020;4:321-335.
37. The R Project for Statistical Computing, https://www.R-project.org/. Accessed September 7, 2022.
38. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med*. 2017;9:34.
39. Guarducci C, Bonechi M, Boccalini G, et al. Mechanisms of resistance to CDK4/6 inhibitors in breast cancer and potential biomarkers of response. *Breast Care (Basel)*. 2017;12:304-308.
40. Li Q, Jiang B, Guo J, et al. INK4 Tumor suppressor proteins mediate resistance to CDK4/6 kinase inhibitors. *Cancer Discov*. 2022;12:356-371.
41. Palafox M, Monserrat L, Bellet M, et al. High p16 expression and heterozygous RB1 loss are biomarkers for CDK4/6 inhibitor resistance in ER(+) breast cancer. *Nat Commun*. 2022;13:5258.
42. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. *Lancet Oncol*. 2016;17:425-439.
43. Tolaney SM, Toi M, Neven P, et al. Clinical significance of PIK3CA and ESR1 mutations in ctDNA and FFPE samples from the MONARCH 2 study of abemaciclib plus fulvestrant. *Cancer Res*. 2019;79(suppl 13):4458.
44. Sant M, Bernat-Peguera A, Felip E, Margelí M. Role of ctDNA in breast cancer. *Cancers (Basel)*. 2022;14:310.
45. Garcia-Pardo M, Makarem M, Li JJN, Kelly D, Leighl NB. Integrating circulating-free DNA (cfDNA) analysis into clinical practice: opportunities and challenges. *Br J Cancer*. 2022;127:592-602.
46. Marabelle A, Fakih MG, Lopez J, et al. Association of tumor mutational burden with outcomes in patients with select advanced solid tumors treated with pembrolizumab in KEYNOTE-158. *Ann Oncol*. 2019;30(suppl 5):V477-V478.
47. Barroso-Sousa R, Jain E, Cohen O, et al. Prevalence and mutational determinants of high tumor mutation burden in breast cancer. *Ann Oncol*. 2020;31:387-394.
48. Cardoso F, Senkus E, Costa A, et al. 4th ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 4). *Ann Oncol*. 2018;29:1634-1657.
49. Karn T, Denkert C, Weber KE, et al. Tumor mutational burden and immune infiltration as independent predictors of response to neoadjuvant immune checkpoint inhibition in early TNBC in GeparNuevo. *Ann Oncol*. 2020;31:1216-1222.
50. Rohanizadegan M. Analysis of circulating tumor DNA in breast cancer as a diagnostic and prognostic biomarker. *Cancer Genet*. 2018;228-229:159-168.
51. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371:2488-2498.
52. Mouhieddine TH, Sperling AS, Redd R, et al. Clonal hematopoiesis is associated with adverse outcomes in multiple myeloma patients undergoing transplant. *Nat Commun*. 2020;11:2996.
53. Bolton KL, Ptashkin RN, Gao T, et al. Cancer therapy shapes the fitness landscape of clonal hematopoiesis. *Nat Genet*. 2020;52:1219-1226.