Target Venue: *Clinical Cancer Research*

Landscape of baseline and acquired genomic alterations in circulating tumor DNA with abemaciclib alone or with endocrine therapy in advanced breast cancer

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Statement of translational relevance

This study investigated genomic alterations in the circulating tumor DNA of patients in the Phase 3 MONARCH 3 and Phase 2 nextMONARCH studies. This study is the first to explore genomic alterations in ctDNA samples from patients with HR+, HER2– ABC treated with abemaciclib +/- NSAI and the relationship between baseline or treatment-emergent genomic alterations and clinical outcomes. The most frequent baseline genomic alterations, similar in both studies, have been previously associated with endocrine resistance and may additionally drive resistance to CDK4/6 inhibitors plus ET. In MONARCH 3, abemaciclib plus NSAI was associated with improved mPFS compared with placebo plus NSAI, regardless of baseline genomic alterations. Acquired alterations potentially associated with resistance to abemaciclib monotherapy or abemaciclib plus NSAI included RB1 and MYC. These findings are hypothesis-generating and further exploration is warranted into mechanisms of resistance to abemaciclib and ET. Understanding potential mechanisms of intrinsic and acquired resistance will help inform future drug development and clinical trials.

ABSTRACT

PURPOSE: To identify potential predictors of response and resistance mechanisms in patients with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2–) advanced breast cancer (ABC) treated with the CDK4/6 inhibitor abemaciclib +/- endocrine therapy (ET), baseline and acquired genomic alterations in circulating tumor DNA (ctDNA) were analyzed and associated with clinical outcomes.

PATIENTS AND METHODS: MONARCH 3: postmenopausal women with HR+, HER2– ABC and no prior systemic therapy in the advanced setting were randomized to abemaciclib or placebo plus nonsteroidal aromatase inhibitor (NSAI). nextMONARCH: women with HR+, HER2– metastatic breast cancer that progressed on/after prior ET and chemotherapy were randomized to abemaciclib alone (two doses) or plus tamoxifen. Baseline and end-of-treatment plasma samples from patients in MONARCH 3 and nextMONARCH (monotherapy arms) were analyzed to identify somatic genomic alterations. Association between genomic alterations and median progression-free survival (mPFS) was assessed.

RESULTS: Most patients had ≥1 genomic alteration detected in baseline ctDNA. In MONARCH 3, abemaciclib+NSAI was associated with improved mPFS versus placebo+NSAI, regardless of baseline alterations. *ESR1* alterations were less frequently acquired in the abemaciclib+NSAI arm than placebo+NSAI. Acquired alterations potentially associated with resistance to abemaciclib +/- NSAI included *RB1* and *MYC*.

CONCLUSION: In MONARCH 3, certain baseline ctDNA genomic alterations were prognostic for ET but not predictive of abemaciclib response. Further studies are warranted to assess whether ctDNA alterations acquired during abemaciclib treatment differ from other CDK4/6 inhibitors. Findings are hypothesis-generating, further exploration is warranted into mechanisms of resistance to abemaciclib and ET.

Introduction

Cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) have changed the treatment landscape of hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2−) advanced breast cancer (ABC) [1]. Three CDK4/6i, palbociclib, ribociclib and abemaciclib, have been approved for use with endocrine therapy (ET), including nonsteroidal aromatase inhibitors (NSAI) or fulvestrant, in the advanced setting [2-6]. Phase 3 studies have demonstrated significant prolongation of progression-free survival (PFS) with abemaciclib when used as initial therapy for ABC in combination with NSAI [6], and PFS and overall survival (OS) in combination with fulvestrant following progression on ET [5, 7]. Additionally, abemaciclib is the only CDK4/6i FDA-approved as monotherapy following disease progression after ET and chemotherapy in the metastatic setting, and for the adjuvant treatment of HR+, HER2−, node-positive, early breast cancer at high risk of recurrence and a Ki-67 score ≥20% [8] (DrugsAtFDA [RRID:SCR_010255]).

Despite the efficacy of CDK4/6i, intrinsic resistance occurs in some patients, while others whose tumors initially respond to therapy may develop resistance during treatment, resulting in disease progression [9]. While putative mechanisms of resistance have been evaluated, most current evidence comes from preclinical studies with limited clinical evidence of acquired genomic alterations associated with resistance [9-14]. Resistance to CDK4/6i currently falls into two main categories: (1) cell cycle alterations, e.g. loss of the retinoblastoma (Rb) tumor suppressor protein, or (2) alterations in upstream oncogenic signal transduction [13]. Greater understanding of the mechanisms of resistance to CDK4/6i will guide development of novel targeted therapeutic strategies aimed at overcoming or circumventing resistance and improving clinical outcomes.

Circulating tumor DNA (ctDNA) analysis is a non-invasive technique used to identify genomic alterations in cancer. This information may be useful for predicting treatment response, identifying mechanisms of resistance, or monitoring disease progression [15, 16]. In this study, genomic alterations were analyzed in ctDNA from patients with HR+, HER2– ABC treated with abemaciclib in the MONARCH 3 and nextMONARCH studies.

MONARCH 3 (NCT02246621) was a Phase 3 study of abemaciclib or placebo plus NSAI in postmenopausal women with HR+, HER2– ABC with no prior systemic therapy in the advanced setting. The primary endpoint of PFS was significantly prolonged in the abemaciclib group (median PFS [mPFS] 28.2 months) versus placebo arm (mPFS 14.8 months) [17]. The Phase 2 nextMONARCH trial (NCT02747004) evaluated the safety and efficacy of abemaciclib plus tamoxifen or two different doses of abemaciclib monotherapy (150 mg or 200 mg) in women with previously treated HR+, HER2– metastatic breast cancer (MBC) that progressed after prior chemotherapy and ET. In the abemaciclib monotherapy arms, mPFS was similar: 6.5 months in the abemaciclib 150 mg arm and 7.4 months in the abemaciclib 200 mg arm [18].

Here, we analyzed baseline and end-of-treatment (EOT) genomic alterations in ctDNA and association with clinical outcomes to identify potential predictors of response and mechanisms of resistance to abemaciclib amongst patients treated with abemaciclib plus NSAI (MONARCH 3) or abemaciclib monotherapy (nextMONARCH).

Methods

MONARCH 3 study design and patients

The MONARCH 3 study design was reported previously [6] and is summarized in **Figure S1**. MONARCH 3 was a Phase 3, randomized, double-blind trial of abemaciclib or placebo plus NSAI in women with HR+, HER2– ABC. The trial enrolled 493 postmenopausal women randomized 2:1 to receive oral abemaciclib (150 mg twice daily [BID]) or placebo, both in combination with NSAI (anastrozole or letrozole).

Eligible postmenopausal women had HR+, HER2– metastatic disease or locoregionally recurrent breast cancer (BC) not amenable to resection or radiotherapy with curative intent. Patients must have had either measurable or non-measurable bone-only disease as defined by Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST V1.1), no prior systemic therapy for advanced disease, adequate organ function, and an Eastern Cooperative Oncology Group performance status (ECOG PS) ≤1. Exclusion criteria included visceral crisis, lymphangitic spread or leptomeningeal carcinomatosis; inflammatory BC; evidence or history of central nervous system (CNS) metastases; or prior treatment with everolimus or a CDK4/6i.

nextMONARCH study design and patients

The nextMONARCH study design was reported previously [19] and is summarized in **Figure S1**. nextMONARCH was a Phase 2, randomized, open-label study that evaluated efficacy and tolerability of abemaciclib +/- tamoxifen in 234 women with previously treated HR+, HER2– MBC that progressed on or after prior ET.

Eligible women had prior treatment with ≥2 chemotherapy regimens (≥1 for MBC) and must have had measurable disease as defined by RECIST V1.1 and ECOG PS ≤1.

Exclusion criteria included presence of visceral crisis; evidence or history of CNS metastases or thromboembolic disease; or prior treatment with a CDK4/6i.

Enrolled patients were randomized 1:1:1 to: (A) abemaciclib 150 mg Q12H plus tamoxifen (n=78), (B) abemaciclib 150 mg Q12H (n=79), or (C) abemaciclib 200 mg Q12H plus prophylactic loperamide (n=77).

Both studies received ethical/institutional review board approval, were conducted in accordance with the Declaration of Helsinki, and patients provided informed consent before enrollment.

Plasma sample collection and ctDNA analysis

As per study protocols and in accordance with country-specific guidelines, plasma samples were to be collected at baseline and EOT (follow-up) from patients enrolled in MONARCH 3 and nextMONARCH. This analysis focusses on the abemaciclib and placebo arms of MONARCH 3 and the abemaciclib monotherapy arms (B and C) of nextMONARCH.

ctDNA analyses were conducted on three populations: the translational research population (TR) - patients with a valid ctDNA sample at baseline; TR2 - patients with a valid ctDNA sample at both baseline and EOT; and TR3 - the subset of MONARCH 3 patients in TR2 with a valid EOT ctDNA sample and progressive disease (PD) (**Figure S1**). For TR3, PD must have occurred while receiving abemaciclib/placebo and NSAI or within 60 days of discontinuation if one drug was stopped early.

Alterations at the gene level that were not present at baseline but acquired by EOT were identified in the TR2 population. Specific genes were also analyzed at the individual variant level, e.g., *ESR1* variants D538G, Y537S, etc. Synonymous mutations were excluded from analysis. Acquired gene alterations in MONARCH 3

patients who discontinued due to PD while on both study drugs, i.e., abemaciclib or placebo plus NSAI, were identified in the TR3 population.

ctDNA was analyzed using the Guardant360® 73-gene next-generation sequencing (NGS)-based assay (Guardant Health, Redwood, CA) [20-22], which has been validated with high rates of sensitivity and specificity [23]. Potential tumor-related (somatic) genomic alterations were identified. Genomic alterations included point mutations (i.e., single nucleotide variants [SNV]), insertions/deletions (INDELs), amplifications (i.e., copy number alterations [CNAs]), and fusions.

Statistical analyses

To assess baseline genomic alterations, data were dichotomized by presence/absence of a somatic alteration and treated as binary variables. To assess acquired genomic alterations, data were further subsetted into patients without a baseline somatic alteration on the gene of interest and then dichotomized by presence/absence of a somatic alteration on that same gene at EOT. Where applicable, rates of acquired genomic alterations by treatment arm were compared using a likelihood ratio chi-square test and p-values were reported accordingly.

Clinical outcomes included PFS and objective response rate (ORR; percentage of patients with a best response of complete [CR] or partial response [PR] as per RECIST V1.1). ORR was reported as the separate percentage of responders +/- detectable genomic alterations. The Kaplan-Meier method was used to estimate mPFS and 95% confidence intervals (CI) in patients +/- detectable genomic alterations and where appropriate, p-values were reported using the log-rank test. Hazard ratios (HR) and 95% CIs were derived from a univariate Cox's proportional-hazards regression model. In MONARCH 3, this analysis modeled the effect of treatment within patients +/- detectable genomic alterations separately. In nextMONARCH, this analysis modeled the effect of presence/absence of detectable genomic alterations.

Additionally for MONARCH 3, the predictive effect of each baseline genomic alteration on PFS was assessed by likelihood ratio test comparing a multivariate Cox's proportional-hazards model with the following factors: treatment arm, indicators for gene alteration(s) at baseline [yes/no] for each of *EGFR, TP53, FGFR1, NF1, CCND1, MYC, PIK3CA* and *ESR1;* and treatment-by-biomarker interaction for the gene of interest to the model with the same factors excluding the treatment-by-biomarker interaction. The predictive effect of any genomic alteration at baseline, alterations in cell cycle genes, and alterations in MAPK genes was assessed by likelihood ratio test comparing a multivariate Cox's proportional-hazards model with the following factors: treatment arm, presence of any alteration in group of genes, and biomarker-by-treatment interaction to the model with the same factors excluding the treatment-by-biomarker interaction.

Data cutoff dates were 31 October 2018 for MONARCH 3 and 28 June 2019 for nextMONARCH. These trials were not powered for retrospective biomarker analyses and no adjustments were made for multiplicity. Statistical analyses were conducted using SAS Version 9.3 or higher or R Version 3.4.4 or higher.

Data Availability

Lilly provides access to all individual participant data collected during the trial, after anonymization, with the exception of pharmacokinetic or genetic data. Data are available to request 6 months after the indication studied has been approved in the US and EU and after primary publication acceptance, whichever is later. No expiration date of data requests is currently set once data are made available. Access is provided after a proposal has been approved by an independent review committee identified for this purpose and after receipt of a signed data sharing agreement. Data and documents, including the study protocol, statistical analysis plan, clinical study report, blank or annotated case report forms, will be provided in a secure data sharing

environment. For details on submitting a request, see the instructions provided at www.vivli.org/ourmember/lilly/.

Results

Patients

In MONARCH 3, 493 patients were randomized (2:1) to receive NSAI plus abemaciclib (n=328) or placebo (n=165) and comprise the intent-to-treat [ITT] population. An evaluable baseline ctDNA sample (TR population) was obtained from 295 patients (201 abemaciclib, 94 placebo) and 210 patients (131 abemaciclib, 79 placebo) had evaluable baseline and EOT ctDNA samples (TR2 population). In nextMONARCH, 156 patients received abemaciclib monotherapy (ITT population). An evaluable baseline ctDNA sample (TR population) was obtained from 139 patients and 79 patients had both evaluable baseline and EOT ctDNA samples (TR2 population; **Figure S1**). Baseline characteristics in both studies were similar amongst the respective ITT and TR populations (**Table S1; Table S2**).

Genomic alterations in baseline ctDNA

81% of patients in MONARCH 3 and 90% of patients in nextMONARCH had at least one genomic alteration detected in baseline ctDNA.

The most frequently altered genes at baseline were *PIK3CA* (37.6%), *TP53* (25.4%), *EGFR* (11.9%), *FGFR1* (11.5%), *NF1* (10.8%), *GATA3* (9.2%), *MYC* (8.8%), and *CCND1* (8.5%) in MONARCH 3 (**Figure 1A**) and *ESR1* (40.3%), *PIK3CA* (34.5%), *TP53* (28.1%), *FGFR1* (22.3%), *GATA3* (20.9%), and *MYC* (20.1%) in nextMONARCH (**Figure 1B**).

In both studies, the most common types of baseline alterations were SNV for patients with *PIK3CA*, *TP53*, *NF1, and ESR1* alterations, CNA for patients with *FGFR1*, *CCND1*, and *MYC* alterations, and INDEL for patients with *GATA3* alterations (**Figure 2A-B**).

At baseline, 44 different *PIK3CA* variants were identified in MONARCH 3 and 69 variants in nextMONARCH. The most frequent baseline *PIK3CA* variants in both studies were common strong activating hotspot mutations, H1047R, E545K, E542K and H1047L and weaker activating mutations including E726K (**Figure S2**) [24, 25].

Association between baseline genomic alterations and clinical outcome

mPFS in the MONARCH 3 abemaciclib and placebo arms was 28.2 and 14.8 months (HR 0.52; 95% CI 0.42-0.66), respectively, in the ITT population, and 38.7 and 16.5 months (HR 0.49; 95% CI 0.33-0.61), respectively, in the TR population (**Figure 3**). mPFS with abemaciclib monotherapy in nextMONARCH was 7.4 months in both the ITT and TR populations (**Figure 4**).

In MONARCH 3, patients treated with abemaciclib had a longer mPFS than those treated with placebo irrespective of whether a baseline alteration was detected (32.8 versus 15.4 months; HR 0.49; 95% CI 0.35-0.69) or not detected (not reached versus 17.5 months; HR 0.25; 95% CI 0.1-0.58). A nominally significant interaction effect between the presence/absence of an alteration and efficacy of abemaciclib plus NSAI versus placebo plus NSAI was observed for *EGFR*, *FGFR1*, *CCND1*, and *PIK3CA* (**Figure 3**); however, these results should be interpreted with caution because of the exploratory nature of the analysis. In the placebo group, alterations in *EGFR, FGFR1, MYC, CCND1, ESR1*, cell cycle-related genes (CCRGs), and *MAPK* pathway genes were associated with a mPFS less than 12 months (**Figure 3; Figure S3A**). In nextMONARCH, mPFS was shorter in patients with a detectable baseline alteration than those with no baseline alteration detected (6.7 versus 13.0 months; HR 0.5; 95% CI 0.26-1.04; **Figure 4**)**.** Baseline genomic alterations in *PIK3CA*, *FGFR1*, *MYC*, *NF1*, *EGFR*, *RB1, CCNE1*, or CCRGs were associated with a mPFS less than 5 months. Patients with detected alterations in *TP53* or *ERBB2* trended towards a shorter mPFS, while patients with a *GATA3* alteration had numerically longer mPFS (**Figure 4; Figure S3B**). Given that there is no control arm in nextMONARCH, these effects cannot be

clearly attributed as prognostic or predictive. A similar trend was also evident for overall survival in nextMONARCH (**Figure S4**). Gene amplifications were the most frequent baseline *EGFR* alterations in MONARCH 3 and nextMONARCH (7.2% and 7.1%, respectively; **Figure S2**).

In MONARCH 3, *ESR1* alterations were rare at baseline but were associated with numerically shorter mPFS in abemaciclib (27.5 months) and placebo (5.7 months) groups compared to those without such alterations (abemaciclib: 38.9 months; placebo: 17.6 months). In nextMONARCH, mPFS was similar with and without *ESR1* alterations detected (6.1 months versus 8.8 months; HR 0.94; 95% CI 0.64-1.39). In nextMONARCH, there was an apparent association between having *ESR1* mutation at baseline and having liver metastases (nominal p=0.0075). *ESR1* mutations, less common at baseline in MONARCH 3, were not associated with liver metastases (nominal p=0.2478).

In MONARCH 3, ORR was numerically higher in patients treated with abemaciclib versus placebo, regardless of whether a baseline alteration was detected (54.3% versus 47.4%) or not (64.9% versus 16.7%; **Figure S5A**). In nextMONARCH, ORR was generally numerically higher in patients without detected alterations, with the exception of ESR1 (detected: 33.9% versus not: 28.9%) and GATA3 alterations (detected: 44.8% versus not: 27.3%; **Figure S5B**).

Regarding baseline mutant allele frequency (MAF), in MONARCH 3, treatment benefit was consistent regardless of highest baseline MAF (highest baseline MAF >median: HR 0.49; ≤median: HR 0.50), although having a highest baseline MAF >median did appear to be prognostic of shorter mPFS overall (Figure S6A). Similarly, in nextMONARCH, the subgroup with highest baseline MAF >median also had a somewhat shorter mPFS (5.2 months vs 9.2 months in the ≤median subgroup) (**Figure S6B**).

Acquired genomic alterations

In MONARCH 3, the most commonly acquired alterations, were *ESR1* (20%), *TP53* (12%), and *EGFR* (8%) in the abemaciclib arm and *ESR1* (32%), *TP53* (10%), and *BRCA1* (6%) in the placebo arm (**Figure 5A**). Acquired alterations more frequent in the abemaciclib versus placebo arm included *RB1* (5% versus 0%, p=0.009), *MYC* (5% versus 0%, p=0.016), *APC* (4% versus 0%, p=0.029) and *BRCA2* (4% versus 0%, p=0.029). In nextMONARCH, alterations in *TP53* (10%), *EGFR* (9%), *RB1* (9%), and *MYC* (9%) were the most commonly acquired. Acquired alterations in *ESR1* (6%) and *AR* (3%) were also found. In MONARCH 3, the most frequent *ESR1* alterations were D538G (9.2% abemaciclib plus NSAI; 24.1% placebo plus NSAI) and Y537S (8.4% abemaciclib plus NSAI; 13.9% placebo plus NSAI). D538G (3.8%) was the most frequent *ESR1 alteration* in nextMONARCH (**Figure 5B**). Acquired *ESR1* mutations were not associated with liver metastases in either nextMONARCH (nominal p=1.0) or MONARCH 3 (nominal p=0.5278).

Certain baseline alterations were undetectable at EOT in a proportion of patients (**Figure 5C**). For example, in MONARCH 3, *PIK3CA* alterations became undetectable in 16.8% of patients treated with abemaciclib compared to 7.6% in the placebo arm. In nextMONARCH this was observed in 4.3% of patients. This should be considered if ctDNA testing is done to identify *PIK3CA* mutations for use of alpelisib.

Acquired alterations in patients with progressive disease

Most patients in the TR2 population of both studies discontinued due to PD: 157 (74.8%) in MONARCH 3 (88 [67.2%] in the abemaciclib arm and 69 [87.3%] in the placebo arm) and 69 (87.3%) in nextMONARCH (**Table S3**). The TR3 population consists of the subset of MONARCH 3 patients in TR2 with a valid EOT ctDNA sample and PD within 2 months of discontinuation of all study treatment (abemaciclib and NSAI; **Figure S1**). As in the TR2 population, *ESR1* alterations were the most frequently

acquired alterations in the TR3 population (abemaciclib: 19.2%; placebo: 30.4%). D538G and Y537S were the most frequently acquired individual *ESR1* mutations in the TR3 population (**Figure S7**). Acquired genomic alterations in the TR3 population are displayed in **Figure S8**.

Association between acquired alterations and PFS

In the MONARCH 3 TR2 population, mPFS was 20.8 months in the abemaciclib and 14.6 months in the placebo group (HR 0.61; 95% CI 0.44-0.84). In the nextMONARCH TR2 population, mPFS was 7.4 months with abemaciclib monotherapy.

In MONARCH 3, mPFS was similar between patients with and without *ESR1* alterations acquired during abemaciclib treatment (20.1 versus 19.1 months; HR 1.11; 95% CI 0.66-1.84). In contrast, in the placebo arm, mPFS was longer in patients with *ESR1* alterations acquired while on treatment compared to those without acquired alterations (23.1 versus 11.1 months; HR 1.66; 95% CI 0.96-2.85) (**Figure 6A**). In nextMONARCH, mPFS was similar between patients with and without *ESR1* alterations acquired during abemaciclib monotherapy (7.2 versus 9.0 months; HR 0.51; 95% CI 0.19-1.36; **Figure 6B)**.

Examination of the association between the most commonly acquired gene alteration (*ESR1*) in MONARCH 3 and the time to second disease progression (PFS2) showed no significant difference between patients with versus without acquired *ESR1* alterations (**Figure S9**).

In the abemaciclib arm of MONARCH 3, mPFS was shorter for patients with alterations in *FGFR1* (HR 0.33, 95% CI 0.16-0.70), *NF1* (HR 0.23, 95% CI 0.09-0.54), and *PDGFRA* (HR 0.44, 95% CI 0.21-0.92) acquired while on treatment compared to those without such acquired alterations (**Table S4**).

Discussion

Abemaciclib has demonstrated efficacy in both the metastatic and adjuvant settings in HR+, HER2– BC [5, 7, 17, 26-28]. However, a small proportion of patients with MBC exhibit primary resistance to abemaciclib and other CDK4/6i, and most develop acquired resistance. Therefore, a greater understanding of the mechanisms of resistance is critically needed [11, 29, 30].

In vitro preclinical studies in BC cell lines treated with CDK4/6i have identified genomic alterations potentially involved in resistance, including loss of *RB1* and amplification of *CCNE1*, *CCNE2*, and *CDK6* [31-34]. However, the clinical relevance of such findings in patients treated with abemaciclib is unclear. This study is the first to explore genomic alterations in ctDNA samples from patients with HR+, HER2– ABC treated with abemaciclib +/- NSAI and the relationship between baseline or treatment-emergent genomic alterations and clinical outcomes. Though direct comparisons between the two studies cannot be made, given the differences in study populations, the analysis from MONARCH 3 provides data from a large, randomized, Phase 3 study, while nextMONARCH allows for analysis in the context of monotherapy rather than combination with ET.

Most patients in MONARCH 3 and nextMONARCH had at least one baseline genomic alteration. While baseline gene alterations were prognostic in the abemaciclib arms of MONARCH 3 and nextMONARCH, in MONARCH 3, patients receiving abemaciclib plus NSAI consistently had improved mPFS compared to those receiving placebo plus NSAI, irrespective of baseline genomic alterations, consistent with results in the ITT population [6, 35].

Alterations in the estrogen receptor (ER) gene *ESR1* were rarely present at baseline in the MONARCH 3 population (5%; initial therapy for advanced disease) but highly

prevalent in the heavily pretreated nextMONARCH population (40%), reflecting the association of *ESR1* mutations with exposure to ET [36]. In previous studies, the detection of *ESR1* mutations has been associated with inferior PFS in patients receiving AI-containing therapies [37, 38]). In MONARCH 3, though the frequency was low, patients in the placebo arm with baseline *ESR1* alterations had a shorter mPFS than those without such alterations. Notably, patients with alterations derived substantial benefit from the addition of abemaciclib to NSAI. In nextMONARCH, mPFS was similar between patients with and without baseline *ESR1* alterations receiving abemaciclib monotherapy suggesting benefit of abemaciclib despite ET resistance in this population [39]. This is similar to MONARCH 2 data, where benefit from abemaciclib plus fulvestrant was observed regardless of *ESR1* mutation status in an ET-resistant population [40].

The *ESR1* alterations most frequently observed in this study occurred within the ligand-binding domain, at D538G and Y537S, consistent with other studies of patients on NSAI [41]. While mPFS was similar between patients with and without *ESR1* alterations acquired during abemaciclib treatment (both studies), mPFS was longer in patients with acquired *ESR1* alterations on placebo plus NSAI (MONARCH 3), suggesting longer exposure to ET monotherapy is associated with the acquisition of *ESR1* alterations. To determine whether the presence of *ESR1* mutations conferred shorter PFS on the next line of therapy after initial disease progression, we evaluated PFS2 in MONARCH 3. No difference in PFS2 was observed between patients with and without acquired *ESR1* alterations in MONARCH 3.

In the PALOMA-3 study of palbociclib or placebo plus fulvestrant,12.8% of patients without a baseline *ESR1* mutation had an acquired mutation at progression, with evidence of selection of *ESR1* Y537S in both arms of the study [12]. In contrast, fewer MONARCH 3 patients with PD acquired *ESR1* alterations in the abemaciclib arm (19.2%) compared to placebo (30.4%) (**Figure S7**), mainly driven by higher rates of

acquisition of the *ESR1* D538G alteration in patients with PD in the placebo arm (24.1%) compared to the abemaciclib arm (9.2%). Fulvestrant has demonstrated antitumor activity in *ESR1*-mutant disease preclinically [42, 43], in the metastatic setting [44], and in patients receiving therapy with aromatase inhibitor (AI) plus palbociclib who experienced rising *ESR1* ctDNA levels and were switched from AI to fulvestrant (while maintaining palbociclib) [45]. Given that abemaciclib may delay PD related to *ESR1* mutation, further studies should evaluate the optimal CDK4/6i partner for selective estrogen receptor degraders (SERDs) and other ET.

Several baseline genomic alterations were associated with mPFS <12 months in the placebo arm of MONARCH 3, including *ESR1*, *MYC*, *CCND1*, *EGFR*, *FGFR1*, CCRGs and MAPK pathway genes. In nextMONARCH, genomic alterations associated with a mPFS <5 months included *CCNE1*, *MYC*, *EGFR*, *FGFR1*, CCRGs, *NF1*, *PIK3CA*, and *RB1*.

Mutations in *TP53*, *RB1*, and *NF1* have been previously associated with poor outcomes in patients with HR+, HER2– ABC, regardless of treatment [46]. Our analyses are the first to suggest baseline *EGFR* alterations (**Figure S2C-D)** may also be associated with poor prognosis in patients with HR+, HER2– ABC, although maintain a benefit with abemaciclib plus NSAI. In the exploratory analyses from MONALEESA-2 and MONALEESA-7 trials, patients with altered receptor tyrosine kinase genes, including *EGFR*, derived a PFS benefit from ribociclib [47, 48].

In the MONALEESA-2 trial, *PIK3CA* (33%) and *TP53* (12%) alterations were found in baseline ctDNA, with prolonged PFS with ribociclib plus letrozole regardless of *PIK3CA* and *TP53* alteration status [47, 49]. Similarly, in our analysis, *TP53* and *PIK3CA* alterations were frequently observed at baseline, and patients with and without *TP53* or *PIK3CA* alterations benefited from combined abemaciclib plus NSAI. In contrast, in nextMONARCH, patients without a detected *TP53* or *PIK3CA* alteration had a longer mPFS than those with an alteration.

Mutations in *FGFR1* and *FGFR2* have been associated with resistance to ET and CDK4/6i [50-52]. In MONALEESA-2, baseline *FGFR1* alterations were associated with a poor prognosis. Patients with baseline *FGFR1* amplification treated with ribociclib plus letrozole had a shorter mPFS (10.6 months) than patients with wild-type *FGFR1* (24.8 months) [51]. While baseline *FGFR1* alterations were associated with a shorter mPFS in both treatment arms of MONARCH 3 and with abemaciclib monotherapy in nextMONARCH, patients in MONARCH 3 benefited from the addition of abemaciclib to NSAI regardless of mutation status.

Limited clinical data on acquired resistance during CDK4/6i treatment has been reported [9, 12]. Acquired genomic alterations potentially associated with emerging resistance to abemaciclib +/- NSAI included alterations in *RB1*, *MYC* or *EGFR*. However, these were seen in <10% of patients and could be impacted by small sample size, therefore further evaluation in a larger patient population is warranted. Acquired *TP53* alterations were found in 10% of patients in both treatment arms of MONARCH 3 and the abemaciclib monotherapy arms of nextMONARCH.

Using whole exome sequencing of metastatic tumor biopsies, Wander et al. [9], identified genomic alterations that could potentially drive resistance to CDK4/6i. These include loss of *RB1*, activating alterations in *AKT1*, *RAS*, aurora kinase A (*AURKA)*, *CCNE2*, *ERBB2* and *FGFR2*, and loss of ER expression. Loss of RB is a mechanism of both intrinsic and acquired resistance to CDK4/6i. However, this is uncommon and does not account for most of the acquired resistance observed in HR+, HER2– ABC. ctDNA analysis from the PALOMA-3 study revealed *RB1* mutations in 5% of patients who acquired a mutation during palbociclib plus fulvestrant treatment, suggesting this is not the predominant mechanism of resistance to CDK4/6i [12]. In this study, acquired *RB1* alterations were detected in <10% of patients receiving abemaciclib +/- NSAI.

In summary, we investigated genomic alterations potentially associated with resistance to abemaciclib +/- NSAI in women with HR+, HER2– ABC using ctDNA analysis from MONARCH 3 and nextMONARCH. The most frequent baseline alterations in our study have been previously associated with endocrine resistance. Importantly, in MONARCH 3, abemaciclib plus NSAI was associated with improved mPFS compared with placebo plus NSAI, regardless of baseline genomic alterations. In addition, potential mechanisms of acquired resistance were explored. Finally, this is the first study to evaluate impact of genomic alterations on CDK4/6i monotherapy. Limitations of this study include that evaluable samples were not available for all patients and that interpretation of nextMONARCH data is limited by the lack of a control arm for comparison, and thus, confirmation if these findings reflect prognostic or predictive association of these alterations is not possible. These findings are hypothesis-generating and need validation in suitably powered prospective studies. Understanding potential mechanisms of intrinsic and acquired resistance will help inform future drug development and clinical trials.

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Abbreviations

ABC, advanced breast cancer; AI, aromatase inhibitor; AR, androgen receptor; AURKA, aurora kinase A; B, baseline; BID, twice daily; BOR, best overall response; CDK4/6, cyclin-dependent kinase 4 and 6; CI, confidence interval; CNA, copy number alteration; CR, complete response; ctDNA, circulating tumor DNA; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; EOT, end-of-treatment; ESR1, estrogen receptor gene; ET, endocrine therapy; FGFR, fibroblast growth factor receptor; HR+, hormone receptor-positive; HR, hazard ratio; HER2−, human epidermal growth factor receptor 2-negative; INDEL, insertions/deletions; ITT, intent-to-treat; mPFS, median progression-free survival; MBC, metastatic breast cancer; N, number of patients; NA, not achieved, NE, not evaluable; NF1, neurofibromatosis type 1; NGS, next-generation sequencing; NSAI, nonsteroidal aromatase inhibitor; ORR, objective response rate; OS, overall survival; PD, progressive disease; PDGFRA, platelet-derived growth factor receptor alpha; PFS, progression-free survival; PFS2, time to second disease progression; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; PR, partial response; QD, once daily; Q12H, once every 12 hours; RB, retinoblastoma; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; SNV, single nucleotide variant; TP53, p53-tumor suppressor protein; TR, translational research

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Figure 1. Gene alterations at baseline. Heatmaps of somatic alterations at baseline by gene (TR population) for MONARCH 3 (A) and nextMONARCH (B). Abbreviations: CNA = copy number alterations; INDEL = insertions/deletions; SNV = single nucleotide variant; TR = translational research.

Figure 2. Frequency of gene alterations at baseline. Bar graphs representing frequency of gene alterations at baseline by gene and type of alteration in MONARCH 3 (A; n=295, TR population) and nextMONARCH (B; n=139, TR population). Abbreviations: CNA = copy number alterations; INDEL = insertions/deletions; SNV = single nucleotide variant; TR = translational research.

Figure 3. **Forest plots of PFS for patients with and without specific genomic alterations at baseline in) MONARCH 3 (TR population).** Cell-cycle related genes consist of *CCND1, CCND2, CDK4, CDK5, CDKN2A, CCNE1, RB1 and TP53.* MAPK genes consist of *ARAF, BRAF, HRAS, KRAS, MAPK1, MAP2K1, MAP2K2, MAP3K1, NRAS* and *RAF1 (CRAF).* CI = confidence interval; ITT = intent-to-treat; NA = not achieved; PFS = progression-free survival; TR = translation research.

Figure 4. **Forest plot of PFS for patients with and without specific genomic alterations at baseline in nextMONARCH (TR population).** Cell-cycle related genes consist of *CCND1, CCND2, CDK4, CDK5, CDKN2A, CCNE1, RB1 and TP53.* CI = confidence interval; ITT = intent-to-treat; NA = not achieved; PFS = progression-free survival; TR = translation research.

Figure 5. Genomic alterations in the abemaciclib and placebo groups in MONARCH 3 and the abemaciclib monotherapy group in nextMONARCH (TR2 population). A) Acquired genomic alterations. *p<0.05 abemaciclib versus placebo in MONARCH 3. B) The frequency of individual ESR1 mutations (found in ≥2 patients) acquired during treatment. C) Genomic alterations detected at baseline but not detected at end-of-treatment. TR2 population consists of patients with a valid ctDNA sample at both baseline and end-of-treatment. Abbreviations: NSAI = nonsteroidal aromatase inhibitor.

Figure 6. **Progression-free survival in patients with and without acquired *ESR1* alterations in MONARCH 3 (A) and nextMONARCH (B).** Abbreviation: CI = confidence interval; HR = hazard ratio.

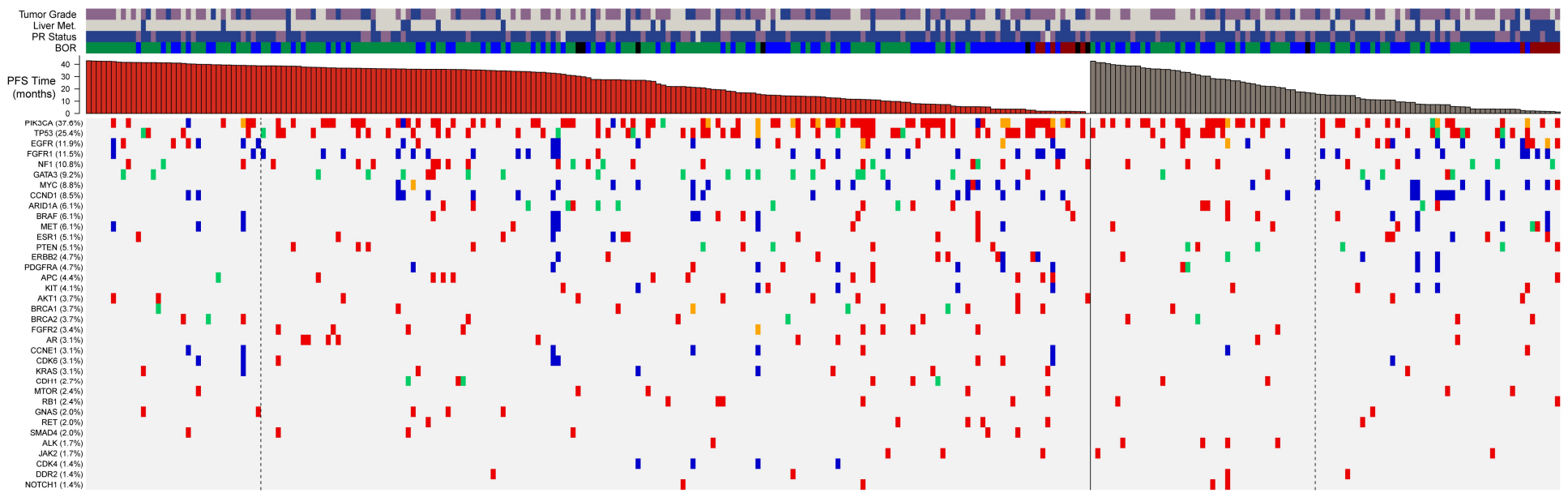


Figure 1A

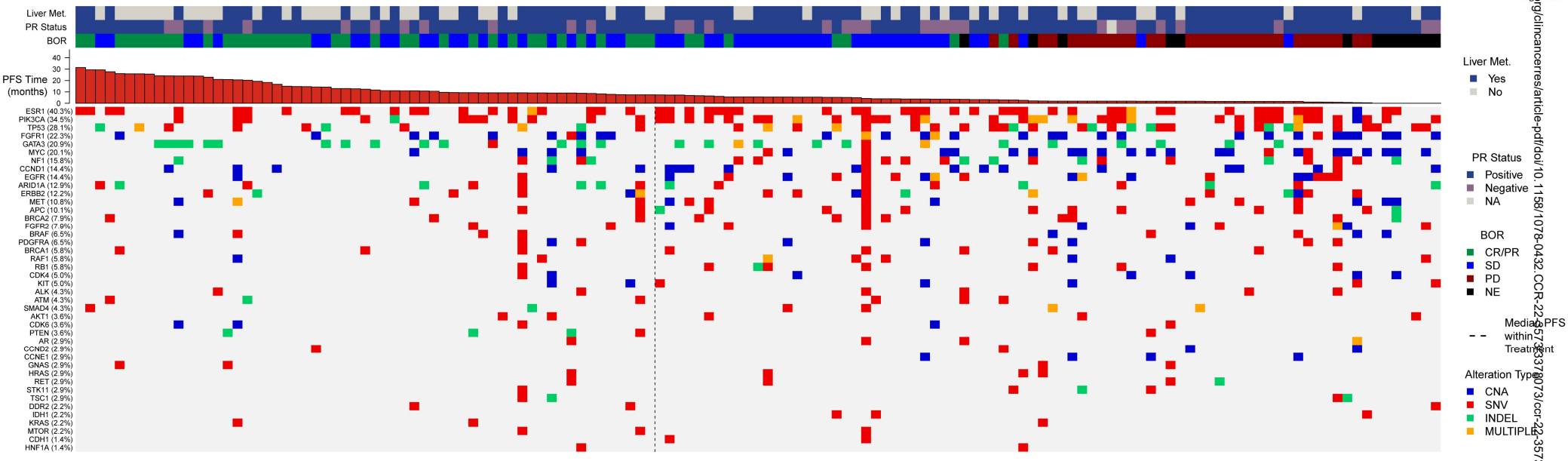
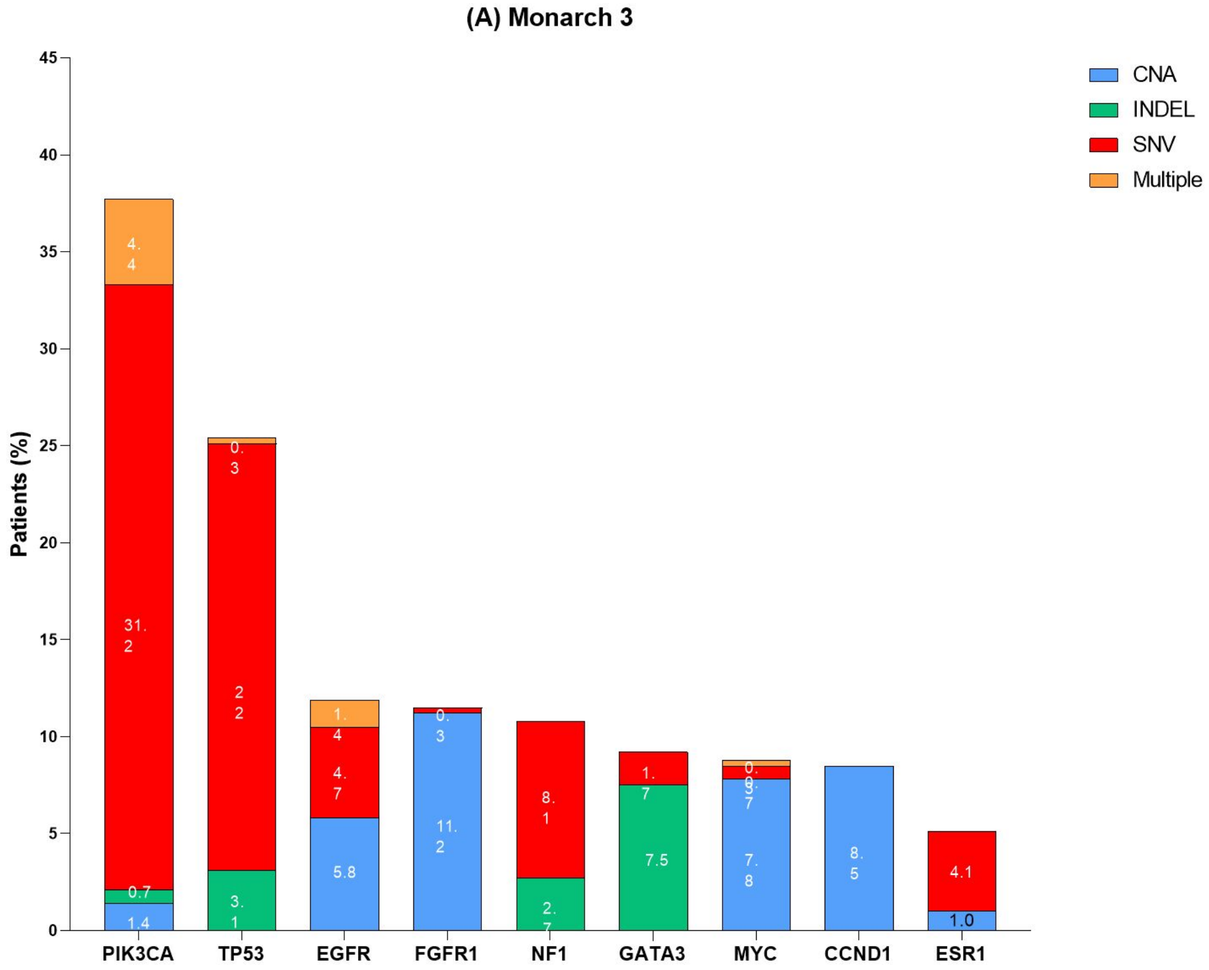


Figure 1B

Figure 2



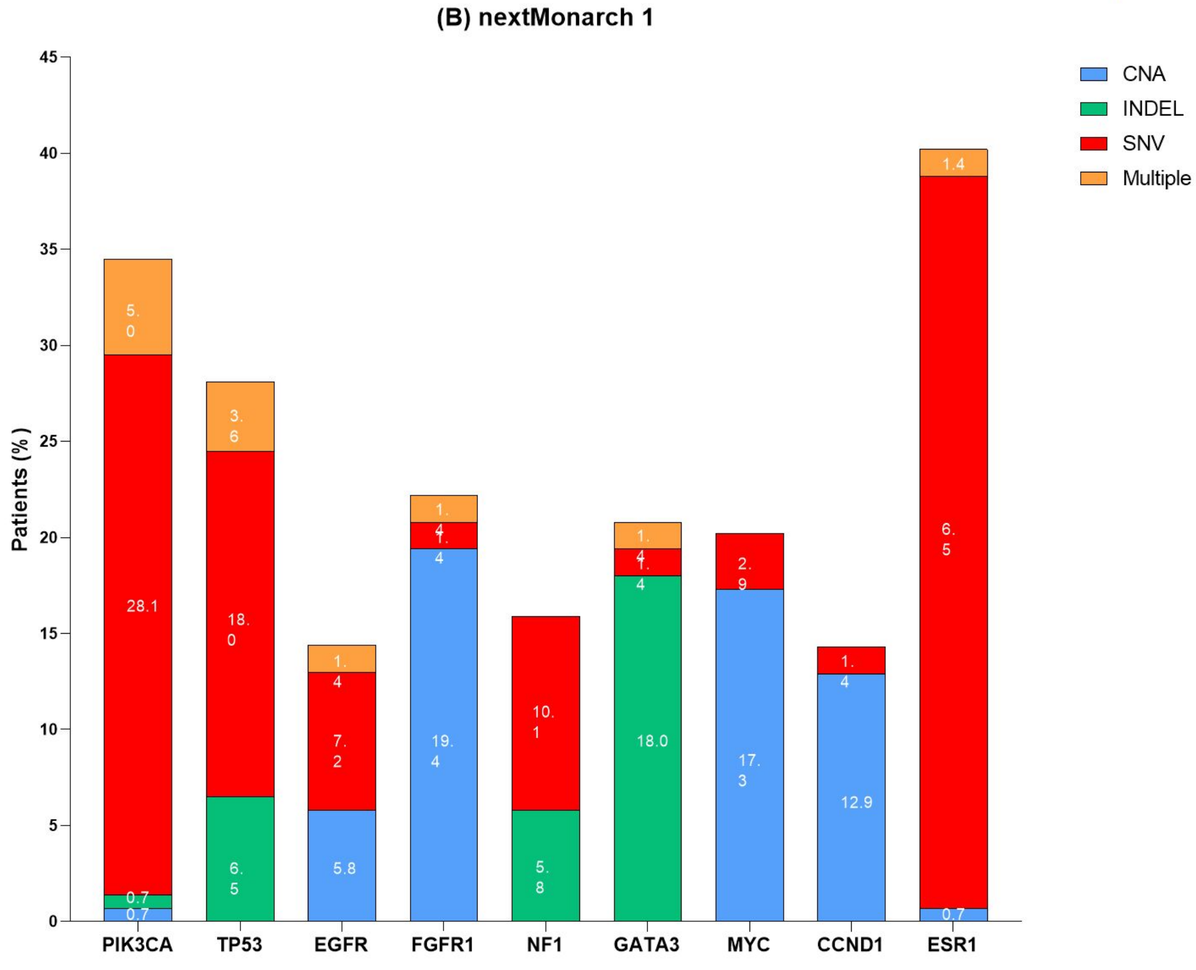
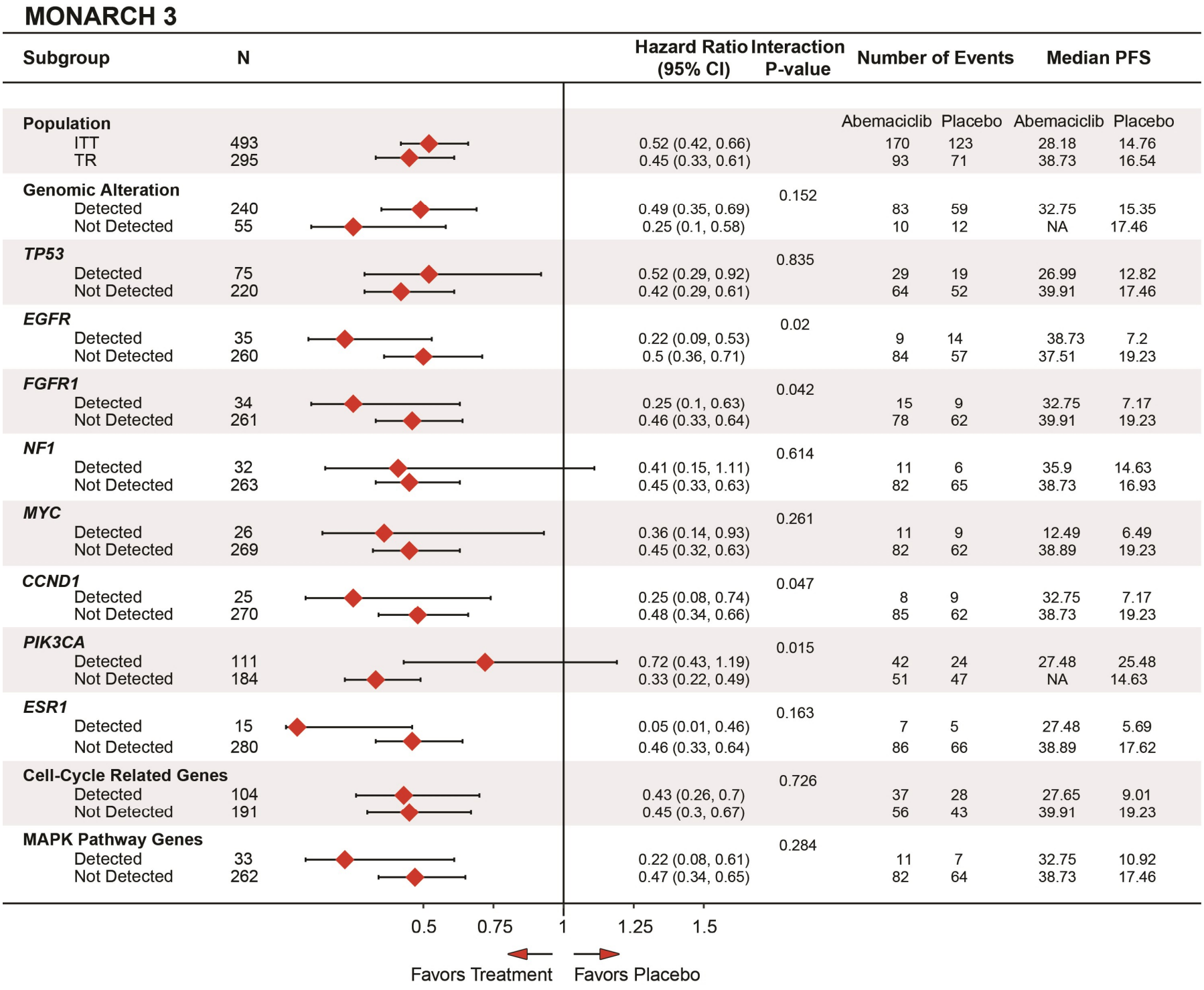
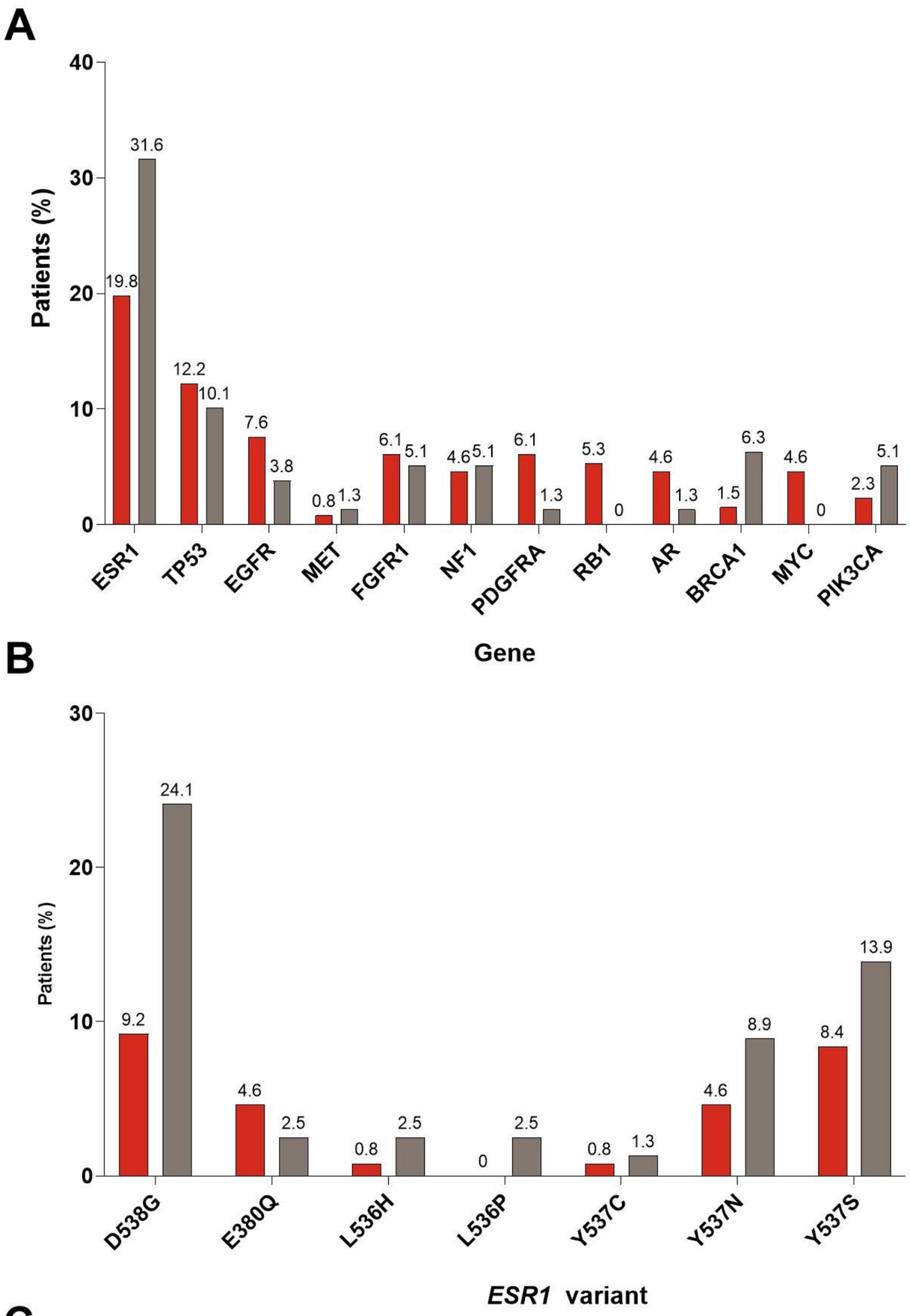
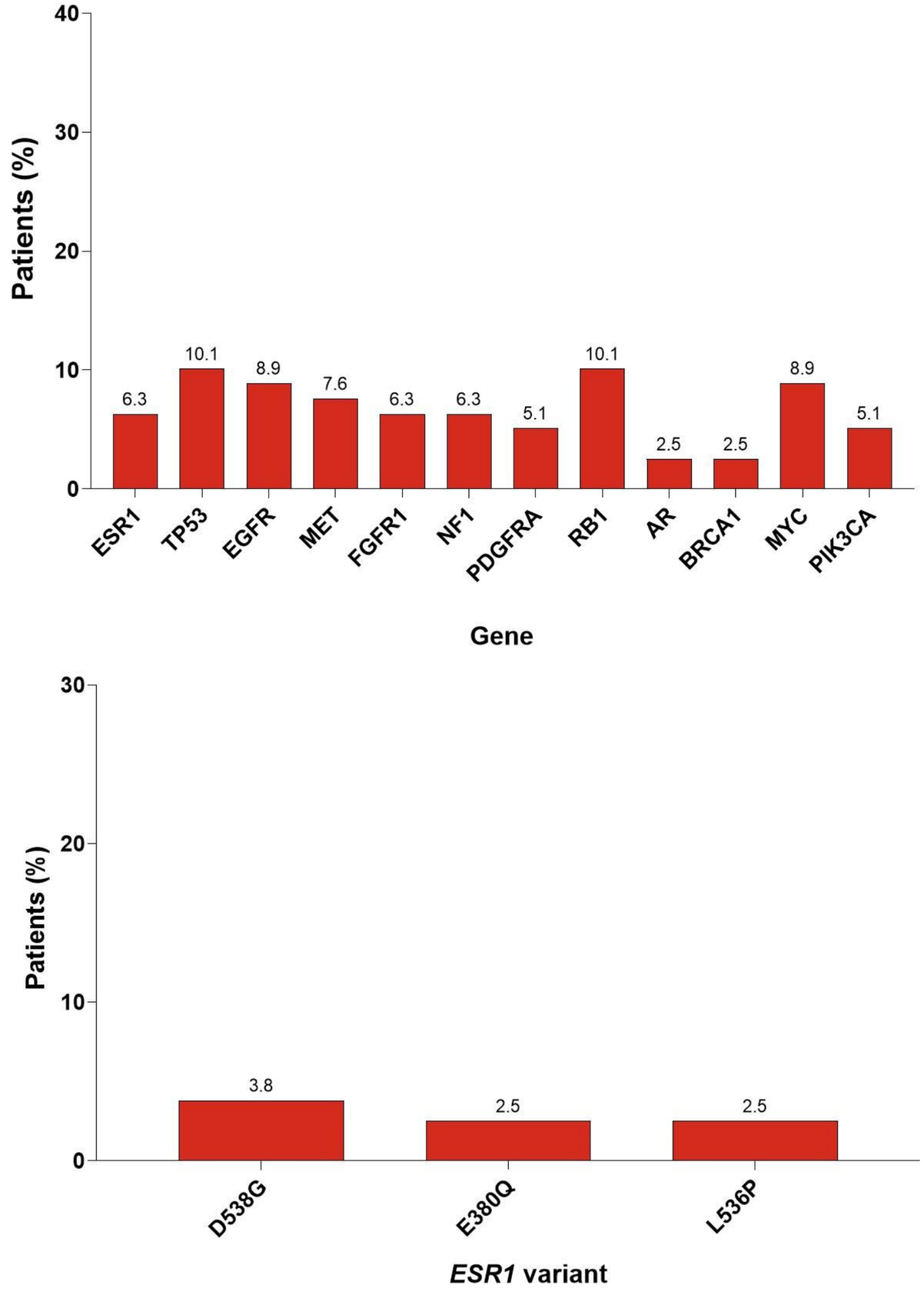


Figure 3

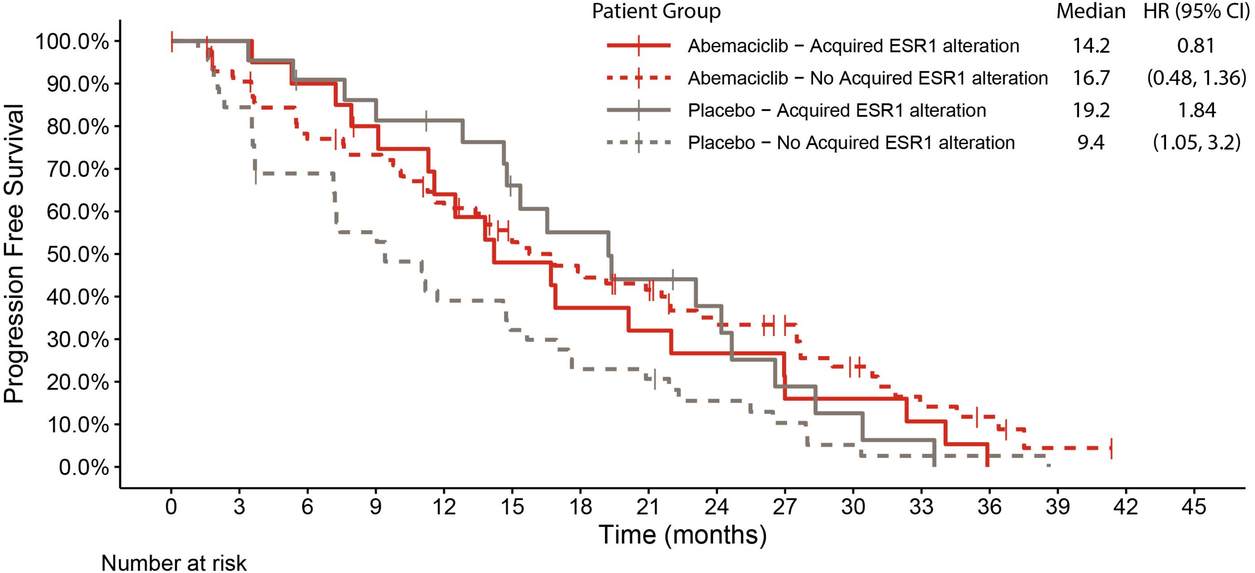


Subgroup	n	n	Hazard Ratio (95% CI)	Log-rank P-value	Number of Events		Median PFS	
	Detected	Not Detected			Detected	Not Detected	Detected	Not Detected
Genomic Alteration	125	14	0.52 (0.26, 1.04)	0.059	100	9	6.71	12.95
ESR1	56	83	0.94 (0.64, 1.39)	0.769	43	66	6.05	8.78
PIK3CA	48	91	0.45 (0.3, 0.67)	< 0.001	42	67	4.06	9.07
TP53	39	100	0.67 (0.44, 1.02)	0.062	30	79	3.72	9.01
FGFR1	31	108	0.51 (0.33, 0.81)	0.003	26	83	3.67	7.53
GATA3	29	110	1.21 (0.77, 1.91)	0.414	24	85	8.78	6.81
MYC	28	111	0.27 (0.16, 0.44)	< 0.001	23	86	1.87	9.01
NF1	22	117	0.46 (0.28, 0.77)	0.003	19	90	3.75	8.22
EGFR	20	119	0.4 (0.23, 0.69)	0.001	17	92	3.52	8.78
ERBB2	17	122	0.59 (0.34, 1.01)	0.051	16	93	5.42	7.53
RB1	8	131	0.41 (0.2, 0.87)	0.016	8	101	4.87	7.53
CCNE1	4	135	0.18 (0.06, 0.53)	< 0.001	4	105	1.84	7.43
Cell-Cycle Related Genes	62	77	0.49 (0.33, 0.71)	< 0.001	50	59	3.72	9.21





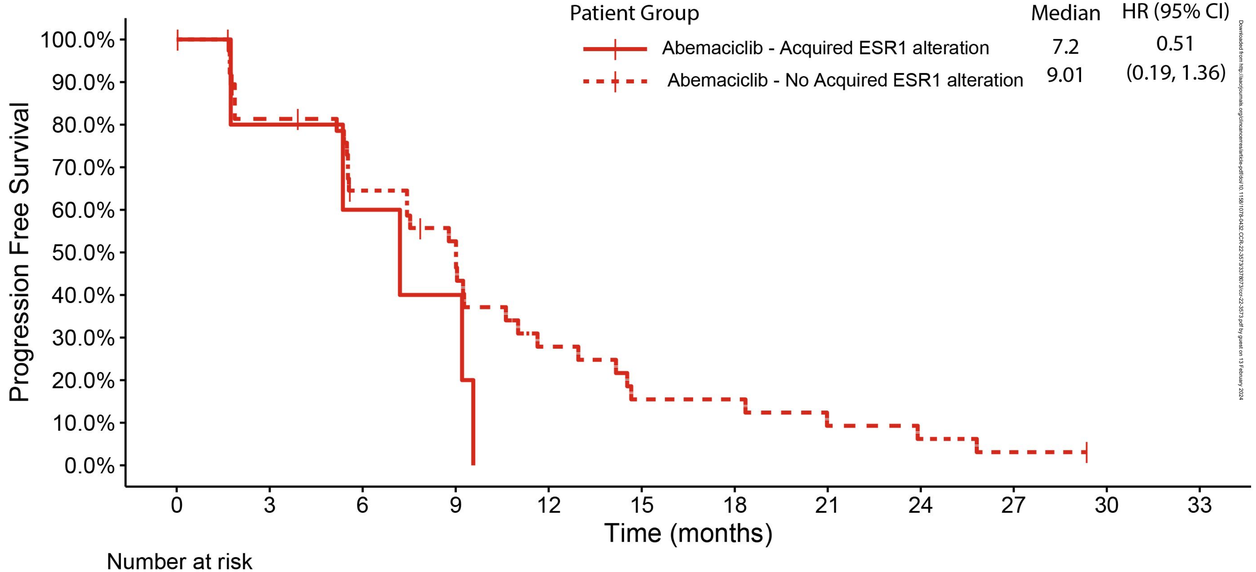
(A) MONARCH 3



Number at risk

———	20	20	18	15	12	9	7	6	5	3	3	2	0	0	0	0
- - -	87	75	63	59	49	38	33	28	20	17	11	6	4	1	0	0
———	22	22	19	18	16	12	10	8	6	3	2	1	0	0	0	0
- - -	45	38	30	24	17	14	10	9	6	4	2	1	1	0	0	0

(B) nextMONARCH



Number at risk

———	5	4	3	2	0	0	0	0	0	0	0	0
- - -	40	30	22	17	9	5	5	3	2	1	0	0