#286 - Mutational analysis of circulating tumor DNA (ctDNA) in patients with ER+/HER2- advanced breast cancer (ABC) receiving palbociclib (P): results from the TREnd trial.

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BACKGROUND

CDK4/6 inhibitors in combination with endocrine therapy (ET) are the mainstay of treatment for ER+ HER2- ABC (1). Despite the effectiveness of these agents, early and acquired resistance is a major clinical challenge. Mechanisms of resistance to palbociclib are multiple, and not yet fully defined (2). Here we show the results of a mutational analysis on ctDNA samples from patients included in the c-TREnd study, the translational cohort of the TREnd trial (NCT02549430) (3)

MATERIALS AND METHODS

- Study population: 46 patients with ER+ HER2- endocrine resistant ABC, who participated in the TREnd trial (NCT02549430) and randomized to receive either P alone or P plus the ET received in the prior line of treatment (Figure 1).
- For each patient, blood samples were collected in EDTA-containing tubes at three time points: before starting treatment (T0), after the first cycle of treatment (T1), and at the time of disease progression prior to the commencement of a new line of therapy (T2).
- ctDNA was extracted using the QIAamp Circulating Nucleic Acid Kit.
- Hybridization and capture were performed using the Illumina TruSight Tumor 170 Kit
- Single nucleotide variants were detected using LoFreq (v2.1.3.1) and annotated using Oncotator (v1.9.9.0) with exploiting both population and cancer databases. To retrieve only somatic mutations both variant allele frequency (VAF) and the annotations in cancer and population databases were taken into account. To characterize variants at all time points, VAFs of low frequency variants undetected by LoFreq were computed after generating pileups using PacBAM (v1.6.0). Tumor mutational burden (TMB) was defined as the sum of silent and non-silent mutations. Tumor fraction (TF) was estimated based on the dispersion of Copy Number Alteration (CNA) genomic profiles.
- Statistical analysis: Progression Free Survival (PFS) was computed as the time from treatment initiation to radiological disease progression or death. The distribution of PFS was estimated using the Kaplan–Meier method and compared with the log-rank test.

Figure 1: CONSORT Diagram

|		Palbociclib+ET (N=18)	Palbociclib (N=14)
Age (years, range)		63.5 (44;82)	64 (52;80)
ECOG Performance Status	0	14 (77.8%)	11 (78.6%)
	1	3 (16.7%)	3 (21.4%)
	2	1 (5.6%)	0 (0%)
Sites of metastasis	Visceral	12 (66.7%)	11 (78.6%)
	Bone only	3 (16.7%)	1 (7.1%)
	Non visceral	3 (16.7%)	2 (14.3%)
Prior lines of ET	1	12 (66.7%)	10 (71.4%)
	2	6 (33.3%)	4 (28.6%)
Duration of most recent ET	≤ 180 days	2 (11.1%)	3 (21.4%)
	> 180 days	16 (88.9%)	11 (78.6%)
Most recent ET	Aromatase inhibitor	9 (50%)	9 (64.3%)
	Fulvestrant	9 (50%)	5 (35.7%)
Prior chemotherapy for ABC	Yes	5 (27.8%)	2 (14.3%)
	No	13 (72.2%)	12 (85.7%)

Table 1: Clinico-pathological characteristics of the study population

REFERENCES

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2) McCartney A et al. Front Oncol 2019 Jul 23;9:666. doi: 10.3389/fonc.2019.00666. eCollection 2019.
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RESULTS

The clinico-pathological characteristics of the study population are illustrated in Table 1. The mutational landscape is shown in Figure 2. The most frequently mutated genes at T0 were *ESR1* (23%), *PIK3CA* (17%), *AR* , *FGFR2* and *TP53* (10%). At T2 we observed the emergence of 9 new mutations in 7 genes (*ESR1*, *AKT1*, *ARID1A*, *BRIP1*, *CCNE1*, *MTOR* and *TP53*).
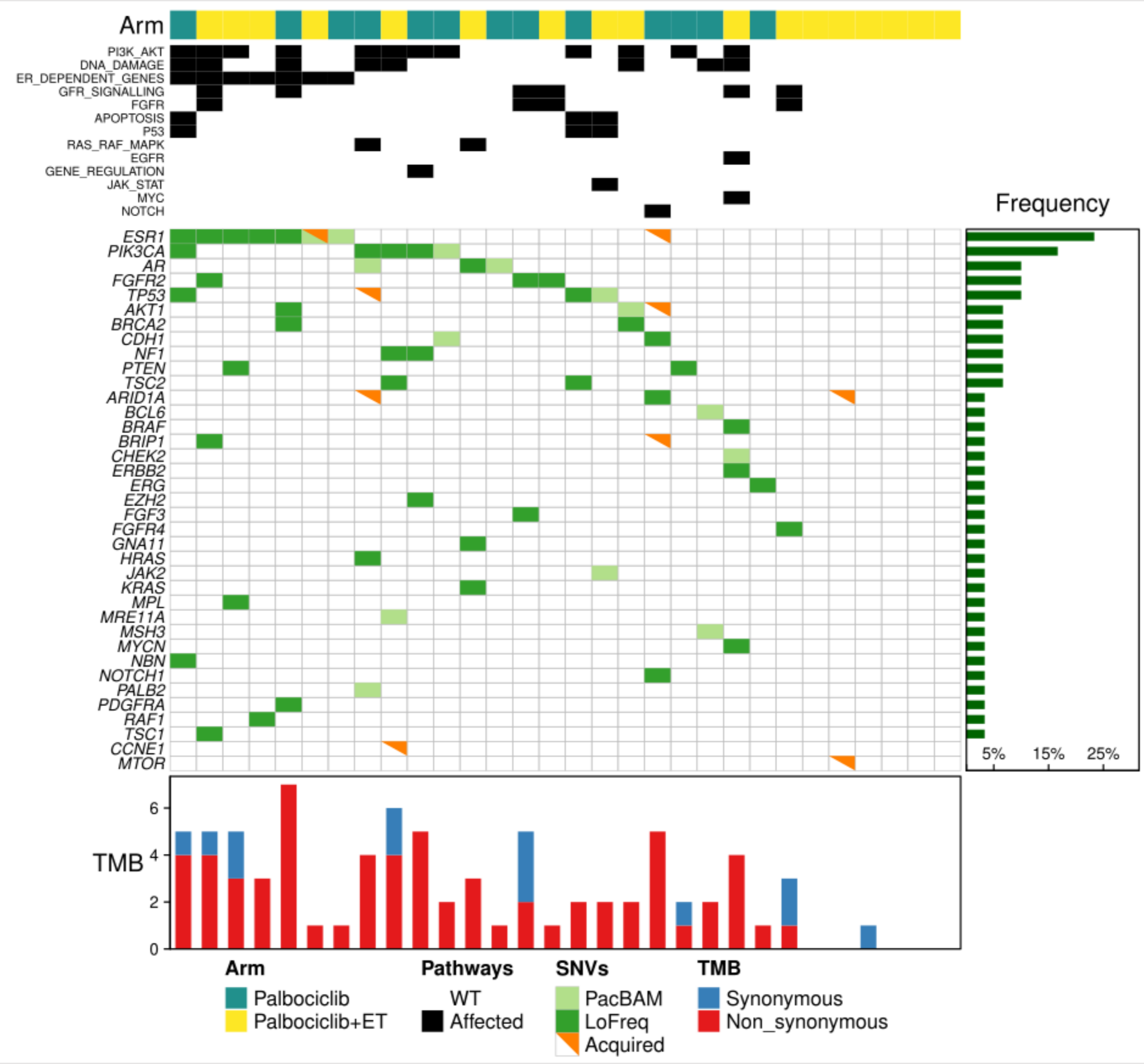
Figure 2: Oncoplot of mutated genes and pathways detected in the ctDNA samples from patients included in the c-TREnd study

TMB and TF at T0 or TF change between T1 and T0 were not associated with PFS. (Figure 3).
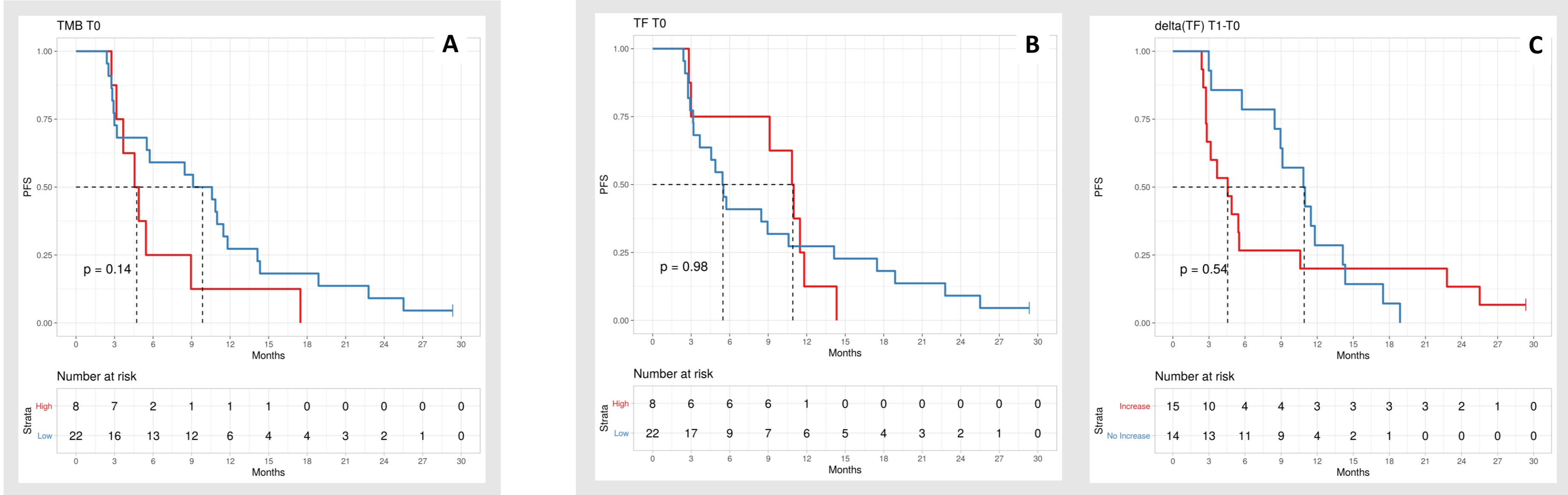
Figure 3: Kaplan-Meier PFS analysis according to : TMB at T0 (A) , TF at T0 (B) and TF changes between T1 and T0 (C)

RESULTS

Mutations in *ESR1* at T0 were associated with poor outcome while mutations in *AR* conferred a better, although not statistically significant, mPFS. *PIK3CA* mutations were not significantly associated with outcome, however in a broader analysis of PI3K pathway (adding *AKT1*, *PTEN*, *TSC1/2* and *BRAF*), a significantly worse mPFS was observed for patients with mutations within the pathway (Figure 4).
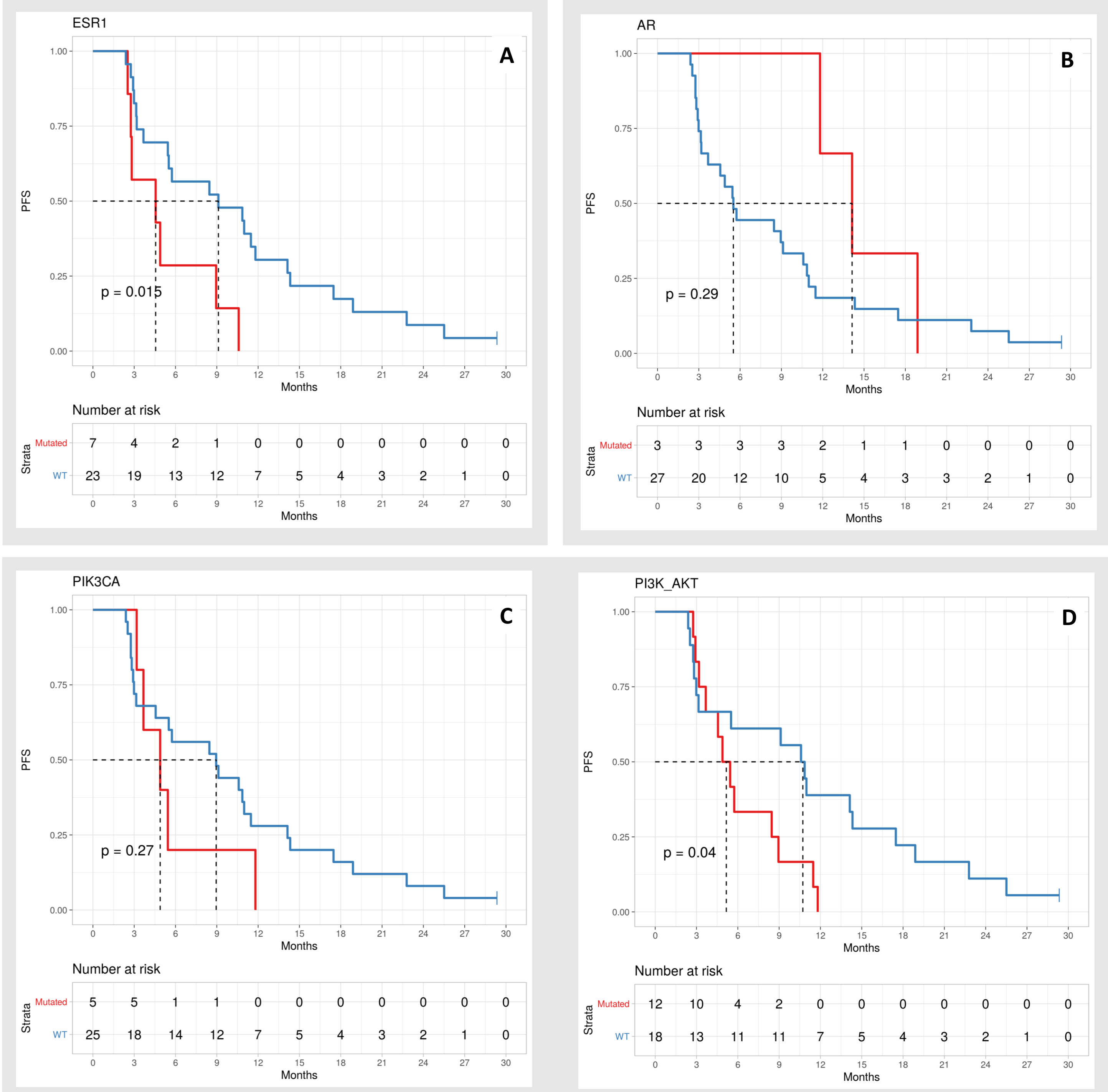
Figure 4: Kaplan-Meier PFS analysis according to *ESR1*, (A) *AR* (B) , *PIK3CA* (C) or PI3K pathway (D) mutational status

CONCLUSIONS

- By employing an untargeted approach, we found new acquired mutations including those in *ARID1A*, *BRIP1* and *CCNE1* genes, which had not been previously described in patients treated with CDK4/6 inhibitors. Confirmatory experiments using digital-droplet PCR are ongoing.
- At baseline, mutations in *ESR1* and in PI3K pathway genes were associated with worse prognosis in patients treated with palbociclib, while TMB and TF were not.

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