Circulating tumor DNA dynamics using a standardized multi-gene panel in advanced breast cancer patients treated with CDK4/6 inhibitors

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Background and objectives

- Changes in ctDNA levels may predict response to a variety of drugs, including CDK4/6 inhibitors (CDK4/6i); however, the best assay and method are still to be defined.
- Paired plasma samples were collected at cycle 1 day 1 (C1) and cycle 2 day 1 (C2).
- In this study, we explored the hypothesis that the early drop of ctDNA after one cycle of CDK4/6i with endocrine therapy (ET) could predict progression free survival (PFS).

Methods

- This is a prospective single-center study in hormone receptor positive/HER2-negative advanced breast cancer patients treated with CDK4/6i and ET.
- Paired plasma samples were collected at cycle 1 day 1 (C1) and cycle 2 day 1 (C2).
- Somatic alterations and variant allele fraction (VAF) were assessed using the 74-gene Guardant360 assay (Guardant Health).
- A VAF ratio (VAFR) was calculated for each alteration with a VAF of ≥ 0.4% at C1 or C2. Molecular response was defined as the mean of all VAFRs (mVAFR).
- VAFs < 0.4% at C1 and C2 were considered to have low-shedding tumors.
- PFS hazard ratios (HR) were calculated using a univariate Cox model.
- PAM50 subtypes and tumor infiltrating lymphocytes (TILs) were determined in a subset.

Results

- 48 patients were treated with ET and palbociclib (89%) or ribociclib (11%). Two patients had missing plasma samples and were excluded from the analysis. The median follow-up was 12.0 months (IQR 6.7-14.6).
- Clinical characteristics are described in **Table 1**. 57% of patients used fulvestrant and 33% an aromatase inhibitor. ctDNA was detected in 96% of patients. Somatic mutations or copy number variations were detected in 51 genes (**Fig. 1 and 2**). PAM50 subtype distribution (n=27) is shown in **Fig. 3**.
- 46% of patients had a decrease in ctDNA after 1 cycle (**Fig. 4**). mVAFR < 0.3 (high-ctDNA responders) (n=12) and low-shedding tumors (n=13) correlated with significantly improved PFS (HR=0.39, p=0.025), especially when compared to patients with ctDNA mVAFR > 1 (HR=0.27, p=0.010, n=12) (**Fig. 5**).
- Within PAM50 tested tumors, non-Luminal (n=5) were low-ctDNA responders (mVAFR > 0.3) (n=3) or low-shedding (n=2); Luminal A or B were high-ctDNA responders (n=8), low-ctDNA responders (n=7) and low-shedding (n=4).
- TILs were increased in low-ctDNA responders relative to high-ctDNA responders (mean 3.3% vs 1.8%).

Results

Figure 1. Somatic mutations at baseline in ctDNA.

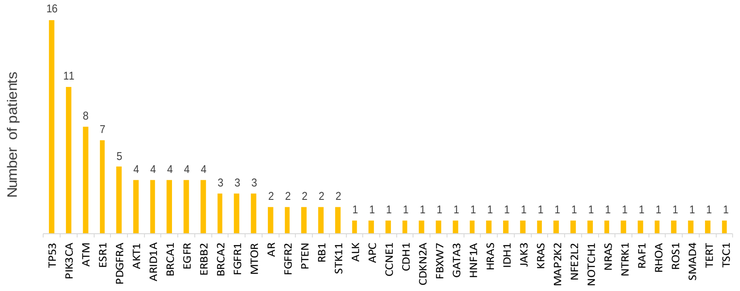


Figure 2. Amplifications identified at baseline in ctDNA.

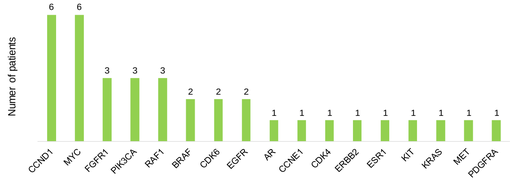


Figure 3. PAM50 distribution (N=27).

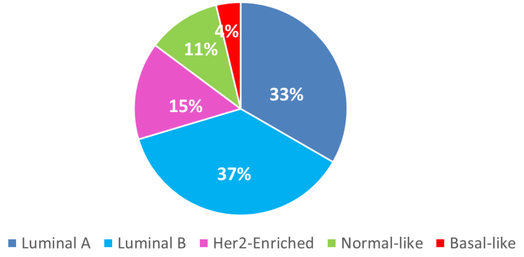
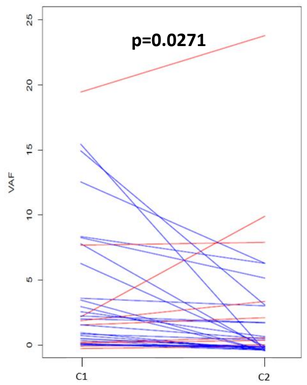


Table 1. Baseline characteristics.

Median age, years (range)	61.5 (39-87)
Treatment line	
•First	22 (48%)
•Second	16 (35%)
•Third or more	8 (17%)
ECOG PS	
•0	18 (39%)
•1	27 (59%)
•2	1 (2%)
Visceral disease	
•No	16 (35%)
•Yes	30 (65%)
Metastatic sites	
•<3	17 (37%)
•≥3	29 (63%)
"De novo" metastatic	
•No	34 (74%)
•Yes	12 (26%)
Hormone-resistance	
•No	30 (68%)
•Yes	14 (32%)

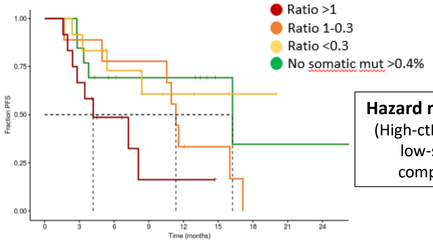
Results

Figure 4. Individual patient mVAF in C1 versus C2.



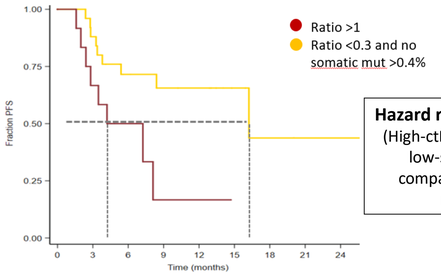
mVAF decreased in 46% of patients
mVAF increased in 26% of patients
mVAF did not change in 28% of patients

Figure 5. PFS according to mVAF ratio (4 groups).



Hazard ratio=0.39, p=0.025
(High-ctDNA responders and low-shedding tumors compared to the rest)

Figure 6. PFS according to mVAF ratio (2 groups).



Hazard ratio=0.27, p=0.025
(High-ctDNA responders and low-shedding tumors compared to low-ctDNA responders)

Conclusions

- ctDNA dynamics are an early surrogate of CDK4/6i + ET efficacy.
- The clinical utility of this biomarker should be tested in prospective trials in which patients with unfavorable ctDNA responses are randomized to alternative treatment strategies.

References and Acknowledgements

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Disclosures

Conflicts of interest: OM has declared travel expenses paid by Roche. AP has declared an immediate family member being employed by Novartis, personal honoraria from Pfizer, Novartis, Roche, MSD Oncology, Lilly and Daiichi Sankyo, travel, accommodations and expenses paid by Daiichi Sankyo, research funding from Roche and Novartis, consulting/advisory role for NanoString Technologies, Amgen, Roche, Novartis, Pfizer and Bristol-Myers Squibb. OTHER AUTHORS: TP reports consulting fees from Roche/Genentech, JC reports personal fees from Roche, Novartis, and Eisai and consulting fees from Roche/Genentech, Celgene, Astra Zeneca, Biothera Pharmaceutical, Merus, and Seattle Genetics, outside the submitted work. FS has declared travel and accommodation expenses paid by Roche, Pfizer and Celgene. NC has declared travel and accommodation expenses paid by Eisai. MV has declared personal honoraria from Pfizer, Novartis, Roche and Daiichi Sankyo, travel, accommodations and expenses paid by Roche and Pfizer, consulting/advisory role for Roche and Novartis. The other authors have nothing to declare.