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WHEN INTERCEPTION NEEDS A SECOND CHANCE: CTDNA DYNAMICS IN ER+/HER2- ADVANCED BREAST CANCER TREATED WITH FIRST LINE CDK4/6 INHIBITORS INSIGHTS FROM THE CICLADES STUDY

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INTRODUCTION

The current standard-of-care for estrogen receptor-positive (ER+), human epidermal growth factor receptor 2 negative (HER2-) advanced breast cancer (ABC) is a combination of endocrine therapy (ET) and cyclin-dependant kinase 4/6 inhibitors (CDK4/6i). Despite proven efficiency, resistance mechanisms are likely to develop in patients receiving aromatase inhibitors as part of their ET regimen, leading to disease progression. Mutations of the *ESR1* gene are known key players in ET resistance, as well as *PIK3CA, AKT1* and *TP53* mutations. Since circulating tumor DNA (ctDNA) has emerged as a valuable tool for real-time monitoring of disease evolution, the CICLADES study (NCT03318263) aimed to evaluate the prognostic and temporal dynamics of these mutations as well as ctDNA-based progression signals.

METHODS

• Patients:
117 patients with ABC, all treated with a first-line combination of ET and CDK4/6i (**Table I**).

• Sampling:
Plasma samples were collected at baseline, then every three months for two years and every six months during the third year. Additional sampling was performed at the time of disease progression (**Figure 1**).

• Molecular analyses:
Baseline and follow-up ctDNA were extracted and sequenced with a custom 35-gene targeted panel (KAPA HyperPETE, Roche Diagnostics, Switzerland).

• Outcomes:
Progression-free survival (PFS) associated with resistance mutations of *ESR1, PIK3CA, AKT1* and *TP53* was evaluated. Other biological parameters such as molecular progression and transient variants were also evaluated as potential markers of tumor evolution dynamics.

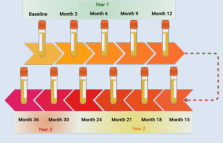


Figure 1: Study sampling scheme from baseline to completion of follow-up at 36 months.

RESULTS

Baseline *ESR1* mutations (n=6, Figure 3.A), combined with those acquired during follow-up (n=26, Table II) were associated with significantly shorter PFS. *PIK3CA, AKT1* and *TP53* mutations also predicted shorter PFS at baseline, but not significantly during follow-up (Table II). While *PIK3CA, AKT1* and *TP53* mutations were largely present at baseline, most *ESR1* mutations emerged under treatment. Molecular progression, defined by ≥50% increase in variant allele frequency (VAF), was detected in 19 patients and preceded radiologic progression in 11, with a median lead time of 2.7 months (Figure 4.A). Thirteen patients exhibited transient low-VAF variants during follow-up, suggestive of subclonal shedding rather than resistance; these did not correlate with clinical progression.

1 – Patients

Characteristic	n (%) or value
Age (years)	Median: 64 (range 32–85)
Disease stage	
Locally advanced	3 (3%)
Metastatic	114 (97%)
Height (median - cm)	162 [145–178]
Weight (median - kg)	71.5 [48–130]
ECOG performance status	
0	45 (39%)
1	41 (35%)
2	14 (12%)
3	4 (3%)
Not documented	13 (11%)
Endocrine therapy	
Anastrozole	24 (21%)
Letrozole	54 (46%)
Exemestane	6 (5%)
Fulvestrant	33 (28%)
CDK4/6 inhibitor	
Palbociclib	108 (92%)
Abemaciclib	4 (4%)
Ribociclib	5 (4%)
Ovarian suppression	
Yes	3 (3%)
No	114 (97%)

Table I: Patient characteristics (n = 117) ; CDK4/6 : cyclin-dependant kinase 4/6

2 – ctDNA baseline analysis

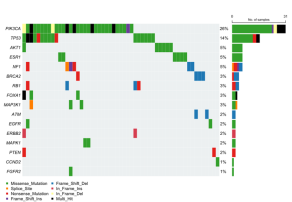
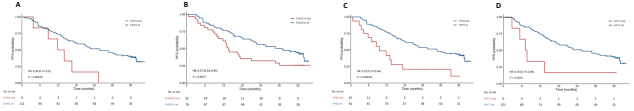


Figure 2: Oncoplot of pathogenic and probably pathogenic somatic alterations detected in the available baseline plasma samples (out of 117 patients).

3 – Progression-Free Survival



Mutation	T.P.	n mut	PFS wt	PFS mut	*P - value*	HR (95% - CI)
ESR1	B	6	25.7 mo	12.9 mo	0.00918	0.34 (0.15 – 0.8)
	F-U	26	28.8 mo	16 mo	0.00638	0.51 (0.31 – 0.83)
PIK3CA	B	31	30.9 mo	15.3 mo	0.0277	0.57 (0.34 – 0.95)
	F-U	47	26.2 mo	18 mo	0.23900	0.76 (0.48 – 1.2)
TP53	B	16	28.7 mo	10.7 mo	0.000421	0.35 (0.19 – 0.64)
	F-U	42	25.5 mo	22.8 mo	0.98900	1 (0.62–1.62)
AKT1	B	6	25.7 mo	7.6 mo	0.01760	0.35 (0.14–0.87)
	F-U	10	25.7 mo	14.2 mo	0.16900	0.6 (0.29–1.25)

Figure 3: Kaplan–Meier estimates of progression-free survival (PFS) in the CICLADES study based on baseline ctDNA status. **(A)** PFS for *ESR1*-mutant versus *ESR1* wild-type. **(B)** PFS for *PIK3CA*-mutant versus *PIK3CA* wild-type. **(C)** PFS for *TP53*-mutant versus *TP53* wild-type. **(D)** PFS for *AKT1*-mutant versus *AKT1* wild-type.

Table II: Progression-free survival (PFS) with 95% confidence intervals (CI), and *P-values*. T.P : Time-Point; B : Baseline; F-U: Follow-up; mo: Months; wt: Wild-Type; mut: mutated; n : number

4 – "POP" variants *vs* molecular progression

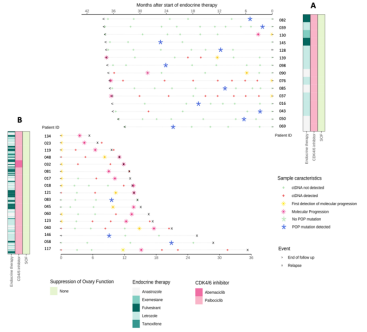


Figure 4: Swimmer plots of ctDNA follow-up based on subclonal shedding. **(A)** Swimmer plot of patients (n = 15) without disease progression. **(B)** Swimmer plot of patients (n = 17) with disease progression before the end of follow-up.

CONCLUSION

In ER+/HER2- ABC, pre-existing *ESR1*, *PIK3CA* and *TP53* mutations, and acquired *ESR1* mutations, are significantly associated with shorter PFS under CDK4/6i-based therapy. Molecular progression may precede radiologic progression by several months, supporting the extension of this concept into a real-world setting, and highlighting a window of opportunity for earlier therapeutic intervention. However, episodes of subclonal shedding underscore the importance of confirmatory sampling before clinical decision-making. These results support longitudinal ctDNA monitoring for real-time disease interception. We thank the patients and their families for their participation to this study.

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