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Mutational profiles from liquid biopsies in Early versus Late Progressors treated with CDK4/6 inhibitors in 1st line Metastatic Breast Cancer

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Background

- Approximately 10% of patients with hormone receptor-positive, HER-2 negative (HR+/HER2-) metastatic breast cancer (MBC) experience **disease progression within 12 months** of initiating first-line treatment with CDK4/6 inhibitors (CDK4/6i).
- Early progressors have a particularly **poor prognosis.**
- There is a need for **personalized strategies** guided by integrated clinical and molecular profiling.

The **objective** of these analysis is to **compare clinical and genomic features between early progressors and late progressors** to uncover mechanisms driving early progression.

Methods

A retrospective analysis of **clinical and plasma-derived circulating tumour DNA (ctDNA) genomic data** from HR+/HER2- MBC patients treated with CDK4/6i enrolled in the **STING molecular screening program** (NCT04932525) from Feb 2021 to Aug 2022 was performed.

- One **post-progression plasma sample** per patient was included selecting only liquid biopsies obtained after radiologic progression on CDK4/6 inhibitors (n = 91). ctDNA was profiled using the **FoundationOne®** Liquid targeted NGS assay.
- **Variants** were analyzed using the *maftools* package. Analyses were restricted to a curated list of **breast cancer–relevant genes.**
- **Differential mutational analysis** between EP and LP was performed using *mafCompare* (Fisher's exact test). Multiple testing correction was applied using the **Benjamini–Hochberg false discovery rate (FDR)**.
- Genes were grouped into manually curated **oncogenic pathways** relevant to breast cancer. For each pathway, we quantified the proportion of patients with ≥1 mutation in that pathway in EP and LP groups and compared proportions using Fisher's exact tests with BH-adjusted FDR.
- **TMB (mut/Mb) and Tumor Fraction Score** were extracted from FoundationOne® reports and compared between EP and LP using Wilcoxon rank-sum tests.
- All analyses were performed in R using *maftools*, *tidyverse*, and *ggplot2*.

Clinicopathological characteristics

Among 116 patients, 91 patients had a liquid biopsy after progression to CDK4/6i.

- The median time between EOT and liquid biopsy was 7 months (2,17) in EP and 6 months in LP (4, 15).
- Clinically, a higher rate of **visceral metasases** was observed in **EP**.
- Downstream analysis shows **no significant differences** for age, menopausal status, hormone sensitivity or tumor histology.

Table 1 : *Clinicopathological characteristics of the complete Cohort (n = 116) and comparison of early progressors (EP) and late progressors (LP). EP defined as progression after 12 months of CDK4/6i.*

Characteristic	**EP**, N = 62[1]	**LP**, N = 54[1]	p-value[2]
Age at biopsy	57 (51, 64)	60 (47, 69)	0.6
Menopausal status			0.4
Post-Menopause	45 (39.5%)	35 (30.7%)	
Pre-Menopause	16 (14.0%)	18 (15.8%)	
Unknown	1	1	
Histology			0.5
Ductal	52 (44.8%)	43 (37.1%)	
Lobular	8 (6.9%)	11 (9.5%)	
Mucinous	1 (0.9%)	0 (0.0%)	
Unknown	1 (0.9%)	0 (0.0%)	
Adjuvant Endocrine Therapy	45 (38.8%)	34 (29.3%)	0.3
Adjuvant Chemotherapy	37 (32.2%)	27 (23.5%)	0.3
Unknown	1	0	
CDK inhibitor			0.4
Abemaciclib	4 (3.4%)	2 (1.7%)	
Palbociclib	48 (41.4%)	47 (40.5%)	
Ribociclib	10 (8.6%)	5 (4.3%)	
Metastatic disease			0.12
De Novo	9 (7.8%)	14 (12.1%)	
Non de novo	53 (45.7%)	40 (34.5%)	
Presence of visceral Metastasis			**0.016**
NO (Non-visceral)	24 (20.7%)	33 (28.4%)	
YES (Lung, Liver)	38 (32.8%)	21 (18.1%)	
Bone only disease			0.087
YES (Bone Only)	16 (13.8%)	22 (19.0%)	
NO (Not Bone only)	46 (39.7%)	32 (27.6%)	
Hormone_sensitivity [3]			0.5
Resistant	32 (42.1%)	22 (28.9%)	
Sensitive	11 (14.5%)	11 (14.5%)	
Unknown	19	21	
Biopsy_timing			**<0.001**
Biopsy_at_progressive disease	51 (44.0%)	28 (24.1%)	
Biopsy_before_progression	11 (9.5%)	26 (22.4%)	

[1] Median (IQR); n (%)
[2] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test
[3] Defined as the absence of progressive disease on or within the first year of completion of adjuvant endocrine therapy

Table 2 : *Clinicopathological characteristics of patients that had liquid biopsies after radiologic progression on CDK4/6i (n = 91)*

Characteristic	**EP**, N = 55[1]	**LP**, N = 36[1]	P-value [2]
Age at biopsy	57 (50, 64)	56 (43, 64)	0.5
Menopausal status			0.2
Post-Menopause	39 (43.8%)	21 (23.6%)	
Pre-Menopause	15 (16.9%)	14 (15.7%)	
Unknown	1	1	
Histology			0.6
Ductal	48 (52.7%)	30 (33.0%)	
Lobular	5 (5.5%)	6 (6.6%)	
Mucinous	1 (1.1%)	0 (0.0%)	
Unknown	1 (1.1%)	0 (0.0%)	
Adjuvant Endocrine Therapy	39 (42.9%)	23 (25.3%)	0.5
Adjuvant Chemotherapy	33 (36.7%)	20 (22.2%)	0.6
Unknown	1	0	
CDK inhibitor			0.5
Abemaciclib	3 (3.3%)	0 (0.0%)	
Palbociclib	45 (49.5%)	31 (34.1%)	
Ribociclib	7 (7.7%)	5 (5.5%)	
Metastatic Disease			0.3
De novo	9 (9.9%)	9 (9.9%)	
Non de novo	46 (50.5%)	27 (29.7%)	
Presence of visceral Metastasis			**0.021**
NO (Non-visceral)	20 (22.0%)	22 (24.2%)	
YES (lung and/or liver)	35 (38.5%)	14 (15.4%)	
Bone_Only_disease			0.1
YES	14 (15.4%)	15 (16.5%)	
NO	41 (45.1%)	21 (23.1%)	
Hormone_sensitivity			0.3
Resistant	26 (44.1%)	18 (30.5%)	
Sensitive	11 (18.6%)	4 (6.8%)	
Unknown	18	14	
Timing (months) between_EOT_and_LB	7 (2,17)	6 (4,15)	> 0.9

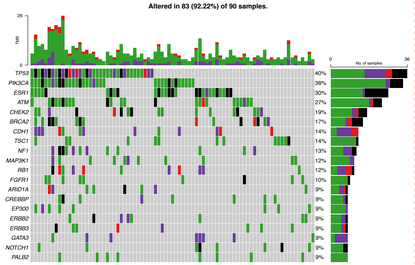
[1] Median (IQR); n (%)
[2] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

Potential biais include the variable timing between the progression on CDK4/6i and the time of liquid biopsy.

- Patients (n = 116) were classified as early progressors (EP) or late progressors (LP), with EP defined as progression occurring within 12 months after initiation of first-line CDK4/6i. **Clinicopathological characteristics** were compared between EP and LP, using a Wilcoxon rank-sum for continuous variables and Chi-squared or Fisher exact tests for categorical variables.

Molecular characteristics

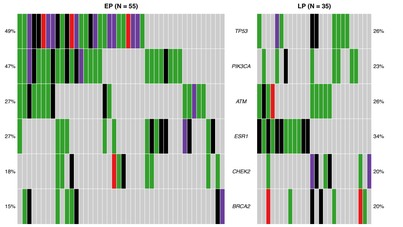
Figure 3 : *Oncoplot, Variant Analysis of the complete Cohort (n = 90; we are losing 1 patient/sample because there are no mutations in the BC gene panel)*



One plasma sample per patient was included, obtained after radiologic progression on CDK4/6i.

- We have selected the most common mutations related to breast cancer.
- TP53 and PIK3CA mutations were the most common mutations identified in the overall cohort (n = 90).
- Molecular alterations were mostly missense mutations.

Figure 4 : *Co - oncoplot, variant analysis comparing EP and LP*



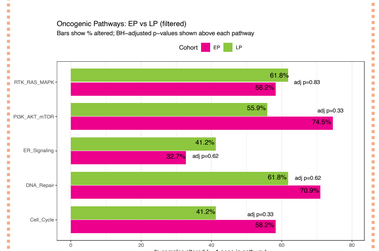
Gene	EP (n/N, %)	LP (n/N, %)	p-value	FDR (adj p)
TP53	26/55 (47.27%)	8/35 (22.86%)	0.022	0.350
PIK3CA	27/55 (49.09%)	9/35 (25.71%)	0.025	0.350
ATM	8/55 (14.55%)	7/35 (20.00%)	0.568	0.883
ESR1	15/55 (27.27%)	12/35 (34.29%)	0.633	0.933
CHEK2	10/55 (18.18%)	7/35 (20.00%)	1.000	1.000
BRCA2	15/55 (27.27%)	9/35 (25.71%)	1.000	1.000

Only 30 samples (21 EP , 9 LP) had copy-number data, and only 26 samples (15 EP , 11 LP) had rearrangement data, which is insufficient for meaningful statistical analysis.

- **EP** exhibit a trend to a higher frequency of **TP53 mutations** (EP 47% vs LP 23%; p=0.022, **FDR 0.350**)
- **EP** exhibit a trend to a higher proportion of **PIK3CA mutations** (EP 49% vs LP 26%; p = 0.025, **FDR 0.350**).
- **ESR1 mutations** were numerically more frequent in **LP** (EP 27% vs LP 34%, p = 0.63). This is consistent with LP having been exposed to aromatase inhibitors for a longer duration.

Results

Figure 5 : *Variant analysis, Pathway analysis comparing EP and LP*

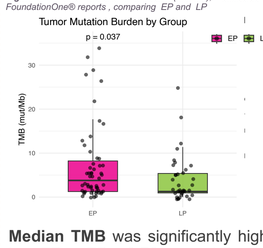


Pathway	Genes
Cell Cycle	TP53, RB1, CCND1, CCND2, CCND3, CCNE1, CDK4, CDK6, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CDKN2C
PI3K–AKT–mTOR	PIK3CA, PIK3R1, PIK3CB, PTEN, AKT1, AKT2, TSC1, TSC2, MTOR, RPTOR, RICTOR, STK11
RTK–RAS–MAPK	ERBB2, ERBB3, ERBB4, EGFR, FGFR1, FGFR2, FGFR3, HRAS, KRAS, NRAS, BRAF, RAF1, MAP3K1, MAP2K4, MAPK1, MAPK3, NF1, ERRFI1
Hormone Signaling	ESR1, ESR2, AR, ESRRG, FOXA1, GATA3, NCOR1, NCOR2

Genes were grouped into **oncogenic pathways relevant to breast cancer.**

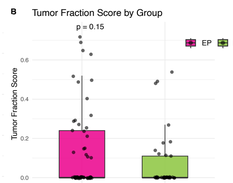
- The **PI3K-AKT-mTOR Pathway** (EP 74% vs LP 56%, adj p = 0,33), the **DNA-repair Pathway** (EP 71% vs LP 62%, adj p = 0,62) and the **Cell-Cycle Pathway** (EP 58% vs LP 41%, adj p = 0,33) **trend to more alterations in EP.**

Figure 6 : *Tumor Mutational Burden (mut/Mb), extracted from FoundationOne® reports , comparing EP and LP*



Median TMB was significantly higher in EP (EP 3.76 vs LP 1.26 mutations/Mb, p=0.037).

Figure 7 : *Tumor Fraction Score, extracted from FoundationOne® reports , comparing EP and LP*



Circulating tumor fraction trends to be higher in EP (mean TF in EP 0.136 vs mean TF in LP 0.0728, p=0.15).

Conclusion

- **Early progression to first-line CDK4/6i in HR+/HER2- MBC** is associated with distinct genomic features.
- Clinically, a higher rate of **visceral metasases** was observed in early progressors.
- Comparisons between EP and LP showed no statistically significant differences after multiple-testing adjustment in individual mutated genes or pathway-level alterations, although EP showed a **descriptive trend toward more PI3K and TP53 alterations**, while LP showed a trend toward more ESR1 mutations.
- EP patients showed **higher median TMB**, while TF differences were not significant, possibly reflecting the higher proportion of non-luminal tumors and more visceral disease in EP.

Although this analysis was performed after progression on CDK4/6i, we hypothesize that **an upfront ctDNA-based genomic profiling could help to identify patients at risk of early recurrence** on CDK4/6i and guide the use of emerging post-CDK4/6i therapies*; however, prospective validation remains necessary.

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