PS1-12-20: Circulating tumor DNA profiling of patients with early-line advanced luminal breast cancer

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INTRODUCTION

Advances in endocrine therapies for hormone receptor-positive (HR+)/HER2-negative (HER2-) advanced breast cancer (ABC) continue to transform care and significantly improve patient outcomes. However, the development of resistance is inevitable, and the integration of molecular and clinical risk stratification into guiding individualized treatment selection remains a key challenge. We therefore used circulating tumor DNA (ctDNA) to assess the genomic landscape of patients receiving early lines of treatment for HR+ ABC in a multicenter cohort of patients in Austria and evaluated whether the integration of baseline ctDNA-derived tumor metrics enhances risk stratification.

METHODS

We collected 225 plasma samples from 184 patients with HR+/HER2- ABC either at diagnosis or following progression on early lines of endocrine treatment. 32 patients had paired samples collected before first-line (1L) and second-line (2L) treatment (**Figure 1**).
ctDNA was analyzed using a 77-gene panel (AVENIO ctDNA Expanded Kit). Tumor fraction was calculated via untargeted aneuploidy assessment using mFAST-SeqS (reported as z-scores) and the highest variant allele frequency (hVAF) detected with the AVENIO assay. When available, ichorCNA was used to more precisely estimate the tumor fraction in plasma samples.
Somatic variants, together with single, binary and three-level composite ctDNA metrics were assessed for their association with progression-free and overall survival (PFS, OS).

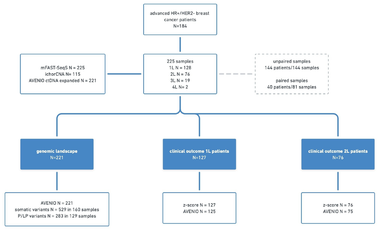


Figure 1. Consort diagram showing the selection of samples for genomic and survival analyses. P/LP pathogenic/likely pathogenic

RESULTS

A total of 529 somatic variants from 68 different genes were identified in 160/221 (72.4%) samples, with a median of two variants per sample (range 1-28 variants). In 1L samples, somatic variants were detected in 65.1% cases, and pathogenic or likely pathogenic variants in 49.2% cases. In 2L samples, somatic variants were detected in 81.3%, and pathogenic or likely pathogenic variants in 69.3% cases. After adjusting for elevated tumor fractions (z-scores ≥ 3) we showed that 2L samples had 80% more somatic variants than 1L samples (IRR 1.80, 95% CI: 1.36-2.38; p < 0.001, mixed-effects negative binomial model).

The genes most frequently mutated for pathogenic or likely pathogenic variants were *PIK3CA* (32.5% and 40.0%), *TP53* (16.7% and 24.0%) and *ESR1* (7.1% and 26.7%), with *ESR1* mutations being significantly more frequent in 2L samples (OR = 4.7, 95% CI 2.2-10.0, $p < 0.001$, cluster-robust logistic regression). Furthermore, in a subset of 31 patients with successfully analyzed paired 1L and 2L samples (**Figure 2**), *ESR1* emerged as the most frequently acquired mutation (25.8%, McNemar test, p = 0.013). Mutations affecting the PI3K pathway (*PIK3CA, AKT1*, and/or *PTEN*) were present in 34.9% of 1L and 44.0% of 2L samples. The co-alteration patterns among pathogenic or likely pathogenic mutations in 1L and 2L samples are shown in **Figure 3**.

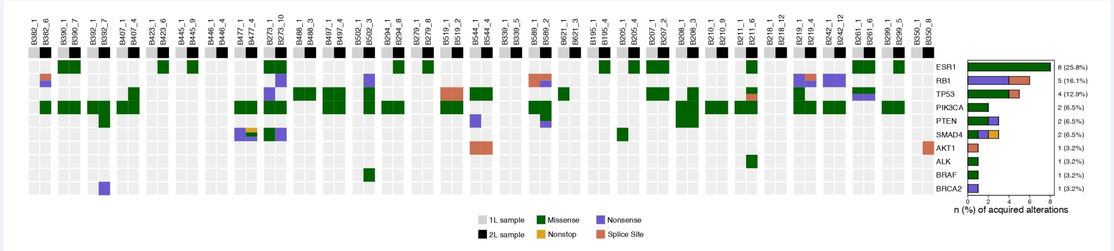


Figure 2. Acquired pathogenic or likely pathogenic somatic alterations detected in a subset of 31 patients with paired samples taken before 1L (gray) and 2L (black) treatment. Bars on the right represent the number of acquired alterations detected in each gene.

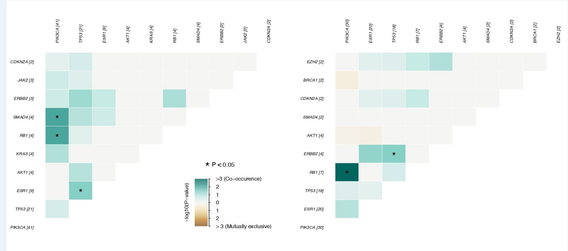


Figure 3. Mutation co-occurrence/exclusivity among the top 10 mutated genes in 1L (left) and 2L (right) samples (pairwise Fisher's Exact test).

The overall tumor fraction was low (median z-score 2.49; range −0.5 to 208.3), with no significant difference between 1L and 2L samples estimated by mFAST-SeqS (adjusted mean difference 6.33, 95%CI: -0.2-12.9; p = 0.058) or ichorCNA (adjusted mean difference -0.003, 95%CI: -0.06-0.07; p = 0.925). However, hVAF was significantly higher in 2L samples (adjusted mean difference 4.74; 95% CI, 1.28–8.20; p = 0.007).

We comprehensively evaluated multiple ctDNA-derived metrics in this patient cohort. In univariable Cox regression analyses, all ctDNA-based measures demonstrated significant associations with PFS and OS, except for *PIK3CA* mutations, which showed no significant association with PFS (**Figure 4**). A three-tier composite variable that stratified patients into low (z-score < 3 and hVAF < 1%), intermediate (z-score ≥ 3 or hVAF ≥ 1%), and high (z-score ≥ 3 and hVAF ≥ 1%) tumor burden demonstrated superior discriminatory capacity and enhanced potential for clinical risk stratification in both 1L (**Figure 5**) and 2L samples. Incorporating this metric into a clinical model consisting of known clinical variables improved prognostic performance (**Figure 6**).

Variable	N	HR (95% CI)	P-value	Uno's C (95% CI)
z-score ≥ 3	48/127	2.48 (1.51-4.08)	<0.001	0.610 (0.544-0.676)
hVAF ≥ 1%	65/125	2.57 (1.53-4.34)	<0.001	0.617 (0.550-0.683)
ctDNA+	90/125	2.34 (1.22-4.49)	0.011	0.579 (0.511-0.648)
z-score ≥ 3 or hVAF ≥ 1%	78/125	3.15 (1.73-5.73)	<0.001	0.632 (0.565-0.699)
ctDNA intermediate (Ref.low)	45/125	2.41 (1.24-4.66)	0.009	0.663 (0.599-0.728)
ctDNA high (Ref.low)	33/125	4.66 (2.38-9.12)	<0.001	
No. of somatic variants	125/125	1.14 (1.06-1.22)	<0.001	0.630 (0.544-0.716)
PIK3CA mutant	41/125	1.25 (0.74-2.09)	0.407	0.524 (0.437-0.612)
TP53 mutant	21/125	2.51 (1.39-4.51)	0.002	0.560 (0.507-0.614)
ESR1 mutant	12/125	2.61 (1.32-5.17)	0.006	0.545 (0.507-0.584)

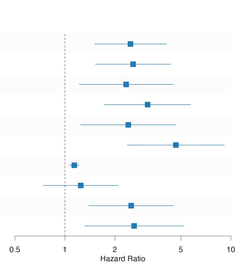


Figure 4. Performance of individual and composite ctDNA TFx metrics, the total somatic variant count and the most frequently mutated genes in relation to PFS. The prognostic discrimination was measured by Uno's C-index.

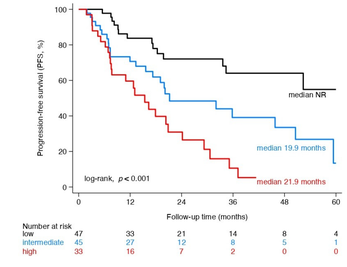
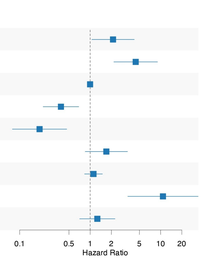


Figure 5. Kaplan-Meier curves illustrating the difference in PFS using a three-level composite variable that achieved the highest prognostic discrimination.

Variable	N	HR (95% CI)	P-value
ctDNA intermediate	45/125	2.11 (1.06-4.22)	0.034
ctDNA high	33/125	4.43 (2.17-9.03)	<0.001
Age at inclusion (years)	125/125	1.00 (0.98-1.02)	0.807
PR positive	95/125	0.38 (0.22-0.69)	0.001
ET + CDKi treatment	115/125	0.19 (0.08-0.47)	<0.001
Liver metastasis	23/125	1.70 (0.85-3.38)	0.133
Number of metastatic organs	125/125	1.11 (0.83-1.49)	0.464
Time to metastasis ≤ 24 months (Ref. de novo)	6/125	10.79 (3.42-34.05)	<0.001
Time to metastasis > 24 months (Ref. de novo)	65/125	1.26 (0.71-2.25)	0.426



Uno's C: 0.758 (95% CI: 0.698-0.818) | AIC: 483.0

Figure 6. Clinical model incorporating the three-level composite ctDNA metric. Increased Uno's C-index and reduced Akaike Information Criterion (AIC) indicate a significantly improved prognostic performance compared to a model based only on common clinical variables.

CONCLUSION

In this prospective multicenter study that included a real-world cohort of patients with HR+/HER2– ABC treated in early lines, we comprehensively evaluated multiple ctDNA-derived metrics, including z-scores generated with mFAST-SeqS, AVENIO-based hVAF, composite variables, as well as the total somatic variant count and the most frequently mutated genes in the cohort. While almost all ctDNA-based measures demonstrated significant associations with PFS and OS both in 1L and 2L patients, a three-tier composite classification that stratified patients into low, intermediate and high tumor burden achieved the highest prognostic discrimination and enhanced the potential for clinical risk stratification.
Our multivariable analyses demonstrate that integrating ctDNA-derived tumor fraction estimates with established clinical variables substantially improves prognostic stratification in patients with HR+/HER2- ABC. While established clinical factors remained significant predictors of outcome, incorporation of the composite ctDNA variable further improved the overall model performance and prognostic discrimination. This improvement suggests that ctDNA-based metrics capture complementary and biologically meaningful information beyond conventional clinicopathological variables, supporting their integration into standardized reporting and interpretation of ctDNA analysis results.
In practical terms, patients characterized as having high ctDNA burden may warrant closer monitoring, earlier escalated therapy, or entry into trials of novel agents, whereas those with low ctDNA burden may be candidates for treatment de-escalation or more conservative management.

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