Comprehensive Characterization of PTEN loss by IHC and *PTEN* alteration by NGS in Metastatic HR-Positive, HER2-Negative Breast Cancer: An Exploratory Analysis of Biomarker Concordance and Co-Occurrence

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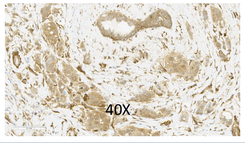
BACKGROUND

- PTEN, a key negative regulator of the PI3K/AKT signaling pathway, can be inactivated in hormone receptor positive (HR+), HER2-negative (HER2−) metastatic breast cancer (mBC).
- While genomic alterations can inactivate PTEN, a subset of tumors lose PTEN protein expression without any *PTEN* genomic alterations, resulting in a discordance between genomic (NGS) and protein-based test (IHC) results.
- The CAPItello-291 trial (NCT04305496) led to the approval of capivasertib (AKT inhibitor) in combination with fulvestrant in patients with HR+/HER2- mBC with alterations in *PIK3CA*, *AKT1*, and/or *PTEN*.
- Prior pre-specified exploratory analysis from the CAPItello-291 study* in patients with PTEN IHC results (using ≥90% of viable malignant cells with no specific cytoplasmic PTEN staining) reported 19% of patient tumor samples (70/367) were PTEN deficient by IHC and 54% (38/70) of these also had a PIK3CA/AKT1/PTEN alteration by NGS.
- Here, we report a prespecified exploratory analysis evaluating the concordance between PTEN loss of expression by IHC and its genomic alteration by NGS, as well as the respective co-occurrences with *PIK3CA* and *AKT1* mutations.

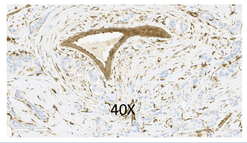
METHODS

- 2,642 breast tumors underwent comprehensive tumor profiling at Caris Life Sciences (Phoenix, AZ) between August 2024 to June 2025.
- All tumors were identified as HR positive and HER2 negative by a combination of IHC and CISH based on ASCO/CAP guidelines.
- Gene alterations were determined by Whole Exome Sequencing.
- PTEN IHC was tested using 6H2.1 antibody and scored by board-certified pathologists.
- **PTEN loss was defined as complete absence of staining (0+, 0%).**

PTEN INTACT BY IHC



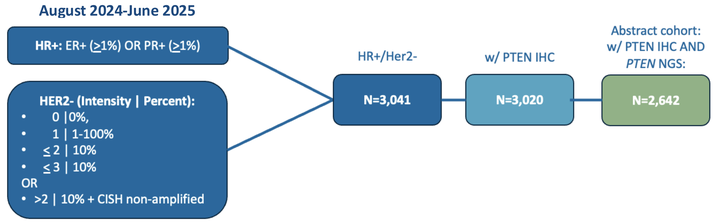
PTEN LOSS BY IHC



RESULTS

Figure 1. Cohort Creation

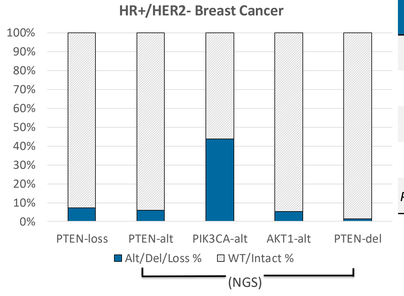
A



B

Characteristic	Overall Population	PTEN-loss	PTEN-intact
N	2,642	194 (7.34)	2,448 (92.7)
Age, median (range)	65 (24-90+)	62 (32-90+)	66 (24-90+)
Gender, N (%)			
Female	2,595	194	2,401
Male	47	0	47

Figure 2. Gene Alteration Prevalence

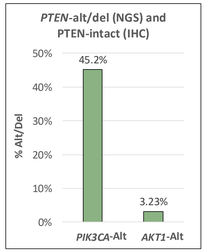


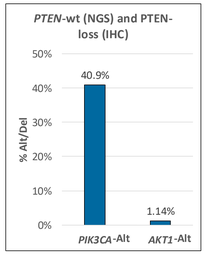
Biomarker	Alt/Del/Loss Pos/Total (%)	WT/Intact Pos/Total (%)
PTEN-loss (IHC)	194/2642 (7.34)	2448/2642 (92.7)
PTEN-alt (NGS)	161/2642 (6.09)	2481/2642 (93.9)
PIK3CA-alt (NGS)	1157/2641 (43.8)	1484/2641 (56.2)
AKT1-alt (NGS)	140/2642 (5.3)	2502/2642 (94.7)
PTEN-del (CNV) (NGS)	39/2584 (1.51)	2545/2584 (98.5)

Among the 2,642 tumors, 194 (7.34%) demonstrated PTEN loss by IHC.

Figure 3. Concordance between PTEN-IHC and *PTEN*-NGS and Prevalence of *PIK3CA*, *AKT1* alterations within PTEN discordant cases

N (%)	*PTEN*-alt/del (NGS)	*PTEN*-wt (NGS)
PTEN-Loss (IHC)	106 (4.01%)	88 (3.33%)
PTEN-Intact (IHC)	93 (3.52%)	2,355 (89.1%)

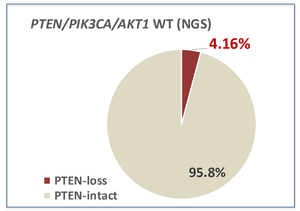




- **The overall concordance between PTEN loss (IHC) and *PTEN* alterations (NGS) was 93.1%** = (106+2355)/(88+2355+106+93)
- In the absence of a definitive reference standard, the **positive percent agreement (PPA)** was **53.3%** and the **negative percent agreement (NPA)** was **96%.**
- Among the discordant cases, two distinct patterns were observed:
  - In tumors with **PTEN-loss by IHC but wild-type (wt) by NGS** (N=88), *PIK3CA* and *AKT1* alterations rates were **40.9%** (36/88) and **1.14%** (1/88), respectively.
  - In tumors with **PTEN-intact by IHC but *PTEN*-alt by NGS** (N=93), *PIK3CA* and *AKT1* alterations rates were **45.2%** (42/93) and **3.23%** (3/93), respectively.

Notably, 4.16% (51/1227) of tumors that were wild-type for *PTEN*, *PIK3CA* and *AKT1* by NGS demonstrated PTEN loss by IHC suggesting PI3K/AKT pathway activation that would have been missed without IHC testing.

Figure 4. PTEN-IHC identified additional 4.16% of cases of PTEN-loss, otherwise not detected by NGS test



CONCLUSIONS

- To our knowledge, this is the largest study highlighting a notable discordance between PTEN expression by IHC and *PTEN* genomic alterations by NGS in HR+/HER2− mBC.
- Our results underscore the utility for a multimodal approach to patient identification and suggest that in addition to NGS panel tests, a PTEN-IHC may identify additional patients with PI3K/AKT pathway activation.
- Compared to the results from the CAPItello-291 study*, this study demonstrates that different IHC PTEN antibody usage, criteria for PTEN deficiency or cut-off for IHC, and NGS test sensitivity can impact patient identification; this needs continued investigation.

REFERENCE

* Jhaveri K, Rugo HS, Oliviera M, et al., Capivasertib–fulvestrant for patients with HR-positive/HER2-negative advanced breast cancer who had relapsed or progressed during or after aromatase inhibitor treatment: exploratory analysis of PTEN deficiency by IHC from the Phase III CAPItello-291 trial. SABCS, 2024: P2-03-19.

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Table Aim 3, N (%)	*PTEN*-Loss	*PTEN*-Intact
***PTEN*-wt/not deleted**	88 (3.33%)	2355 (89.1%)
***PTEN*-mt/deleted**	106 (4.01%)	93 (3.52%)

Table Aim 3, N (%)	*PTEN*-Loss	*PTEN*-Intact
***PTEN*-wt**	112 (4.12%)	2443 (89.9%)
***PTEN*-alt**	84 (3.09%)	77 (2.84%)