Detection of actionable gene mutations in breast cancer by amplicon-based next-generation sequencing liquid biopsy

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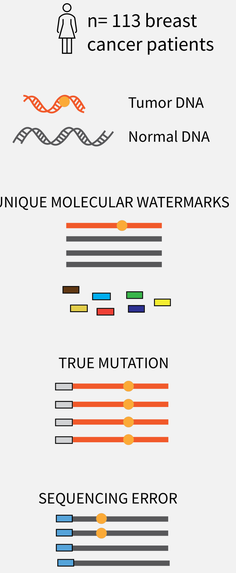
Introduction

- *PIK3CA* mutations occur in 30-40% of breast cancer patients.
- The PI3K-inhibitor, alpelisib, is approved for the treatment of hormone receptor (HR)-positive, HER2-negative breast cancers with *PIK3CA* mutations.
- High test sensitivity of mutation detection in plasma cell-free DNA (cfDNA) would support the clinical application of non-invasive liquid biopsy in breast cancer, especially to identify patients with actionable mutations.
- We applied an ultra-sensitive amplicon-based next-generation sequencing platform technology (AmpliMark™) to detect mutations in cfDNA from breast cancer patients as quality evaluation.

Materials and Methods

- Detection of actionable gene mutations was assessed in the plasma cfDNA from the blood of breast cancer patients (n = 113, 82.3% metastatic), both treated and untreated.
- A primer-based target capture panel with >8 genes and 6 microsatellite loci based on the AmpliMARK™ technology, was validated using standard reference material (Horizon HD829, Tru-Q and cell lines).
- Unique molecular watermarks enable error-correction for the sensitive and accurate detection of mutations.
- Genes include, but are not limited to: *AKT1, CDH1, ERBB2, ESR1, GATA3, MYC, PIK3CA, PTEN, TP53*

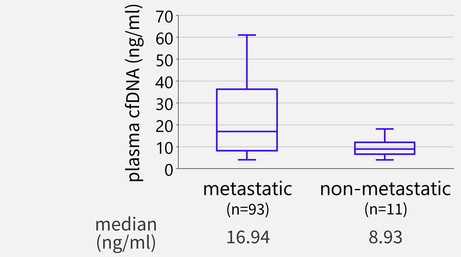
  MSI loci: BAT25, BAT26, NR21, NR24, NR27, MONO27



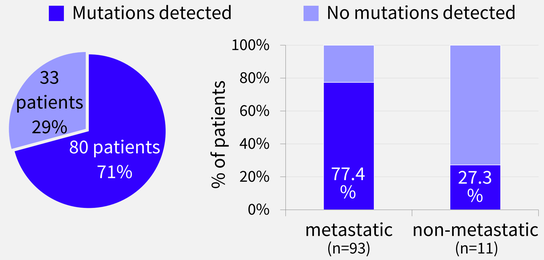
Results

(A) AmpliMARK™ is a sensitive method for the detection of actionable mutations in breast cancer.

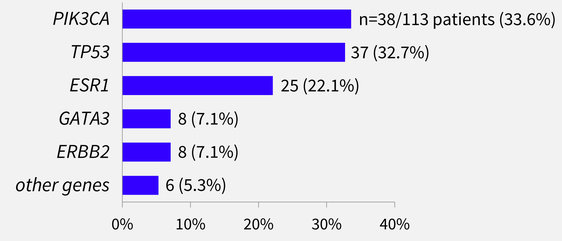
I. Plasma cfDNA concentration is higher in metastatic samples .



II. Mutations were detected in 71% of patients, and were more commonly detected in patients with metastatic breast cancer.

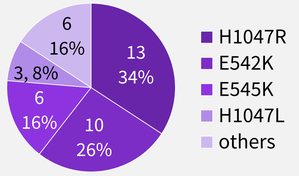


III. *PIK3CA* and *ESR1* were among the most frequently mutated genes, demonstrating a high rate of detection of actionable mutations in breast cancer.

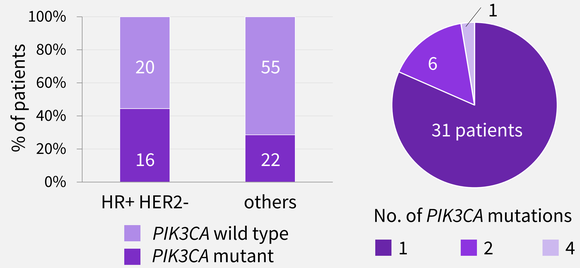


(B) Analysis of *PIK3CA* mutations detected in the plasma cell-free DNA.

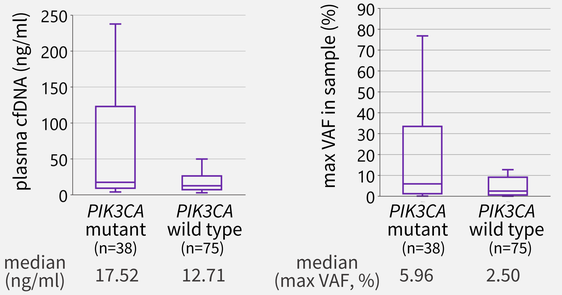
I. Known *PIK3CA* hotspot mutations were the most frequently detected *PIK3CA* mutations in plasma cfDNA from breast cancer patients.



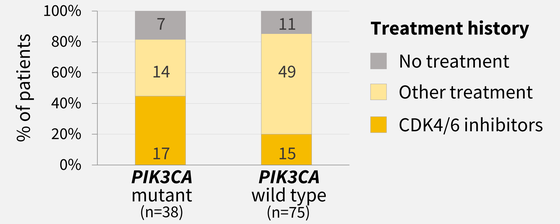
II. *PIK3CA* mutations were most common in HR+ HER2- breast cancer patients. 7 (of 38) patients with *PIK3CA* mutations had >1 *PIK3CA* mutation and might be more sensitive to PIK3CA inhibition.

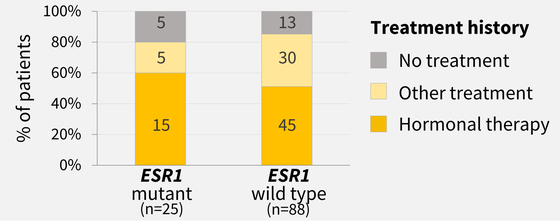


III. *PIK3CA* mutations were associated with higher plasma cfDNA concentrations and higher maximum variant allele frequency (VAF), suggestive of higher tumor burden in patients with *PIK3CA* mutations.



(C) *PIK3CA* and *ESR1* mutations were more frequently detected in patients treated with CDK4/6 inhibitors or with hormonal therapy, respectively.





Conclusion

- We report the application of liquid biopsy coupled with AmpliMARK™ for the detection of actionable mutations in breast cancer at frequencies similar to external tissue studies.
- Our technology would enable the high-sensitivity and non-invasive identification of breast cancer patients who might be sensitive to PI3K inhibition (e.g. by alpelisib), including patients who might be ultra-sensitive due to the presence of >1 *PIK3CA* mutation, or patients who might have acquired *PIK3CA* resistance mutations after CDK4/6 inhibition.

Acknowledgements

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