Highly Sensitive and Specific Analysis of *PIK3CA* Mutations in Formalin-Fixed, Paraffin-Embedded (FFPE) Samples using MALDI-TOF Mass Spectrometry

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INTRODUCTION

PIK3CA mutations are detected in approximately 40% of hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) metastatic breast cancer. According to recent clinical data, the dual inhibition of estrogen receptor (ER) and PI3K-α subunit increases overall response rate in *PIK3CA* mutated patients over ER blockade alone. Therefore, recent NCCN and ESMO guidelines recommend genomic testing for *PIK3CA* mutations in routine clinical practice for therapy selection. In this study we evaluated and validated a new MALDI-TOF-based, targeted *PIK3CA* panel.

MATERIALS & METHODS

For assessing the sensitivity and specificity of the novel ClearSEEK™ PIK3CA Panel (Agena Bioscience, San Diego, CA) 10 ng of commercial DNA standards harboring 11 *PIK3CA* mutations (SensID, Germany) accountable for 90% of known actionable mutations were analyzed at variant allele frequencies (VAF) ranging from <1% to 12% on the MALDI-TOF-based MassARRAY® System (Agena Bioscience). In total, the ClearSEEK Panel covers 20 *PIK3CA* variants across exons 8, 10, 21 (previously: exons 7, 9, and 20; see table 1).

For verification, on average 15 ng DNA from 48 archived formalin-fixed, paraffin-embedded (FFPE) specimens from patients with metastatic Breast Cancer were analyzed with the ClearSEEK Panel. Allele calls were generated by the automated software report within the MassARRAY workflow.

The *PIK3CA* mutation profiles had previously been assessed by the Tumor Hotspot MASTR Plus assay (Multiplicom, Belgium) on the MiSeq NGS system (Illumina, San Diego, CA).

Retrospectively, all samples were also analyzed with the iPLEX® Pro Exome QC Panel (Agena Bioscience) for assessing the number of amplifiable DNA copies and the level of template fragmentation.

NT change	AA change	NT change	AA change
c.1258T>C	p.C420R	c.1636C>A	p.Q546K
c.1624G>A	p.E452K	c.1636C>G	p.Q546E
c.1624G>C	p.E542Q	c.1637A>C	p.Q546P
c.1633G>A	p.E545K	c.1637A>G	p.Q546R
c.1633G>C	p.E545Q	c.1637A>T	p.Q546L
c.1634A>C	p.E545A	c.3139C>T	p.H1047Y
c.1634A>G	p.E545G	c.3139C>A	p.H1047N
c.1634A>T	p.E545V	c.3140A>C	p.H1047P
c.1635G>C	p.E545D	c.3140A>G	p.H1047R
c.1635G>T	p.E545D	c.3140A>T	p.H1047L

Table 1 (above): List of the 20 clinically relevant *PIK3CA* mutations which are covered by the ClearSEEK PIK3CA Panel, a 3-well multiplex reaction assay on the MassARRAY System.

Sample #	PIK3CA mutation NGS	PIK3CA mutation MassARRAY	DNA input; Qubit (Exome QC) [ng]
BR1	Q546K	Q546K	
BR2	H1047R	H1047R	
BR3	E545K	E545K	
BR4			
BR5	Q546R	Q546R	
BR6	E542K	E542K	
BR7	E545K	E545K	
BR8		H1047R	15 (3.5)
BR9		H1047R	15 (3.0)
BR10			
BR11			
BR12			
BR13			
BR14			
BR15			
BR16			
BR17			
BR18			
BR19	E545A	E545A	
BR20	H1047L	H1047L	
BR21			
BR22			
BR23			
BR24			

Sample #	PIK3CA mutation NGS	PIK3CA mutation MassARRAY	DNA input; Qubit (Exome QC) [ng]
BR25			
BR26			
BR27			
BR28	E542K	E542K	
BR29	H1047R	H1047R	
BR30			
BR31			
BR32			
BR33			
BR34			
BR35	E545K	E545K	
BR36			
BR37			
BR38		Q546K	20 (<1.0)
BR39			
BR40			
BR41	E545G	E545G	
BR42			
BR43			
BR44			
BR45	Q546R	Q546R	
BR46	Q546K	Q546K	
BR47	H1047Y	H1047Y / E545Q	20 (15)
BR48	Q546P	Q546P	

Figures (below): Fragmention charts and copy number tables as created by the Exome QC report. A) BR8, B) BR9, and C) BR38: Samples show high level of degradation. D) BR47: Intact, non-fragmented DNA. E) BR47: Mass spectra for *PIK3CA* E545Q and H1047Y mutations (specific mass peaks indicated by blue arrows).

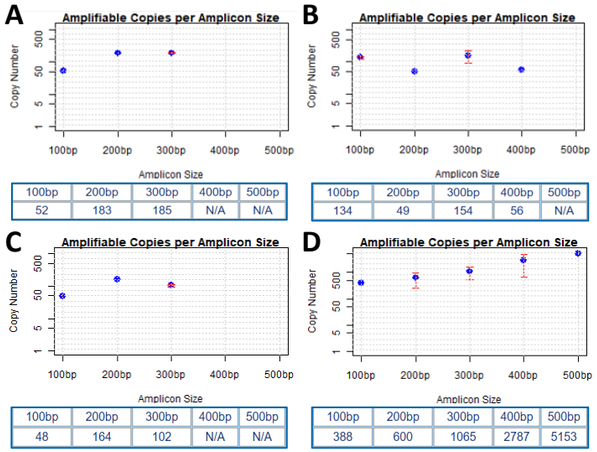
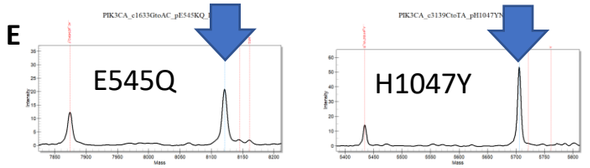


Table 2 (above): *PIK3CA* mutations detected across 48 FFPE derived DNA samples by two technologies, NGS (MASTR Plus assay on MiSeq) and the MassARRAY System (ClearSEEK PIK3CA Panel). Only overlapping assay content was analyzed. Empty cells indicate wild-type calls for these variants. Discrepant results are highlighted in red color. DNA input as assessed by Qubit and Exome QC are shown for discrepant samples only, for better visibility.



RESULTS

Sensitivity for the 11 *PIK3CA* mutations tested was 100% at 3% VAF. At 1.5% VAF the sensitivity dropped to 75% and at 0.8% VAF, 5 mutations were detected in >50% of the cases (46% sensitivity), namely the frequent variants E542K, E545K, H1047L, H1047R, and H1047Y. The specificity for the 11 tested mutations was 100%.

According to previous NGS analysis, 16 of the 48 FFPE samples were expected to carry panel-overlapping *PIK3CA* mutations which were all confirmed by the ClearSEEK Panel. Interestingly, in four samples (BR8, BR9, BR38, and BR47) additional mutations were detected by the ClearSEEK Panel on the MassARRAY System which were wild-type on NGS.

The overall concordance between MassARRAY and NGS was 92% (44/48). All results are summarized in table 2. From the discrepant samples, both, BR8 and BR9 presented a *PIK3CA* H1047R mutation with a VAF of <1% and >10%, respectively. Retrospective analysis of sample quality and integrity with the Exome QC Panel revealed a significantly lower abundance of amplifiable DNA molecules than previously determined by Qubit. Further, the DNA was highly degraded in both samples, resulting in DNA fragments not exceeding fragment sizes of 400 bp (figures A and B).

In sample BR38, a *PIK3CA* Q546K mutation was detected on the MassARRAY. Also here, the concentration of amplifiable DNA was extremely low and fragmented (figure C).

The low abundance of amplifiable DNA molecules and high level of degradation in BR8, BR9, and BR38 may explain why the mutations detected by MassARRAY were missed by NGS.

Lastly, in BR47 a secondary mutation, *PIK3CA* E545Q, was detected with the ClearSEEK Panel (figure E). This DNA sample was of good quantity and quality (figure D). In this case a retrospective analysis of the NGS raw data may be advisable.

CONCLUSIONS

Our results suggest that the analysis of *PIK3CA* mutations on the MassARRAY System using 10 ng DNA shows a good sensitivity with a limit of detection between 1% and 2%. Using a NGS validated set of human FFPE samples, data presented here suggest that the MALDI-TOF-based assay could detect mutations with minimal VAF as well as in samples with limited and degraded DNA, a frequent situation in breast cancer *PIK3CA* testing with archived or aged FFPE samples.