Detection of PIK3CA mutations in tumor tissue and cf-DNA plasma samples of patients with metastatic breast cancer using a novel highly sensitive commercially available CE-IVD kit

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ABSTRACT

Introduction: Oncolipsy PIK3CA CE-IVD kit is intended to be used for the detection of *PIK3CA* mutations based on the combination of allele-specific priming, asymmetric PCR, and melting curve analysis. The aim of the present study is to: a) evaluate the performance of the novel CE-IVD kit both in FFPEs and plasma-cfDNA samples from patients with metastatic breast cancer (MBC) and directly compared the derived results with two commercially available assays, the cobas® PIK3CA Mutation Test and a ddPCR PIK3CA mutation test (Bio-Rad).

Patients and methods: 30 primary tumors (FFPEs) and 25 plasma-cfDNA samples from patients with HR positive and HER2 negative MBC were analyzed. Genomic DNA was isolated from FFPEs using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany), while cfDNA was extracted from 2.00 mL of plasma using the QIAamp DSP cNA Kit (Qiagen, Hilden, Germany).

Results: Direct comparison between the Oncolipsy *PIK3CA* CE-IVD kit and the cobas® *PIK3CA* Mutation Test using the same gDNA isolated from FFPEs revealed a concordance of 20/29 (69%) for *PIK3CA* p.E542K, 24/30 (80%) for *PIK3CA* p.H1047R and 28/30 (93.3%) for *PIK3CA* p.E545K mutation. The corresponding concordance between the Oncolipsy *PIK3CA* CE-IVD kit and ddPCR was 19/29 (86.2%) for *PIK3CA* p.E542K, 26/30 (86.7%) for *PIK3CA* p.H1047R and 18/30 (60%) for *PIK3CA* p.E545K mutation (Table 1). Direct comparison between the Oncolipsy *PIK3CA* CE-IVD kit and ddPCR assays using the same gDNA isolated from plasma samples revealed a concordance of 13/15 (86.7%) for *PIK3CA* p.E542K, 21/25 (84%) for *PIK3CA* p.H1047R and 26/26 (100%) for *PIK3CA* p.E545K mutation (Table 2).

Conclusions: The commercially available Oncolipsy *PIK3CA* CE-IVD kit is highly sensitive and specific for the detection of four *PIK3CA* hotspot mutations in primary tumors and plasma-cfDNA.

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INTRODUCTION

- PI3K (Phosphoinositide 3-kinase) signaling is deregulated in a variety of cancers. The three main PIK3CA hotspot mutations, E545K and E542K in exon 9 and H1047R in exon 20, are detected approximately in 40% of hormone receptor positive (HR+) breast cancer (BC), mainly in the helical and kinase domains of the PIK3CA gene. Beyond BC, PIK3CA mutation detection is also very important in other types of cancer like lung cancer, colorectal cancer, anal squamous cell carcinoma and pancreatic cancer.
- In recent years, novel therapeutics targeting specific driver mutations in PIK3CA have revolutionized the treatment of BC. Alpelisib is the only PIK3 inhibitor that has been approved by both the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) for use in combination with fulvestrant in patients with HR-positive, HER2-negative, PIK3CA-mutated, advanced or MBC following progression on or after treatment with an endocrine-based regimen.
- Oncolipsy PIK3CA kit (Pharmassist Ltd, Greece) is based on the combination of allele-specific priming, asymmetric PCR, and melting curve analysis. The CE-IVD kit can detect the presence of four PIK3CA hotspot mutations (E542K, E545K, E545Q, H1047R).
- The aim of the present study is to: a) evaluate the performance of the novel CE-IVD kit both in FFPEs and plasma-cfDNA samples from patients with metastatic breast cancer (MBC) and directly compared the derived results with two commercially available assays, the cobas® PIK3CA Mutation Test and a ddPCR PIK3CA mutation test (Bio-Rad).

METHODS AND MATERIALS

30 primary tumors (FFPEs) and 25 plasma-cfDNA samples from patients with HR positive and HER2 negative MBC were analyzed. Genomic DNA was isolated from FFPEs using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany), while cfDNA was extracted from 2.00 mL of plasma using the QIAamp DSP cNA Kit (Qiagen, Hilden, Germany).

Figure 1. Oncolipsy *PIK3CA* kit (Pharmassist Ltd, Greece) and a typical graph of melting curve analysis interpretation results.

RESULTS

Tumor biopsy samples (Table 1)

E545K		Oncolipsy PIK3CA kit		
		+	-	Total
Cobas PIK3CA kit	+	4	2	8
	-	0	24	24
	Total	4	26	30
Concordance: 28/30, 93.3% (k= 0.762, p<0.001) substantial agreement				
		Oncolipsy PIK3CA kit		
		+	-	Total
ddPCR	+	4	12	16
	-	0	14	14
	Total	4	26	30
Concordance: 18/30, 60.0% (k= 0.237, p=0.044<0.05) fair agreement				

E542K		Oncolipsy PIK3CA kit		
		+	-	Total
Cobas PIK3CA kit	+	0	0	0
	-	9	20	29
	Total	9	20	29
Concordance: 20/29, 69% (k=0.000)*				
		Oncolipsy PIK3CA kit		
		+	-	Total
ddPCR	+	8	3	11
	-	1	17	18
	Total	9	20	29
Concordance: 19/29, 86.2% (k= 0.696, p<0.001) substantial agreement				

H1047R		Oncolipsy PIK3CA kit		
		+	-	Total
Cobas PIK3CA kit	+	8	1	9
	-	5	16	21
	Total	13	17	30
Concordance: 24/30, 80% (k=0.577, p < 0.001) moderate agreement				
		Oncolipsy PIK3CA kit		
		+	-	Total
ddPCR	+	12	3	15
	-	1	14	15
	Total	13	17	30
Concordance: 26/30, 86.7% (k=0.733, p<0.001) substantial agreement				

Liquid biopsy samples (Table 2)

E545K		Oncolipsy PIK3CA kit		
		+	-	Total
ddPCR	+	1	0	1
	-	0	25	25
	Total	1	25	26
Concordance: 26/26, 100% (k= 1.000, p< 0.001) perfect agreement				

E542K		Oncolipsy PIK3CA kit		
		+	-	Total
ddPCR	+	1	1	2
	-	1	12	13
	Total	2	13	15
Concordance: 13/15, 86.7% (k= 0.423, p=0.101) moderate agreement				

H1047R		Oncolipsy PIK3CA kit		
		+	-	Total
ddPCR	+	2	0	2
	-	4	19	23
	Total	6	19	25
Concordance: 21/25, 84% (k= 0.432, p=0.009<0.05) moderate agreement				

- In FFPEs samples, *PIK3CA* p.E545K mutation was detected in 4/30 (13.3%) samples, the p.E542K mutation in 9/29 (31%) samples, and the p.H1047R mutation in 13/30 (43.3%) samples. The p.E545Q mutation was not detected in any of the FFPE samples tested.
- Direct comparison between the Oncolipsy *PIK3CA* CE-IVD kit and the cobas® *PIK3CA* Mutation Test using the same gDNA isolated from FFPEs revealed a concordance of 20/29 (69%) for *PIK3CA* p.E542K, 24/30 (80%) for *PIK3CA* p.H1047R and 28/30 (93.3%) for *PIK3CA* p.E545K mutation. The corresponding concordance between the Oncolipsy *PIK3CA* CE-IVD kit and ddPCR was 19/29 (86.2%) for *PIK3CA* p.E542K, 26/30 (86.7%) for *PIK3CA* p.H1047R and 18/30 (60%) for *PIK3CA* p.E545K mutation (Table 1).
- Direct comparison between the Oncolipsy *PIK3CA* CE-IVD kit and ddPCR assays using the same gDNA isolated from plasma samples revealed a concordance of 13/15 (86.7%) for *PIK3CA* p.E542K, 21/25 (84%) for *PIK3CA* p.H1047R and 26/26 (100%) for *PIK3CA* p.E545K mutation (Table 2).

CONCLUSIONS

The commercially available Oncolipsy *PIK3CA* CE-IVD kit is highly sensitive and specific for the detection of four *PIK3CA* hotspot mutations in primary tumors and plasma-cfDNA clinical samples.

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