FEASIBILITY STUDY OF A ctEGFR PROTOTYPE ASSAY ON THE FULLY AUTOMATED IDYLLA™ PLATFORM

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INTRODUCTION

To predict the response to EGFR tyrosine kinase inhibitor (TKI) therapy in non-small cell lung cancer (NSCLC) patients, formalin-fixed paraffin-embedded (FFPE) tumor tissue is routinely tested for the presence of somatic mutations in the epidermal growth factor receptor (EGFR) gene. Sufficient tumor tissue is not always available and ctEGFR testing from plasma is an alternative approach for the detection of EGFR mutations.

METHODS

Idylla™ (Biocartis) is a fully integrated molecular detection platform that combines speed and ease of use with high sensitivity and high multiplexing capabilities. In terms of ctDNA testing, it overcomes the time-consuming step of ctDNA extraction from plasma. After the addition of proteinase K and 2 ml of human plasma into the cartridge, the complete process of ctDNA extraction, real-time PCR, data analysis and reporting is fully automated (Fig. 1). The ctEGFR prototype assay allows the detection of mutations including insertions and deletions in exons 18, 19, 20 and 21. A study was performed at BioPath Innovations SA (Athens, Greece) to compare the results obtained by the ctEGFR prototype assay with results obtained on the same plasma samples by NGS (Ion PGM platform with Ion AmpliSeq™ panel targeting EGFR exons 18, 19, 20 and 21). For each amplicon/exon, NGS target coverage was 2000 reads and the assay could detect allelic frequencies down to 2-5%. Discrepant samples were further tested with the cobas® EGFR Mutation Test v2.

RESULTS

Sixty-four NSCLC plasma samples were tested with both assays. One sample failed on NGS. Overall, 34 mutations were detected by NGS and confirmed by the ctEGFR prototype assay. In 33 samples, NGS detected no mutation. The ctEGFR prototype assay detected 8 additional mutations of which 7 mutations were detected in samples which were scored WT by NGS (Fig. 2). Retesting with the cobas EGFR Mutation Test v2 confirmed the presence of these mutations. Analytical sensitivity was assessed for 20 mutations using plasma spiked with synthetic targets. Analytical sensitivities ranging from 1 to 4% were obtained for the tested mutations (Fig. 3). Inclusivity was demonstrated for 49 mutations in total (Fig. 4). The average turnaround time of a run of the ctEGFR prototype assay was <2h 40 min and the hands-on time for loading the cartridge was <2 min.

CONCLUSION

This study shows that the Idylla™ platform enables the development of a prototype ctEGFR assay with high sensitivity and ease of use combined with a fast turnaround time for the testing of 49 relevant EGFR mutations in 2 ml of plasma.

DISCLOSURES
All authors are full-time employees of the indicated companies.

Figure 1: ctEGFR work flow

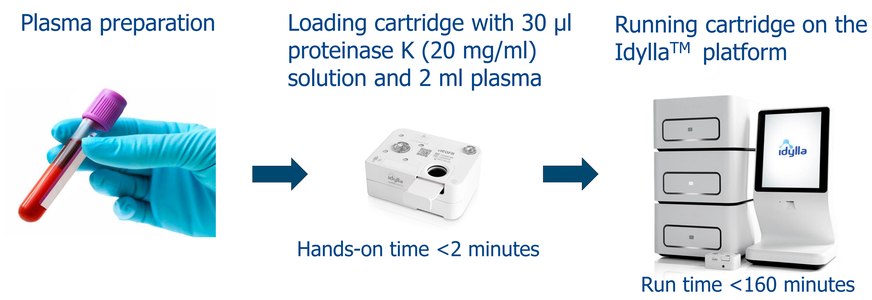

Figure 2: Concordance between EGFR genotyping of plasma with Idylla™ ctEGFR prototype assay and NGS

		NGS									
		L858R	Ex 19 del	T790M	Ex 20 insertion	S768I	L861Q	G719A/C/S	L858R/ T790M	Ex19 del/ T790M	No mutation
Idylla	L858R	**7**									**2***
	Ex 19 del		**12**								**4***
	T790M			**1**							
	Ex 20 insertion										**1***
	S768I					**1**					
	L861Q										
	G719A/C/S										
	L858R/T790M	**1***									
	Ex19 del/T790M									**6**	
	No mutation										**28**

** The presence of these mutations was confirmed by the cobas® EGFR Mutation Test v2*

Figure 3: LOD table of the Idylla™ ctEGFR prototype assay

	EGFR Mutation	LOD (% mutant in 10,000 WT cps/ml plasma)
Exon 18	LOD c.2156G>C (G719A)	3%
	LOD c.2155G>A (G719C)	1%
	LOD c.2155G>T (G719S)	2%
Exon 19	LOD c.2239_2248delinsC (del9)	2%
	LOD c.2239_2251delinsC (del12)	1%
	LOD c.2235_2249del (del15)	1.5%
	LOD c.2236_2250del (del15)	1.5%
	LOD c.2240_2257del (del18)	3%
	LOD c.2238_2258del (del21)	1%
	LOD c.2236-2256del (del21)	2%
	LOD c.2253-2276del (del24)	4%
Exon 20	LOD c.2369C>T (T790M)	1.5%
	LOD c.2303G>T (S768I)	1%
	LOD c.2307_2308insGCCAGCGTG (insASV(9))	2%
	LOD c.2309_2310insCCAGCGTGGAT (insASV(11))	2%
	LOD c.2311_2312insGCGTGG ACA (insSVD)	1%
	LOD c.2310_2311insGGT (insG)	1%
	LOD c.2319_2320insCAC (insH)	2%
Exon 21	LOD c.2573T>G (L858R)	1%
	LOD c.2582T>A (L861Q)	2%

Figure 4: EGFR mutations detected by Idylla™ ctEGFR prototype assay

EGFR exon	Mutation	AA mutation	NT mutation
Exon 18	G719A	p.Gly719Ala	c.2156G>C
	G719C	p.Gly719Cys	c.2155G>T
	G719C2	p.Gly719Cys(2)	c.2154_2155delinsTT
	G719S	p.Gly719Ser	c.2155G>A
Exon 19	Deletion 9	p.Leu747_Ala750delinsPro	c.2238_2248delinsGC
			c.2239_2248delinsC
		p.Leu747_Ala750delinsSer	c.2240_2248del
		p.Leu747_Glu749del	c.2239_2247del
	Deletion 12	p.Leu747_Thr751delinsPro	c.2239_2251delinsC
		p.Leu747_Thr751delinsSer	c.2240_2251del
	Deletion 15	p.Glu746_Ala750del	c.2235_2249del
			c.2236_2250del
		p.Leu747_Thr751del	c.2239_2253del
			c.2240_2254del
			c.2238_2252del
		p.Glu746_Thr751delinsAla	c.2237_2251del
		p.Glu746_Thr751delinsIle	c.2235_2252delinsAAT
		p.Glu746_Thr751delinsVal	c.2237_2252delinsT
		p.Lys745_Ala750delinsThr	c.2234_2248del
		p.Glu746_Thr751delinsLeu	c.2236_2253delinsCTA
		p.Glu746_Thr751delinsVal	c.2237_2253delinsTA
		p.Glu746_Thr751delinsAla	c.2235_2251delinsAG
		p.Glu746_Thr751delinsGln	c.2236_2253delinsCAA
		p.Ile744_Ala750delinsValLys	c.2230_2249delinsGTCAA
	Deletion 18	p.Leu747_Pro753delinsSer	c.2240_2257del
		p.Glu746_Ser752delinsVal	c.2237_2255delinsT
		p.Leu747_Ser752del	c.2239_2256del
		p.Glu746_Thr751del	c.2236_2253del
		p.Leu747_Pro753delinsGln	c.2239_2258delinsCA
		p.Glu746_Ser752delinsAla	c.2237_2254del
		p.Glu746_Ser752delinsAsp	c.2238_2255del
		p.Glu746_Ser752delinsIle	c.2236_2256delinsATC
		p.Glu746_Ser752delinsVal	c.2237_2256delinsTT
			c.2237_2256delinsTC
			c.2235_2255delinsGGT
	Deletion 21	p.Leu747_Pro753del	c.2238_2258del
		p.Glu746_Ser752del	c.2236_2256del
	Deletion 24	p.Ser752_Ile759del	c.2253_2276del
Exon 20	T790M	p.Thr790Met	c.2369C>T
	S768I	p.Ser768Ile	c.2303G>T
	InsG	p.Asp770_Asn771insGly	c.2310_2311insGGT
	InsASV(9)	p.Val769_Asp770insAlaSerVal	c.2307_2308insGCCAGCGTG
	InsASV(11)	p.Val769_Asp770insAlaSerVal	c.2309_2310delinsCCAGCGTGGAT
	InsSVD	p.Asp770_Asn771insSerValAsp	c.2311_2312insGCGTGGACA
	InsH	p.His773_Val774insHis	c.2319_2320insCAC
Exon 21	L858R	p.Leu858Arg	c.2573T>G
			c.2573_2574delinsGT
			c.2573_2574delinsGA
	L861Q	p.Leu861Gln	c.2582T>A

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