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Biocartis Idylla proves Fast and Accurate in Detecting Oncogenic KRAS and EGFR Mutations in Paraffin Embedded Tumor Samples

David Graf Brohawn[1], Xiaofeng Mu[2,4], Zhuang Tong[3], Elizabeth Rao[5], Ye Wang[4] and Xinmin Li[1*]

[1]UCLA Technology Center for Genomics & Bioinformatics, Department of Pathology & Laboratory Medicine, 650 Charles E Young Dr., Los Angeles, CA 90095, USA

[2]Academy of Medical Engineering and Translational Medicine, Tianjin University, China

[3]Cancer Hospital of China Medical University, Liaoning Cancer Hospital and Institute, No.44 Xiaoheyan Road, Dadong District, Shenyang 110042, Liaoning Province, China

[4]Clinical Laboratory, Qingdao Central Hospital, The Second Affiliated Hospital of Medical College of Qingdao University, China

[5]John Hopkins University, 333 Cassell Dr # 3000, Baltimore, MD 21224

[*]Corresponding author: Xinmin Li, UCLA Technology Center for Genomics & Bioinformatics, 650 Charles E Young Dr., Los Angeles, CA 90095, USA, Tel: 310-825-3664; E-mail: xinminli@mednet.ucla.edu

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Abstract

Many modern therapies target oncogenic mutations identified in a patient's biopsied tumor cells. The process of identifying these mutations can be costly, labor-intensive, and requires significant technical expertise. Biocartis' Idylla system, a real time PCR-based machine, streamlines oncogenic DNA mutation testing, directly from formalin-fixed paraffin embedded tissue with only 2 minutes hands-on time The instrument reports the presence or absence of clinically-relevant oncogenic mutations in KRAS, EGFR, BRAF, and/or NRAS in ≤ 2.5 hours per sample.

In this study, we tested how quickly and accurately the Biocartis Idylla detect oncogenic KRAS and EGFR mutations previously identified in 46 formalin-fixed paraffin embedded human patient biopsy samples. The Idylla successfully detected all of the oncogenic mutations with 100% accuracy.

Based on these results, the Biocartis Idylla holds great clinical utility. It is an ideal tool in any clinical testing environment to provide a cost effective, quick and accurate test for identifying frequently observed oncogenic mutations in KRAS and EGFR.

Keywords: Oncogenic; KRAS; EGFR; Mutations

Introduction

The International Agency for Research on Cancer (IARC) reported 14.1 million new cancer cases and 8.2 million cancer-related deaths worldwide in 2012 [1]. The IARC projects 3.9 million new cancer cases and 1.9 million cancer-related deaths in Europe alone in 2018 [2]. Lung cancer is the second most common malignant tumor, and the most lethal type of cancer [3]. Colorectal cancer is the third most common type of cancer globally, and among the most lethal [4]. These cancers represent enormous challenges to modern healthcare, and warrant continual development of technologies that offer better detection, diagnosis, and therapeutic options.

Oncogenic DNA mutations have been identified in hundreds of human genes, reflecting significant heterogeneity in the genetic underpinnings of different cancers. A large proportion of these genes encodes proteins responsible for regulating cell growth, differentiation, and/or apoptosis. Among those are KRAS (Kirsten Rat Sarcoma Viral Oncogene Homolog) and EGFR (Epidermal Growth Factor Receptor), two of the most frequently mutated genes in lung and colorectal cancer. Between 15-25% of patients with non-small-cell lung cancer (NSCLC) harbor KRAS mutations [5], and approximately 15-40% of NSCLC tumors harbor EGFR mutations [3]. Several very effective tyrosine kinase inhibitors (e.g., Erlotinib and Afatinb) specifically target EGFR mutations in the NSCLC patients, and two anti-EGFR monoclonal antibodies can prolong survival for colorectal cancer patients when their tumor cells carry no KRAS mutations [6]. These findings underscore our need for technologies that can rapidly identify oncogenic DNA mutations in EGFR and KRAS in patient biopsy or formalin-fixed paraffin embedded (FFPE) samples, enabling informed decisions about what personalized therapy has the best chance of halting each patient's tumor progression.

From a technical perspective, multiple steps are carried out to identify oncogenic DNA mutations in a patient's cancer cells. These steps vary based on whether the biopsy specimen is frozen or a formalin-fixed paraffin embedded (FFPE) sample, and the number and type of mutations tested. Most clinical specimens are converted into FFPE samples, as these are amenable to sectioning, histological staining, pathological examination, and long-term storage at room temperature. Typically, pathologists demarcate what cells in the heterogeneous tumor are likely cancerous based on morphological features, and order downstream PCR- or NGS-based tests to detect oncogenic DNA mutations in those cells.

The wet and dry lab steps necessary to identify oncogenic DNA mutations in cancer cells within a FFPE sample generally involve 1) isolating the demarcated cancer cells fixed in paraffin from the slide, 2) removing paraffin wax, 3) applying a protease treatment, 4) isolating and purifying DNA, 5) quantifying the extracted DNA, and 6) performing DNA mutation testing using a PCR- or NGS-based workflow and downstream bioinformatics analyses. These steps can be costly and time consuming, especially if the final step involves next generation sequencing (NGS) based methods. Further, proper execution of this whole process requires technicians with considerable wet lab and/or bioinformatics skills.

Biocartis developed the Idylla, a real time PCR-based machine, to streamline oncogenic DNA mutation testing in FFPE (and other biological) samples. The instrument accepts single-gene sample cartridges that test the presence or absence of clinically-relevant oncogenic mutations in KRAS, EGFR, BRAF, and/or NRAS in ≤ 2.5 hours.

In a recent study comparing the Biocartis Idylla against competitors' platforms for KRAS mutation testing, the Biocartis Idylla ranked best for ease of use, fewest number of handling steps from molecular

analysis to report, and least amount of required technical expertise for operation [7]. Given these attractive features, we tested how quickly and accurately the Biocartis Idylla detected oncogenic KRAS and EGFR mutations that were previously identified in 46 FFPE human patient biopsy samples.

Materials and Methods

We obtained 46 FFPE human biopsy samples. These included 7 colorectal and 24 lung tumors from the tissue bank of UCLA Translational Pathology Core Laboratory, and 3 colorectal and 12 lung tumors from Qingdao Central Hospital with varying tumor purities for testing. Each sample was known to carry either an EGFR or KRAS mutation, as previously identified using PCR-based technology during their clinical testing (Table 1).

Sample	Gene	Nucleotide change	Amino acid change
Colon 1	KRAS	c.38G>A	p.G13D
Colon 5	KRAS	c.182A>G	p.Q61R
Colon 6	KRAS	c.38G>A	p.G13D
Colon 7	KRAS	c.38G>A	p.G13D
Colon 8	KRAS	c.35G>T	p.G12V
Colon 9	KRAS	c.38G>A	p.G13D
Colon 10	KRAS	c.34G>T	p.G12C
Lung 1	EGFR	c.2303_2311dupGCGTGGACA	p.S768_D770dup
Lung 2	KRAS	c.35G>T	p.G12V
Lung 3	EGFR	c.2573T>G	p.L858R
Lung 4	EGFR	c.2235_2249del	p.E746_A750del
Lung 5	KRAS	c.35G>T	p.G12V
Lung 7	KRAS	c.35G>A	p.G12D
Lung 8	KRAS	c.34G>T	p.G12C
Lung 9	EGFR	c.2237_2255delinsT	p.E746_S752delinsV
Lung 10	EGFR	c.2156G>C, c.2303G>T	p.G719A, p.S768I
Lung 13	EGFR	c.2236_2250del, c.2369C>T	p.E746_A750del, p.T790M
Lung 14	EGFR	c.2236_2250del	p.E746_A750del
Lung 17	KRAS	c.35G>T	p.G12V
Lung 19	KRAS	c.35G>T	p.G12V
Lung 20	EGFR	c.2237_2255delinsT	p.E746_S752delinsV
Lung 22	KRAS	c.34G>T	p.G12C
Lung 24	KRAS	c.35G>T	p.G12V
Lung 25	KRAS	c.35G>T	p.G12V
Lung 27	KRAS	c.34G>T	p.G12C
Lung 28	KRAS	c.35G>A	p.G12D
Lung 32	KRAS	c.183A>C	p.Q61H
Lung 35	EGFR	c.2573T>G	p.L858R
Lung 38	EGFR	c.2235_2249del	p.E746_A750del

Lung 39	KRAS	c.34G>T	p.G12C
Lung_S1	EGFR	c.2573T>G	p.L858R
Lung_S2	EGFR	Exon 19 Deletion	Exon 19 Deletion
Lung_S3	EGFR	c.2573T>G	p.L858R
Lung_S4	EGFR	c.2573T>G	p.L858R
Lung_S5	EGFR	Exon 19 Deletion	Exon 19 Deletion
Lung_S6	EGFR	Exon 19 Deletion	Exon 19 Deletion
Lung_S7	EGFR	c.2573T>G	p.L858R
Lung_S8	EGFR	Exon 19 Deletion	Exon 19 Deletion
Lung_S9	EGFR	Exon 19 Deletion	Exon 19 Deletion
Lung_S10	EGFR	c.2573T>G	p.L858R
Lung_S11	EGFR	c.2573T>G	p.L858R
Lung_S12	EGFR	c.2573T>G	p.L858R
Lung_S13	EGFR	Exon 19 Deletion	Exon 19 Deletion
Colon_S14	KRAS	c.35G>A	p.G12D
Colon_S15	KRAS	c.38G>A	p.G13D
Colon_S16	KRAS	c.35G>A	p.G12D

Table 1: Summary of the Tested Samples and Mutations.

Following Biocartis' manufacturer protocol, we input ≥50mm² of FFPE tissues from a 5 μM glass slide containing >10% tumor cells onto filter paper soaked with nuclease free water, placed it onto the "lysis pad" in a KRAS or EGFR test cartridge, and inserted the closed cartridge into the Biocartis Idylla. Sample and assay information were entered into the Idylla software interface. The remaining steps (from paraffin removal through mutation detection) were automated within the machine. We reviewed each sample's result in the Biocartis Explore web application (https://idyllaexplore.biocartis.com/) after they were automatically uploaded upon completion.

Results

The Biocartis Idylla successfully detected mutations at the same genomic coordinates as what was previously reported for all 46 samples. Figure 1 shows the real-time PCR traces recorded for sample "Lung 2" that underwent KRAS testing. Table 2 shows the expected mutation vs. the detected mutation for all samples.

We defined false positives as any KRAS or EGFR mutation detected in a sample's DNA by the Biocartis Idylla that was not previously detected using next generation sequencing based technologies. We defined false negatives as any KRAS or EGFR mutation detected in a sample's DNA by next generation sequencing based technologies that were not detected by the Biocartis Idylla. We did not have any false positives or false negatives.

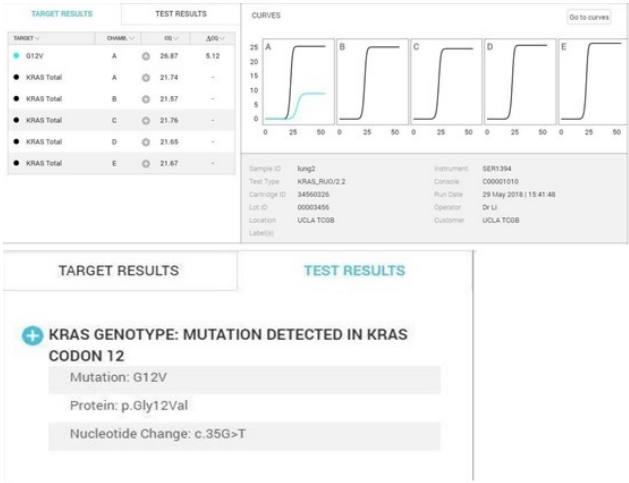

Figure 1: An example of KRAS G12V mutation reported by Idylla software. The top pane shows Lung 2's real-time PCR traces across the Biocartis Idylla's 5 chambers (named A-E). Each chamber tests a portion of the 21 total KRAS mutations. This assay revealed Lung 2 was positive for the KRAS c. 35G>T mutation (p.G12V) as summarized in the bottom pane.

Sample	Gene	Expected mutation	Detected mutation	Reported amino acid change
Colon 1	KRAS	c.38G>A	c.38G>A	p.G13D
Colon 5	KRAS	c.182A>G	c.182A>G/c.182A>T	p.Q61R/p.Q61L
Colon 6	KRAS	c.38G>A	c.38G>A	p.G13D
Colon 7	KRAS	c.38G>A	c.38G>A	p.G13D
Colon 8	KRAS	c.35G>T	c.35G>T	p.G12V
Colon 9	KRAS	c.38G>A	c.38G>A	p.G13D
Colon 10	KRAS	c.34G>T	c.34G>T	p.G12C
Lung 1	EGFR	c.2303_2311dupGCGTGGACA	Exon 20 Insertion	N/A
Lung 2	KRAS	c.35G>T	c.35G>T	p.G12V
Lung 3	EGFR	c.2573T>G	c.2573T>G, c.2573_2574delinsGT, c. 2573_2574delinsGA	p.L858R
Lung 4	EGFR	c.2235_2249del	Exon 19 deletion	N/A
Lung 5	KRAS	c.35G>T	c.35G>T	p.G12V
Lung 7	KRAS	c.35G>A	c.35G>A	p.G12D
Lung 8	KRAS	c.34G>T	c.34G>T	p.G12C
Lung 9	EGFR	c.2237_2255delinsT	Exon 19 deletion	N/A
Lung 10	EGFR	c.2156G>C, c.2303G>T	c.2156 G>C/c.2155 G>T ; c. 2154_2155delinsTT/c.2155 G>A c.2303 G>T	Mutation 1: p.G719A/ p.G719C/p.G719S Mutation 2:p.S768I
Lung 13	EGFR	c.2236_2250del, c.2369C>T	Exon 19 deletion c2369 C>T	Mutation 1: N/A Mutation 2: p.T790M
Lung 14	EGFR	c.2236_2250del	Exon 19 Deletion	N/A
Lung 17	KRAS	c.35G>T	c.35G>T	p.G12V
Lung 19	KRAS	c.35G>T	c.35G>T	p.G12V
Lung 20	EGFR	c.2237_2255delinsT	Exon 19 deletion	N/A
Lung 22	KRAS	c.34G>T	c.34G>T	p.G12C
Lung 24	KRAS	c.35G>T	c.35G>T	p.G12V
Lung 25	KRAS	c.35G>T	c.35G>T	p.G12V
Lung 27	KRAS	c.34G>T	c.34G>T	p.G12C
Lung 28	KRAS	c.35G>A	c.35G>A	p.G12D
Lung 32	KRAS	c.183A>C	c.183A>C/c.183A>T	p.Q61H
Lung 35	EGFR	c.2573T>G	c.2573T>G, c.2573_2574delinsGT, c. 2573_2574delinsGA	p.L858R
Lung 38	EGFR	c.2235_2249del	Exon 19 Deletion	N/A
Lung 39	KRAS	c.34G>T	c.34G>T	G12C
Lung_S1	EGFR	c.2573T>G	c.2573T>G,c.2573_2574delinsGT, c. 2573_2574delinsGA	p.L858R

Lung_S2	EGFR	Exon 19 Deletion	Exon 19 Deletion	N/A
Lung_S3	EGFR	c.2573T>G	c.2573T>G,c.2573_2574delinsGT, c.2573_2574delinsGA	p.L858R
Lung_S4	EGFR	c.2573T>G	c.2573T>G,c.2573_2574delinsGT, c.2573_2574delinsGA	p.L858R
Lung_S5	EGFR	Exon 19 Deletion	Exon 19 Deletion	N/A
Lung_S6	EGFR	Exon 19 Deletion	Exon 19 Deletion	N/A
Lung_S7	EGFR	c.2573T>G	c.2573T>G,c.2573_2574delinsGT, c.2573_2574delinsGA	p.L858R
Lung_S8	EGFR	Exon 19 Deletion	Exon 19 Deletion	N/A
Lung_S9	EGFR	Exon 19 Deletion	Exon 19 Deletion	N/A
Lung_S10	EGFR	c.2573T>G	c.2573T>G,c.2573_2574delinsGT, c.2573_2574delinsGA	p.L858R
Lung_S11	EGFR	c.2573T>G	c.2573T>G,c.2573_2574delinsGT, c.2573_2574delinsGA	p.L858R
Lung_S12	EGFR	c.2573T>G	c.2573T>G,c.2573_2574delinsGT, c.2573_2574delinsGA	p.L858R
Lung_S13	EGFR	Exon 19 Deletion	Exon 19 Deletion	N/A
Colon_S14	KRAS	c.35G>A	c.35G>A	p.G12D
Colon_S15	KRAS	c.38G>A	c.38G>A	p.G13D
Colon_S16	KRAS	c.35G>A	c.35G>A	p.G12D

Table 2: Summary of expected and detected mutations by Idylla.

Biocartis idylla workflow	**Average time requirement**	**Next generation sequencing based workflow**	**Average time requirement**
Scratching tumor cells from sample FFPE slide	5 minutes	Scratching tumor cells from sample FFPE slide	5 minutes
Preparing sample cartridge and initiating assay	5 minutes	Paraffin removal and proteinase K digestion	1.5 hours
Paraffin removal and proteinase K digestion	≤2.5 hours	DNA isolation	20 minutes
DNA isolation		DNA quantification	10 minutes
qPCR		Illumina Tru Seq custom Amplicon next generation sequencing workflow	7 hours
Data analysis		MiSeq 2x150 run (Recommended for Illumina TruSeq Custom Amplicon libraries)	24 hours
		Demultiplexing, Alignment, Variant calling	2 Hours
Total time per sample	≤2 hours 40 minutes		35 hours 5 minutes

Table 3: Comparison of required time for each step between Idylla workflow and NGS workflow.

In addition to 100% accuracy, the Biocartis Idylla KRAS or EGFR workflow requires <3 hours total time for each sample with only 10 minutes hands on time. This is significantly faster and has fewer steps relative to using a targeted NGS-based workflow to identify EGFR and KRAS mutations within FFPE samples, as summarized in Table 3.

Due to the assay's qPCR primer design, the machine listed several possibilities for the exact nucleotide and/or corresponding amino acid change at mutation sites in 12 of our samples with point mutations. Further, for all samples with an EGFR indel, it reported the indel without listing the indel's nucleotides.

Discussion

Many modern personalized therapies target specific oncogenic DNA mutations identified in a patient's tumor cells. The importance of rapid and accurate oncogenic DNA mutation detection in a patient's tumor cells is paramount to this process. In this study, we tested how quickly and accurately the Biocartis Idylla could detect common oncogenic KRAS and EGFR mutations in 46 FFPE human biopsy samples. We found 100% concordance between PCR-based Idylla results and clinically tested results.

For a single patient's FFPE sample, the Biocartis Idylla was more than 13 times faster in identifying clinically actionable EGFR or KRAS mutations compared to using a standard targeted NGS-based workflow. This finding mirrored a recent report stating the Biocartis Idylla ranked best for ease of use, fewest number of handling steps from molecular analysis to report, and least amount of required technical expertise for operation compared to competing KRAS mutation detection workflows [7]. The Biocartis Idylla successfully detected mutations at the same genomic coordinates as what was previously reported for all of our 46 samples. While the Idylla listed several possibilities for the exact nucleotide change underlying non-synonymous mutations and indels in a subset of our samples, we are unaware of any clinical scenario where knowing the exact nucleotide change would aid or alter treatment strategy.

Conclusion

The Biocartis Idylla is a valuable instrument for rapid clinical testing of common oncogenic mutations in KRAS, EGFR, BRAF, and/or NRAS. Its main strengths include 1) ease of use and minimal technical skill requirements for operation, 2) low sample input requirements, 3) automation of FFPE sample DNA isolation through PCR-based testing, 4) quick turnaround time, and 5) an intuitive web based software program for viewing DNA mutation results, requiring minimal "dry lab" skills. Given it's favorable features compared to other oncogenic DNA mutation detection technologies [7] and 100% accuracy in detecting mutations in this study, the Biocartis Idylla holds great clinical utility. It is an ideal tool in any clinical testing environment to provide a cost effective, quick and accurate test for identifying frequently observed oncogenic mutations in KRAS and EGFR.

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