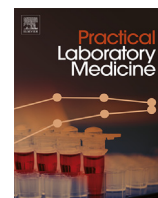


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Rapid *EGFR* mutation testing in lung cancer tissue samples using a fully automated system and single-use cartridge

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A B S T R A C T

Introduction: Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor (*EGFR*) gene in non-small cell lung cancer (NSCLC) patients predicts response to *EGFR* tyrosine kinase inhibitors (TKIs). The Idylla™ system (Biocartis, Mechelen, Belgium) is a fully integrated, cartridge-based platform that provides automated sample processing and real-time PCR-based mutation detection in a single-use cartridge. This study evaluated the Idylla™ *EGFR* Mutation Assay cartridges against next-generation sequencing (NGS) using formalin fixed, paraffin embedded (FFPE) lung cancer tissue samples.

Methods: Thirty-four FFPE lung adenocarcinoma tissue samples were tested on the Idylla™ system. 21 had at least one mutation in *EGFR* and 13 had no *EGFR* mutation as determined by NGS analysis using the Ion AmpliSeq 50-gene Cancer Hotspot Panel v2 (Thermo Fisher Scientific). One 10 μm FFPE tissue section was used for each Idylla™ test and all cases met the Idylla™ minimum tumor content requirement (≥10%).

Results: Idylla™ results were in complete agreement with those obtained by NGS for *EGFR* mutations targeted by the Idylla™. NGS identified two additional *EGFR* mutations that are not targeted by the Idylla™ in two samples (E709V and V774M). No *EGFR* mutations were detected by the Idylla™ in samples determined by NGS as having wild-type *EGFR*.

Conclusion: The fully automated Idylla™ system offers rapid and reliable testing for clinically actionable mutations in *EGFR* directly from FFPE tissue sections. Its simplicity and ease of use compared to other available molecular techniques make it suitable for routine clinical use in a variety of settings.

1. Introduction

Lung cancer remains the leading cause of cancer deaths [1]. The management of lung cancer has been aided by the better understanding of molecular mechanisms involved in its initiation and progression and increased usage of molecular testing. Mutations in the tyrosine kinase domain of the epidermal growth factor receptor (*EGFR*) gene are observed in approximately 15% of non-small cell lung cancer (NSCLC) patients in the US [2]. In advanced NSCLC, the presence of an activating *EGFR* mutation confers a more favorable prognosis and strongly predicts response to *EGFR* tyrosine kinase inhibitors (TKIs) while other *EGFR* mutations confer resistance to these therapies. The recent guidelines from the College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC) and the Association for Molecular Pathology (AMP) recommend universal testing of lung cancer patients with advanced-stage cancers with an adenocarcinoma component, using molecular diagnosis for activating “hot-spot” mutations in *EGFR* exons 18 to 21 with at least 1% prevalence (ie, codons 709 and 719, exon 19 deletion 768, and exon 20 insertions 790, 858, and 861) [3]. In some lung cancer patients with advanced disease, *EGFR* testing should be available as soon as possible to allow fast management decisions and

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determine the need for a first-line therapy with EGFR TKIs in patients with *EGFR*-mutated NSCLC.

The Idylla™ system (Biocartis, Mechelen, Belgium) is a fully integrated, cartridge-based platform that provides automated sample processing (deparaffinization, tissue digestion and DNA extraction) and real-time PCR-based mutation detection with all reagents included in a single-use cartridge. The Idylla™ EGFR Mutation Assay uses a real-time PCR chemistry based on the PlexPrime™ and PlexZyme™ (also known as MNazyme) technology that allows multiplex mutation detection with high sensitivity and specificity [4]. With this technology, each primer is designed to have a 5' target-recognition region, a short 3' target-specific sequence complementary to the mutation of interest, and a distinct insert sequence that is mismatched to the target. This results in production of allele-specific amplicons that are detected in real time by allele-specific PlexZyme™ enzymes and a universal fluorescent probe, allowing for detection of a broad range of mutations in a single reaction.

This retrospective study aimed at evaluating the Idylla™ EGFR Mutation Assay cartridges (Research Use Only) against next-generation sequencing (NGS) using lung cancer tissue samples.

2. Methods

2.1. Study samples

The study included thirty-four archived formalin-fixed paraffin-embedded (FFPE) resected or biopsy tissue samples obtained from patients with adenocarcinoma of the lung at the Dartmouth-Hitchcock Medical Center. NGS analysis was previously performed on these samples using the Ion AmpliSeq 50-gene Cancer Hotspot Panel v2 (Thermo Fisher Scientific). For each case, one 10 µm unstained FFPE tissue section was obtained as a roll and all cases met the Idylla™ minimum tumor content requirement of 10%. The study was approved by the Committee for the Protection of Human Subjects of the Dartmouth-Hitchcock Medical Center and Dartmouth College and the results obtained were not used for diagnostic purposes of any kind.

2.2. Idylla™ testing

The Idylla™ EGFR Mutation Assay (RUO) is intended for detection of mutations in the *EGFR* oncogene including exon 18 (G719 A/S/C), exon 21 (L858R, L861Q), exon 20 (T790M, S768I) mutations, exon 19 deletions and exon 20 insertions (Table 1).

Sample preparation was performed by placing a single 10 µm FFPE tissue section between two small filter papers (10 mm, Whatman filter papers, GE Healthcare) wetted with nuclease-free water and the resulting "sandwich" was inserted into the desired cartridge, which was loaded on the instrument for a total run time of approximately two and a half hours.

Idylla™ cartridges contain all necessary reagents to perform sample preparation and real-time PCR amplification and detection. Briefly, the FFPE tissue section is subjected to a combination of reagents, enzymes, heat, and high intensity focused ultrasound (HIFU) that collectively result in deparaffinization, disruption of tissue, and lysis of cells within the closed cartridge. Nucleic acids are subsequently subjected to PCR amplification and fluorescent detection of cartridge-specific target sequences. Results of a detected mutation include details regarding the protein and nucleotide change.

2.3. Next-generation sequencing (NGS)

Targeted NGS testing was previously performed on study specimens using the Ion AmpliSeq 50-gene Cancer Hotspot Panel v2 (Thermo Fisher Scientific) as previously described [5]. Based on diagnostic confirmation of H&E staining by an anatomic pathologist, consecutive slides of the samples were enriched by manual macrodissection.

Table 1
Mutations detectable by the Idylla™ *EGFR* Mutation Assay.

Exon	Mutation
Exon 18	G719A (c.2156G > C) G719C (c.2155G > T; c.2154_2155delisTT)G719S (c. c.2155G > A)
Exon 19	Del 9 (c.2238_2248delinsGC; c.2239_2248delinsC; c.2240_2248del; c.2239_2247del Del 12 (c.2239_2251delinsC; c.2240_2251del) Del 15 (c.2235_2249del; c.2236_2250del; c.2239_2253del; c.2240_2254del; c.2238_2252del; c.2237_2251del; c.2235_2252delinsAAT; c.2237_2252delinsT; c.2234_2248del; c.2236_2253delinsCTA; c.2237_2253delinsTA; c.2235_2251delinsAG; c.2236_2253delinsCAA;c.2230_2249delinsGTCAA) Del 18 (c.2240_2257del; c.2237_2255delinsT; c.2239_2256del; c.2236_2253del; c.2239_2258delinsCA; c.2237_2254del; c.2238_2255del; c.2237_2257delinsTCT; c.2236_2255delinsAT; c.2236_2256delinsATC; c.2237_2256delinsTT; c.2237_2256delinsTC;c.2235_2255delinsGGT) Del 21 (c.2238_2258del; c.2236_2256del) Del 24 (c.2253_2276del)
Exon 20	T790M (c.2369C > T) S768I (c.2303G > T)insG (c.2310_2311insGGT) insASV9 (c.2307_2308insGCCAGCGTG) insASV11 (c.2309_2310delinsCCAGCGTGGAT) insSVD (c.2311_2312insGCGTGGACA) insH (c.2319_2320insCAC)
Exon 21	L858R (c.2573T > G; c.2573_2574delinsGT; c.2573_2574delinsGA) L861Q (c.2582T > A)

3. Results

NGS testing results showed that 21 samples had at least one mutation in *EGFR* and the remaining 13 had no *EGFR* mutation. The Idylla *EGFR* Mutation Assay detected all 23 of the *EGFR* activating mutations identified by NGS (Table 2). These included L858R (8 samples), G719A (2 samples), G719C (2 samples), S768I (3 samples), and exon 19 deletions (8 samples). NGS identified two additional *EGFR* mutations that are not targeted by the Idylla™ in two samples (E709V and V774M). No *EGFR* mutations were detected by the Idylla™ in samples determined by NGS as having wild-type *EGFR*. The Idylla™ system produced results rapidly with a turnaround time of approximately 2.5 h.

4. Discussion

In this retrospective study, we evaluated the performance of the Idylla™ system against targeted NGS using lung cancer tissue specimens. Our results showed that Idylla™ system is a reliable and cost effective platform (approximately 3 min of labor) for rapid testing of clinically actionable mutations in *EGFR* directly from FFPE tissue specimens. Idylla™ results were in complete concordance with previous NGS testing.

The Idylla™ assay allows for a minimal sample requirement of one 10 µm FFPE tissue section and a minimum tumor content of 10%. Additionally, unlike most molecular methods currently available for somatic mutation testing, Idylla™ does not require separate sample pre-processing steps such as deparaffinization, FFPE tissue digestion, or DNA extraction. Moreover, the closed cartridge system minimizes the potential for contamination. The simplicity of the Idylla™ testing makes it especially appropriate for centers that lack strong molecular expertise or infrastructure.

Previous studies evaluating the Idylla™ *EGFR* Mutation Assay in lung adenocarcinoma showed excellent concordance with other available molecular techniques including PCR, digital PCR, pyrosequencing, and NGS [6–10]. Samples from these studies included surgical and cytology samples as well as lung biopsies and extracted DNA preparations. In a study comparing Idylla™ against NGS, 20 of 25 samples where assessment by NGS was unfeasible gave valid results on the Idylla™ system [11]. Similarly, we tested 6 samples previously determined to have insufficient DNA quantity for NGS analysis (QNS samples) on the Idylla™ system. For five of these samples, we added extracted DNA directly into the cartridge and were able to generate valid results on the Idylla™ system, indicating enhanced robustness of this assay compared to NGS (data not shown).

NGS has revolutionized the field of cancer diagnostics due to its capability to detect increased numbers of variants in numerous genes and its reliability. Yet, NGS requirements for infrastructure and significant technical and bioinformatics skills as well as the high cost inhibit its routine use in diagnostic centers. In addition, NGS turnaround time typically ranges from 5-14 days, which can hinder the delivery of effective first line treatments to cancer patients. The Idylla™ system can complement NGS by providing rapid and cost effective mutation analysis that allows initiation of a specific treatment while awaiting more comprehensive genome profiling by NGS. This is probably more crucial in lung cancer patients with rapid clinical deterioration for whom *EGFR* testing results should be available as soon as possible to determine eligibility to receive treatment with *EGFR* antagonists.

Compared with NGS, one limitation of the Idylla™ *EGFR* assay is that it is only intended to detect certain actionable mutations and therefore uncommon mutations, many of which have undetermined clinical significance, will be missed. Additionally, the system does

Table 2

Idylla™ *EGFR* results compared with NGS results for *EGFR* mutation-positive samples. Variants with an asterisk (*) are not detectable by the Idylla™ system by design.

Sample	Tumor Content (%)	NGS	Idylla™
1	35	L858R	L858R
2	60	L858R	L858R
3	30	L858R	L858R
4	20	L858R	L858R
5	20	L858R	L858R
6	70	L858R	L858R
7	15	L858R	L858R
8	20	L858R	L858R
9	70	K745_A750del (Exon 19)	Exon 19 deletion
10	15	E746_A750del (Exon 19)	Exon 19 deletion
11	20	E746_A750del (Exon 19)	Exon 19 deletion
12	30	E746_A750del (Exon 19)	Exon 19 deletion
13	25	K745_E749del (Exon 19)	Exon 19 deletion
14	50	K745_A750del (Exon 19)	Exon 19 deletion
15	30	E746_S752>A (Exon 19)	Exon 19 deletion
16	90	K745_A750>T (Exon 19)	Exon 19 deletion, S768I
17	40	G719A	G719A/S/C
18	20	G719C, E709V*	G719A/S/C
19	10	G719C, S768I	G719A/S/C, S768I
20	30	G719A, S768I	G719A/S/C, S768I
21	95	S768I, V774M*	S768I

not provide quantitative information related to variant allele fraction (VAF).

In conclusion, the Idylla EGFR Mutation Assay is a simple, fully automated and reliable test that provides rapid turnaround time allowing for timely management decisions in the care of patients with lung cancer. The Idylla™ system is especially appropriate for clinical laboratories that lack molecular diagnostics expertise and infrastructure.

Declaration of competing interest

M. Rabie Al-Turkmani – received travel funds from Biocartis to present study findings at a national meeting.
MAS, SJD, DCG, CCB, KS, KHD – no conflicts of interest to report.
Gregory J. Tsongalis – Biocartis supported this study by providing cartridges.

CRedit authorship contribution statement

M. Rabie Al-Turkmani: Data curation, Writing - original draft. **Michael A. Suriawinata:** Investigation, Methodology. **Sophie J. Deharvengt:** Data curation, Investigation, Methodology. **Donald C. Green:** Data curation, Methodology, Software. **Candice C. Black:** Investigation, Project administration, Supervision, Writing - review & editing. **Keisuke Shirai:** Resources, Project administration, Supervision, Writing - review & editing. **Konstantin H. Dragnev:** Resources, Formal analysis, Project administration, Supervision, Writing - review & editing. **Gregory J. Tsongalis:** Conceptualization, Formal analysis, Project administration, Funding acquisition, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plabm.2020.e00156>.

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