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EGFR Point of Care Clinical Testing using Idylla Platform Decreases Laboratory Turnaround Time in Advanced Stage Non-Small Cell Lung Cancer, as Compared to New Generation Sequencing

Norbert Bany^{1,2}, Curtis Hughesman⁴, Kelly McNeil BSc⁴, Barbara Melosky^{1,5}, Deepu Alex^{2,3}, Diana N Ionescu^{2,3}

¹ Faculty of Medicine, University of British Columbia; ² Department of Pathology, BC Cancer; ³ Department of Pathology and Laboratory Medicine, UBC; ⁴ Cancer Genetics and Genomics, BC Cancer; ⁵ Medical Oncology, BC Cancer; ⁶ Vancouver Coastal Health Research Institute.

Corresponding author: Diana N. Ionescu; **e-mail:** dionescu@bccancer.bc.ca

Abstract

Background: Patients with advanced-stage non-small cell lung cancer (NSCLC) benefit from a short time-to-treatment (TTT) due to disease severity. Patients at BC Cancer with NSCLC undergo OncoPanel testing, a next-generation sequencing assay, for potential oncogenic drivers prior to treatment as outlined by CAP-AMP-IASLC guidelines. Genetic testing via OncoPanel takes more than two weeks and commonly contributes to an increased TTT. The novel ultra-rapid Idylla EGFR testing platform may decrease TTT in patients who are EGFR mutation positive (M+) due to the mutual exclusivity of actionable mutations. This study evaluates the lab turnaround time (TAT) of the Idylla EGFR testing platform and compares it to that of the OncoPanel.

Methods: A group of patients (N = 235) with stage IIIB or stage IV lung adenocarcinoma diagnosed between November 1, 2020 and May 1, 2021 had both OncoPanel and Idylla EGFR testing. The time at which the sample was received in the lab, the time of Idylla EGFR test reporting, and the time of OncoPanel reporting were

recorded for each patient. Differences in the lab TAT between the OncoPanel and Idylla EGFR test were compared using a paired t-test within the cohort.

Results: The mean lab TAT for the Idylla and OncoPanel tests were 3.4 days (Range: 0-8 days) and 15.8 days (Range: 12-31 days), respectively. It was observed that the lab

TAT of the Idylla EGFR test was faster by an average of 12.4 days (Range: 6-29 days, $p < 0.01$, 95% CI: [11.9, 12.8] days) than the OncoPanel TAT (N=235).

Conclusions: The lab TAT of the Idylla EGFR test is significantly shorter than of OncoPanel testing. In patients who are EGFR M+, molecular testing could be completed considerably faster using the Idylla EGFR testing platform since further genetic testing is unlikely to yield additional actionable information. Using the Idylla EGFR test as part of a reflexive molecular testing repertoire in advanced-stage NSCLC patients could thus reduce patient TTT.

Keywords: NGS, Idylla, EGFR, lung cancer, NSCLC

Background

Patients with advanced-stage non-small cell lung cancer (NSCLC) benefit from a short time-to-treatment (TTT) due to disease severity and often advanced stage at presentation. In more recent years, treatments for NSCLC have been advanced with the introduction of molecular testing in the treatment algorithm. For patients with advanced-stage NSCLC, genetic testing should be performed before delivering treatment as outlined in the guidelines set by the College of American Pathologists (CAP), Association for Molecular Pathology (AMP), and the International Association for the Study of Lung Cancer (IASLC) (1).

EGFR mutations are particularly common in patients with advanced NSCLC, especially in those with adenocarcinoma histology (2, 3). The incidence of *EGFR* mutations in lung cancer differs between Caucasian (range: 7%–36%) and Asian patients (range: 20%–76%) (2). As EGFR remains the most prevalent targetable oncogenic mutation in NSCLC, its rapid identification in advanced-staged NSCLC would significantly shorten time to treatment for these patients. Since most targetable molecular drivers are mutually exclusive, a positive EGFR result will inform the clinician of the best course of action at an earlier time point, and avoid further, more comprehensive and expensive testing.

The Idylla (Biocartis, Mechelen, Belgium)TM platform is a fully-automated, real-time PCR-based molecular testing system

with applications to a wide range of clinical settings, where all the reagents required for sample preparation and PCR detection are provided in a single cartridge that can then be easily and rapidly loaded into the system. The system requires as little as 25 nanograms of tumor DNA, which is evaluated for single gene molecular changes, such as EGFR mutations, in less than three hours. This assay is validated in the BC Cancer Genetics and Genomics Laboratory (CGL) for clinical reporting. This study's objective is to evaluate the TAT of the Idylla EGFR testing platform and compare it to that of the OncoPanel.

Methods

This prospective study was performed on patients diagnosed with NSCLC and referred to BC Cancer to undergo OncoPanel clinical testing, a next-generation sequencing (NGS) assay, which screens 41 of the most common potential oncogenic genes, including EGFR. Genetic testing via OncoPanel takes more than two weeks to complete from the time the tumor sample is received in the laboratory to the time at which the genetic report is released to the ordering physician (lab TAT). OncoPanel testing commonly contributes to an increased TTT due to its lengthy TAT. The aim of the study is to compare TAT for patients with lung adenocarcinomas having EGFR testing performed by Idylla and compared to OncoPanel, the existing clinical mutational testing at BC Cancer, at the time of this study.

Case Selection

The samples were obtained from a group of patients (N = 235) with stage IIIB or stage IV lung adenocarcinoma diagnosed between November 1, 2020 and May 1, 2021 that had both OncoPanel and Idylla EGFR testing. Among these samples, specimens submitted for the identification of EGFR T790M resistant mutations, and samples with less than 500 nanograms of total extracted DNA were excluded, in order to ensure sufficient DNA for both Idylla and OncoPanel testing, and to focus this study on identification of *de novo* EGFR mutations. Prospective, real life clinical testing was performed on 235 advanced stage NSCLC patients, identified using the above criteria, and were concomitantly tested using the Idylla platform, and the clinically requested next generation sequencing (NGS) OncoPanel. The time at which the sample was received in the lab, the time of Idylla EGFR test reporting, and the time of OncoPanel reporting were recorded for each patient.

Statistical Analysis

Differences in the TAT between the OncoPanel and Idylla EGFR test were compared using a paired t-test over each patient. The analysis was performed using base R (version 4.0.2).

Results

The mean lab TAT for the Idylla and OncoPanel tests were 3.4 days (Range: 0-8 days) and 15.8 days (range: 12-31 days), respectively. It was observed that the lab TAT of the Idylla EGFR test was faster by an average of 12.4 days (range: 6-29 days, $p < 0.01$, 95% CI: [11.9, 12.8] days) than OncoPanel TAT (Figure 1).

This TAT included specimen accessioning at referral laboratory, microtome cutting and H&E slide preparation, tumor content assessment and DNA extraction and preparation, qPCR, and automated analysis.

For a select few cases, in view of clinical urgency, tumor tissue was loaded directly to the Idylla cartridge without extraction. This allowed for same day resulting and explains the Idylla TAT range of 0-8 days. However, for the vast majority of cases, in order to accurately compare results between Idylla and OncoPanel, extracted DNA was used for both tests.

After the tests were performed, results were released on the patient's medical record on the same day or the subsequent day to allow for appropriate treatment planning.

Fig. 1: Comparison of Idylla and NGS TATs (N=235). Distributions do not overlap, demonstrating a unanimously lower TAT in favor of the Idylla EGFR test.

Discussion

This study demonstrates that the Idylla EGFR test TAT is significantly shorter than NGS testing TAT. As the average Idylla TAT is 3.4 days, compliance with the CAP-ILSLC-AMP molecular testing guidelines to have EGFR results within 10 working days from receipt of specimens in the laboratory can be easily achieved. Due to the mutual exclusivity of major oncogenic drivers in NSCLC, a positive Idylla EGFR test would lead to the completion of pretreatment tumor testing and could thereby improve TTT by 12 days on average in this patient population. Additionally, in a reflex but stepwise testing

scenario, cases positive for EGFR by this ultra-rapid panel would not require further, more comprehensive and more expansive testing (due to lack of incremental information), thereby saving NGS testing costs on up to 30.3% of lung adenocarcinoma patients, depending on the EGFR mutation incidence of the tested population (2, 3). The Idylla testing platform can be easier implemented and validated than other sequencing platforms in a variety of clinical settings, as it only requires limited infrastructure and training. Further research into the cost of implementing Idylla EGFR testing is required as patients who are EGFR mutation negative will undergo both Idylla and NGS testing. While the Idylla platform displays improved time and tumor material requirements as compared to NGS, there is a reduction in its comprehensiveness concerning the genes it can test as well as the mutations it can detect (Table 1-EGFR testing by Idylla and OncoPanel).

Other factors contribute to the lengthening of TTT in NSCLC, which are often independent of the testing laboratory, and independent of the technology used for testing. These factors include time for receiving the

tumor block from originating hospital to the reference laboratory, interpretation and reporting time, etc. While several steps including evaluation of tissue cellularity and tumor content, DNA extraction and time to releasing the report to the ordering physician may overlap in length, others show greater variability, and therefore explain the overall decrease in TTT and Idylla TAT; the main contributing factors are shorter sequencing time using Idylla, as compared to any of the commercially available platforms (2.5 hours versus a few days) and shorter reporting time due to the analysis of one gene by Idylla versus multiple genes using NGS.

The number of mutations that can be detected by Idylla is predetermined by specific exonic regions that include hotspot variants (*BRAF*, 7; *EGFR*, 51; *KRAS*, 21) (4). Idylla requires minimal expertise for test performance and its turnaround time and hands-on time are the main advantages various other characteristics of these technologies, including required upfront extraction, minimal FFPE (formalin fixed paraffin embedded) tissue input, and type of output of results are summarized in Table 1.

Table 1. Practical characteristics of EGFR Mutation Assay and OncoPanel Assay

	Idylla EGFR	OncoPanel
Sequencing Time	150 min	Few Days
Hands-on Time	10 min	Hours
EGFR Mutations	51 SNV/del/ins	Full exon coverage
Upfront DNA Extraction	Yes	Yes
Minimal Input: FFPE	1×5µm FFPE section and ≥10% tumor cells	6×5µm FFPE section and ≥20% tumor cells
Data Processing	Proprietary algorithm	Lab Developed Algorithm
Expertise	Low	High
Output	Somatic hotspot mutations in EGFR	Any EGFR alterations requiring curation
Bioinformatics Assessment Needed	No	Yes

The analysis of the mutation detected is automatically performed by the Idylla platform and a report including the presence and type of EGFR mutation is generated. The NGS platform requires extensive bioinformatics analysis, highly trained personnel performing data analysis and interpretation of the results generated by the machine. Such characteristics suggest that the Idylla platform could be implemented in non-expert centers where no sequencing machines or expert technicians are currently available (5). Therefore, even pathology laboratories without molecular expertise could successfully use this method as long as a pathologist confirms the presence of a tumor on the slide, prior to testing (6-8). This high success rate depends on appropriate triaging and tissue handling, as tissue input is a pivotal determinant of the assay sensitivity and performance (9).

If reflex-testing using this technology may not be feasible, Idylla's shorter TAT

over NGS testing could be beneficial in various clinical scenarios. For example, in patients with poor performance status, identification of EGFR mutation 12 days earlier may represent a lifesaving clinical step and should be considered on a case-by-case basis. Due to the difference in response to immunotherapy reported in NSCLC adenocarcinomas harboring driver mutations, timely identification of EGFR negative patients, in the right clinical scenario, may contribute to decreased immunotherapy TTT.

Conclusions

Molecular EGFR testing is completed considerably faster using the Idylla EGFR testing platform and this allows faster time to treatment for EGFR positive patients allowing rapid access to targeted therapy, and for EGFR negative patients allowing rapid access to immunotherapy.

Abbreviations:

EGFR – epidermal growth factor receptor

NSCLC – non-small cell lung cancer

TTT – time-to-treatment

CAP – College of American Pathologists

AMP – Association for Molecular Pathology

IASLC – International Association for the Study of Lung Cancer

CGL – Cancer Genetics and Genomics Laboratory

NGS – next-generation sequencing

TAT – turnaround time

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