Original Article

Evaluation of the Idylla™ EGFR Mutation Test on formalin-fixed, paraffin-embedded tissue of human lung cancer

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Background: Epidermal growth factor receptor (*EGFR*) mutation detection is essential for the therapy of lung cancer. A sensitive, specific, and cost-effective standardized method to quickly and accurately detect *EGFR* mutations is urgently needed.

Methods: We evaluated the Idylla™ EGFR Mutation Assay for *EGFR* mutations in formalin-fixed, paraffin-embedded (FFPE) tumor samples from 232 lung cancer patients, and compared the results with amplification refractory mutation system (ARMS) (n=146) and next-generation sequencing (NGS) (n=86). The surgical tumor sections and cell blocks derived from the same FFPE section were compared. Overall concordance, specificity, sensitivity, cost-effectiveness and turnaround time were compared among the three methods.

Results: The overall concordance between Idylla and ARMS was 89.51% [95% confidence interval (CI): 83.31% to 93.64%] and the specificity of Idylla was 88.68% (95% CI: 80.69% to 93.76%). A concordance of 97.67% (95% CI: 91.41% to 99.86%) was obtained between Idylla and NGS, the specificity of Idylla was 96.30% (95% CI: 86.16% to 99.36%). Compared to the ARMS and NGS, the Idylla™ system significantly reduces the turnaround time. Combining labor, equipment, reagents and time costs, Idylla is more affordable.

Conclusions: Clinically urgent cases with adequate cellularity, can first perform Idylla to detect critical markers, then perform NGS for a comprehensive mutation analysis. Besides, with limited molecular expertise or infrastructure, the Idylla has the potential to extend *EGFR* testing to more pathology laboratories in primary hospitals.

Keywords: Idylla; epidermal growth factor receptor (*EGFR*); lung cancer; next-generation sequencing (NGS); amplification refractory mutation system (ARMS)

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Introduction

Lung cancer is one of the most common malignant tumors with the fastest increase in morbidity and mortality. Its prognosis is relatively poor, with a 5-year survival rate ranging from 10% to 20%, depending on the stage of the disease at diagnosis (1). Lung cancer is mainly divided into two categories: small cell lung cancer (SCLC) and non-SCLC (NSCLC), and NSCLC accounts for nearly 85%

of lung cancers (2). The majority of NSCLC patients are diagnosed at an advanced stage, only about 20–30% of patients with an early diagnosis of cancer can receive radical surgery, and many of them have a high risk of recurrence (25–70%) due to the presence of preoperative micrometastases (3). However, conventional chemotherapy and radiotherapy have shown limited efficacy (4).

About 10–15% Caucasian and over 30–50% Asian NSCLC patients have epidermal growth factor receptor (*EGFR*) gene mutations (5-7). The NSCLC patients associated with *EGFR* mutations have a good prognosis with *EGFR* tyrosine kinase inhibitors (TKIs). Deletion of exon 19 and the exon 21 L858R point mutation are the most common mutations in *EGFR*, accounting for about 85–90% (8). Most of the *EGFR* mutations are sensitive to TKIs. However, some mutations may show resistance to TKIs (9,10). Hence, guidelines are required to determine the *EGFR* mutational status in advanced NSCLC before the TKI therapy. For example, the *T790M* mutation in *EGFR* exon 20 plays a key role in the development of drug resistance to TKIs. Therefore, *T790M* should be monitored during disease progression. With the development of research, third-generation TKIs have been designed to target the *T790M* mutation and are used to treat patients who develop this resistance mutation (11-13). To make a long story short, *EGFR* detection plays an important role in guiding the selection of targeted drugs.

A variety of technologies, such as Sanger sequencing, next-generation sequencing (NGS) and amplification refractory mutation system (ARMS) have been developed to assess the *EGFR* mutational status of formalin-fixed, paraffin-embedded (FFPE) NSCLC tissue samples (14). Each of these methods has its advantages and disadvantages. NGS results are more comprehensive but often at the expense of longer turnaround time due to test complexity, and it needs a certain amount of DNA. However, the tumor tissue is not always satisfactory, it tends to be small and the tumor content may be limited. ARMS is quick and requires less amount of DNA but it is known to explore only a small number of certain hot spot *EGFR* mutations. The typical turnaround time in clinical practices is five days for ARMS and two weeks for NGS. The Idylla™ EGFR Mutation Test (Biocartis, Belgium) is a new diagnostic test for the qualitative detection of exons 18, 19, 20 and 21 in the *EGFR* gene. The system is fully automated, polymerase chain reaction (PCR)-based mutation testing system supporting hands-free processing of all steps, starting with tissue input to molecular results. The turnaround time is about 2.5 hours, which is faster and more convenient than NGS and ARMS. To date, there are only a few published studies describing routine testing of FFPE tissue samples with the Idylla™ system. Most of them only compared Idylla results with NGS. Lee *et al.* found the Idylla™ EGFR Mutation Test has reduced sensitivity for the T790M mutation compared with NGS and droplet digital PCR (ddPCR) methods (15). Hawkins *et al.* found the Idylla™ EGFR Assay is sensitive to extracted DNA and can be reliably applied to cytologic specimens, enabling its implementation as an ancillary first-line test for patients with NSCLC (16). However, different investigators hold different opinions on the accuracy of Idylla™ testing.

This study compared the NGS/ARMS used in our laboratory with the Idylla™ EGFR Mutation Test to describe Idylla's potential clinical utility for delivering precision medicine to Chinese NSCLC patients. We present this article in accordance with the STARD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1293/rc).

Methods

Patients and samples

This study was a retrospective study. FFPE tumor samples from 232 patients with lung cancer obtained between 2018 and 2020 in the Pathology Department of the Shanghai Cancer Center were collected prospectively. Lung cancer was diagnosed according to the pathology results in Shanghai Cancer Center. Tumor contents were determined by some sections stained with hematoxylin and eosin. Serial 5 μm tumor sections were used for *EGFR* mutation detections. Some section was used for ARMS or NGS according to our routine procedures and other sections was used for testing on the Idylla™ platform. All of the assays were processed independently, and were blinded to the mutation status, as determined by both the ARMS and the NGS. At the end of the study, the pooled results were compared. If the results were inconsistent, the results were verified by Sanger sequencing. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Ethics Committee of Fudan University Shanghai Cancer Center (No. 050432-4-2108*). This is a retrospective study and the data are anonymous, the requirement for informed consent was therefore waived.

Table 1 Mutations detected by the Idylla™ EGFR Mutation Test

Exon	Mutation	Nucleotide variation
18	G719A	c.2156G>C
	G719C	c.2155G>T; c.2154_2155delinsTT
	G719S	c.2155G>A
19		c.2238_2248delinsGC
	Del9	c.2239_2248delinsC
		c.2240_2248del
		c.2239_2247del
	Del12	c.2239_2251delinsC
		c.2240_2251del
	Del15	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
	Del18	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
	Del21	c.2238_2258del
		c.2236_2256del
	Del24	c.2253_2276del
20	T790M	c.2369C>T
	S768I	c.2303G>T
	insG	c.2310_2311insGGT
	insASV9	c.2308_2309insGCCAGCGTG
	insASV11	c.2308_2311delinsCCAGCGTGGAT
	insSVD	c.2311_2312insGCGTGGACA
	insH	c.2319_2320insCAC
21	L858R	c.2573T>G
		c.2573_2574delinsGT
		c.2573_2574delinsGA
	L861Q	c.2582T>A

EGFR, epidermal growth factor receptor.

Idylla™ EGFR Mutation Test

The Idylla™ EGFR Mutation Test is intended for detection of 51 different *EGFR* oncogene mutations on exon18, 19, 20 and 21 (*Table 1*). Two or three 5 µm FFPE tissue sections were inserted into the desired cartridges and loaded into the instrument with a total running time of approximately 2.5 hours and an actual hands-on time of <2 minutes. After the FFPE tissue section was inserted into the cartridge, a combination of chemical reagents, enzymes and heat lysed the cells. The test results were directly displayed on the Idylla™ Console in the form of reports, including positive, negative, or invalidity. When an invalid result occurred, a new cassette needed to be replaced to retest the sample.

NGS

We isolated genomic DNA from eight 5 µm FFPE tissue sections via the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's specifications. The capture-based lung cancer research panel (Burning Rock Biotech, Guangzhou, China), which included all exons in 68 genes was used to analyze the genomic DNA. The DNA was then mapped to 300 bp using a Covaris S220 Focused ultrasonicator (Covaris, Woburn, Massachusetts), followed by hybridization with a capture probe bait, magnetic bead hybridization selection, and PCR amplification. QIAcel Advanced (Qiagen) was then used to assess the size range. The available indexing samples were then sequenced on the Nextseq500 system (Illumina, San Diego, CA, USA).

ARMS

The FFPE DNA kit (Qiagen) was used to extract

Table 2 Characteristics of the 232 patients with lung cancer

Characteristics	N (%)
Gender	
Male	136 (58.62)
Female	96 (41.38)
Age (years)	
≤59	95 (40.95)
>59	137 (59.05)
Sample nature	
Surgical sample	215 (92.67)
Cell block	17 (7.33)
% of tumor cells	
>50%	121 (52.16)
26–50%	98 (42.24)
10–25%	6 (2.59)
<10%	7 (3.02)

DNA from three to four sections of 5 μm thick FFPE tissues. The *EGFR* mutation was detected by the ARMS commercial regent (Amoy, Xiamen, China) according to the manufacturer's protocol. Amplification conditions were conducted on the Applied Biosystems ABI 7500 PCR instrument (Thermo Fisher Scientific, Waltham, MA, USA) as follows: an initial denaturation at 95 ℃ for 5 minutes; 15 cycles, including denaturation at 95 ℃ for 25 seconds, annealing at 64 ℃ for 20 seconds, extension at 72 ℃ for 20 seconds; 31 cycles, including denaturation at 93 ℃ for 25 seconds, annealing at 60 ℃ for 35 seconds, extension at 72 ℃ for 20 seconds. FAM and HEX (or VIC) signals were collected at 60 ℃.

Statistical analysis

Agreement between Idylla and the comparison methods was evaluated on the basis of point estimates for overall, positive, and negative percentage diagnostic agreement together with 95% two-sided Wilson's score method.

Results

Prospective study cohort

Two hundred and thirty-two FFPE tumor samples from 232 patients with lung cancer were collected prospectively (*Table 2*). Patient median age was 62 years (range, 30–92 years). Most samples were surgical resections (n=215, 92.67%). In most cases, the tumor cell content of FFPE tissue sections was greater than 25% (n=219, 94.40%).

Comparison between Idylla™ EGFR Mutation Test and ARMS

The *EGFR* mutational status of 146 prospective clinical FFPE samples from patients with lung cancer was tested with Idylla™ System, and results were compared with the assessments made by AmoyDx Human EGFR Gene Mutation Detection Kit.

Among the 146 lung cancer FFPE samples, three "invalid" calls were reported, and the success rate of Idylla was 97.95% (143/146) while that of ARMS was 100% (146/146). Two "invalid" cases were cell blocks with total tissue area of less than 25 mm^2, in which the amount of DNA did not meet the requirements of Idylla. One case was from another hospital, the specimen may not meet the requirement of Idylla due to inappropriate fixing method or FFPE preservation method. These three "invalid" samples were excluded from the data set. The Idylla™ EGFR Mutation Test identified *EGFR* mutations in 143 samples (*Table 3*). Deletions in exon 19 were detected in 38 samples, 3 of which were associated with one or two other mutations. The *EGFR p.L858R* mutation was reported in 41 samples, and 1 sample was associated with another mutation. An *EGFR p.G719X* mutation was detected in 8 samples, 5 of which were associated with one or two other mutations. Eleven samples had the *EGFR p.L861Q* mutation, and 4 of them were associated with other mutations. All 3 samples carrying the *EGFR p.S768I* mutation were associated with one or two other mutations. *T790M* was detected in 5 samples, all associated with one or two *EGFR* variants. Insertions of exon 20 were found in 5 samples. Finally, no mutations were found in 43 cases. Results showed that this dataset contained 143 samples for consistency analysis. In 15 patients out of 143 patients, the testing results were inconsistent. Of these 15 discordant samples, 12 were considered as wild-type/mutant-type inconsistent samples. For the other 3 samples, there were differences in the number of mutations detected. Among the 15 cases, there were 5 cases of cell blocks (3 cases were positive by ARMS method, negative by Idylla; 1 case detected one more mutation than ARMS and 1 case detected one mutation less than ARMS), 10 cases of surgical specimens (6 cases were

Table 3 Accuracy of Idylla™ EGFR Mutation Test compared with ARMS

Idylla	ARMS															
	19del	L858R	G719X	L861Q	Exon20ins	S768I	T790M	L858R/T790M	L858R/S768I	19del/T790M	19del/T790M/S768I	L861Q/T790M	G719X/L861Q	G719X/S768I	WT	Total
19del	32									1†					2†	35
L858R		38							1†						1†	40
G719X			3													3
L861Q				7												7
Exon20ins					5											5
S768I																0
T790M																0
L858R/T790M								1								1
L858R/S768I																0
19del/T790M										2						2
19del/T790M/S768I										1†						1
L861Q/T790M												1				1
G719X/L861Q													3			3
G719X/S768I														2		2
WT	2†	3†	1†			1†	1†			1†					34	43
Total	34	41	4	7	5	1	1	1	1	5	0	1	3	2	37	143

†, discordant samples. EGFR, epidermal growth factor receptor; ARMS, amplification refractory mutation system; WT, wild type.

Table 4 Comparison between results of the Idylla™ EGFR Mutation Test and results of ARMS or NGS

	ARMS			NGS		
	Mutation	WT	Total	Mutation	WT	Total
Idylla						
Mutation	94	3	97	52	0	52
WT	12	34	46	2	32	34
Total	106	37	143	54	32	86
Idylla performance						
Positive agreement	88.68% (95% CI: 80.69% to 93.76%)			96.30% (95% CI: 86.16% to 99.36%)		
Negative agreement	91.89% (95% CI: 76.98% to 97.88%)			100% (95% CI: 86.66% to 100%)		
Overall agreement	89.51% (95% CI: 83.31% to 93.64%)			97.67% (95% CI: 91.41% to 99.86%)		

EGFR, epidermal growth factor receptor; ARMS, amplification refractory mutation system; NGS, next-generation sequencing; WT, wild type; CI, confidence interval.

Table 5 Accuracy of Idylla™ EGFR Mutation Test compared with NGS

Idylla	NGS							
	19del	L858R	G719X	L861Q	19del/T790M	c.2314_2319dup (p.Pro772_His773dup)	WT	Total
19del	20							20
L858R		28						28
G719X			3					3
L861Q				1				1
19del/T790M					1			1
Not in Idylla panel						1[†]		1
WT	1[†]						31	32
Total	21	28	3	1	1	1	31	86

[†], discordant samples. EGFR, epidermal growth factor receptor; NGS, next-generation sequencing; WT, wild type.

positive by ARMS, negative by Idylla; 3 cases were positive for Idylla, negative by ARMS and 1 case detected only one mutation point which ARMS detected two).

Overall, the Idylla results for 128 of the 143 samples were fully consistent with the ARMS results [overall concordance of 89.51%; 95% confidence interval (CI): 83.31% to 93.64%]. The estimated technical sensitivity was 88.68% (95% CI: 80.69% to 93.76%), while the estimated technical specificity was 91.89% (95% CI: 76.98% to 97.88%) (*Table 4*).

Comparison between Idylla™ EGFR Mutation Test and NGS

The NGS cohort had 86 cases, no "invalid" call was reported, and the success rate of Idylla was 100%. The Idylla identified *EGFR* mutations in 86 samples (*Table 5*). Deletions in *exon 19* were detected in 21 samples, 1 of which was associated with *T790M* mutation. The *EGFR p.L858R* mutation was reported in 28 samples. A *G719X* mutation was detected in 3 samples. One sample harbored an *L861Q* mutation. No mutation was found in 32 cases.

Among the 86 cases, there were two discordant cases. The concordance of Idylla with NGS therefore was 97.67% (95% CI: 91.41% to 99.86%). The two samples were small lung biopsies from lung adenocarcinoma, all of which were detected mutant by NGS and negative by Idylla. A mutation detected by NGS but designated wild type by Idylla in one case was *19del*. The other case had a mutation detected by

Table 6 Discordant results between the Idylla™ EGFR Test and ARMS

Sample	Tissue type	Tumor cell (%)	Idylla	ARMS	Sanger (verify)
1	Cell block	10–20	WT	T790M	T790M
2	Cell block	80	19del	T790M/19del	T790M/19del
3	Cell block	60–70 atypical cells	WT	T790M/19del	T790M/19del
4	Cell block	70	19del/T790M/S768I	T790M/19del	T790M/19del
5	Surgical sample	50–60	19del	WT (ARMS does not contain the mutation point)	19del
6	Surgical sample	80	L858R	WT	WT
7	Surgical sample	30	WT	L858R	L858R
8	Surgical sample	60	WT	19del	19del
9	Surgical sample	60	L858R	L858R/S768I	L858R/S768I
10	Surgical sample	80	19del	WT	19del
11	Cell block	40 atypical cells	WT	G719X	G719X
12	Surgical sample	70	WT	S768I	S768I
13	Surgical sample	20–30	WT	19del	19del
14	Surgical sample	10	WT	L858R	L858R
15	Surgical sample	30–40	WT	L858R	L858R

EGFR, epidermal growth factor receptor; ARMS, amplification refractory mutation system; WT, wild type.

Table 7 Discordant results between the Idylla™ EGFR Test and NGS

Sample	Tissue type	Tumor cell (%)	Idylla	NGS	Sanger (verify)
1	Surgical sample	40	WT (Idylla panel does not contain the mutation point)	c.2314_2319dup	c.2314_2319dup
2	Surgical sample	50	WT	19del	19del

EGFR, epidermal growth factor receptor; NGS, next-generation sequencing; WT, wild type.

NGS which was not covered by the Idylla panel.

The estimated technical sensitivity was 96.30% (95% CI: 86.16% to 99.36%), while the estimated technical specificity was 100% (95% CI: 86.66% to 100%) (*Table 4*).

Discordant results

The detection results were inconsistent for 15 samples in ARMS (*Table 6*) and two in NGS (*Table 7*). In NGS, one discordant result was the result of *c.2314_2319dup* (*p.Pro772_His773dup*) that was not contained in the design of the Idylla™ EGFR Mutation Test. In one case, ARMS does not contain the mutation point detected by Idylla. Fifteen samples had mutations detected by the ARMS or NGS and missed by Idylla, two samples had mutations detected by Idylla but missed by ARMS. The percentage of tumor cells was <60% in most discordant samples. All these samples were fine-needle biopsy samples, cell blocks and samples from other hospitals.

Comparison of the turnaround time and costs between Idylla, ARMS and NGS

In our laboratory, the turnaround time is two weeks for NGS testing and five days for ARMS. Both of these approaches suffer from labor intensive procedures, especially NGS, which includes DNA isolation, library preparation, sequencing and bioinformatics analyses. Idylla

system does not need to extract DNA from tissue sections, just put the wax roll directly into the machine, the average time from sample to result is only about 2.5 hours. This greatly reduces the detection time and shorten the time patients wait for results.

Economic costs are also important. We calculated the reagent costs for different technologies (list prices not including salary and equipment): ARMS (CNY1,200), NGS (CNY1,500) and Idylla (CNY1,800) per sample. The reagent cost of Idylla was a bit higher. However, NGS and ARMS tests require considerable investments on instrumentation, staff training in laboratory skills, bioinformatics analyses, data interpretation and reporting. By comparison, the Idylla™ test is far easier to perform and interpret.

In summary, combining labor, equipment, reagents and time costs, Idylla is more affordable.

Discussion

After the first generation of *EGFR* TKI came out, the detection of *EGFR* mutations in NSCLC has become a systematic and mandatory clinical practice in either newly diagnosed NSCLC patient or patients with resistance mutations (11,17). At least 50% of Asian NSCLC patients have these mutations, all of them may benefit from the targeted therapies. As a result, *EGFR* testing is now an integral part of lung cancer pathology, and the clinical need for rapid testing in advanced lung cancer patients is quite high (18).

There are many methods used to detect *EGFR* mutations. In this study, the Idylla™ EGFR Mutation Test results were compared with the results of *EGFR* mutations detected in ARMS and NGS in FFPE tissue samples (including surgical samples, fine-needle biopsy samples and cell blocks). In total, 232 NSCLC FFPE samples were selected and tested. Samples with invalid test results (n=3) obtained by the Idylla™ EGFR Mutation Test were excluded. As a result, the ARMS dataset contained 143 samples for the consistency analysis. For Idylla, 15 of these 143 samples, the *EGFR* mutation test result was inconsistent with the ARMS results. The overall concordance between the Idylla and ARMS was 89.51% (95% CI: 83.31% to 93.64%), with a sensitivity of 88.68% (95% CI: 80.69% to 93.76%), specificity of 91.89% (95% CI: 76.98% to 97.88%). The 15 discordant results were described fully in the Results section (*Table 6*). In 13 discordant samples, the ARMS identified mutations not detected by Idylla and two discordant samples the other way around. The NGS data set contained 86 samples for concordant analysis. Discordance between results of the Idylla and NGS was observed for two of these 86 samples (*Table 7*). The overall concordance between the Idylla and NGS was 97.67% (95% CI: 91.41% to 99.86%), with a sensitivity of 96.30% (95% CI: 86.16% to 99.36%), specificity of 100% (95% CI: 86.66% to 100%).

Currently, the majority of centers are probably using NGS-based tests. NGS attracts huge attention due to the continuous advancement in the technology, it seems that the time of single gene testing is over. One approach to improving time-to-treatment would be through decreasing lab turnaround time for genetic testing. Clinician should determine the most suitable technique according to the ordering of clinical, sample quality, the main purpose for the molecular testing and economic factors. In our lab, NGS is predominant, and ARMS is used for some patients who need rapid results. In this study, the consistency of Idylla and ARMS was 89.51%, lower than that with NGS 97.67%. It may be because the specimens of ARMS had more cell blocks and fine-needle biopsy samples, in which the tumor tissue was small and the tumor cells had not been enriched while the NGS samples were all surgical specimens with more tumor tissue. In this study, Idylla had a low concordance rate in FFPE cell block samples (12/17, 70.6%) while a few authors have reported positive experiences on cytology samples. The concordance and diagnostic sensitivity were both up to 95% (19,20). The reason for this discrepancy is that their cytology samples also including cell pellet, supernatant or residual cytology specimens in CytoLyt and precapture NGS libraries. The tissue quality of these samples was much better than FFPE cell blocks and improve the success rate of detection. Our failed FFPE cell blocks had very small density of tumor cells (*Tables 6*,7), DNA used for Idylla was insufficient. Moreover, Cazzato *et al.* reported formaldehyde can affect the DNA double helix, sometimes severely damaging the quality of the DNA used after FFPE. All of these reactions can potentially alter the correct sequences during the later processes, such as PCR and NGS and work was being devoted to possible strategies to reduce single-nucleotide variant (SNV) and other fixation artifacts (21). FFPE was also one of the reasons for affecting the results of cell block sequencing.

Furthermore, Idylla has many advantages compared to ARMS and NGS. We must acknowledge that due to the rapid progression of tumors and ensuring that patients get the most appropriate treatment available at a time when

they are sufficiently well enough to tolerate side effects, clinicians need to rapidly treat patients with metastatic NSCLC with molecular therapies. Thus, clinicians are urged to develop rapid technological methods, the faster the detection of mutation of *EGFR* genes, the better the disease management (22). An important advantage of the Idylla™ EGFR Mutation Test is the rapid turnaround time. For the analysis by ARMS, in our laboratory, the experimental protocol of ARMS includes cutting tissue sections, DNA extraction, sequencing, and final analysis. The average time from sample to result is about five days. The turnaround time of NGS is usually 14 days, often affecting doctors to provide effective treatment to cancer patients in a timely manner. For the analysis by the Idylla™ system, the average time from sample to result is only about 2.5 hours. Several studies found that Idylla™ EGFR Test was, on average, 9–12 days faster than NGS (23,24). Although most patients would be negative for *EGFR* mutations, the probability of carrying an *EGFR*-mutation-positive tumor is higher in certain patient populations, such as non-smokers. The true value of ultra-rapid *EGFR* testing in these patient subsets is the prompt identification of a targetable mutation and subsequent quick match to the appropriate treatment. If a positive result is obtained, the Idylla™ EGFR Test may be sufficient. If the test result is negative, a more expansive panel is needed.

Cost issues are also an important factor. The cost of Idylla reagents was slightly higher. However, for ARMS and NGS, nearly 60% of the total costs were labor-related, and 40% were related to kit costs. For Idylla, about 99.0% of the total cost is related to consumables and reagents needed for test. Labor-related is 1.0% of total cost. Several studies have shown that the Idylla™ platform has the shortest practical operation time compared to traditional molecular methods (25). Therefore, depending on the hand-on time and equipment, the Idylla may reduce the cost for some institutions.

Another advantage of Idylla is that it requires only one 10 μm thick FFPE tissue section, whereas in the NGS method, at least four 10 μm tissue sections are required for DNA extraction in our laboratory. This is really important for NSCLC biopsy specimens, with many biomarkers that need to be detected. In addition, detection of *EGFR* mutations in decalcified lung cancer bone metastasis is challenging using NGS, all those samples in our lab showing invalid results. Boureille *et al.* (26) demonstrated that the Idylla™ EGFR Mutation Test shows a good performance on decalcified bone samples and could be used as a first step. Idylla is an important complement to NGS and ARMS in our laboratory. At present, the biggest advantages of Idylla are easy to operate and short turnaround time. Several studies have found that only 79% of *EGFR* positive advanced lung cancer patients in the US commenced on appropriate TKI therapy, while 71% of advanced lung cancer patients in England receiving TKI therapy on identification of an *EGFR* mutation. The authors conclude that it is due to the timeliness of reporting *EGFR* mutation. Furthermore, in Germany, that up to 20% of *EGFR* mutation positive patients started chemotherapy instead of TKI therapy because they were clinically unable to wait for NGS results to be made available (27-29). However, in our study, Idylla had limited sensitivity in detecting FFPE cell block samples and samples with low percentage of tumor cells. A false outcome may cause the patient to fail to receive the most accurate treatment and enjoy the benefit of *EGFR* mutation test. Our study findings are in line with other studies. Bennett *et al.* (30) suggested that integrated cellular-molecular pathology laboratories should be equipped, empowered, and appropriately funded to determine the best analytical approach depending upon the individual specimen/patient. Finall *et al.* (31) discussed issues around integrating rapid PCR testing alongside NGS in multidisciplinary care pathways. Dual testing for stage IV non-squamous, NSCLC patients have the potential to improve care and survival outcomes by provides guidance to the right test at the right time. Recently, National Health Service (NHS) England guidance that describes a salvage testing pathway for patients with advanced lung cancer who would not survive to see the potential beneficial repercussions of NGS-based mutation detection. It allows local testing to continue by rapid PCR methods in a context of genomic testing in centralized laboratory hubs (32).

Conclusions

The Idylla™ platform needs minimal hands-on time (2 minutes) and delivers results in less than 2.5 hours, it can be easily integrated into any clinical molecular diagnostic laboratory, enabling rapid screening for critical molecular markers. Given its limited sensitivity in detecting cell block samples, clinically urgent cases with adequate cellularity can first do Idylla to detect critical markers, then do NGS for a comprehensive mutation analysis. Besides, with very little molecular expertise or infrastructure, the Idylla has the potential to extend *EGFR* testing to more pathology laboratories in primary hospitals.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1293/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Ethics Committee of Fudan University Shanghai Cancer Center (No. 050432-4-2108*). This is a retrospective study and the data are anonymous, the requirement for informed consent was therefore waived.

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