Original Article

Comparison of next-generation sequencing and immunohistochemistry analysis for targeted therapy-related genomic status in lung cancer patients

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Background: Some drugs that target molecular pathways are available for the targeted treatment of lung cancer. Multiple tests are needed to detect the status of the known molecular targets to determine whether the patients can respond to the drugs. An integrated platform for various gene alteration detection including both mutations and rearrangements is necessary for patients, especially those without enough tissue.

Methods: In our study, detections of *EGFR* mutations, *ALK* rearrangement, *ROS1* rearrangement, and alterations of other nine important lung cancer-related genes were integrated into a single next-generation sequencing (NGS) platform. The NGS analysis was performed in 107 cases of non-small cell lung cancer (NSCLC). Meanwhile, hot spots such as *EGFR* L858R, *EGFR* E746-A750Del mutations and gene rearrangement of *ALK* and *ROS1* were detected by immunohistochemical (IHC) staining.

Results: NGS could explore various gene mutations and gene rearrangements with a reduced experiment time and lower amounts of tumor tissues than multiple IHC staining experiments. NGS results were more informative and reliable than IHC staining for *EGFR* gene alterations, especially for the exon 19 region. NGS could also increase the positive rate of *ALK* rearrangement and decrease the false positive results of *ROS1* rearrangements detected by IHC staining.

Conclusions: NGS is effective for confirmation the status of various important lung cancer-related gene alterations. Furthermore, NGS is necessary for the confirmation of the IHC results of *ALK* and *ROS1* rearrangements.

Keywords: Lung cancer; *EGFR* mutations; *ALK* rearrangement; *ROS1* rearrangement; next-generation sequencing (NGS); immunohistochemistry (IHC)

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Introduction

The treatment of human cancer is shifting toward precision medicine, of which molecularly targeted therapy aimed at the genomic status of the tumor in each patient is a typic modality. Several drugs that target molecular pathways are available for patients with non-small cell lung cancer (NSCLC) harboring the relevant gene alterations.

Approximately 35% of NSCLC patients contain *EGFR* gene mutations (1), which are predictors of response to *EGFR*-tyrosine kinase inhibitors (TKIs) and predominantly located in exons 18 to 21 (2-6). In practical works, relevant mutations in patients are detected by direct sequencing (7), amplification refractory mutation system (ARMS) (7), digital PCR (dPCR) (8,9), next-generation sequencing

(NGS), etc. (10,11). Immunohistochemistry (IHC) with mutation-specific antibodies (E746-A750Del and L858R) is also used to detect *EGFR* mutations and predict the response to *EGFR-TKIs* (12-15).

ALK/ROS1 inhibitors is another group of targeted drugs utilized mostly for the treatment for lung cancer patients with *ALK*- or *ROS1*-rearrangement (16,17). EML4-*ALK* fusion is present in approximately 4–6% of all NSCLC patients (18,19), and *ROS1* rearrangements are present at an even lower frequency (1–2%) (18-20). In clinical application, the results of the *ALK* rearrangement by fluorescence *in situ* hybridization (FISH) and IHC are compared with each other to find the exact result (21). Positive staining of *ROS1* by IHC should be double-checked by molecular assays to exclude false-positive cases because the specificity of IHC testing is not good enough (22). Massively parallel NGS assays are used in some clinical diagnostics to test for gene rearrangements (23,24).

In addition to the alterations of the three most common genes described above, some other lung cancer-related genes also play important roles. The *EGFR* pathway through RAS-RAF-MEK-MAPK and PI3K-AKT-mTOR can be activated by mutations in *KRAS*, *NRAS*, *BRAF*, *ERBB2*, *RET*, *MET*, *FGFR1*, or *PIK3CA*, resulting in resistance to TKIs (25-28), and *TP53*-inactivating mutations could disrupt its key function and are associated with poor prognosis (29).

If all the above molecular alterations need to be detected, a large amount of tumor tissue is necessary, with the exception of NGS. NGS has a considerable advantage in genetic mutation detection, but its sensitivity and accuracy of gene rearrangement detection are not clear. The aim of this study is to integrate the gene mutation and rearrangement alterations in a single targeted NGS panel to investigate the relevant gene alterations, and assess the accuracy of the NGS platform based on the integrated panel.

Methods

Patients and sampling

Formalin-fixed, paraffin-embedded (FFPE) tumor tissues from 107 NSCLC patients were collected from the Department of Pathology, Peking University First Hospital between April 1, 2018 and July 31, 2018. Characteristics of these patients were listed in *Table 1*. Thirty-three cases were core needle biopsies, 16 cases were bronchoscopic biopsies, and 58 cases were surgical resection specimens. All specimens were fixed in 10% neutral buffered formalin overnight. Sectioned tissues were routinely embedded in paraffin. For the patients with *ALK* or *ROS1* fusion, response to the targeted drugs were recorded. The present study was approved by the Ethics Committee of Peking University First Hospital {No. 2016[1111]}.

IHC detection

IHC staining tests were performed to explore the molecular status of *EGFR* (L858R and E746-A750del) on 65 samples, *ALK* rearrangement on 101 samples, and *ROS1* rearrangement on 92 samples (*Figure 1*). IHC staining of tumor tissues was performed on 4-μm sections using the standard procedure and primary monoclonal antibodies against *EGFR* L858R (clone: 43B2, 1:200, Cell Signaling Technology), *EGFR* E746-A750del (clone: 6B6, 1:200, Cell Signaling Technology, Danvers, MA), *ALK* (clone: D5F3, 1:200, Ventana, Tucson, AZ), and *ROS1* (clone: D4D6, 1:200, Cell Signaling Technology). The experiments were performed by standard protocols. A positive result was interpreted as moderate to strong staining of the membrane and/or cytoplasm in >10% tumor cells.

Nucleic acid extraction from tissue samples

DNA was extracted from all the FFPE samples using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilton, Germany) according to the manufacturer's instructions. The DNA was quantified using a Qubit Fluorometer 3.0 (Thermo Scientific, USA). A total mass of more than 20 ng and most fragments above 500 bp were suitable for the following NGS experiments. RNA was extracted from 12 cases with sufficient tissue using the RNeasy FFPE Kit (Qiagen, Hilton, Germany) according to the manufacturer's instructions. The quantity and purity of the extracted RNA was measured using the NanoDrop ND-2000 Spectrophotometer (Thermo Scientific, MA, USA). A concentration higher than 20 ng/μL and an OD260/280 between 1.9 to 2.0 (*Figure 2*) is considered qualified for the quantitative PCR (qPCR) tests.

ARMS

ARMS PCR was performed on 40 cases using the AmoyDx™ Human *EGFR* Mutation Detection Kit (Amoy Diagnostics, Xiamen, China) for the detection of *EGFR*

Table 1 Characteristics of the patients and specimens in our study (n=107)

Variables	Number of patients (%)	Total DNA (median)
Sex		–
Male	59 (55.1)	
Female	48 (44.9)	
Age, years		–
Median	65	
Range	37–85	
Histological type		–
ADC	93 (86.9)	
SCC	12 (11.2)	
ASC	2 (1.9)	
Differentiation		–
Well	1 (0.9)	
Moderate	44 (41.1)	
Poor	25 (23.4)	
Undefined	37 (34.6)	
Specimens type		
Core needle biopsies	33 (30.8)	68.4–6,000.0 ng (800.5)
Bronchoscopic biopsies	16 (15.0)	162.0–2,478.0 ng (1,021.0)
Surgical resection	58 (54.2)	56.8–28,000.0 ng (4,900.0)

ADC, adenocarcinoma; SCC, squamous cell carcinoma; ASC, adenosquamous cell carcinoma.

mutations in DNA sample, and was also performed on 12 RNA samples using the AmoyDx™ Human *EML4-ALK/ROS1* Gene Fusions Detection Kit (Amoy Diagnostics) for the detection of *ALK/ROS1* rearrangements. Each sample was detected with an external control assay and a mutation assay in the same well, while each run contained a negative control and a positive control. The amplification was set up according to the manufacturer's instructions using the ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA). The final run files were also analyzed and interpreted according to the manufacturer's instructions.

NGS platform and analysis pipeline

DNA library preparation and NGS sequencing for these cancer samples were performed according to the manufacturer's recommended protocols of the SGI OncoAim® Lung Cancer Targeting Gene Detection Kit (Singlera Genomics Inc., Shanghai, China). Target regions were captured by designed probes targeting all exons of ten genes, including *ALK*, *BRAF*, *ERBB2*, *EGFR*, *FGFR1*, *MET*, *KRAS*, *NRAS*, *PIK3CA* and *TP53*, and potential gene rearrangement/fusion of *ALK*, *ROS1* and *RET*.

The 150 bp paired-end sequencing was performed using a NextSeq 500 Sequencer (Illumina, Inc., San Diego, CA, USA) in combination with the NextSeq™ 500 High Output Kit (Illumina). Bioinformatics analysis of NGS sequence data was performed according to the guidelines of the OncoAim® Kit (Singlera), with read mapping, quality control, variant calling, and genotyping performed automatically using the Tools Kit supplied in the OncoAim® Kit (Singlera). Hg19/GRCh37 was used as the reference human genome sequence for aligning reads. Variant functional annotation was performed using the ENSEMBL Variant Effect Predictor tool. The minimum confidence threshold for variant and insertion/deletion (indel) calling was set to 5%.

Statistics

Comparisons of gene mutation frequency between adenocarcinoma (ADC) samples and squamous cell carcinoma (SCC) samples were performed using the chi-square test using SPSS v19.0 software. Cohen's κ was calculated to assess the consistency of IHC staining and NGS. Cohen's κ <0.4 was considered weak, 0.4≤ Cohen's κ <0.75 was considered moderate, and Cohen's κ ≥0.75 was considered strong. Significance was assumed for a P value of less than 0.05.

Results

DNA mutations in all patients

A total of 107 NSCLC patients, including 93 ADC, 12 SCC, and 2 adenosquamous carcinoma (ASC) cases, were enrolled in this study. DNA variations were found in 95 of the 107 carcinoma samples (88.79%). In total, 193 mutations in all ten genes and 12 gene rearrangements/fusions of all three genes in the NGS panel were observed (details of gene mutations and gene fusions detected by NGS in all 107 samples are available online: http://fp.amegroups.cn/cms/e5583e904e56e6a4d53175b2dbefdc56/jtd.2019.12.25-1.pdf). Overall, both the *EGFR* gene and the *TP53* gene

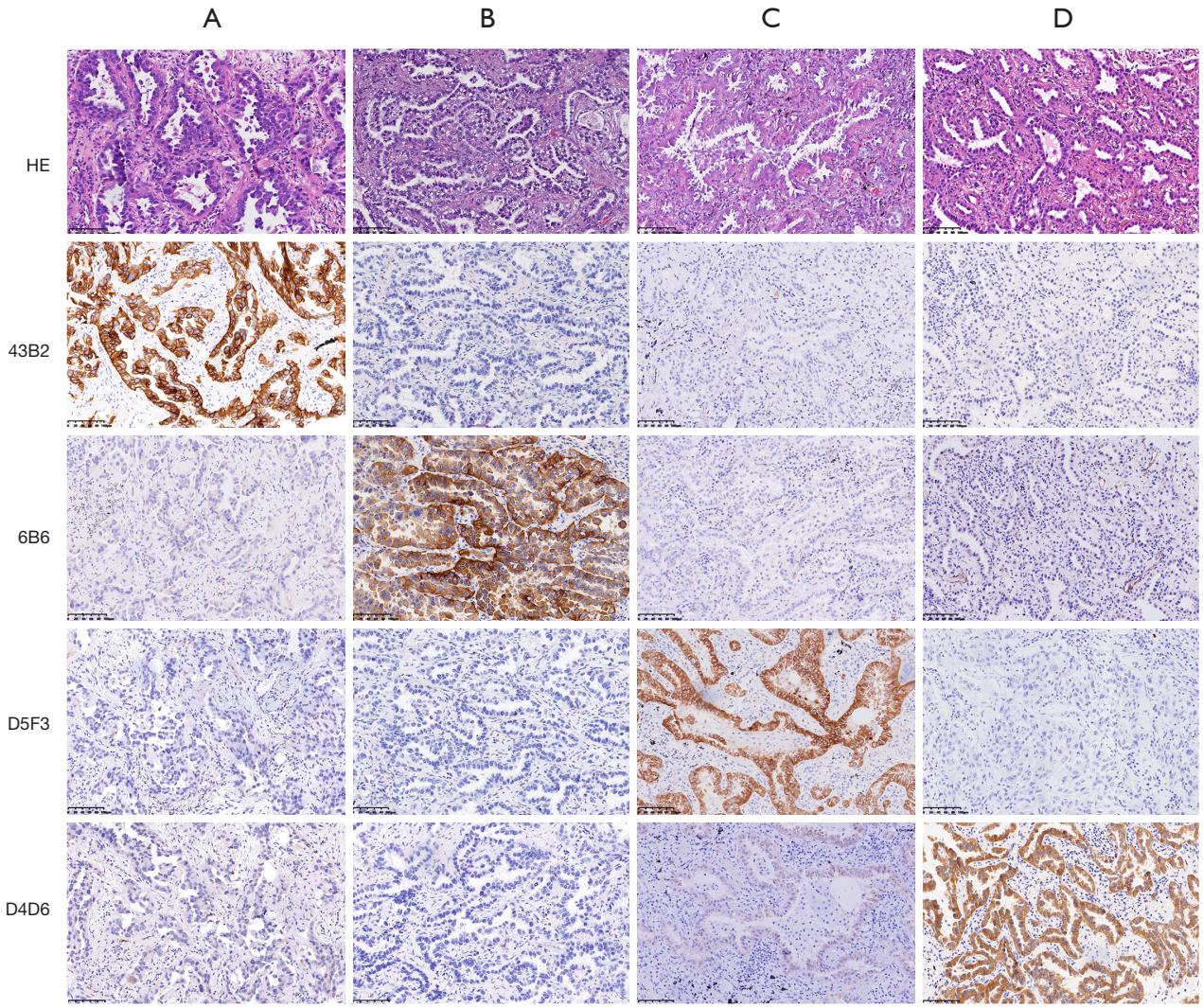

Figure 1 Immunohistochemical staining of EGFR, ALK, and ROS1 in four different cases of lung adenocarcinoma, all of which were consistent with NGS results. Diffuse and strong cytoplasmic and membranous staining of tumor cells with EGFR L858R-specific antibody 43B2 (row A) and EGFR E746_A750-specific antibody 6B6 (row B), and diffuse and strong cytoplasmic staining of tumor cells with ALK (D5F3) antibody (row C) and ROS1 (D4D6) antibody (row D), which were negative for the other three antibodies in each case. Scale bar, 100 μm. NGS, next-generation sequencing.

showed 69 mutations in our cases, which are much higher than that of other genes (*Table 2*). The hot spot mutation *EGFR* L858R and E746_A750del was found in 25 samples (23.4%) and 9 samples (8.4%) respectively, occurred with the two highest frequency. If we consider all types of exon 19 deletions of *EGFR* as one hot spot, we found 17 samples presented 5 types of *EGFR* 19Del, including c.2235_2249del (9 samples), c.2236_2250del (5 samples), c.2240_2257del (1 sample), c.2240_2254del (1 sample), c.2237_2253del (1 sample). Most *EGFR* mutations were found to be located in the *EGFR* kinase domain exon 19–21 in all patients. None or one mutation was observed in all SCC patients for the detected genes except TP53. A total of 11 mutations in the *TP53* gene were found in ten SCC patients (10/12, 83.33%), which is more frequent than that in ADC patients (49/93, 52.69%, P=0.0440). The 12 gene fusions were all

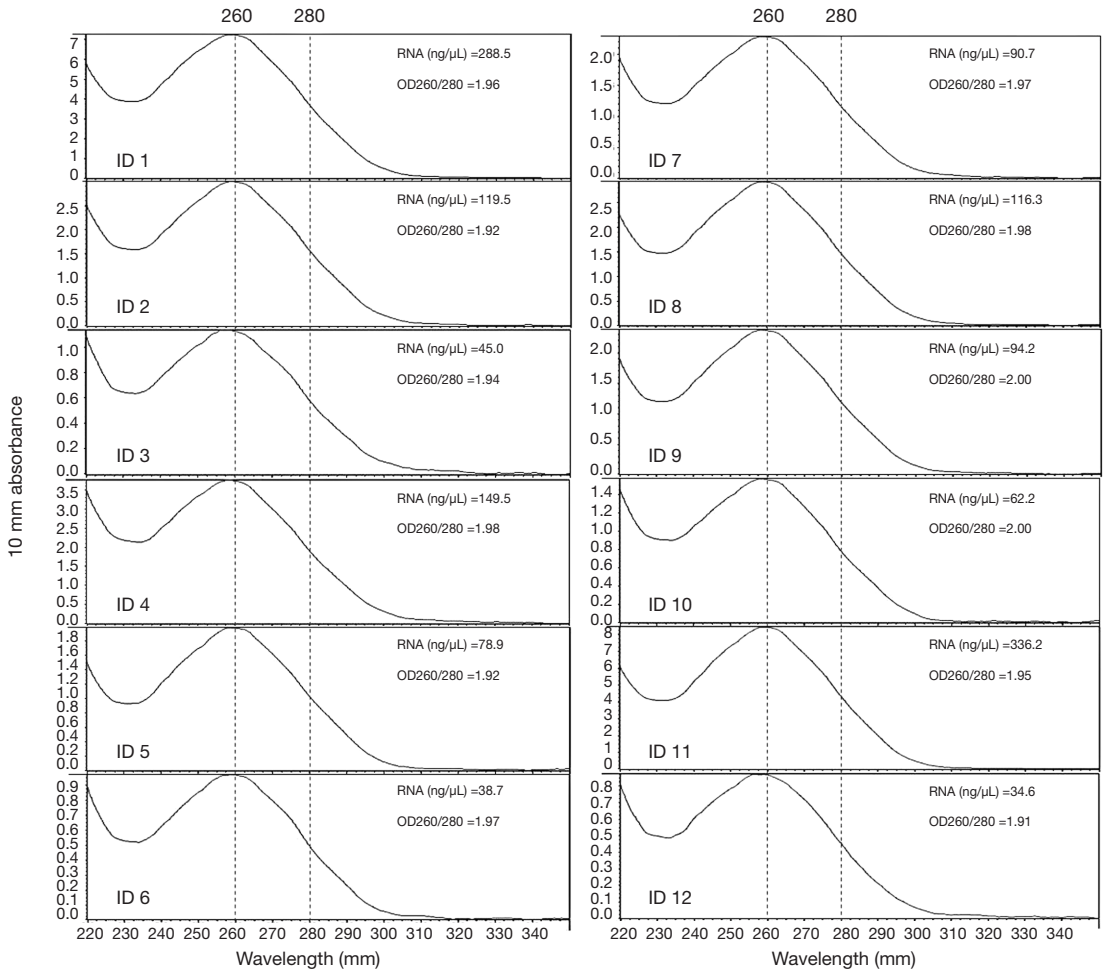

Figure 2 The quantity and purity of RNA extracted from 12 cases.

found in ADC samples (*Table 2*).

Real-time PCR (ARMS) confirmed the sensitivity of NGS

To assess the sensitivity and specificity of NGS for related gene alterations, we used ARMS to double check the gene status of *EGFR* for 40 cases, and *ALK* and *ROS1* for 12 cases (*Table 3*). For one individual (ID 4), an *EGFR* G719C mutation found by NGS was not confirmed by ARMS. For patient ID 5 and ID 37, *EGFR* G719X mutations were observed by ARMS without distinguishing which nucleotide (adenine, thymine, or cytosine) replaced the guanine, whereas the results of NGS showed that guanine in 719 was changed into an adenine (G719A). Similarly, *EGFR* exon 20Ins was observed by ARMS in sample ID 6, and NGS discovered the inserted amino acid sequence and its position, which was shown as M766MASV. For other cases, the results of gene status of both mutations and fusions in the scope of the PCR kit detected by ARMS were completely consistent with that of NGS.

Concordance between NGS and IHC detection

High concordance between NGS and IHC on the detection of hotspots *EGFR* mutations

The results of IHC staining showed that 23.08% of samples were positive for *EGFR* L858R, of which 98.46% (64/65) samples showed consistent results between IHC staining and NGS. One sample was negative by IHC but positive by NGS, and this sample was confirmed to be positive for L858R by ARMS (sample ID 38). In the 65 samples, 9 samples showed either an c.2235_2249del or c.2236_2250del

Table 2 Summary of gene mutations and fusions observed by NGS in all 107 samples

Gene	Spot_Num	Alt_Num	Sample_Num (ADC/SCC/ASC)	Frequency (%)
Mutation				
ALK	9	9	7 (6/1/0)	6.54
BRAF	5	5	5 (4/1/0)	4.67
EGFR	23	68	57 (55/1/1)	53.27
ERBB2	6	7	7 (7/0/0)	6.54
FGFR1	6	6	6 (6/0/0)	5.61
KRAS	7	11	11 (11/0/0)	10.28
MET	6	6	5 (4/1/0)	4.67
NRAS	1	1	1 (1/0/0)	0.93
PIK3CA	11	11	9 (8/1/0)	8.41
TP53	63	69	61 (49/10/2)	57.01
Fusion				
ALK	6	6	5 (5/0/0)	4.67
RET	3	3	2 (2/0/0)	1.87
ROS1	3	3	2 (2/0/0)	1.87

Spot_Num: the number of spots for gene mutation or break points for gene fusion observed in every gene. Alt_Num: the number of mutations or fusions observed for each gene. Sample_Num: the number of altered samples (mutation or fusion) for each gene. Frequency: the frequency of the altered gene (mutation or fusion) in this cohort. NGS, nest generation sequencing; ADC, adenocarcinoma; SCC, squamous cell carcinoma; ASC, adenosquamous cell carcinoma.

alteration, which were documented as positive for E746_A750del in the results of NGS. The same 9 samples were observed to be positive for E746_A750del by IHC staining as well, while all of the remaining 56 samples were negative for E746_A750del by either IHC or NGS, showing a high concordance between these two methods (*Table 4* and *Figure 3*).

NGS could find more *ALK* fusion samples than IHC staining

ALK rearrangement detection by IHC revealed two positive samples (2/101, 1.98%), whereas 5 positive samples were found by NGS (5/101, 4.95%). Among these *ALK*-positive samples, positive results by both IHC and NGS were shown in only one case (*Figure 3*), of which the disease was controlled stable after receiving therapy with alectinib (*Table 5*). For 5 of the 6 patients with *ALK* rearrangement revealed by either NGS or IHC, their tumors were all controlled stable or regressed after treatment with *ALK* inhibitors like crizotinib and alectinib (*Table 5*), indicating the facticity of all these results of *ALK* rearrangement. Based on this hypothesis, sensitivity (83.33%) of NGS was much higher than that (33.33%) of IHC (*Table 4*). The Cohen's κ value of 0.265 suggested a low concordance between these two methods in *ALK* detection.

NGS could exclude the false positivity of *ROS1* fusion detection by IHC

A total of 11 samples were positive for *ROS1* rearrangement according to IHC staining (11/92, 11.96%), of which only two samples were demonstrated to be positive by NGS (2/92, 2.17%). These two samples were found stable of their disease, and one of them was receiving the treatment of crizotinib. Among the nine samples with *ROS1* rearrangement by IHC only (*Table 5*), 6 were demonstrated to harbor *EGFR* mutations in exons 18 to 21 by NGS or ARMS PCR, 2 of which (patient ID 1 and 4) were demonstrated to be negative for *ROS1* rearrangement by PCR amplification. The other three individuals, one was lost to follow-up, one still survived with a short follow-up time, and the last one progressed 3 months after the initial diagnosis, though treated with crizotinib, and died soon (*Table 5*). It was suggested that *ROS1* rearrangement detected

Table 3 Gene alterations of *EGFR*, *ALK* and *ROS1* detected by ARMS and compared with NGS (ARMS/NGS)

Sample ID	Gene alterations
1*	E746-A750Del/E746-A750Del
2*	L858R/L858R
3*	L858R, T790M/L858R, T790M
4*	S768I/G719C, S768I
5*	G719X, L861Q/G719A, L861Q
6*	Exon 20Ins/exon 20Ins
7*	L861Q/L861Q
8*	*ALK* fusion/*ALK* fusion
9*	*ROS1* fusion/*ROS1* fusion
10*	–/–
11*	–/–
12*	–/–
13	Exon 20Ins/exon 20Ins
14	L858R/L858R
15	–/–
16	L858R/L858R
17	E746-A750Del/E746-A750Del
18	E746-A750Del/E746-A750Del
19	–/–
20	L858R/L858R
21	L858R/L858R
22	L858R/L858R
23	E746-A750Del/E746-A750Del
24	E746-A750Del/E746-A750Del
25	L858R/L858R
26	E746-A750Del/E746-A750Del
27	L858R/L858R
28	–/–
29	E746-A750Del/E746-A750Del
30	L858R/L858R
31	E746-A750Del/E746-A750Del
32	L858R/L858R
33	L858R/L858R
34	E746-A750Del/E746-A750Del
35	L858R/L858R
36	L858R/L858R
37	G719X, S768I/G719A, S768I
38	L858R/L858R
39	–/–
40	–/–

Samples [1–12] indicated by asterisk (*) had both *EGFR* mutation and *ALK*/*ROS1* fusion results detected by ARMS. Other samples [13–40] only had EGFR mutation results based on ARMS. ARMS, amplification refractory mutation system.

by IHC only were suspicious. Based on this hypothesis, a little lower specificity (90%) of IHC was observed than that (100%) of NGS (*Table 4*). The NGS results of *ROS1* rearrangement were significantly different from the IHC results (Cohen's κ 0.281, P=0.013).

Discussion

In this study, the NGS panel included not only the mutations found frequently in lung cancer but also 3 "druggable" fusion genes (*ROS1*, *ALK*, and *RET*) by a single NGS test with only 50 ng DNA. The genetic status in our study was in accordance with those in previous studies of NSCLC. For example, *EGFR* and *TP53* mutated most frequently among these related genes (30,31), L858R and 19Del were the most common hot spots of the *EGFR* gene in NSCLC (6), and *TP53* mutations were more prevalent in SCC patients than in ADC patients (31). Additionally, a high concordance was found between the results observed by ARMS and NGS, but more detailed information was revealed by NGS. All these items suggested the high reliability of the NGS experiments employed in our study.

For the results of *EGFR* L858R and E746-A750Del detected by NGS test, IHC staining showed almost the same results for these two molecular statuses. Previous studies (32,33) used two different rabbit monoclonal antibodies recognizing the *EGFR* mutations in exon 19 (E746-A750Del) and exon 21 (L858R) and showed a high sensitivity (91.5–93%) and specificity (100%) of IHC staining for L858R in comparison with direct sequencing.

Table 4 Consistency of IHC staining and NGS for *EGFR* (E746-A750Del, L858R), *ALK*, and *ROS1* detection

Variable	E746-A750Del	L858R	*ALK*	*ROS1*
No. of samples detected by IHC	65	65	101	92
Positive for both	9	15	1	2
Positive for IHC & negative for NGS	0	0	1	9
Positive for NGS & negative for IHC	0	1	4	0
Positive for neither	56	49	95	81
Cohen's κ	1	0.958	0.265	0.281
P value for κ	0.000*	0.000*	0.097	0.013*
Sensitivity for IHC, %	100	93.75	33.33	100
Specificity for IHC, %	100	100	100	90
Sensitivity for NGS, %	100	100	83.33	100
Specificity for NGS, %	100	100	100	100

*, indicates a significant P value that is <0.05. IHC, immunohistochemistry; NGS, next-generation sequencing.

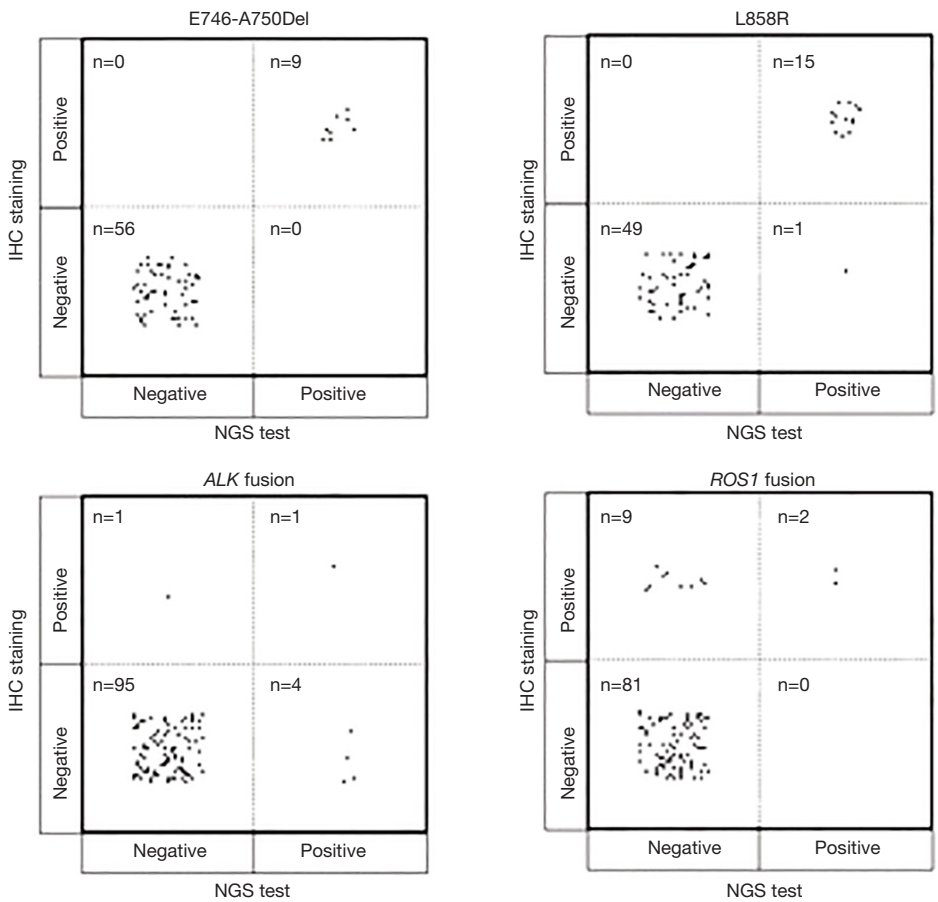

Figure 3 Results of IHC staining and NGS for the *EGFR* E746-A750Del, L858R, *ALK*, and *ROS1* detection. "n" in the figure indicates the number of samples. IHC, immunohistochemistry; NGS, next-generation sequencing.

Table 5 The treatment and follow-up of the patients with gene fusion by IHC and/or NGS

Sample ID	Sex	Age, years	EGFR status	Medicine treated	PFS until now (months)	Disease status
ALK fusion observed by IHC only						
15	Male	37	Wild type	Crizotinib	33	Stable
ALK fusion observed by NGS only						
57	Male	62	Wild type	Crizotinib	19	Stable
60	Male	54	Wild type	Alectinib	6	Stable
77	Male	47	Wild type	Crizotinib	18	Stable
90	Female	44	E282K	None	18	Stable
ALK fusion observed by IHC and NGS						
8	Male	46	Wild type	Alectinib	18	Stable
ROS1 fusion observed by IHC only						
1	Female	59	19Del	None	22	Stable
4	Male	76	S768I		Lost to follow-up	
19	Female	79	Wild type	None	42	Stable
20	Female	62	L858R	None	30	Stable
37	Female	51	G719A, S768I	EGFR-TKI	18	Stable
41	Female	72	Wild type		Lost to follow-up	
54	Male	80	19Del	EGFR-TKI	3	Stable
75	Female	62	Wild type	Crizotinib	3	Dead
82	Female	68	L858R		Lost to follow-up	
ROS1 fusion observed by IHC and NGS						
9	Male	64	Wild type	Crizotinib	16	Stable
86	Female	66	Wild type	None	18	Stable

IHC, immunohistochemistry; NGS, next-generation sequencing.

Our present study suggested that both IHC and NGS techniques are reliable for the clinical use of *EGFR* L858R detection. The unique type of DNA change for the mutation of L858R may contribute to the high consistency between IHC and direct or NGS sequencing. However, direct sequencing can find several types of DNA changes around the *EGFR* E746-A750 region, some of which could give different amino changes when detected with the antibodies used in IHC staining, resulting in a lower sensitivity (63–77%) of E746-A750Del by IHC compared with direct sequencing in the previous studies (32,33) mentioned above. According to the NGS results, we could distinguish the different types of alterations in this region. We could also easily detect the exacted DNA changes occurring in the E746-A750Del, which could be observed by IHC staining with the mutation-specific antibody, and improved the consistency of IHC and NGS to 100% in our study. At the same time, more information, including single nucleotide variants (SNVs) in exons 18–21 and other types of *EGFR* 19Del, could be determined by NGS, thereby predicting the response to the *EGFR-TKIs* more reliably, rather than IHC staining (34).

The results of the *ALK* fusion detection by NGS showed an acceptable frequency of 4.7%, which was similar to the reported rate of 4–8% (18,19). Detection of *ALK* rearrangement by protein-based IHC staining usually showed a higher sensitivity than other methods, such as DNA-based FISH and mRNA-based RT-PCR (35). The criteria for FISH positive results are artificial and depend too much on the subjectivity of the pathologist (18). The

RT-PCR method relies on primers that cannot consider all types of *ALK* fusion. However, any effective fusion will result in the fusion protein, which can be observed by IHC staining. NGS shows a higher sensitivity than FISH in detecting *ALK* rearrangements (36). However, no matter whether IHC staining, NGS, FISH or RT-PCR was used, it cannot achieve an accuracy rate of 100% on the *ALK* rearrangement detection (37). In our study, one sample was *ALK* positive for IHC staining but negative for NGS, of which the break point of *ALK* in this sample may be outside of the panel used in the NGS test. And this *ALK* fusion was confirmed to be true positive by the response of the patient to the targeted therapy. In contrast, *ALK* fusion identified by NGS but negative by IHC staining were found in four individuals, most of which the reality of these gene fusion was confirmed by their responses to the targeted treatment. Based on these results, *ALK* positive by either IHC or NGS deserved serious consideration. While in the situation of *ALK* negative identified by IHC or NGS only, more targeted methods like FISH and RT-PCR should be performed to avoid missing the potential opportunity to benefit from the targeted drugs.

In our study, 11 samples (11/92, 11.96%) were positive for *ROS1* by IHC staining, of which 9 samples were *ROS1* negative by NGS. The positive rate of *ROS1* by IHC staining in our study was unrealistically higher than that detected by NGS (1.87%), as well as that reported in existing publications (1–2%) (18-20). It was extremely rare that patients harbor both *EGFR* mutation and *ROS1* fusion. There was only one reported case that simultaneous L858R mutation and *ROS1* fusion occurred in a single NSCLC patient with intrinsic gefitinib resistance (38). In our cohort, more than a half of samples with *ROS1* fusion identified by IHC only showed simultaneous *EGFR* mutation by NGS or ARMS PCR, 2 of whom responded to the *EGFR-TKI*, which made the IHC results extremely suspicious. In previously published studies (18,39), *ROS1* positive rates were commonly presented with different results when multiple methods were used in one cohort, and it has been suggested that IHC was not sensitive enough to determine *ROS1* rearrangement. *ROS1* staining could be observed in certain nonneoplastic conditions, including normal pneumocytes and bronchiolar metaplasia, which might result in false-positive staining for *ROS1* rearrangement detection by IHC staining (40,41). Our results were in concordance with the conclusion that the sensitivity of *ROS1* detection by IHC staining is much better than its specificity (22,39). IHC is an effective screening method for avoiding other redundant analyses in patients with *ROS1* negative results (39). However, for *ROS1*-positive samples detected by IHC staining, it is necessary to perform other analyses, such as FISH or NGS, for further validation.

The mutation frequencies of the remaining genes detected by NGS was in accordance with those in reported studies of NSCLC, such as KRAS (8.93–30%), MET (5%), ERBB2 (1.79–4%), FGFR1 (1–3%), NRAS (<2%), PIK3CA (5.36%) and BRAF (1.79–6%) (30). These results suggest that NGS could comprehensively investigate the status of many lung cancer-related genes in addition to popular druggable genes (*EGFR*, *ALK*, and *ROS1*).

Conclusions

Various gene mutations and gene rearrangements could be detected by a single platform of NGS, which showed high performance and cost-effectiveness. NGS could provide more informative and reliable results than IHC staining for *EGFR* gene alterations, especially for the exon 19 region. For gene fusion detection, NGS increased the positive rate of *ALK* and decreased the false-positive results of *ROS1* compared with IHC staining. Thus, we suggest that in the samples with *ALK* negative and/or *ROS1* positive observed by IHC only, it is necessary to perform other analyses like NGS for confirmation.

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Footnote

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