Improvement strategies for successful next-generation sequencing analysis of lung cancer

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Aim: We aimed to improve the success rate of NGS (next-generation sequencing) analysis through improved strategies of lung cancer sampling. **Materials & methods:** The improvement strategies are as follows. Surgically resected specimens were preferentially submitted in cooperation with pathologists and surgeons. In bronchoscopic samples, the size of the sample collection device and the number of samples collected was increased. **Results:** The strategies increased the success rate of NGS analysis of DNA from 69.3 to 91.1%, and that of RNA from 64.6 to 90.0%. **Discussion:** The introduction of strategies aimed at improving the success of NGS analysis resulted in an improvement in the success rate and brought us closer to the delivery of effective precision medicine in cancer therapy.

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Keywords: lung cancer • next-generation sequence • precision medicine • sampling • success rate

Lung cancer is a leading cause of cancer-related deaths worldwide [1]. In the past decades, genetic and genomic profiling of non-small-cell lung cancer (NSCLC) has progressed considerably, and our understanding of the underlying molecular mechanisms of the disease has improved leading to the development of strategies for genome-guided targeted therapies [2,3]. For many advanced NSCLC patients harboring certain actionable driver mutations including activating *EGFR* mutations, *ALK* fusion genes, and *ROS1* fusion genes, targeted tyrosine kinase inhibitors provide significant improvement in survival and quality of life [4,5]. In addition, it is strongly recommended that more extensive molecular profiling be performed to detect rare mutations; including *BRAF*, *KRAS*, *MET* mutations and *RET* fusion genes, for which the corresponding targeted therapies are available or options for off-label treatment in clinical trials exist [6,7]. At the same time, the identification of those patients who have the actionable driver mutations using next-generation sequencing (NGS) represents a rapid method of identifying genetic biomarkers in clinical practice. The advances in NGS in clinical diagnostics has revolutionized clinical medicine including the field of lung cancer [8]. NGS analysis has enabled us to overcome several specific limitations of direct sequencing and allele-specific molecular testing.

To provide effective treatment options for advanced NSCLC patients harboring rare targetable driver mutations, a nationwide biomarker screening project was launched in Japan in 2013. The lung cancer genomic screening project for individualized medicine (LC-SCRUM) in Japan is a nationwide cancer genomic screening network for the development of novel molecular targeted therapies for lung cancer [9,10]. From February, 2013 up to May, 2019, a total of 263 institutions have been participating in the project and a total of 7775 lung cancer patients have been enrolled. The project was initially started with the screening of *RET/ROS1/ALK* fusion genes by RT-PCR and fluorescent *in situ* hybridization, and from 2015, a targeted NGS analysis with Oncomine™ comprehensive assay has been implemented. Based on the achievements of LC-SCRUM-Japan, in June 2019, Oncomine Dx

Figure 1. NGS analysis success rate. The success rates of DNA, RNA-based NGS analysis from 70 centers participating in the lung cancer genomic screening project for individualized medicine Japan project as presented at the lung cancer genomic screening project for individualized medicine plenary session in Japan in November 2018. Our facility was the third one from the bottom.

LC-SCRUM: Lung cancer genomic screening project for individualized medicine; NGS: Next-generation sequencing.

Target Test CDx System has received reimbursement coverage from Japan's Ministry of Health, Labor and Welfare and is now commercially available in Japan.

In the workflow of LC-SCRUM-Japan, the secretariat reported about the yields of extracted DNA and RNA, and the results of NGS analysis. We have been concerned about the low success rate of NGS analysis in our institution, which was announced in November 2018 (Figure 1). To overcome this challenge, we drastically revised the sampling procedures and reviewed the sample quality assessment methods in an attempt to improve the success rate of NGS analysis in our institution. In this study, we report the improvement strategies for NGS analysis in the LC-SCRUM project.

Materials & methods

Patients

LC-SCRUM-Japan is a prospective, nationwide clinical and genomic screening program for lung cancer (UMIN ID: UMIN000010234). In this program, a total of 7775 NSCLC patients were enrolled between February 2013 and May 2019. All patients provided written informed consent for the enrolment in the LC-SCRUM-Japan program. LC-SCRUM initially started genome screening for *RET*, *ROS1*, and *ALK* fusion genes using RT-PCR and FISH at the SRL Incorporation (Tokyo, Japan) which is a CLIA (Clinical Laboratory Improvement Amendments) certified laboratory. From 2015, the NGS assay, called Oncomine comprehensive assay (version 1), was adopted for genome screening of *EGFR* mutation-negative NSCLC patients in collaboration with 17 pharmaceutical companies; this constituted the first phase of LC-SCRUM-Japan. From 2017, all NSCLC patients, irrespective of *EGFR* mutations, were subjected to the genome screening which constituted the second phase of LC-SCRUM-Japan. The second

Figure 2. Improvement strategy for transbronchial sampling.

†The actual content of tumor cells cannot be assessed.

stage registration of NSCLC patients ended by May 2019, and after the maintenance of the screening system, the target of screening was expanded to Asia. The third phase has now started.

We retrospectively reviewed records of all patients who were enrolled in the second phase of LC-SCRUM-Japan in our institution. We divided the patients into two groups, those before January 2019 (called the ‘before group’), when the improvement approaches were introduced and those after January (called the ‘after group’). Clinical information pertaining to each group and the success rate of LC-SCRUM-Japan were collected. We divided the patients into groups starting from January 2019 because we reviewed and improved our bronchoscopy procedures following the report on NGS success rate at the LC-SCRUM plenary meeting in November 2018.

Improvement strategies for NGS analysis

We introduced the following improvements in the sampling method in our institution beginning in January 2019. First, the surgical biopsy specimens were preferentially submitted in collaboration with pathologists and surgeons. Second, during sampling using transbronchial biopsy, the standard biopsy forceps (FB-231D.A; Olympus, Tokyo, Japan) with a 5.0 mm cup opening was used instead of the one with narrower cup opening [11]. Third, during sampling using endobronchial ultrasound with real-time guided transbronchial needle aspiration (EBUS-TBNA), whenever possible, the biopsies were performed using a 19-gauge needle and the samples submitted for NGS were collected at least twice. Fourth, before and after sampling with transbronchial biopsy for NGS analysis, specimens were also collected for pathological analysis to confirm that the freshly obtained samples contained tumor cells (Figure 2). Finally, all the samples were submitted following specimen evaluation by pathologists. In all the sampling methods, we used rapid on-site evaluation of tumor cells [12].

DNA & RNA extraction & NGS reports

In this study, DNA and RNA samples were extracted from fresh frozen specimens or pleural effusions. These samples were analyzed using a targeted NGS assay, the Oncomine comprehensive assay version 3 (OCA v3; Thermo Fisher Scientific, MA, USA) at the laboratories of SRL incorporated. A target region containing 161 genes was amplified using multiplex PCR for DNA and RNA, and the somatic mutations in the region were detected. Hotspot mutations (single nucleotide variants; SNVs, deletions and insertions) and copy number variations (CNVs) were detected in the DNA-based sequences, and fusion gene alterations were detected in RNA-based sequences. The secretariat of LC-SCRUM-Japan reported on the concentration of extracted DNA and RNA, and the result of NGS analysis. Of note, fusion gene analysis was not planned for the cases with small cell lung cancer, and only DNA-based NGS analysis was performed. Of the multiple somatic mutations analyzed in the OCA v3. panel, 20 mutations, which have been reported to be associated with pathogenesis of lung cancer and to have the corresponding therapeutic agents including unapproved drugs, were reported to each attending physician. These mutations included *RET*, *ALK* and *ROS1* fusion genes; *FGFR* 1–4 gene mutations, amplification and fusions; *MET* and *ERBB2* gene mutations and amplification; *AKT1*, *BRAF*, *HRAS*, *KRAS*, *NRAS*, *EGFR* and *PIK3CA* gene mutations; and *NTRK1-3* fusion genes and *NRG1* fusion gene. When the extracted amounts of DNA and

Table 1. Clinical characteristics of enrolled patients and sampling methods.

Characteristic	Before group (n = 88)	After group (n = 45)	p-value
Age (years)			
Median	65 (36–83)	65 (32–81)	NS (0.686)
Gender			
Male	62 (70.5)	26 (57.8)	NS (0.176)
Female	26 (29.5)	19 (42.2)	
Smoking			
Never	19 (21.6)	11 (24.5)	NS (0.23)
Former	50 (56.8)	19 (42.2)	
Current	19 (21.6)	15 (33.3)	
Histology			
Ad	59 (67.0)	36 (80.1)	NS (0.124)
Sq	10 (11.4)	2 (4.4)	
NSCLC	13 (14.8)	2 (4.4)	
SCLC	6 (6.8)	5 (11.1)	
Stage			
IIIA,B	6 (6.8)	3 (6.7)	NS (0.974)
IV	82 (93.2)	42 (93.3)	
Sampling methods			
Bronchoscopy:	70 (79.5)	28 (62.2)	0.0284
– Transbronchial niopsy	60 (85.7)	22 (78.6)	
– EBUS-TBNA	10 (14.3)	6 (21.4)	
CT-guided needle biopsy	7 (8.0)	2 (4.4)	
Pleural effusion	1 (1.1)	1 (2.3)	
Surgical biopsy	10 (11.4)	14 (31.1)	
Data represented as n (%), unless otherwise stated. Data collected from July 2017 to December 2019. CT: Computed tomography; EBUS-TBNA: Endobronchial ultrasound-guided transbronchial needle aspiration; NS: Not significant; NSCLC: Non-small-cell lung cancer; SCLC: small cell lung cancer.			

RNA did not meet the specified criteria for NGS analysis, it was reported that the amount of the nucleic acids was insufficient. In this study, we defined analysis failure when NGS analysis was not accomplished for reasons including insufficient DNA or RNA sample volumes.

Comparison of success rates of NGS analysis before & after introduction of the improved strategies

We compared the success rate of NGS analysis, the method of collecting submitted samples, and the amount of DNA and RNA before and after the introduction of the improvement strategies based on the electronic medical records and the secretariat reports.

Statistical analysis

The R commander statistical functions package, Easy R (EZR), was used for statistical analyses such as two-sample t-test, Kruskal–Wallis rank sum test and Pearson's χ^2 test [13].

Results

A total of 133 patients enrolled in our institution in the second phase of LC-SCRUM-Japan, including 88 in the before group and 45 in the after group. The clinical characteristics of the patients are listed in Table 1. While there were no significant differences between the two groups in terms of age, sex, smoking history, lung cancer histology and clinical stage, there appeared to be an increased frequency of submission of surgical samples for NGS following the introduction of the improvement strategies. All bronchoscopic samples from the after group were confirmed to contain tumor cells by pathological analysis of formalin-fixed paraffin embedded (FFPE) specimens before and after the submission of frozen specimens. We suspended the submission of samples if they did not contain tumor cells either before or after the samples were submitted.

Figure 3. DNA and RNA yields of success and failure groups and by each sampling method. (A) Comparison of DNA and RNA yields ($\mu\text{g}/\mu\text{l}$) with the failure and success groups in the before group. (B) Comparison of DNA and RNA yields ($\mu\text{g}/\mu\text{l}$) among sampling methods. * $p < 0.05$; ** $p < 0.05$. CT: Computed tomography.

The effect of the DNA and RNA yields on the success and failure of the NGS analysis were compared in the before group. The mean and median yields of DNA and RNA in the success group were 0.10 and 0.08 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.39 $\mu\text{g}/\mu\text{l}$), and 0.05 and 0.07 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.97 $\mu\text{g}/\mu\text{l}$), respectively. While in the failure group, they were 0.01 and 0.01 $\mu\text{g}/\mu\text{l}$ (range, 0.01 $\mu\text{g}/\mu\text{l}$), and 0.02 and 0.01 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.97 $\mu\text{g}/\mu\text{l}$), respectively (Figure 3A). In the success group, significantly higher amounts of both DNA and RNA were obtained from the samples. Furthermore, the DNA and RNA yields were compared according to sampling methods in the before group (Figure 3B). The mean and median yields of DNA and RNA were 0.05 and 0.02 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.39 $\mu\text{g}/\mu\text{l}$), and 0.03 and 0.01 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.23 $\mu\text{g}/\mu\text{l}$) in the bronchoscopic samples ($n = 70$); 0.04 and 0.03 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.08 $\mu\text{g}/\mu\text{l}$), and 0.03 and 0.01 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.10 $\mu\text{g}/\mu\text{l}$) in computed tomography (CT)-guided samples ($n = 7$); and 0.19 and 0.20 $\mu\text{g}/\mu\text{l}$ (range, 0.04–0.36 $\mu\text{g}/\mu\text{l}$), and 0.16 and 0.10 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.59 $\mu\text{g}/\mu\text{l}$) in surgical samples ($n = 10$). There was only one sample from pleural effusion in our study and the DNA and RNA yield in the pleural effusion sample was 0.37 and 0.97 $\mu\text{g}/\mu\text{l}$, respectively. The DNA and RNA yields from surgical samples tended to be higher than those obtained by other means.

After the introduction of the improvement strategies, the success rate of NGS analysis of DNA increased from 69.3% to 91.1%, and that of RNA increased from 64.6% to 90.0% (Table 2). There was a significant increase in the success rate of NGS for both DNA and RNA analyses. In bronchoscopic samples, excluding EBUS-TBNA samples, the NGS analysis success rate improved from 58.3% to 95.5% for DNA analysis, and from 56.7% to 78.9% for RNA analysis. In samples obtained by EBUS-TBNA, the rate of DNA analysis was maintained at 100%, but the success rate of RNA analysis improved from 50% to 100%. Although the number of CT-guided biopsy samples submitted decreased in the after group and the two specimens submitted for RNA analysis failed, both were successfully analyzed for DNA. There were no significant changes in the before and after groups in the surgical biopsy samples, and the success rates of both DNA and RNA analyses were optimum.

Table 2. Change in next-generation sequencing analysis success rate.

Variable	Before group	After group	p-value
DNA NGS analysis (%)			
Total	61/88 (69.3)	41/45 (91.1)	0.0049
Sampling methods			
Bronchoscopy			
Transbronchial biopsy	35/60 (58.3)	21/22 (95.5)	
EBUS-TBNA	10/10 (100)	6/6 (100)	
CT-guided needle biopsy	5/7 (71.4)	2/2 (100)	
Pleural effusion	1/1 (100)	1/1 (100)	
Surgical biopsy	10/10 (100)	14/14 (100)	
RNA NGS analysis (%)			
Total	53/82 (64.6)	36/40 (90.0)	0.0127
Sampling methods			
Bronchoscopy			
Transbronchial biopsy	33/58 (56.7)	15/19 (78.9)	
EBUS-TBNA	4/8 (50)	5/5 (100)	
CT-guided needle biopsy	5/5 (100)	0/2 (0)	
Pleural effusion	1/1 (100)	1/1 (100)	
Surgical biopsy	10/10 (100)	12/13 (92.3)	

CT: Computed tomography; EBUS-TBNA: Endobronchial ultrasound-guided transbronchial needle aspiration; NGS: Next-generation sequencing.

Figure 4. Comparison of DNA and RNA yields ($\mu\text{g}/\mu\text{l}$) between before and after groups in transbronchial biopsy and endobronchial ultrasound-guided transbronchial needle aspiration samples.* $p < 0.05$.

EBUS-TBNA: Endobronchial ultrasound-guided transbronchial needle aspiration; NS: Not significant.

We compared the yields of DNA and RNA from transbronchial biopsy and EBUS-TBNA samples (Figure 4). In the transbronchial biopsy samples, the mean and median DNA and RNA yields in the before group were 0.05 and 0.01 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.39 $\mu\text{g}/\mu\text{l}$), and 0.03 and 0.01 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.12 $\mu\text{g}/\mu\text{l}$), respectively. While the mean and median DNA and RNA yields in the after group were 0.09 and 0.09 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.25 $\mu\text{g}/\mu\text{l}$), and 0.02 and 0.01 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.08 $\mu\text{g}/\mu\text{l}$), respectively. After the introduction of the improvement strategies, there was a significant improvement in the DNA yield but no significant change in the RNA yield. In the EBUS-TBNA samples, the mean and median DNA and RNA yields in the before group were 0.07 and 0.08 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.10 $\mu\text{g}/\mu\text{l}$), and 0.05 and 0.01 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.23 $\mu\text{g}/\mu\text{l}$), respectively, while that in the after group were 0.19 and 0.19 $\mu\text{g}/\mu\text{l}$ (range, 0.07–0.31 $\mu\text{g}/\mu\text{l}$), and 0.20 and 0.04 $\mu\text{g}/\mu\text{l}$ (range, 0.01–0.87 $\mu\text{g}/\mu\text{l}$),

Figure 5. The list of detected targetable driver mutations in this study. The number of patients is represented by a bar graph and the mutation profile is shown in the table.

respectively. The DNA and RNA yields from EBUS-TBNA samples showed a trend similar to that observed in transbronchial biopsy samples.

Reported targetable driver mutations were detected in 50 out of the 133 patients screened. A total of 53 mutations were found, as shown in Figure 5. Three patients had multiple genetic mutations at the same time and their mutation profiles were as follows [2]: *EGFR* Ex.19 del and T790M; *EGFR* Ex.19 del and *MET* amplification; and *EGFR* Ex.19 del and *PIK3CA* E545K. Four (three patients with *KIF5B-RET* fusion and one with *EML4-ALK* fusion) out of the 50 patients with targetable driver mutations were successfully enrolled in clinical trials of unapproved drugs.

In the after group, two patients (one with *KIF5B-RET* fusion and one with *CD74-ROS1* fusion) were found to have poor quality of initial transbronchial biopsy samples, including low tumor cell content. However, they underwent successful NGS analysis, either by switching to surgical biopsy or EBUS-TBNA samples for biomarker retrieval. Repeated and improved biopsies allowed these patients to be administered drugs that matched their targetable driver mutations.

Discussion

NGS analysis begins with clinical ordering, followed by wet bench operations such as DNA extraction and library creation, followed by dry bench operations for sequence data processing, and proceeds through a series of workflow in which the data is fed back to the clinic [14,15]. This study focused on improving the pre-analytic and the earliest phase. The clinical NGS workflow has several processes, and involves several people. Therefore, for NGS success, it is essential for every element to work well. Sampling is the first and one of the most important steps in this process. It is important for the physicians involved in sampling to meticulously collect samples for the NGS analysis and share all relevant information pertaining to the sampling with the pathologist [15,16]. In LC-SCRUM-Japan, the protocol required the submission of fresh frozen tissue samples, and therefore the evaluation of tumor cell content in the submitted samples was problematic. In our study, we collected samples before and after the submission of samples, and made efforts to submit fresh samples that were highly likely to contain tumor cells by evaluation of the FFPE specimens before and after submission of samples. Although formalin is widely used as a fixative, it has a negative effect on the integrity of the DNA and causes nucleotide transitions and DNA fragmentation [17–19]. There are several reports that FFPE samples are comparable to fresh tissue samples and that NGS analysis (including

RNA analysis) is viable in these samples [20–23]. However, it is certain that damage to nucleic acids is reduced when formalin is not used. When fresh tissues from bronchoscopic samples are used for NGS analysis, one approach is to obtain tissue samples before and after the procedure, as in our study, and to indirectly confirm the presence of tumor cells. Another way to evaluate tumor cells in fresh tissue is to identify them by touching the cut surface of fresh tissues samples to a slide followed by analysis [24,25].

An increase in the success rate of NGS analysis with bronchoscopic samples contributed to the increase in the success rate of NGS because of the introduction of improvement strategies. For EBUS-TBNA samples, the success rate of RNA NGS analysis improved from 50 to 100% (Table 2). Previous studies have shown that DNA NGS analysis can be sufficiently successful if sufficient tumor cells are visible in EBUS-TBNA samples [26–29]. Only one of these studies examined RNA NGS from EBUS-TBNA samples, and their analysis showed that yield of RNA extracted from EBUS-TBNA FFPE samples was less than that from CT-guided needle biopsy or transbronchial biopsy, with a 82% success rate (9/11) for RNA NGS analysis [26]. In our study, in the after group, the success rate for RNA NGS analysis was 100% (5/5). We used a 19-gauge needle and attempted sample collection at least twice. All the previous studies used a 22-gauge needle for sampling [26–29]. The amount of EBUS-TBNA samples was clearly higher after the introduction of the improvement strategies than before (Supplementary Figure 1), and submitting fresh tissue samples may have contributed to the improved success rate of RNA NGS analysis. Previous reports suggest that EBUS-TBNA using 22-gauge may be sufficient to achieve successful DNA NGS analysis, but successful RNA NGS analysis may require the use of a 19-gauge needle or an increase in the volume of the sample submitted. However, there was no significant improvement in the RNA yield from bronchoscopic samples even after the introduction of improvement strategies. The success rate of RNA-NGS analysis was significantly improved, but utilization of RNA stabilization solution may be necessary to increase the RNA yields from bronchoscopic samples [30,31].

There are few reports on the feasibility of CT-guided biopsy samples for NGS analysis [26]. CT-guided biopsy samples tend to be larger than bronchoscopic or EBUS-TBNA samples, and the success rate of NGS analysis was comparable to that with bronchoscopic or EBUS-TBNA samples, although less so than with surgical biopsies [26]. In this study, we did not plan any improvement strategy for CT-guided sampling. In the after group, only two CT-guided biopsy samples were submitted. The DNA NGS analysis was successful, but RNA NGS analysis failed for both. We reviewed the hematoxylin and eosin stained slides of the CT-guided samples at the time of submission of fresh frozen sample, and found that the variable tumor cell content was approximately 30% in both samples. In one case, we reviewed two sections of CT-guided biopsy sample and found that there were no tumor cells in one section with predominantly fibrocytes and inflammatory cells. Even in the one case in the before group, in which the DNA NGS analysis failed, we found that the variable tumor cell number was very low compared with the size of the sample (Supplementary Figure 2). Although the size of CT-guided sample is relatively large, it may contain fewer tumor cells and more fibrous tissue as the needle is inserted from the body surface into the lesion. Prior to submission for NGS analysis, additional microdissection may be necessary to increase the tumor cell content.

A surgical specimen is the most ideal specimen for NGS analysis if sufficient tumor volume can be secured and a variety of tumor tissues are represented, including macroscopically removed necrotic areas. In the present study, both before and after groups showed good success rates for both DNA and RNA NGS in surgical samples. Only in one case in the after group, the RNA NGS failed. In this case, the tumor lesion was very small (Supplementary Figure 3). It is often difficult to obtain surgical specimens before treatment for advanced lung cancers [26]. Frozen samples are collected after confirmation by the pathologist. Cooperation between the surgeon and pathologist is essential to link fresh frozen surgical samples to NGS analysis. The National Cancer Institute initiated Biospecimen Preanalytical Variables (BPV) program revealed the effects of a delay to fixation (DTF) or time in fixative (TIF) on the quantity and quality of DNA and RNA isolated from FFPE samples [32]. Exposure of surgical samples to formalin for TIF over 72 h or DTF for over 12 h significantly impairs the quantity and quality of DNA and RNA [32,33]. Both, the DTF by the surgeon and TIF by the pathologist significantly affect the quantity and quality of DNA and RNA. In addition, in the case of collection and preservation of fresh samples, since the surgical method of cutting and excising the specimen greatly influences the pathological diagnosis of the surgical specimen, the sampling of resected the specimen must be preserved with the cooperation of the pathologist.

Among the 50 patients with targetable driver mutations detected by LC-SCRUM-Japan screening in this study, all three patients detected with the *KIF5B-RET* fusion gene enrolled in the study at a relatively early stage following diagnosis [34]. For *EGFR* mutations, *BRAF* V600E mutation, *ALK* fusion gene, and *ROS1* fusion gene, there were approved molecular targeted drugs that were covered by insurance [3–6]. For *KIF5B-RET* fusion gene, however,

there were no insurance-approved drugs and only investigational drugs were available during the study period. In general clinical practice, genomic detection methods do not exist except for the screening in LC-SCRUM-Japan. Therefore, in the case of these three patients, successful gene screening through LC-SCRUM-Japan enabled the delivery of effective treatments that matched their driver gene mutations. The development of molecular targeted drugs against *KRAS* mutations has recently led to the emergence of promising agents against these mutations [35,36]. AMG 510 is in Phase I/II trials for *KRAS* G12C mutation. Several *KRAS* mutations (second in number to *EGFR* mutations) were detected in this study (Figure 3) and we are close to delivering promising therapies to patients with these mutations.

Conclusion

In conclusion, to improve the success rate of NGS analysis, we improved the sampling method by collecting as many samples as possible and also strengthened the cooperation with pathologists and surgeons during sample collection. Clinical sampling is the entry point to the NGS workflow, and success of the NGS workflow depends on good sample collection at this stage, and thus this is the most important step. There is no time to lose in the treatment of patients with advanced lung cancer. It is indispensable to clarify the genetic profile of the lung cancer as soon as possible from the time of initial diagnosis to deliver the best precision medicine. In the future, the development and clinical introduction of promising molecular targeted drugs are expected to advance rapidly, and the significance of successful NGS analysis will increase. In this context, we, the physicians, need to work closely with the pathologists, surgeons and bioinformaticians to ensure a smooth NGS workflow to realize the promise of precision medicine for the patient.

Summary points

- Next-generation sequencing (NGS) analysis is now essential in the practice of precision medicine, and failure of NGS analysis can delay the introduction of therapy.
- Although NGS analysis is based on a multi-step work flow, clinical sampling is the first and most important step.
- For successful NGS analysis, it is necessary to obtain a sufficient quantity of clinical samples with sufficient tumor cells.
- Because the initial diagnosis of advanced-stage lung cancer is mainly based on bronchoscopy, in which only relatively small-sized samples can be obtained, special efforts are required to obtain sufficient specimens for NGS analysis.
- By increasing the size of the bronchoscopic biopsy forceps, or by increasing the number of samples collected, efforts to obtain a sufficient amount of samples can result in sufficient success in NGS analysis of bronchoscopic specimens.
- Cooperation between the surgeons and pathologists is essential when using surgical biopsy specimens rather than bronchoscopic specimens.
- Before submitting clinical specimens for NGS analysis, it is essential that the pathologists review the tumor content of the submitted samples.
- Since submission of insufficient samples may delay treatment, it is necessary to perform a repeated biopsy for NGS analysis rather than submission of insufficient samples.
- The clinical introduction of upcoming targetable driver mutations, such as *KRAS* G12C mutation and *RET* fusion genes, is emerging, and the success of pretreatment NGS analysis is increasingly relevant.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fon-2020-0332

Author contributions

K Kunimasa was responsible for the concept and design of the study. S Matsumoto, K Goto, K Nishino, H Kuhara, M Tamiya, T Inoue, T Kawamura, H Kawachi, K Kuno, T Kimura, T Maniwa and J Okami were responsible for acquisition of the data. H Nakamura, SI Nakatsuka and T Kumagai were responsible for the analysis of the data. All the authors were responsible for interpretation of the data, and drafting the manuscript. All the authors gave their final approval of the version to be published and agree to be 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.

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Ethical conduct of research

The authors state that this study was approved by the Institutional Review Board at Osaka International Cancer Institute (#19018). In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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