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ORIGINAL ARTICLE

Real-world data on NGS using the Oncomine DxTT for detecting genetic alterations in non-small-cell lung cancer: WJOG13019L

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Abstract

Considering the increasing number of identified driver oncogene alterations, additional genetic tests are required to determine the treatment for advanced non-small-cell lung cancer (NSCLC). Next-generation sequencing can detect multiple driver oncogenes simultaneously, enabling the analysis of limited amounts of biopsied tissue samples. In this retrospective, multicenter study (UMIN ID000039523), we evaluated real-world clinical data using the Oncomine Dx Target Test Multi-CDx System (Oncomine DxTT) as a companion diagnostic system. Patients with NSCLC who were tested for a panel of 46 genes using the Oncomine DxTT between June 2019 and January 2020 were eligible for enrollment. Patients from 19 institutions affiliated to the West Japan Oncology Group were recruited. The primary endpoint

of the study was the success rate of genetic alteration testing in four driver genes (*EGFR*, *ALK*, *ROS1*, and *BRAF*) using the Oncomine DxTT. In total, 533 patients were enrolled in the study. The success rate of genetic alteration testing for all four genes was 80.1% (95% CI 76.5%-83.4%). Surgical resection was associated with the highest success rate (88.0%), which was significantly higher than that for bronchoscopic biopsy (76.8%, $P = .005$). Multivariate analysis revealed a significant difference for surgical resection alone ($P = .006$, 95% CI 1.36-6.18, odds ratio 2.90). Although the success rate of genetic alteration testing immediately after Oncomine DxTT induction was not sufficient in this study, optimizing specimen quantity and quality may improve the use of driver gene testing in clinical settings.

KEYWORDS

next-generation sequencing gene panel, non-small-cell lung cancer, Oncomine Dx, turnaround time

1 | INTRODUCTION

Lung cancer is the most common type of cancer worldwide and the leading cause of cancer-related death.[1] Approximately 85% of individuals with lung cancer have non-small-cell lung cancer (NSCLC), with 70% of NSCLC tumors being inoperable, locally advanced, or metastatic at diagnosis.[2] Molecular targeting agents such as epidermal growth factor receptor (*EGFR*)-tyrosine kinase inhibitors (TKIs), anaplastic lymphoma kinase (*ALK*)-TKIs, c-ros oncogene 1 (*ROS1*)-TKIs, and v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*)-TKIs have markedly improved progression-free survival (PFS) and overall survival (OS) in patients with NSCLC who are positive for the corresponding genetic alterations.[3-7] Testing for these driver oncogene alterations in advanced NSCLC, especially in non-squamous-cell carcinoma, is essential for making informed treatment decisions. Conventional single-gene tests have been performed to identify patients that are responsive to molecular targeting agents.[3-7] However, with the increasing number of identified driver oncogene alterations, more genetic tests are required to determine the treatment for advanced NSCLC. Therefore, tissue utilization for analyzing multiple single-gene tests has increased, whereas the completion rates of genetic tests have decreased.[8]

Next-generation sequencing (NGS) can help identify multiple driver oncogenes simultaneously, enabling the analysis of limited amounts of biopsied tissue samples. The Oncomine Dx Target Test Multi-CDx System (Ion Torrent PGM Dx Sequencer; Thermo Fisher Scientific) is an NGS panel for NSCLC testing that was approved by the Ministry of Health, Labour and Welfare of Japan in February 2019. It is a qualitative, in vitro diagnostic test that uses high-throughput parallel sequencing to detect sequence variations in 46 genes using DNA and RNA isolated from formalin-fixed paraffin-embedded (FFPE) specimens or from fresh, frozen tumor samples. In Japan, the Oncomine Dx Target Test Multi-CDx System has been approved for use as a companion diagnostic platform for targeted therapies to identify alterations in four driver genes: *EGFR*, *ALK*, *ROS1*, and *BRAF* (p.V600E).

The Oncomine Dx Target Test Multi-CDx System can reliably identify mutations in multiple genes simultaneously; however, in several cases, an assessment cannot be performed because of the presence of an insufficient amount or low quality of extracted nucleic acid. Without accurate identification results, the opportunities for implementing appropriate molecular targeting therapies against advanced NSCLC may be missed. Therefore, we designed a multicenter retrospective study (West Japan Oncology Group [WJOG] 13019L) to analyze real-world data using the Oncomine Dx Target Test Multi-CDx System in patients with NSCLC. Additionally, we evaluated factors related to the success rate of the Oncomine Dx Target Test Multi-CDx System.

2 | MATERIALS AND METHODS

2.1 | Eligibility

Patients with NSCLC whose diagnoses were confirmed using histological or cytological methods, and who had undergone testing for 46 genes using the Oncomine Dx Target Test Multi-CDx System between June 2019 and January 2020 were eligible for enrollment. The study protocol was approved by the ethics review boards of all participating institutions, and the trial was registered in the UMIN database with the ID 000039523.

2.2 | Study design

This multicenter, retrospective study recruited patients at 19 institutions affiliated with the WJOG in Japan. The primary endpoint was to evaluate the success rate of genetic alteration testing in four

driver genes (*EGFR*, *ALK*, *ROS1*, and *BRAF*) using the Oncomine Dx Target Test Multi-CDx System. As for *EGFR* mutations, *EGFR* exon 19 deletion and *EGFR* exon 21 L858R mutation were evaluated as the primary endpoints. In the present study, we defined analysis "success" as cases that were successfully reported as positive or negative for each gene mutation by Oncomine Dx Target Test Multi-CDx System testing, and analysis "failure" as cases reported as "no call" or "invalid". The success rate was defined as the percentage of cases that were determined as "success" among all cases submitted for Oncomine Dx Target Test Multi-CDx System testing. The secondary endpoints included the success rate of genetic alteration testing in four driver genes (*EGFR*, *ALK*, *ROS1*, and *BRAF*) depending on the sample condition, identification of mutational frequencies in the 46 genes in samples classified successfully, and evaluation of time from sample submission to the determination of results (turnaround time). For sample evaluation, the following information was recorded: method and site of sample collection, number of biopsies and tissue fixation method, tumor evaluation by a pathologist, and whether or not macro-dissection was performed.

2.3 | Genetic alteration tests

In the present study, at least five slide-mounted sections (4- to 5-μm thick) of biopsy samples prepared from formalin-fixed and paraffin-embedded (FFPE) specimens or from pleural effusion cell pellets were submitted to SRL Laboratories (Tokyo, Japan) or LSI Medience Laboratories (Tokyo, Japan). Genetic alteration tests were performed using the Oncomine Dx Target Test Multi-CDx System based on Thermo Fisher's Ion AmpliSeq technology. Both laboratories have the International Organization for Standardization (ISO) 15 189 and College of American Pathologists (CAP) certification for quality assurance.

2.4 | Baseline information and assessments performed

Clinical information related to several parameters was obtained. The sample information included the results of the Oncomine Dx Target Test Multi-CDx System for mutations including *EGFR* exon21 L858R, *EGFR* exon19 deletion, the *ALK* and *ROS1* fusion genes, $BRAF^{V600E}$, and those in other genes; the dates of sample collection, Oncomine Dx Target Test Multi-CDx System submission, and result determination; tissue fixation conditions (fixation solution [10% neutral buffered formalin or others] and fixation time [less than 24 h, 24-48 h, 48 h or more]); tumor content evaluation by a pathologist; whether macro-dissection was performed; and the collection method and site: (i) bronchoscopy (forceps size FB-233D, FB-231D, or other), number of samples, use of guide sheath or not, (ii) CT-guided biopsy, echo-guided biopsy (size of a biopsy needle 14G, 16G, 18G, or other), number of samples, (iii) volume of pleural fluid collected, and (iv) surgical resection (surgical technique, time, and temperature control from removal to fixation).

Other clinical information included sex, age at the time of specimen collection, clinical stage (I, II, III, IV, or postoperative recurrence) at the time of specimen collection and Oncomine Dx Target Test result submission, histological type, PD-L1 expression status, and history of smoking, surgery, and radiotherapy for lung cancer.

2.5 | Statistical analysis

Patient background was analyzed for all enrolled cases and the full-analysis set (FAS). Summary statistics were calculated for the patient background. The primary endpoint was to evaluate the success rate of genetic alteration testing in four driver genes (*EGFR*, *ALK*, *ROS1*, and *BRAF*). Binomial and ordinal logistic regression models were constructed utilizing successful sample identification as the outcome and test conditions as explanatory variables including specimen collection method, tumor cell content, fixation conditions, and the use of macro-dissection. The effect of test conditions on the success of identification was expressed as an odds ratio, and the two-tailed 95% confidence interval was used to evaluate statistical significance. The presence of genetic mutations in the panel of 46 genes was calculated. The turnaround time (days) from specimen submission to the determination of results was tabulated. The Kruskal-Wallis test was used to evaluate statistically significant differences in the detection of mutations based on the sample collection method. The chi-square test was used to evaluate surgical or other biopsy methods at each fixation time and significant differences in the detection of each gene mutations were determined based on macro-dissection.

3 | RESULTS

3.1 | Patient characteristics

In total, 533 patients were enrolled in the study conducted between April 2020 and June 2020. The baseline characteristics of the patients are summarized in Table 1. The median age was 72 years (range 25-94 years) and 345 patients (64.7%) were male. The percentages of patients with adenocarcinoma detected histologically or those with stage IV disease were 73.2% and 46.0%, respectively. PD-L1 status was evaluated in 497 patients; among these, 133 (25.0%) showed more than 50% PD-L1 expression. Evaluation of patient smoking history showed that 138 (25.9%) had never smoked, whereas 394 patients (74.1%) had a history of smoking.

3.2 | Sample conditions

The sample conditions are summarized in Table 2. All 533 patient samples were eligible for analysis. The median time from sampling

TABLE 1 Baseline characteristics of the patients included in the study ($n = 533$)

Characteristic	*n*	%
Age (years)		
Median	(72)	
Range	(25-94)	
Sex		
Male	345	64.7
Female	188	35.3
ECOG performance status		
0	243	45.6
1	237	44.5
2	32	6.0
3	19	3.6
4	2	0.4
Tumor histology		
Adenocarcinoma	390	73.2
Squamous cell carcinoma	94	17.6
Not otherwise specified (NOS)	19	3.6
Others	30	5.6
Disease stage		
I	117	22.0
II	48	9.0
III	110	20.6
IV	245	46.0
Postoperative recurrence	13	2.4
PD-L1		
≥50%	133	25.0
1-49%	171	32.1
<1%	193	36.2
Unknown	36	6.8
Smoking history		
Current	135	25.4
Former	259	48.7
Never	138	25.9

to Oncomine Dx Target Test Multi-CDx System testing was 10 days, and the interquartile range (IQR) was 6.0 to 23.0 days. Fixation conditions included the use of 10% neutral buffered formalin solution for 95.5% samples, and fixation time was less than 24 h, between 24 and 48 h, or more than 48 h for 57.2%, 13.3%, and 13.3% of samples, respectively. In 15.9% of the samples, the tumor cell content was less than or equal to 30% as determined by a pathologist. Macro-dissection was performed in 57.4% of cases and these samples had a tumor cell content of less than or equal to 30%. Specimens were collected via bronchoscopy, CT-guided biopsy, and surgical resection in 54.7%, 10.6%, and 29.0% of cases, respectively. In cases where bronchoscopy was performed, transbronchial lung biopsy (TBLB) and endobronchial ultrasonography with a guide sheath (EBUS-GS) was the most commonly used method (88.4%), followed by endobronchial

TABLE 2 Sample conditions as obtained from the patients ($n = 533$)

Sample condition	*n*	%
Period from sampling to analysis (days)		
Median [IQR]	10.0 [6.0-23.0]	
Fixation solution		
10% neutral buffered formalin solution	509	95.5
Unknown	24	4.5
Fixation time		
<24 hours	305	57.2
24-47 hours	113	21.2
≥48 hours	71	13.3
Unknown	44	8.3
Tumor cell content		
<10%	8	1.5
10-30%	77	14.4
31-49%	46	8.6
≥50%	31	5.8
Unknown	371	69.6
Macro-dissection (tumor cell content ≤30%)		
Yes	49	57.4
No	35	41.2
Unknown	1	1.2
Sampling method		
Bronchoscopy	285	54.7
(TBLB/EBUS-GS)	252	
(EBUS-TBNA)	31	
CT-guided biopsy	55	10.6
Ultrasound-guided biopsy	18	3.5
Pleural effusion	12	2.3
Surgical resection	151	29.0
Number of biopsies by bronchoscopy		
Median [IQR]	5.0 [3.0-8.5]	
Biopsy site of CT-guided biopsy		
Primary tumor	46	83.6
Metastatic tumor (bone)	2	3.6
Metastatic tumor (lymph node)	2	3.6
Metastatic tumor (liver)	2	3.6
Metastatic tumor (pleura)	1	1.8
Others	2	3.6
Size of biopsy needle		
16G	4	3.9
17G	3	2.9
18G	51	49.5

(Continues)

TABLE 2 (Continued)

Sample condition	*n*	%
20G	6	5.8
21G	10	9.7
22G	15	14.6
Others	14	13.6
Surgical resection technique		
Lobectomy	89	58.9
Partial resection	21	13.9
Metastatic site (lymph node)	16	10.6
Others	25	16.6

Abbreviations: EBUS-GS, endobronchial ultrasonography with a guide-sheath; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; IQR, inter-quartile range; TBLB, transbronchial lung biopsy.

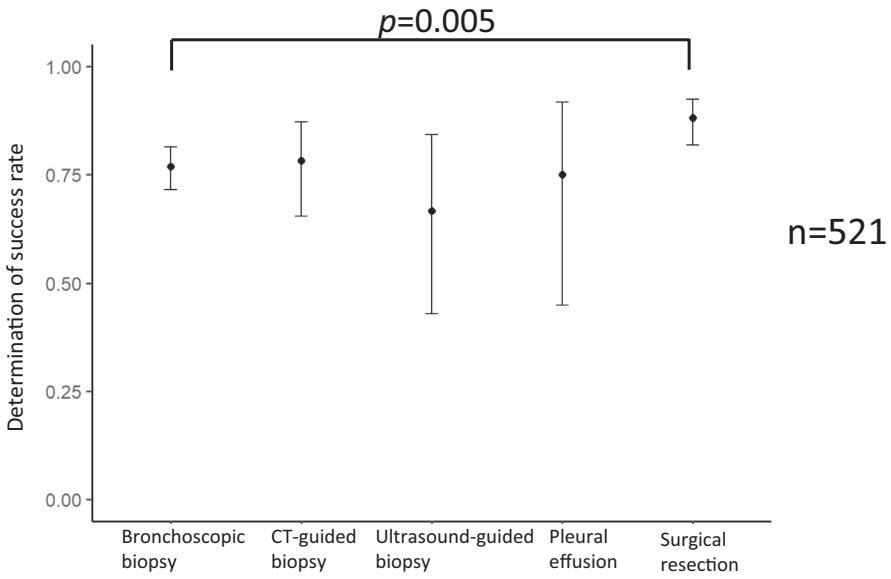
ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) (10.9%). The median number of biopsies performed via bronchoscopy was 5.0, and the IQR was 3.0 to 8.5. Guide sheaths were used concomitantly in 77.0% of the cases. The primary tumor was the most common biopsy site using CT-guided biopsy (83.6%). The most commonly used biopsy needle size was 18G (49.5%). The most common surgical resection techniques were lobectomy of the primary tumor (58.9%), followed by partial resection of the primary tumor (13.9%) or lymph node metastases (10.6%).

3.3 | Success rate of genetic alteration testing

The success rate of genetic alteration testing is summarized in Table 3. The success rate of genetic alteration testing for all four

TABLE 3 Success rate of genetic alteration testing in the four genes ($n = 533$)

Gene	Success rate (%) [95% confidence interval]	Number of patients (n)
Four genes (*EGFR*, *ALK*, *ROS1*, *BRAF*)	80.1 [76.5-83.4]	427
EGFR (exon 19 del, L858R)	85.4 [19.3-44.1]	455
ALK	89.9 [87.0-92.3]	479
ROS1	89.9 [87.0-92.3]	479
BRAF	85.0 [81.7-87.9]	453



Collection methods	Success, n (%)
Bronchoscopic biopsy (n=285)	219 (76.8)
CT-guided biopsy (n=55)	43 (78.2)
Ultrasound-guided biopsy (n=18)	12 (66.7)
Pleural effusion (n=12)	9 (75.0)
Surgical resection (n=151)	133 (88.0)

FIGURE 1 The success rate of genetic alteration testing was evaluated for different sample collection methods for the 521 eligible patients. Surgical resection had the highest success rate of genetic alteration testing (88.0%), which was significantly greater than that for bronchoscopic biopsy (76.8%) ($P = .005$)

driver genes (*EGFR*, *ALK*, *ROS1*, and *BRAF*), which was the primary endpoint, was 80.1% (95% CI 76.5%-83.4%). The success rates of genetic alteration testing for each driver gene were as follows: 85.4% (95% CI 82.1%-88.3%) for *EGFR* mutations (exon 19 deletion or exon 21 L858R), 89.9% (95% CI 87.0%-92.3%) for the *ALK* fusion gene, 89.9% (95% CI 87.0%-92.3%) for the *ROS1* fusion gene, and 85.0% (95% CI 81.7%-87.9%) for the *BRAF* mutation. Analysis of sample collection methods indicated that 88.0% of surgical resections had the highest success rate of genetic alteration testing, which was significantly higher than that for bronchoscopic biopsy (76.8%, $P = .005$) (Figure 1). The success rate of genetic alteration testing was higher for a fixation time of 24 to 48 h (87.6%) than that for samples fixed for less than 24 h (78.0%) or more than 48 h (81.7%) ($P = .09$). The success rate of genetic alteration testing using surgically resected samples or other sampling methods by fixation times was 87.5% ($n = 35$) and 76.6% (n = 203) ($P = .12$), 90% ($n = 63$) and 83.7% (n = 36) ($P = .33$), 83.3% ($n = 20$), and 80.9% ($n = 38$) ($P = .80$) for fixation times of less than 24 h, 24-48 h, and more than 48 h, respectively. The success rate of genetic alteration testing tended to be higher for surgically resected samples.

Tumor cell content was analyzed in 162 samples (30.4%). There was no difference in success rate between the two groups without macro-dissection (tumor cell content of ≤30% or >30%), 68.5% and 71.4%, respectively ($P = .78$). However, regardless of the tumor cell content, the success rate of genetic alteration testing tended to be higher when macro-dissection was performed, even though the difference was not statistically significant (83.7% vs 68.5%, $P = .11$ in tumor cell content ≤30% and 82.1% vs 71.4%, $P = .30$ in tumor cell content >30%) (Table S1 and Supporting Digital Content 1). There was no difference in the success rate of test using RNA (*ALK* and *ROS1*), regardless of whether macro-dissection was performed, whereas the success rate of test using DNA (*EGFR* and *BRAF*) was increased with macro-dissection. (Table S2 and Supporting Digital Content 2). A binomial logistic regression model was evaluated with successful genetic determination of NSCLC as the outcome and sample conditions (specimen collection method, tumor cell content, fixation conditions, and whether macro-dissection was performed) as explanatory variables. The results of multivariate analysis showed a significant difference for surgically resected samples alone ($P = .006$, 95% CI 1.36-6.18, odds ratio 2.90) (Table 4).

3.4 | Turnaround time

The median time from sample submission to the confirmation of results was 11 days and the IQR was 8-14 days.

3.5 | Detected genes

In the present study, 311 of 390 adenocarcinoma cases showed successful analysis of all four driver genes (*EGFR*, *ALK*, *ROS1*, and *BRAF*). Among the 311 adenocarcinoma cases, all detected cancer-related genes are shown in Figure 2: *EGFR* mutations included the exon 21 L858R mutation in 18.6% and the exon 19 deletion in 9.0% of samples. The *EGFR* exon 21 L858R mutation included duplicated cases of E709K ($n = 1$), E709G ($n = 3$), L868 M ($n = 1$), and PIK3CA ($n = 2$) as compound mutations. Additionally, the prevalence of uncommon *EGFR* mutations was 1.9%. Other mutations included those for *ALK* (1.0%), *ROS1* (1.3%), $BRAF^{V600E}$ (0.3%), *KRAS* (14.1%, *KRAS* G12C in 4.2% of samples), *MET* (2.9%, *MET* exon 14 skipping in 1.6% of samples), and *RET* in 0.3% of samples. In Figure 2, "negative" indicates that no genetic alteration was detected. Among the cases in which all four driver genes (*EGFR*, *ALK*, *ROS1*, and *BRAF*) were analyzed successfully, all the detected genes for 77 squamous cell carcinoma cases are shown in Figure S1 (Supporting Digital Content 3.). The mutations for *EGFR* exon 21 L858R and exon 19 deletion, *KRAS* G12C, *FGFR2*, *FGFR3*, and *NRAS* were detected in one patient (1.3%) each, and *PIK3CA* was detected in two patients (2.6%). Among the cases in which all four driver genes (*EGFR*, *ALK*, *ROS1*, and *BRAF*) were evaluated successfully, all detected genes for not otherwise specified (NOS) are shown in Figure S2 (Supporting Digital Content 4), with *KRAS* mutations identified in four patients (36.4%) (*KRAS* G12C in 18.2%), *MET* exon 14 skipping in one patient (9.1%), and *HRAS* mutations in one patient (9.1%).

4 | DISCUSSION

This is the first large-scale, real-world study to implement NGS as a companion diagnostic system in clinical practice; it revealed the NSCLC genetic mutation profile using the Oncomine Dx Target Test Multi-CDx System. The success rate of genetic alteration testing of

TABLE 4 Multivariate analysis of sample conditions and success rate of genetic alteration testing

Sample condition	Odds ratio	Confidence interval	*P* value
CT-guided biopsy vs Bronchoscopy	1.05	0.45-2.45	.91
Ultrasound-guided biopsy vs bronchoscopy	0.53	0.18-1.54	.24
Surgical resection vs bronchoscopy	2.90	1.36-6.18	.006
Fixation time 24-48 h vs <24 h	1.93	0.80-4.67	.14
Fixation time >48 h vs <24 h	0.93	0.45-1.88	.83
Macro-dissection (tumor cell content ≤30%) yes vs no	1.27	0.65-2.47	.49

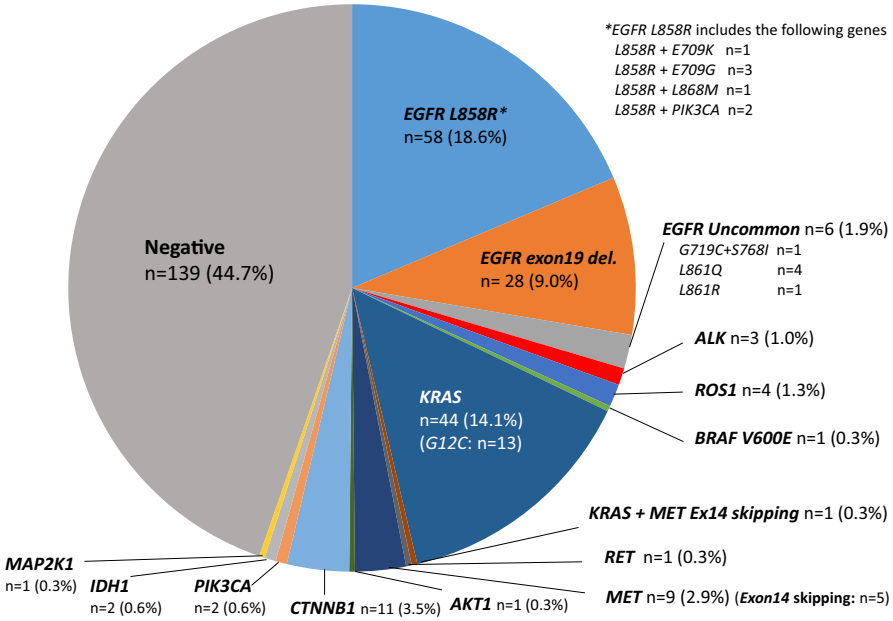


FIGURE 2 Of the 390 adenocarcinoma cases, all four driver genes (*EGFR*, *ALK*, *ROS1*, and *BRAF*) were successfully evaluated in 311 cases. All detected genes of 311 adenocarcinoma cases are shown. "Negative" indicates that no genetic alteration was detected. The *EGFR* L858R mutation ($n = 58$, 18.6%) included the following alleles: *L855R*+E709K ($n = 1$), L858R+E709G ($n = 3$), L858R+L868 M ($n = 1$), and L858R+PIK3CA ($n = 1$). The *KRAS* gene mutations ($n = 44$, 14.1%) included the following alleles: *KRAS*+PIK3CA ($n = 1$)

the four driver genes *EGFR*, *ALK*, *ROS1*, and *BRAF* was 80.1% (95% CI 76.5%-83.4%).

The results of the study show that among the sample conditions analyzed, surgical resection alone showed a statistically significant association with a high success rate of genetic alteration testing using multivariate analysis. Samples collected via surgical resection are larger in volume than those collected using nonsurgical methods such as bronchoscopy. Earlier, based on an analysis of 167 patients, Ariyasu et al reported that the success rate of mutation detection using the Oncomine Dx Target Test Multi-CDx System was the highest when using surgically resected samples.[9]

Previously conducted global clinical trials (E2201 study) using the Oncomine Dx Target Test CDx System reported a 72% detection success rate for studies evaluating *BRAF* mutations.[10] Here, we showed that the success rates of genetic alteration testing in all four genes (*EGFR*, *ALK*, *ROS1*, and *BRAF*) and the *BRAF* mutation alone were 80.1% and 85.0%, respectively. Although direct comparisons cannot be made because of differences in patient backgrounds, the pathology department or contract laboratories in Japan determine the appropriate volume of tumor samples that can be obtained to perform macro-dissection before submission for the Oncomine Dx Target Test, which may be one of the reasons for the increased success rate of mutation detection. Several reports using the Oncomine Dx Target Test have been published from Japan. According to these, in clinical practice specimens from cases with high tumor cell content or surgically resected samples are actively submitted for evaluation using the Oncomine Dx Target Test.[9]

In a systematic review, the frequency of *EGFR* mutations in Japanese NSCLC patients was reportedly 45% (range 21%-68%).[11] In our study, the frequency of *EGFR* mutations was rather low (29.5%); however, the high percentage of patients with a history of smoking (74.1%) and male sex (64.7%) may be associated with the low frequency of *EGFR* mutation. Additional factors may include selection bias because some patients enrolled in this study were evaluated using the Oncomine Dx Target Test Multi-CDx System after confirmation of *EGFR* mutation-negative status using single-plex tests for the *EGFR* mutation.

Nemoto et al reported that a higher tumor cell count is associated with a higher success rate of mutation detection using the Oncomine Dx Target Test CDx System.[12] In our study, we analyzed the data of tumor cell content and found that there was no difference in the success rate between the two groups without macro-dissection (tumor cell content of ≤30% or >30%), at 68.5% and 71.4%, respectively ($P = .78$). However, the success rate of genetic alteration testing tended to be higher when macro-dissection was performed, even though the difference was not statistically significant (83.7% vs 68.5%, $P = .11$ in tumor cell content ≤30% and 82.1% vs 71.4%, $P = .30$ in tumor cell content >30%). Regardless of the percentage of tumor cell content, increasing the tumor cell content by performing macro-dissection may contribute to improving the success rate of genetic alteration testing in driver genes using the Oncomine Dx Target Test Multi-CDx System. In the present study, there was no difference in the success rate of testing using RNA (*ALK* and *ROS1*), regardless of whether macro-dissection was performed, whereas the success rate of testing using DNA (*EGFR* and *BRAF*) was increased with macro-dissection. As for the discrepancy in improvement of success rate between DNA and RNA, it is possible that the success rate of analysis using DNA was affected by the rate of tumor cell content or quantity of tumor samples; in contrast, the success rate of analysis using RNA was affected by the quality of tumor samples rather than by the tumor cell content.

The present study does have some limitations. We have not confirmed the concordance between the Oncomine Dx Target Test Multi-CDx System and single-plex genetic alteration testing. However, previous reports have shown a high concordance for *EGFR*, *ALK*, and *ROS1* between the Oncomine Dx Target Test Multi-CDx System and single-plex testing.[13] Another limitation is the absence of data on the exact number of submitted slides and the amount of

DNA or RNA input in the submitted samples for Oncomine Dx Target Test Multi-CDx System testing. Additionally, our study is a retrospective analysis that was conducted immediately after approval of the Oncomine Dx Target Test Multi-CDx System. Subsequently, several modifications were made for conducting NGS tests, including those using the Oncomine Dx Target Test Multi-CDx System at each hospital. Therefore, the success rate of the Oncomine Dx Target Test Multi-CDx System may be greater than that described here.

In conclusion, although the success rate after the initial introduction of the Oncomine Dx Target Test Multi-CDx System was not sufficient in this study, optimizing the quantity and quality of the specimens could help make this system the first choice for driver gene testing in clinical settings.

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DISCLOSURE

The authors have no conflict of interest.

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REFERENCES

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. *CA Cancer J Clin*. 2011;61:69-90. https://doi.org/10.3322/caac.20107
2. Cojean I, LeChevalier T. Chemotherapy of stage IIIB and IV non-small-cell lung cancer. *Ann Oncol*. 1995;6(suppl 3):S41-S44. https://doi.org/10.1093/annonc/6.suppl_3.s41
3. Soria JC, Ohe Y, Vansteenkiste J, et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med*. 2018;378:113-125. https://doi.org/10.1056/NEJMoa1713137
4. Solomon BJ, Mok T, Kim DW, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med*. 2014;371:2167-2177. https://doi.org/10.1056/NEJMoa1408440
5. Peters S, Camidge DR, Shaw AT, et al. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med*. 2017;377:829-838. https://doi.org/10.1056/NEJMoa1704795
6. Shaw AT, Ou SH, Bang YJ, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med*. 2014;371:1963-1971. https://doi.org/10.1056/NEJMoa1406766
7. Planchard D, Smit EF, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously untreated $BRAF^{V600E}$-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. *Lancet Oncol*. 2017;18:1307-1316. https://doi.org/10.1016/S1470-2045(17)30679-4
8. Yu TM, Morrison C, Gold EJ, et al. Multiple biomarkers testing tissue consumption and completion rates with single-gene tests and investigational use of Oncomine Dx Target Test for advanced non-small-cell lung cancer: A single-center analysis. *Clin Lung Cancer*. 2019;20:20-29.e8. https://doi.org/10.1016/j.cllc.2018.08.010
9. Ariyasu R, Uchibori K, Ninomiya H, et al. Feasibility of next-generation sequencing test for patients with advanced NSCLC in clinical practice. *Thorac Cancer*. 2021;12:504-511. https://doi.org/10.1111/1759-7714.13786
10. Planchard D, Besse B, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously treated BRAF (V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. *Lancet Oncol*. 2016;17:984-993. https://doi.org/10.1016/S1470-2045(16)30146-2
11. Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMapII). *Am J Cancer Res*. 2015;5:2892-2911.
12. Nemoto D, Yokose T, Katayama K, et al. Tissue surface area and tumor cell count affect the success rate of the Oncomine Dx Target Test in the analysis of biopsy tissue samples. *Thorac Cancer*. 2021;12:194-200. https://doi.org/10.1111/1759-7714.13743
13. Food and Drug Administration. Summary of Safety and Effectiveness Data 2017. https://www.accessdata.fda.gov/cdrh_docs/pdf16/p160045b.pdf. Accessed September 29, 2021.

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