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
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.

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.
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, BRAFV600E 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 mutation 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|

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

| Sample condition          | n   | %   |
|---------------------------|-----|-----|
| Period from sampling to analysis (days) | 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 | 57.4|
|                           | No  | 41.2|
|                           | Unknown | 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 | 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)
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

| 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                    |

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)

| 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)     |
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 \( \leq 30\% \) 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 \( \leq 30\% \) 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\textsuperscript{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

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**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 \( \leq 30\% \)) yes vs no | 1.27       | 0.65-2.47           | .49          |
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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