Comparison of next-generation sequencing and cobas® EGFR Mutation Test v2 in detecting EGFR mutations

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Research Article

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Abstract

Purpose

Targeting oncogenic driver mutations, such as sensitizing mutations in the epidermal growth factor receptor gene (EGFR), significantly improves the prognosis of patients with advanced non-squamous non-small-cell lung cancer (NS-NSCLC). As the number of genetic mutations that must be tested increases, the Oncomine Dx Target Test (ODxTT), which can simultaneously detect multiple cancer-related genes, is becoming the main test used, in preference to single-molecule testing. In this study, we evaluated the performance of ODxTT and cobas® EGFR Mutation Test v2 (cobas® EGFR), one of the single-molecule tests, in detecting EGFR mutations.

Materials and methods

Samples from 211 patients who had been diagnosed with NS-NSCLC were tested simultaneously or sequentially with the cobas® EGFR Mutation Test and ODxTT. We compared the success rates and detection rates of both tests and evaluated their equivalence by determining the concordance rate and k-coefficient of the two tests.

Results

The success rate in detecting EGFR mutations was 95.7% for ODxTT and 100% for cobas® EGFR. EGFR mutations were detected in 26.5% of samples with ODxTT and in 28.0% with cobas® EGFR. For the 200 samples successfully analyzed with both tests, the concordance rate and k-coefficient were 97.5% and 0.938, respectively. ODxTT failed to detect two exon 19 deletion mutations (p.E746_P753delinsVS and p.E746_P753delinsLS), and cobas® EGFR failed to detect three instances of an exon 19 deletion (p.L747_P753delinsS), L861R, and an exon 20 insertion (p.P772_H733insV).

Discussion

The success rate of ODxTT is slightly inferior to that of cobas® EGFR. ODxTT shared a high concordance rate and k-coefficient with cobas® EGFR in detecting EGFR mutations, but discordant results between the two tests were observed in a few cases, mainly due to the difference of detectable EGFR variants. Therefore, the advantages and limitations of each test must be clarified to ensure that genomic testing methods are used properly.

Introduction

Non-small-cell lung cancer (NSCLC) is diagnosed at an advanced clinical stage in a large proportion of patients, entailing a poor clinical outcome. These patients are candidates for systemic chemotherapy, which may improve their survival and quality of life, but chemotherapy is still considered palliative. Since the introduction of targeted therapies for NSCLC, which were developed to block aberrant oncogenic signaling a decade ago, these therapies have opened a new era in the management of advanced NSCLC, improving survival outcomes [1]. Targeting oncogenic driver mutations, such as sensitizing mutations in the epidermal growth factor receptor gene (EGFR) and fusions of echinoderm microtubule-associated protein-like 4 (EML4) and anaplastic lymphoma kinase (ALK), rearrangements of the ROS proto-oncogene 1 (ROS1), the BRAF V600E mutation, and the MET exon 14 skipping, has significantly improved the prognosis of advanced NSCLC, with higher response rates and longer progression-free survival compared with those achieved with conventional cytotoxic chemotherapy. These therapies have been approved for clinical use for NSCLC [2–7]. Among the genetic mutations with prognostic value, EGFR mutations are most frequently detected, occurring in 45% of patients with advanced non-squamous NSCLC (NS-NSCLC) in Japan [8]. Several clinical trials evaluating EGFR-tyrosine kinase
inhibitors (TKIs) have been conducted, and the median overall survival (OS) of patients with EGFR-activating mutations treated with EGFR-TKI has reached 4–5 years in recent clinical trials [9–11].

Conventionally, platinum-based chemotherapy has been the basic treatment for NSCLC patients without driver mutations. However, its efficacy had reached a plateau, with a median OS of 1 year [12–15]. Recently, the development of immune checkpoint inhibitors (ICIs) has changed the treatment of advanced NSCLC dramatically. ICIs provide long-term survival in some patients with advanced NSCLC, and ICI monotherapy or the combination of ICIs with cytotoxic chemotherapy has become the standard treatment for advanced NSCLC without driver mutations [16, 17]. The response to ICI monotherapy is about 20% in unselected NS-NSCLC patients [18], but the effect is limited in patients with some driver mutations [18–20], such as those in EGFR.

Therefore, molecular biomarker testing has become crucial when decisions on treatment are made, especially in patients with advanced NS-NSCLC, because the efficacy of targeted therapies can be predicted. Conventionally, the real-time reverse transcription–polymerase chain reaction (RT–qPCR)-based cobas® EGFR Mutation Test v2 (cobas® EGFR: Roche Molecular Systems, Pleasanton, CA, USA), which can identify 42 different EGFR mutations in exons 18, 19, 20, and 21, has been widely used as a single companion diagnostic test for therapies with EGFR-TKIs. However, as the number of genetic mutations that must be tested increases, the ability of a companion molecular test to screen each genetic mutation simultaneously or sequentially is approaching its limit.

Next-generation sequencing (NGS) is a recently developed, massive, parallel, large-scale sequencing technology that is becoming a key technique for the simultaneous screening of multiple cancer-related genes [21]. Oncomine Dx Target Test (ODxTT; Thermo Fisher Scientific, Waltham, MA, USA), which simultaneously evaluates 46 cancer-related genes, was the first NGS panel for NSCLC testing approved by the US Food and Drug Administration, in June 2017 [22]. In daily clinical practice in Japan, ODxTT is often performed if a sufficient amount of tumor tissue can be collected. NGS is a promising technology for the simultaneous detection of multiple genes, but it has several limitations. It requires specimens containing nucleic acids of sufficient quality and quantity. Poor-quality or too little tumor sample can cause the failure of an NGS analysis. For ODxTT, the estimated tumor content of the biopsy sample is recommended to be >30% of the total cells, which is higher than the tumor content of 5% recommended for cobas® EGFR. We also previously reported that a sufficient amount of tissue is required to successfully exploit the NGS technology with ODxTT [23, 24]. ODxTT is based on amplicon sequencing, and it is a highly targeted approach to the analysis of genetic variations using PCR, with sets of primers for exons or hotspots in selected genes. Therefore, ODxTT cannot detect untargeted gene mutations, but is designed to detect various EGFR mutations in exons 3, 7, 12, 15, 18, 19, 20, and 21 (Supplementary Table 1).

In this study, we evaluated the consistency of cobas® EGFR and ODxTT in detecting EGFR mutations and the effect of the EGFR mutation frequencies on the number of variants detected with the two methods.

**Patients And Methods**

**Patients**

The study participants included 211 patients who were pathologically diagnosed with NS-NSCLC, and were subjected for ODxTT testing between September 2019 and August 2021 at the Kanagawa Cancer Center Hospital, Japan. The tissue sampling procedures included transbronchial biopsy (TBB) with endobronchial ultrasonography with a guide sheath (EBUS-GS), endobronchial biopsy (EBB) with direct-vision forceps, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), computed tomography (CT)-guided biopsy, and surgical resection from the lung or other sites. When the tumor samples were judged by the pathologist to contain sufficient cancer cells, a biomarker analysis was performed with ODxTT, regardless of the patient characteristics or clinical background. Samples in which only ODxTT was performed were retrospectively tested with cobas® EGFR, using the residual specimens originally used for ODxTT.
After April 2021, ODxTT and cobas® EGFR were performed simultaneously on the same specimens. cobas® EGFR was performed on all but two samples for which there was no residual specimen. We retrospectively reviewed the medical records of the patients and evaluated the patient characteristics, sampling methods, staging, and the results of genetic tests. When EGFR mutations detected with ODxTT and cobas® EGFR were discordant, we reviewed the clinical course of treatment and the treatment outcome, and the results of a genetic analysis with Oncomine Comprehensive Assay v3 (OCA v3; Thermo Fisher Scientific) using fresh-frozen tissues, which was performed in a lung cancer genomic screening project for individualized medicine in Japan (LC-SCRUM) (UMIN ID: UMIN000010234). We obtained ethical approval for the study from the Kanagawa Cancer Center Hospital Japan (2019EKI-48), and patient confidentiality was maintained. Written informed consent was obtained from all subjects when undergoing ODxTT.

Tumor specimens and genetic analysis of EGFR mutations

The tumor samples were fixed in 10% neutral-buffered formalin solution for 6–24 h, embedded in paraffin wax, and processed for histopathological examination with routine histology techniques. The histological diagnosis was made by pathologists according to the World Health Organization (WHO) classification of lung tumors [25].

After the pathological diagnosis of NSCLC, we routinely screened for therapy-predictive biomarkers. We assessed three pathological factors that potentially influence the success rate of NGS analysis in clinical practice: the tissue surface area, the tumor cell count, and the tumor content ratio. In general, the estimated tumor content in a biopsy sample is recommended to be >30% of the total cells for ODxTT. Since January 2020, we added to our local submission criteria a tissue surface area of ≥1 mm². When there was sufficient tissue, 10–30 glass slides stuck with 5 µm thickened tissue piece were prepared for ODxTT from each tumor samples, depending on the tumor cell count and tissue area per slide. More five sections of 10 µm thickness were simultaneously or sequentially cut from each specimen for cobas® EGFR. These samples were submitted to SRL Laboratories (Tokyo, Japan), a Japanese commercial laboratory, at which the ODxTT and cobas EGFR testing was performed.

In this study, we defined analytical “success” as samples that were successfully reported as positive or negative for EGFR mutations and BRAF V600E with DNA sequencing and for ALK and ROS1 with RNA sequencing using ODxTT, and analysis “failure” as samples reported as “no call” or “invalid” for these four driver mutations.

Statistical analysis

The patient's background, the analytical success rate, and the detection rate for EGFR mutations with each test were analyzed for all enrolled patients. The analytical success rates and detection rates for EGFR mutations achieved with ODxTT and cobas® EGFR were compared with Pearson's χ² test. Statistical significance was defined as a P value of < 0.05. The equivalence between ODxTT and cobas® EGFR in detecting EGFR mutations was evaluated with concordance rates and the κ-coefficient. The concordance rates and κ-coefficients were determined for samples successfully analyzed with both ODxTT and cobas® EGFR. A κ-coefficient of >0.80 was considered to indicate excellent agreement between the methods.

Results

Patients’ characteristics

A total of 211 patients who were pathologically diagnosed with NS-NSCLC and were analyzed with ODxTT were reviewed in this study. The patient characteristics are summarized in Table 1. The mean age of the patients was 71 years. Of the 211 patients, 140 (66.4%) were men, and 150 (71.1%) were smokers. The distribution by stage was as follows: stage I, 23 patients; stage II, 27 patients; stage III, 42 patients; stage IV, 111 patients; and postoperative recurrence, eight patients. Tumor tissues were sampled with large EBUS-GS (FB-231D; Olympus Medical Systems, Tokyo, Japan; n = 89), small
EBUS-GS (FB-233D; Olympus Medical Systems; n = 12), EBB (n = 7), EBUS-TBNA (n = 25), CT-guided biopsy (n = 10), or lung surgical resection (n = 47). Most patients were diagnosed with adenocarcinoma (83.4%), followed by “not otherwise specified” (NOS; 8.5%) and NSCLC (5.3%). The success rate of DNA sequencing for ODxTT was 95.7% (202/211), that of RNA sequencing was 97.6% (206/211), and that of both types of sequencing was 94.7% (200/211) across all patients.

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ODxTT</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Success (n = 211)</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>71 (38–90)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>140/71</td>
</tr>
<tr>
<td>Stage (I/II/III/IV/r)</td>
<td>23/27/42/111/8</td>
</tr>
<tr>
<td>Smoker/Non-smoker</td>
<td>150/61</td>
</tr>
<tr>
<td>Smoking Index</td>
<td>560 (0–2940)</td>
</tr>
<tr>
<td>Tissue sample</td>
<td></td>
</tr>
<tr>
<td>Large EBUS-GS TBLB</td>
<td>89</td>
</tr>
<tr>
<td>Small EBUS-GS TBLB</td>
<td>12</td>
</tr>
<tr>
<td>EBB</td>
<td>7</td>
</tr>
<tr>
<td>EBUS-TBNA</td>
<td>25</td>
</tr>
<tr>
<td>CT-guided biopsy</td>
<td>10</td>
</tr>
<tr>
<td>Lung surgery</td>
<td>47</td>
</tr>
<tr>
<td>Other</td>
<td>15</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>176</td>
</tr>
<tr>
<td>NOS</td>
<td>18</td>
</tr>
<tr>
<td>NSCLC</td>
<td>11</td>
</tr>
<tr>
<td>Pleomorphic</td>
<td>4</td>
</tr>
<tr>
<td>Adenosquamous</td>
<td>2</td>
</tr>
</tbody>
</table>

ODxTT, Oncomine Dx Target Test; r, recurrence; EBUS-GS, endobronchial ultrasonography with a guide sheath; TBLB, transbronchial lung biopsy; EBB, endobronchial biopsy; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; NOS, not otherwise specified.

**Test Success Rate And Frequency Of Driver Mutations From Each Genetic Analysis**

The success rate of *EGFR* mutation detection for ODxTT was 95.7% (202/211); two samples were diagnosed as “insufficient quantity” and seven were diagnosed as “no-call”. There was insufficient sample left to perform cobas® EGFR
testing in two patients, but cobas® EGFR was performed in the remaining 209 patients. The cobas® EGFR test was successfully analyzed in all of these patients, with no failure (P = 0.0025).

The frequency of EGFR mutations detected with each test was 26.5% for ODxTT and 28.0% for cobas® EGFR, across all patients (P = 0.7429) (Fig. 1A, B). EGFR mutations were only detected in adenocarcinomas. When limited to adenocarcinomas, the frequency of EGFR mutations detected was 31.8% (56/176) with ODxTT and 33.5% (59/176) with cobas® EGFR (P = 0.7332). Of the 59 samples in which EGFR mutations were detected with cobas® EGFR, none were detected in four samples with ODxTT due to “no-call”. Compound mutations, defined as double mutations in the EGFR kinase domain, were detected in 8.9% (5/56) of samples with ODxTT and in 5.1% (3/59) of samples with cobas® EGFR (P = 0.418).

**Agreement of cobas® EGFR and ODxTT in the detection of EGFR mutations**

Among the 200 samples analyzed with both tests, discordant results, in which an EGFR mutation was only detected with only one assay, were obtained for five samples. The concordance rate and κ-coefficient were 97.5% (95% confidence interval [CI], 94.3–99.2%) and 0.938 (95% CI, 0.859–0.971), respectively (Table 2). ODxTT failed to detect two exon 19 deletions (p.E746_P753delinsVS and p.E746_P753delinsLS), which were confirmed with OCA (Table 3). In contrast, cobas® EGR failed to detect three instances of an exon 19 deletion (p.L747_P753delinsS), L861R, and an exon 20 insertion (p.P772_H733insHV). Two compound EGFR mutations were detected only with ODxTT, and both involved mutations L858R and E709X.

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>cobas® EGFR</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Negative</td>
<td>No sample</td>
<td>Total</td>
</tr>
<tr>
<td>ODxTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>53</td>
<td>3</td>
<td>0</td>
<td>56</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>142</td>
<td>2</td>
<td>146</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>150</td>
<td>2</td>
<td>211</td>
</tr>
</tbody>
</table>

ODxTT, Oncomine Dx Target Test; cobas® EGFR, cobas® EGFR Mutation Test v2.
Of the three patients in whom discordant results involved an exon 19 deletion, an EGFR-TKI (osimertinib) was administered to two as the first-line treatment and was administered to the other patient as a second-line therapy, and these three patients showed tumor shrinkage. One patient with an EGFR exon 20 insertion was treated with cytotoxic chemotherapy with an ICI. One patient with the L858R and E709G mutations was transferred to another hospital. The other patient with the L858R and E709K mutations was treated with osimertinib and displayed tumor shrinkage.

**Discussion**

In this study, we evaluated the performance of ODxTT and cobas® EGFR in detecting EGFR mutations, which is essential for the assessment of advanced NS-NSCLC patients, because the frequency of EGFR mutations associated with this disease is high. cobas® EGFR is a highly sensitive and reliable test that is commonly used as a single test for detecting EGFR mutations. Therefore, it is important to know whether ODxTT, the first approved NGS panel testing in Japan, is inferior to cobas® EGFR. In this comparison, we detected some differences in the success rates of ODxTT and cobas® EGFR, but the two tests showed high concordance on the samples successfully analyzed with both tests. However, discordant results were observed in several cases, although EGFR-TKI caused tumor shrinkage, even in some discordant cases.
Genotyping tumors at the time of diagnosis is essential for determining the optimal first-line treatment for advanced NSCLC. Among the oncogenic driver mutations of this cancer, those in \textit{EGFR} are among the most important genomic mutations and their detection is essential, especially in female and nonsmoker patients, who frequently carry them \cite{26,27}. In the BRAVE study, a multicenter retrospective observational study of single biomarker testing in advanced NSCLC patients in Japan, the proportions of patients who underwent individual biomarker testing for \textit{EGFR}, \textit{ALK}, and \textit{ROS1} were 97.5\%, 88.1\%, and 67.3\%, respectively \cite{28}. A previous study showed that as the number of single biomarker tests performed for each driver mutation increased, the number of single tests that could be ordered decreased accordingly \cite{29}. To solve these clinical problems, NGS testing, which can analyze multiple genes simultaneously, must be widely accessible.

Both ODxTT and cobas® EGFR are widely used in clinical practice for the detection of \textit{EGFR} mutations in NSCLC tumor samples, but using these two tests simultaneously is not permitted by the Japanese national healthcare policy. Previously, the cobas® EGFR test was mainly used, but it has been replaced by the NGS test, which can simultaneously detect \textit{EGFR} mutations and other driver mutations. In general, compared with NGS, PCR-based tests have few failures, the results are obtained rapidly, and even small samples can be tested. Therefore, depending on the patient's background, cobas EGFR may be prioritized in daily clinical practice. The success rate when screening for genomic changes with cobas® EGFR was 100\%, which was higher than the success rate with ODxTT (about 95\%) in present study. An NGS analysis, which can detect a large number of genes, requires a large amount of tumor sample, and the success rates of NGS analyses based on small samples can be low \cite{30}. In our previous study, we suggested that taking larger samples increases the success rate of mutation detection with ODxTT and that the success rate varies according to the biopsy device used \cite{23,24}. In the present study, 10 of the 11 patients who were unsuccessfully tested with ODxTT were sampled with bronchoscopy, and six of these were sampled using small-forceps with a small EBUS-GS. These findings indicate that clinicians must strive to ensure that a sufficient amount of tumor cells is retrieved for successful analysis with ODxTT.

In this study, the detection rate for \textit{EGFR} mutations was 26.5\% with ODxTT and 28.0\% with cobas® EGFR. These frequencies are rather low compared with the frequency of ~45\% previously reported in Japanese NSCLC patients \cite{8}. In a retrospective study of 390 patients with adenocarcinoma whose genetic changes were analyzed with ODxTT (West Japan Oncology Group [WJOG] 1309L) \cite{31}, the frequency of \textit{EGFR} mutations was 29.5\%, similar to our results. This trend is attributable to the large number of males (66.4\%) and smokers (71.1\%) enrolled in our study. Moreover, ODxTT was only performed for patients with sufficient tumor tissue to meet the submission criteria, which may have affected the selection bias. High concordance was observed between ODxTT and cobas® EGFR in detecting \textit{EGFR} mutations among the patients successfully analyzed with both tests, but the results for five samples were discordant. cobas® EGFR missed one mutation, a deletion in exon 19, probably because the percentage of mutated DNA in this sample was low (7.5\%), and the cobas® EGFR assay is only reliable when the sample contains >5\% mutant DNA. In the remaining four cases, ODxTT failed to detect two mutations, both deletions in exon 19 (p.E746_P753delinsVS and p.E746_p753delinsLS), which is not detectable variant type by ODxTT. Conversely, cobas® EGFR failed to detect mutation L861R in two samples and an insertion in exon 20 (p.P772_H733insV), which are not detectable variant by cobas® EGFR. cobas® EGFR was unable to detect E709X, which was detected with ODxTT in a compound mutation with L858R in two samples in the present study. Importantly, the third-generation EGFR-TKI osimertinib was used in three patients with discordant results for exon 19 deletions, all of whom showed a partial clinical response.

Currently, two gene-panel tests, FoundationOne CDx (F1CDx) and the NCC OncoPanel, in addition to ODxTT, have been approved in Japan \cite{32}. ODxTT is categorized as a hot-spot panel test based on amplicon sequences, and amplifies each targeted site with PCR and primers spanning part of the coding region. Therefore, ODxTT can be used with a small amount of DNA or RNA but can only detect mutations at the targeted mutational hotspots. F1CDx and the NCC OncoPanel have been approved as comprehensive genome profile tests, which use the hybrid capture method, and can
detect mutations, amplifications, and homozygous deletions in the entire coding regions of the targeted genes, together with rearrangements of the targeted oncogenes included in each panel. Therefore, using the hybrid capture method, F1CDx and the NCC OncoPanel can detect rare variants that cannot be detected with hot-spot panel tests, including ODxTT and cobas® EGFR. Actionable genetic aberrations, including rare EGFR mutations, were identified with the NCC OncoPanel even in patients with NSCLC in whom no EGFR mutations or ALK fusions were detected with single companion diagnostics [33]. It is noteworthy that these patients in whom EGFR mutations were detected with the NCC OncoPanel were treated with EGFR-TKIs, with demonstrable therapeutic effects [33, 34]. Based on these facts, even when a companion diagnostic test detects no actionable gene mutations, an NGS analysis, such as with a CGP method, should be considered, especially in patients who are likely to have some genetic mutations, such as young people and nonsmokers.

The present study had several limitations. First, there was potential for selection bias because the study was conducted at a single institution and only patients for whom ODxTT was performed were included. ODxTT was mainly performed on tumor samples that met the submission criteria of our institution. Patients who were diagnosed with cytology alone or for whom there was insufficient tumor sample for ODxTT were not included in the study. Therefore, the frequency of EGFR mutations in this study referred to a selected cohort of patients, rather than all NS-NSCLC patients. Secondly, a relatively small sample of patients with EGFR mutations was used to evaluate the rate of discrepancies between the two tests in detecting EGFR mutations, so the false-negative rate was not accurate. Furthermore, neither test detected all of the variants of EGFR, so both tests produced false-negative results for some rare EGFR variants. Finally, because we evaluated only EGFR mutations, the consistency between ODxTT and a single test for other mutations has still to be examined.

**Conclusion**

The success rate of ODxTT is acceptable in clinical practice, but slightly inferior to that of cobas® EGFR. However, the performance of ODxTT in detecting EGFR mutations is similar to that of cobas® EGFR. Nonetheless, discordant results between the two tests were observed in a few cases, mainly because the two tests differ in their ability to detect EGFR variants. Therefore, the advantages and limitations of each test must be clarified to ensure that genomic testing methods are used properly.

**Declarations**

**Acknowledgments**

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**Author contributions**

Collection of clinical data: SM, KS, TI, SK, RU, TK, TK, and HS.

Interpretation of results: SM, and TY.

Writing and editing of the manuscript: SM, TY, KS, and HS.

All authors reviewed the manuscript.

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Availability of Data and Material (ADM)

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was conducted in accordance with the provisions of the Declaration of Helsinki, and was approved by the Ethics Committee of the Kanagawa Cancer Center Hospital, Japan (2019EKI-48). Informed consent was obtained from the patient.

Consent for publication

Not applicable.

Competing interests

Shuji Murakami reports personal fees from AstraZeneca, Chugai Pharmaceutical, Boehringer Ingelheim, Taiho Pharmaceutical, and Ono Pharmaceutical.

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Haruhiro Saito reports grants from Chugai Pharmaceutical and AstraZeneca, and personal fees from Ono Pharmaceutical, Nippon Boehringer Ingelheim, MSD, and Novartis Pharma.

The other authors report no conflicts of interest.

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Figures
Figure 1

Frequency of driver mutations detected with ODxTT and cobas® EGFR (A) Detection rate of driver mutations with ODxTT. (B) Detection rate of *EGFR* mutations with cobas® EGFR.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalTable1.docx](#)