Analytical and clinical validation of the Oncomine Dx Target Test to assess HER2 mutation status in tumor tissue samples from patients with non-small cell lung cancer treated with Trastuzumab Deruxtecan in the DESTINY-Lung01 and DESTINY-Lung02 studies

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Analytical and Clinical Validation of the Oncomine Dx Target Test to Assess *HER2* Mutation Status in Tumor Tissue Samples From Patients With Non–Small Cell Lung Cancer Treated With Trastuzumab Deruxtecan in the DESTINY-Lung01 and DESTINY-Lung02 Studies

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• ***Context.***—**Trastuzumab deruxtecan (T-DXd), a human epidermal growth factor receptor 2 (HER2)–targeted therapy, has demonstrated durable anticancer activity in patients with advanced, metastatic *HER2* (also known as *ERBB2*)–mutant (*HER2*m) non–small cell lung cancer (NSCLC) who have limited treatment options and poor prognosis.**

Objective.—**To analytically validate and assess the clinical utility of the Oncomine Dx Target (ODxT) Test as a companion diagnostic to identify patients with *HER2*m NSCLC.**

Design.—**Tumor samples from patients in DESTINY-Lung01 and DESTINY-Lung02 studies were retrospectively analyzed alongside commercially procured samples using the ODxT test and compared to the assays used for screening in these clinical trials.**

Results.—**Positive percent agreement (PPA) and negative percent agreement (NPA) between the ODxT Test and TruSight Tumor 170 assay when testing DESTINY-Lung01 and commercially procured samples met prespecified thresholds (PPA and NPA ≥90%) for analytical accuracy (100% and 99.1%). The ODxT Test results were highly concordant with clinical trial assays (CTAs) used in DESTINY-Lung01 (PPA and NPA, 98.0% and 100%) and DESTINY-Lung02 (PPA and NPA, 96.7% and 100%) to identify activating *HER2* mutations in tumor samples. Confirmed objective response rates were similar between patients with *HER2*m tumors identified by the ODxT Test and by CTAs in DESTINY-Lung01 (58.3% and 54.9%) and DESTINY-Lung02 (53.6% and 53.8%). Response duration was 12.0 and 9.3 months for patients identified by the ODxT Test and CTAs, respectively, in DESTINY-Lung01.**

Conclusions.—**The ODxT Test detected *HER2* mutations in NSCLC with high analytical and clinical accuracy and identified *HER2*m populations with response rates similar to populations identified by CTAs, supporting clinical utility of the ODxT Test to inform treatment decisions for *HER2*m NSCLC.**

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In non–small cell lung cancer (NSCLC), tumors with alterations in the human epidermal growth factor receptor 2 (*HER2* [also known as *ERBB2*]) gene make up a distinct molecular subtype. The abnormal activation of HER2 in cancer results from gene mutation, gene amplification, or by overexpression of the protein.[1] Among NSCLCs, HER2 protein overexpression has been reported in approximately 10% to 15% of tumors, with overexpression present in up to 30% of lung adenocarcinomas.[2] *HER2* mutations occur in 2%–4% of lung cancer cases, and *HER2* amplification occurs

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in 1%–3% of cases.[1] Studies have shown *HER2*-mutant (*HER2*m) NSCLC to be associated with younger age, female sex, a history of never smoking, poor prognosis, and an increased risk of brain metastasis.[3–6] Standard of care for metastatic NSCLC includes chemotherapy or immunotherapy; however, the effectiveness of such therapies in second or later lines is limited.[7,8] There is, therefore, a need for HER2-targeted therapies to treat patients with *HER2*m NSCLC.[7–12]

Trastuzumab deruxtecan (T-DXd) is the first HER2-targeted therapy to show a clinically meaningful benefit in patients with *HER2*m NSCLC. T-DXd has been approved in several countries for patients with previously treated *HER2*m NSCLC. In the United States, it received accelerated approval as the first HER2-directed treatment for patients with unresectable NSCLC or *HER2*m NSCLC, as detected by a US Food and Drug Administration (FDA)–approved test in those who have received a prior systemic therapy.[13] Reliable identification of patients whose tumors contain *HER2* mutations is critical for identifying those who may derive optimal therapeutic benefit from T-DXd. Therefore, the contemporaneous submission and approval of a companion diagnostic (CDx) in vitro assay was required for the FDA approval of T-DXd for the treatment of *HER2*m NSCLC.

As targeted therapies for the treatment of NSCLC continue to be developed, the challenge for physicians is how to use a limited amount of tumor tissue available for an increasing number of single-gene or small-panel biomarker tests that identify targetable alterations.[14] The feasibility of employing next-generation sequencing (NGS) for evaluating multiple biomarkers simultaneously and providing targeted treatment guidance for lung cancer and other solid tumors is being investigated in clinical trials and is increasingly being deployed in clinical settings.[14]

However, many NGS-based tests require personnel and infrastructure that can perform a highly complex workflow. Due to this complexity, NGS-based testing is often implemented in a centralized testing model where samples are sent from the hospital where the patient is being treated to a third-party (or reference) laboratory that can run the NGS-based test. This type of model leads to longer turnaround time, challenges with sample traceability, and loss of local expertise with molecular results. Therefore, solutions that can remove much of that complexity and allow local implementation are desirable.

An approved and distributed NGS test eliminates the need for hospitals and laboratories to send samples to specialized NGS testing facilities, thereby decreasing the turnaround time from biopsy to biomarker identification and treatment decisions.

The Oncomine Dx Target Test (ODxT Test) by ThermoFisher Scientific was the first targeted NGS in vitro diagnostic test for NSCLC. The ODxT Test uses targeted high-throughput, parallel-sequencing technology to detect single nucleotide variants (SNVs), deletions, and insertions in 23 genes. The test can also detect copy number variations but is not adequately validated.[15]

The ODxT Test can detect genomic alterations in both DNA and RNA from formalin-fixed, paraffin-embedded (FFPE) tumor samples with as little starting material as 10 ng. It is validated to qualitatively identify the presence of gene variants by generating positive, negative, or "no call" results based on a cutoff value and variant calling parameters. Its suitability for use in situ in hospitals and associated laboratories removes the need to ship samples to specialized laboratories and decreases the turnaround time from sample collection to test result. Given these advantages, the ODxT Test was chosen to be further developed as a CDx to identify patients whose tumors have activating *HER2* (*ERBB2*) mutations in NSCLC.

The purpose of the current study was to provide evidence in support of the effectiveness of the ODxT Test by analytically validating and assessing the clinical utility of the ODxT Test as a CDx test for identifying patients with NSCLC whose tumors contain *HER2* mutations and who may therefore benefit from HER2-targeted treatment. The validation was conducted using tissue from patients enrolled in the DESTINY-Lung01 and DESTINY-Lung02 clinical trials and from commercially procured NSCLC tumor samples; analyses were performed while DESTINY-Lung02 was ongoing, but before approval of the ODxT Test as a companion diagnostic for T-DXd (the submission for which was supported by these data). Of note, the population cohorts from the 2 studies that supplied samples differed with respect to ethnicity: patients in the DESTINY-Lung01 cohort were most frequently White (44.0% [40 of 91]) or Asian (34.1% [31 of 91]), while the majority of those in the DESTINY-Lung02 cohorts were Asian (63.2% [96 of 152]).

Herein are presented the results from ODxT Testing across NSCLC tumor samples from the DESTINY-Lung01/02 trials and commercially procured samples and analyses of its clinical validity for identifying patients who may respond to T-DXd treatment.

METHODS

Study Design

DESTINY-Lung01 (NCT03505710) was an open-label, phase 2 study that evaluated the efficacy and safety of T-DXd in patients with previously treated unresectable and/or metastatic *HER2*m or HER2-overexpressing NSCLC.[16] DESTINY-Lung02 (NCT04644237) was a randomized, phase 2 study that evaluated the efficacy and safety of 2 doses of T-DXd in patients with pretreated metastatic *HER2*m NSCLC.[17] Patients were enrolled at sites in Asia, Europe, and North America for both studies.[16,17] Tumor samples were analyzed retrospectively from patients who were enrolled in DESTINY-Lung01 or DESTINY-Lung02 and had NSCLC with activating *HER2* mutations according to local testing, and from commercially procured samples, in order to test the analytical accuracy and clinical validity of the ODxT Test.

Study Oversight

DESTINY-Lung01 and DESTINY-Lung02 trials were approved by the institutional review board at each site and conducted in accordance with the International Council for Harmonization Good Clinical Practice guidelines, the Declaration of Helsinki, and local regulations regarding the conduct of clinical research. All the patients provided written informed consent before participation in both trials, which also covered the use of tissue samples for the ODxT Test.[16,17]

Tumor Samples and Assays

FFPE NSCLC tumor samples were obtained for 86 of 91 patients enrolled from the DESTINY-Lung01 *HER2*m cohort (Supplemental Figure 1, see the supplemental digital content containing 2 figures and 5 tables at https://meridian.allenpress.com/aplm in the June 2025 table of contents.) and for 94 of 102 patients enrolled from the DESTINY-Lung02 5.4-mg/kg arm (Supplemental Figure 2; the present analysis was restricted to samples from patients who received the approved 5.4 mg/kg dose; therefore, samples from the DESTINY-Lung02 6.4-mg/kg arm were not evaluated). Tumor samples were provided as FFPE blocks or FFPE slides or freshly collected wet tissue shipped in 70% alcohol after formalin fixation. The wet tissues were processed into FFPE blocks at a central lab; FFPE blocks were stored at ambient temperature while FFPE slides were stored at 4°C.

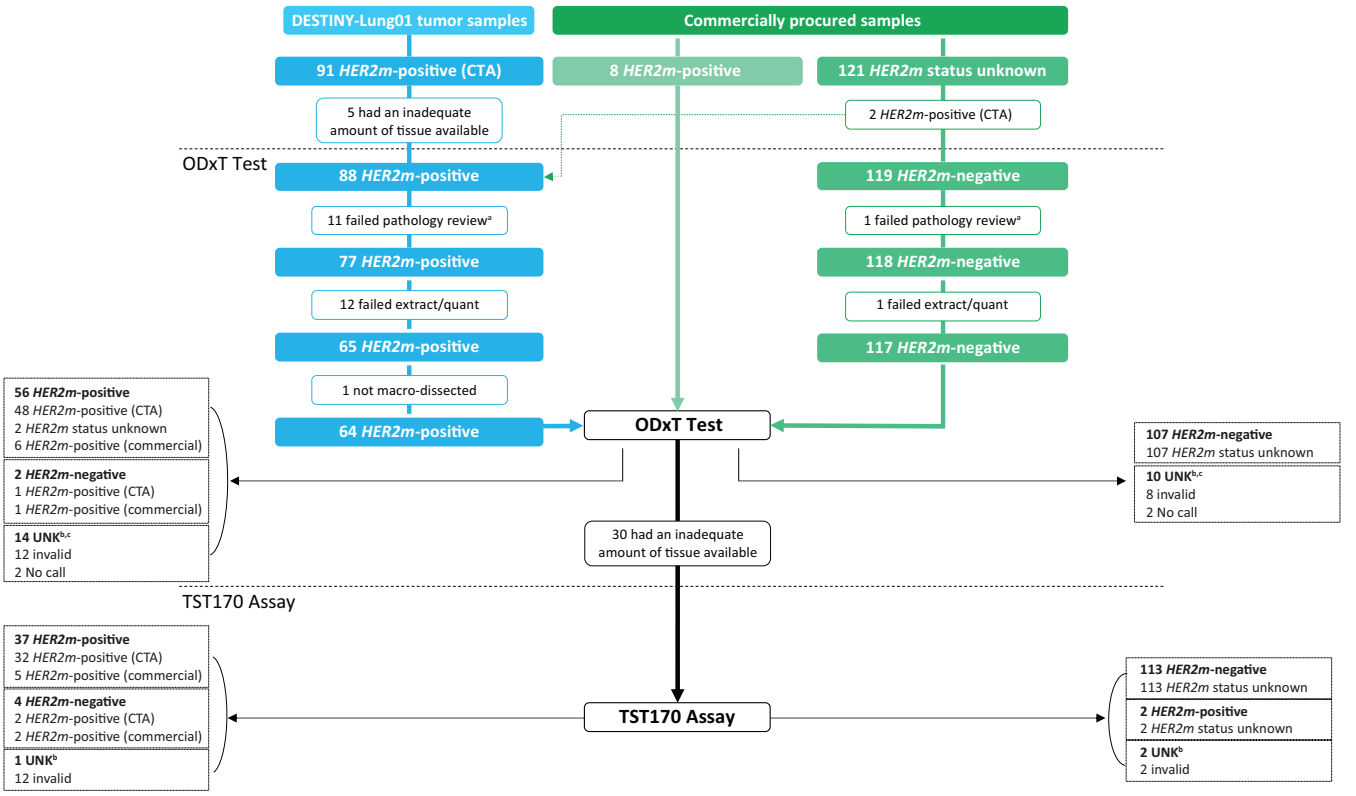

Figure 1. *Sample accountability flow chart for DESTINY-Lung01 and commercially procured samples. Abbreviations: CTA, clinical trial assay; Extract, extraction; HER2, human epidermal growth factor receptor 2 (ERBB2) gene; ODxT, Oncomine Dx Target; Quant, quantification; UNK, unknown.* [a]*Samples that failed pathology review were those obtained by fine-needle aspiration and samples with low or no tumor tissue as defined by assay user guidelines and study protocols.* [b]*An invalid result was obtained due to unmet DNA library quality control metrics (AQ20MRL and/or DNA percent reads).* [c]*A no-call result was obtained due to inadequate DNA library coverage reads (≤347 reads).*

An additional 121 FFPE stage-matched NSCLC tumor samples with mutation status unknown were procured from commercial vendors (BiolVT, Westbury, New York; Audubon Biosciences, Houston, Texas; BIO-OPTIONS, Brea, California; BioChain Institute, Newark, California) for analytical and clinical validation studies. Eight *HER2*m-positive NSCLC FFPE samples from patients with similar stages of disease were procured from commercial vendors (BiolVT; BioChain Institute) for analytical accuracy testing only.

Tumor tissue samples underwent a pathology evaluation for tumor content to determine whether they were suitable for use with the ODxT Test before *HER2* mutation analysis. With an approximate turnaround time of 3–5 days, the starting material volume needed for the ODxT Test is 10 ng with minimum tumor content of 10%. Only core needle biopsy or resection samples were accepted for ODxT Test analysis—fine-needle aspiration or cell block preparations were not permitted.

The suitable tumor tissue samples were extracted using a column-based isolation method and quantified by fluorimetry to determine if the nucleic acid yield met the minimum concentration requirement: DNA ≥0.83 ng/μL. For library preparation, the ODxT Test requires a minimum input of 10 ng of DNA for target amplification and uses Ion AmpliSeq (Thermo Fisher Scientific, Waltham, Massachusetts), which is an amplicon-based enrichment method for targeted NGS that was performed on the Ion PGM Dx System (Thermo Fisher Scientific). The sequenced data were processed through an analysis pipeline that called HER2 variants by comparing against specific hotspot regions that were aligned to Hg19. After *HER2* mutation analysis, tumor tissue samples were classified as *HER2*m-positive (limit of detection ~5% for both insertions and SNVs), *HER2*m-negative, no call (sample provided inadequate DNA library coverage reads [≤347 reads], strand bias, or poor quality score), or invalid (sample did not meet DNA library quality control metrics [AQ20 mean read length (MRL) <98 bp and/or DNA percent reads <0.7]). Statistical analyses were conducted to determine the minimum coverage required to accurately call SNVs and insertion/deletion (indels) variants. Sample accountability for all samples obtained from DESTINY-Lung01 and DESTINY-Lung02 are shown in Figures 1 and 2, respectively.

There were 41 eligible activating *HER2* mutations predicted to be oncogenic or likely oncogenic based on a review of the literature, information in the public precision oncology knowledge base (eg, PubMed, CKB, OncoKB, ClinVar, DoCM, and CIViC), or investigation in clinical studies that evaluated efficacy and safety of experimental anti-HER2 agents (https://ckb.jax.org; Supplemental Table 1). Inclusion criteria for DESTINY-Lung01 and DESTINY-Lung02 required patients to have an eligible activating *HER2* mutation (SNVs or exon 20 insertions) as outlined in Supplemental Table 2. The *HER2* mutations that qualified patients for enrollment differed between DESTINY-Lung01 (27 eligible mutations) and DESTINY-Lung02 (43 eligible mutations). DESTINY-Lung01 included 1 eligible mutation that was not specified in the DESTINY-Lung02 sequencing panels (Ser310Pro). DESTINY-Lung02 included 16 eligible mutations that were not included in the DESTINY-Lung01 sequencing panels (Ala775_Gly776insTyrValMetAla, Ala775_Gly776insVal, Arg896Cys, Arg896His, Val777_Gly778insGlyCysPro, Gly776_Val777insValGlyCys, Ile767Met, Ile767Phe, Leu755_Glu757delinsSer, Leu755_Thr759del, Leu755Ala, Leu869Arg, Thr798Ile, Thr862Ile, Val777_Gly778insCysVal, Val842Ile).

Analytical validation of the ODxT Test was carried out by comparing results from the ODxT Test with a reference NGS assay, TruSight Tumor 170 (TST170; Illumina, San Diego, California). TST170 was used as an orthogonal reference assay for analytical accuracy in this study based on guidance from the FDA. TST170 uses a different NGS platform, had low usage during enrollment in DESTINY-Lung01, and could be validated by the testing laboratory prior to the analytical accuracy study for the ODxT Test. For this study, samples from patients in DESTINY-Lung01 and commercially procured samples were first assessed using the ODxT Test and, if adequate tissue

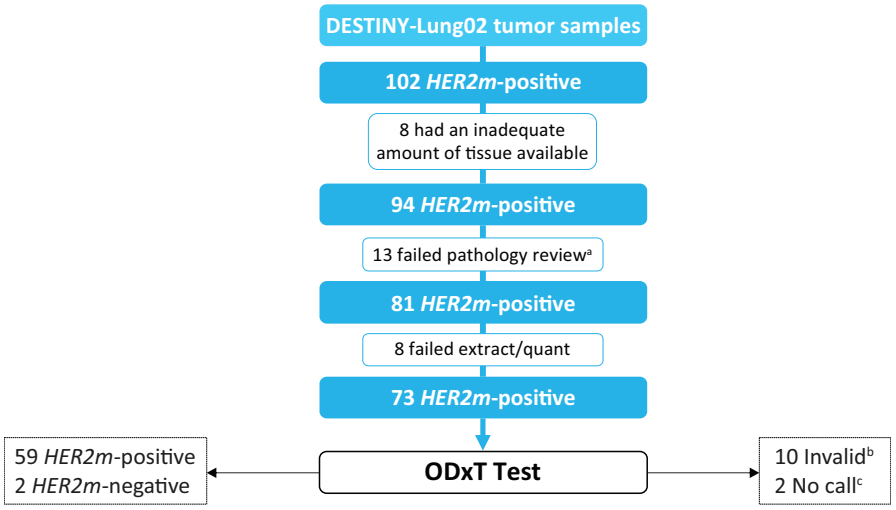

Figure 2. *Sample accountability flow chart for DESTINY-Lung02. Abbreviations: Extract, extraction;* HER2*, human epidermal growth factor receptor 2 (*ERBB2*) gene; ODxT, Oncomine Dx Target; Quant, quantification. [a]Samples that failed pathology review were those obtained by fine-needle aspiration and samples with low or no tumor tissue as defined by assay user guidelines and study protocols. [b]An invalid result was obtained due to unmet DNA library quality control metrics (AQ20MRL and/or DNA percent reads). [c]A no-call result was obtained due to inadequate DNA library coverage reads (≤347 reads).*

remained, were then tested with the TST170 assay. Samples from DESTINY-Lung02 were not used for analytical validation as the validation was conducted before the completion of DESTINY-Lung02. *HER2*m results from the ODxT Test (investigational use only assay [23 genes, >600 variants, DNA and RNA]) were compared with those from TST170 (170 genes, DNA and RNA) to determine whether the ODxT Test met a prespecified threshold for agreement.[18]

Clinical validation of the ODxT Test was achieved by comparing the CTAs used in screening for DESTINY-Lung01 and DESTINY-Lung02 against ODxT Test results. CTAs used to screen tumor samples for enrollment in DESTINY-Lung01 and DESTINY-Lung02 are summarized in Supplemental Table 3. From these study participants, available archival and/or freshly collected tumor tissue processed into FFPE samples (n = 180) were used for clinical validation. In both the DESTINY-Lung01 and DESTINY-Lung02 studies, only FFPE tissue samples were accepted. In DESTINY-Lung02, only participants with sufficient tissue samples were eligible for the study. A fresh tissue sample was defined as "recently collected," either as an archival tissue in FFPE or as a wet tissue sample. Study sites were offered fresh tissue collection kits that enabled the placement of wet tissue in 70% alcohol, with fixed tissue later embedded in a central laboratory. Commercially procured samples (with mutation status unknown; n = 121) were also included, for which results from the ODxT Test were compared against results from 1 of 2 representative CTAs also performed: the Oncomine Comprehensive Assay v3 (Thermo Fisher Scientific; n = 65) or the OncoPanel assay (Brigham & Women's Hospital, Boston, Massachusetts; n = 56).

Analytical Methods

Concordance, positive percent agreement (PPA), and negative percent agreement (NPA) between the ODxT Test and comparator assays were assessed to validate the analytical and clinical accuracy of the ODxT Test. Analytical accuracy was quantified by using the alignment of results from the ODxT Test with an accepted testing standard. In this case, the ODxT Test was compared with the TST170 assay for detecting *HER2*m NSCLC tumors in DESTINY-Lung01 tumor samples and commercially procured NSCLC FFPE samples. Prespecified requirements for demonstrating analytical accuracy were a PPA ≥90% and NPA ≥90% when excluding unknown results. Clinical accuracy was quantified by using the alignment of results from the ODxT Test with tests used to enroll patients in DESTINY-Lung01 and DESTINY-Lung02. Results from CTAs were compared with those from the ODxT Test for *HER2*m status in NSCLC tumor samples from DESTINY-Lung01 and DESTINY-Lung02, and commercially procured NSCLC FFPE samples. PPA and NPA had to meet FDA requirements (PPA 95% CI lower bound ≥85% and NPA ≥98% when excluding unknown results) to demonstrate clinical accuracy.

To evaluate the clinical utility of the ODxT Test in identifying patients who are likely to benefit from treatment with T-DXd, confirmed objective response rate (ORR) and duration of response (DoR) were compared between patients who were classified as having *HER2*m NSCLC determined by CTAs (31 assays used in DESTINY-Lung01 and 23 assays used in DESTINY-Lung02) and those classified post hoc based on ODxT Test results. DESTINY-Lung01 efficacy analyses were based on the primary analysis of the *HER2*m cohort (n = 91; data cutoff, May 3, 2021). DESTINY-Lung02 efficacy analyses were based on the prespecified early cohort interim analysis (n = 52), which included patients who were randomized at least 4.5 months prior to the data cutoff (March 24, 2022).

RESULTS

Patient Demographics

There were no notable differences in demographics between patients whose tumor samples were evaluable compared with the overall population and with patients whose tumor samples were unevaluable (Supplemental Table 4). Of note, differences in ethnicity rates can be attributed to where the patients in DESTINY-Lung01 and DESTINY-Lung02 studies were enrolled; the majority of those enrolled in each study (61% and 94%, respectively) were from Asia or Europe. A total of 322 samples that were commercially procured or obtained from patients enrolled in DESTINY-Lung01 (*HER2*m cohort) and DESTINY-Lung02 (5.4-mg/kg arm) underwent a pathology review to determine their eligibility for use in this clinical validation study of the ODxT Test (Figures 1 and 2). Eighty-six samples from patients with *HER2*m NSCLC (determined by a CTA) were obtained from the DESTINY-Lung01 *HER2*m cohort (N = 91) and 94 *HER2*m (per CTA) samples were obtained from the DESTINY-Lung02 5.4-mg/kg arm (N = 102). Approximately two-thirds of samples tested from each trial were primary tissue samples, while the other third were metastatic tissue samples. A total of 129 NSCLC samples were commercially procured for analysis. Eight commercially procured samples were *HER2*m-positive and only used for analytical accuracy, and 121 commercially procured samples had an unknown *HER2* mutation status.

Analysis of potential associations between patient demographics or clinical characteristics and sample evaluability with the ODxT Test was performed to determine whether there was a statistical association detected between whether the ODxT Test result was missing and any of the characteristics (statistically speaking, whether the results were missing at random). Variables with *P* values <.20 in that association test were tumor content ($P < .001$), specimen type ($P = .05$), and patient age

Table 1. Concordance of the ODxT Test With the TST170 Assay[a]: Samples From DESTINY-Lung01 and Commercially Procured Samples

	TST170 Assay				
ODxT Test	Positive	Negative	Unknown	Test Not Performed[b]	Total
Positive	38	1	1	16	56
Negative	0	108	1	0	109
Unknown	1	8	1	14	24
Test not performed	0	0	0	31	31
Total	39	117	3	61	220

Abbreviations: ODxT, Oncomine Dx Target; TST170, Illumina TruSight Tumor 170 next-generation sequencing assay.

[a] Human epidermal growth factor receptor 2 (*HER2* [*ERBB2*]) gene single nucleotide variant and exon 20 insertion.

[b] Clinical samples were first evaluated using the ODxT Test. Remaining tissue sample was then tested using the TST170 assay. Some tumor samples tested by the ODxT Test did not provide enough leftover material for the subsequent TST170 assay.

($P = .07$) in the DESTINY-Lung01 study. The statistical procedure named multiple imputation was performed to assign values to the participants missing an ODxT Test result, using those 3 variables and response information in the modeling. The resulting estimates of concordance and assay agreement were consistent with the ones estimated with the observed data (Supplemental Table 5). The missing results did not bias the estimates of concordances or drug efficacy.

Pathology Review of Tumor Samples

Tumor samples unevaluable for use with the ODxT Test were reported from both trials. In DESTINY-Lung01, tumor samples from 43 of 91 patients (47.3%) were unevaluable: 5 patients had no tissue sample available, 11 failed the pathology review, 12 did not meet DNA extraction/quantification thresholds, 1 was not macro-dissected, 2 were deemed no call, and 12 were deemed invalid. In DESTINY-Lung02, tumor samples from 41 of 102 patients (40.2%) were unevaluable: 8 patients had no tissue sample available, 13 failed the pathology review, 8 did not meet DNA extraction/quantification thresholds, 2 were deemed no call, and 10 were deemed invalid.

Analytical Accuracy of the ODxT Test

High concordance was observed between the ODxT Test and the TST170 reference assay among samples from DESTINY-Lung01 and commercially procured NSCLC FFPE tumor samples (Table 1). Percent agreement calculations met the prespecified requirements for demonstrating analytical accuracy (PPA ≥90% and NPA ≥90% when excluding unknown results), with a PPA of 100% and NPA of 99.1% (Table 2).

Clinical Accuracy of the ODxT Test

High concordance was also seen between CTAs and the ODxT Test in DESTINY-Lung01, DESTINY-Lung02, and commercially procured samples (Table 3). The resulting percent agreements (Table 4) were in line with FDA requirements for demonstrating testing accuracy in a clinical setting (PPA 95% CI lower bound ≥85% and NPA ≥98% when excluding unknown results). The PPA and NPA calculations were 98.0% and 100%, respectively, when excluding unknown results in DESTINY-Lung-01. The PPA and NPA calculations were 96.7% and 100%, respectively, when excluding unknown results in DESTINY-Lung02. Concordance of the commercially procured samples were captured within the PPA and NPA calculations of DESTINY-Lung01 and DESTINY-Lung02. Three patients (1 patient in DESTINY-Lung01 and 2 patients in DESTINY-Lung02) identified by CTAs as positive for *HER2*m tumors were assessed as negative for *HER2*m tumors by the ODxT Test (Table 3).

Clinical Efficacy Analysis Based on ODxT Test Results

Confirmed ORR and DoR were similar for patients with *HER2*m NSCLC identified by local CTAs and a central ODxT Test in both the DESTINY-Lung01 and DESTINY-Lung02 trials (Table 5). The DESTINY-Lung01 *HER2*m cohort had a confirmed ORR by independent central review of 54.9% (95% CI, 44.2%–65.4%) in 91 patients with *HER2*m NSCLC identified by CTAs compared with 58.3% (95% CI, 43.2%–72.4%) in 49 evaluable patients with *HER2*m NSCLC identified by the ODxT Test. The median DoR in patients identified by CTAs and the ODxT Test within the DESTINY-Lung01 *HER2*m cohort was 9.3 months (95% CI, 5.7–14.7 months) and 12.0 months (95% CI, 5.54–18.23 months), respectively.

The confirmed ORR by blinded independent central review in the DESTINY-Lung02 5.4-mg/kg arm at interim analysis was 53.8% (95% CI, 39.5%–67.8%) in 52 patients with *HER2*m NSCLC identified by CTAs compared with 53.6% (95% CI, 33.9%–72.5%) in 29 evaluable patients identified by the ODxT Test. DoR results within the DESTINY-Lung02 were immature at the time of analysis data cutoff.

Of the 3 patients identified as positive for *HER2*m tumors by CTAs but assessed as negative by the ODxT Test, 2

Table 2. Agreement Between the ODxT Test and the TST170 Assay[a] From 49 Evaluable DESTINY-Lung01 and 129 Commercially Procured Samples

Measure of Agreement	Excluding Unknown[b] Results		Including Unknown[b] Results	
	Agreement % (n of Total)	95% CI[c]	Agreement % (n of Total)	95% CI[c]
PPA	100 (38 of 38)	90.8–100	97.4 (38 of 39)	86.5–99.9
NPA	99.1 (108 of 109)	95.0–100	92.3 (108 of 117)	85.9–96.4

Abbreviations: ODxT, Oncomine Dx Target; NPA, negative percent agreement; PPA, positive percent agreement; TST170, Illumina TruSight Tumor 170 next-generation sequencing assay.

[a] Human epidermal growth factor receptor 2 (*HER2* [*ERBB2*]) gene single nucleotide variant and exon 20 insertion.

[b] Invalid or no-call samples by the ODxT Test.

[c] Calculated using Clopper-Pearson method.

Table 3. Concordance of CTAs With the ODxT Test: Samples From DESTINY-Lung01/DESTINY-Lung02 and Commercially Procured[a] Samples

ODxT Test	CTA Positive	CTA Negative[b]	CTA Total
DESTINY-Lung01 *HER2*m Cohort and Commercially Procured Samples			
Positive	50	0	50
Negative	1	107	108
Unknown	13	10	23
Test not performed	29	2	31
Total	93[c]	119	212[d]
DESTINY-Lung02 5.4-mg/kg Arm and Commercially Procured Samples			
Positive	59	0	59
Negative	2	107	109
Unknown	12	10	22
Test not performed	29	2	31
Total	102[c]	119	221

Abbreviations: CTA, clinical trial assay; *HER2*, human epidermal growth factor receptor 2 (*ERBB2*) gene; *HER2*m, *HER2* mutant; ODxT, Oncomine Dx Target.

[a] One hundred twenty-one formalin-fixed, paraffin-embedded stage-matched non–small cell lung cancer tumor samples with unknown human epidermal growth factor receptor 2 (*HER2* [*ERBB2*]) mutation status.

[b] Negative results from commercial samples were screened by the Oncomine Comprehensive Assay v3 (OCAv3) and OncoPanel.

[c] Out of 121 HER2 status unknown commercial samples used to evaluate clinical accuracy, 2 were *HER2* mutation–positive and included in the 91 samples from DESTINY-Lung01, totaling 93. The analysis with DESTINY-Lung02 included the 119 *HER2* mutation–negative samples and excluded the 2 positive samples.

[d] The 8 commercially procured *HER2*m-positive samples were not included in the concordance analyses of CTAs and the ODxT Test because they were only used for analytical accuracy as per the study design.

did not respond to treatment (1 each from DESTINY-Lung01 and DESTINY-Lung02), while the third (from DESTINY-Lung02) was not part of the early efficacy analysis cohort.

DISCUSSION

Analytically and clinically validated in vitro diagnostic and laboratory-developed tests can be used to determine genomic alterations and provide guidance on the most suitable approach for treating patients with cancer.[19] The ODxT Test is an in vitro diagnostic NGS assay approved in the United States and Japan as a CDx test for detecting activating *HER2* mutations in the tumors of patients with unresectable or metastatic NSCLC before treatment.[20,21] High levels of concordance between CTAs and the ODxT Test are required for the ODxT test to be considered a reliable in vitro diagnostic assay for detecting *HER2* mutations in NSCLC tumor samples. This study demonstrated that the ODxT Test had high analytical and clinical accuracy for detecting *HER2* mutations in tumor samples from patients with NSCLC in the DESTINY-Lung01 and DESTINY-Lung02 trials and may serve as a reliable CDx test to identify patients who are likely to benefit from treatment with T-DXd.

Percent agreements between the ODxT Test and the TST170 assay, an NGS assay used as a reference for analytical accuracy, met prespecified requirements of 90% or higher, indicating high concordance of analytical accuracy between these tests. This validates the analytical accuracy and reliability of the ODxT Test to predict the *HER2* mutation status of patients, which is an important consideration in developing recommendations for diagnostic methods. Regarding cases that did not agree, in the DESTINY-Lung01 study, 1 sample classified as *HER2*m by a CTA used to enroll participants into the clinical trial was subsequently reported as negative for *HER2* mutations by the ODxT Test and TST170 assay. However, further review of the tissue sample determined that the material used to test with the ODxT Test was not from the same FFPE tissue block that provided tissue to test with the enrolling CTA. In addition, review of the raw data generated by the ODxT Test concluded that the sample sequenced had no presence of *HER2* mutation. These findings, along with the negative results reported by the TST170 assay, support the likelihood that the discordant result reported by the ODxT Test is a true negative result and not a false negative result. Any discordance may also be explained by heterogeneity in the tumor samples.

Table 4. Agreement Between CTAs and the ODxT Test From Evaluable DESTINY-Lung01/DESTINY-Lung02 and Commercially Procured Samples

Measure of Agreement	Excluding Unknown[a] Results (DESTINY-Lung01 *HER2*m Cohort: n = 49) Agreement % (n of total)	95% CI[b]	Including Unknown[a] Results (DESTINY-Lung01 *HER2*m Cohort: n = 62) Agreement % (n of total)	95% CI[b]
	DESTINY-Lung01 *HER2*m Cohort and Commercially Procured Samples			
PPA	98.0 (50 of 51)	89.6–100	78.1 (50 of 64)	66.0–87.5
NPA	100 (107 of 107)	96.6–100	91.5 (107 of 117)	84.8–95.8
	DESTINY-Lung02 5.4-mg/kg Arm and Commercially Procured Samples			
	Excluding Unknown[a] Results (DESTINY-Lung02 5.4-mg/kg Arm: n = 61)		**Including Unknown[a] Results (DESTINY-Lung02 5.4-mg/kg Arm: n = 73)**	
PPA	96.7 (59 of 61)	88.7–99.6	80.8 (59 of 73)	69.9–89.1
NPA	100 (107 of 107)	96.6–100	91.5 (107 of 117)	84.8–95.8

Abbreviations: CTA, clinical trial assay; *HER2*, human epidermal growth factor receptor 2 (*ERBB2*) gene; *HER2*m, *HER2* mutant; NPA, negative percent agreement; ODxT, Oncomine Dx Target; PPA, positive percent agreement.

[a] Invalid or no call by the ODxT Test.

[b] Calculated using the Clopper-Pearson method.

Table 5. Confirmed ORR and DoR in Samples With ODxT Test Results: DESTINY-Lung01/DESTINY-Lung02					
	DESTINY-Lung01 *HER2*m Cohort (CTA Positive)				
	ODxT Test Positive (n = 48)	ODxT Test Negative (n = 1)	ODxT Test Evaluable (n = 49)	ODxT Test Unevaluable[a] (n = 42)	CTA Positive Total (N = 91)
Confirmed ORR[b], n (%) [95% CI]	28 (58.3) [43.2–72.4]	0 [0.0–97.5]	28 (57.1) [42.2–71.2]	22 (52.4) [36.4–68.0]	50 (54.9) [44.2–65.4]
Median DoR [95% CI][c], months	12.0 [5.54–18.23]	—	—	—	9.3 [5.7–14.7]
	DESTINY-Lung02 5.4-mg/kg Arm (CTA Positive)				
	ODxT Test Positive (n = 28)	ODxT Test Negative (n = 1)	ODxT Test Evaluable (n = 29)	ODxT Test Unevaluable[a] (n = 23)	CTA Positive Total (N = 52)
Confirmed ORR[b], n (%) [95% CI]	15 (53.6) [33.9–72.5]	0 [0.0–97.5]	15 (51.7) [32.5–70.6]	13 (56.5) [34.5–76.8]	28 (53.8) [39.5–67.8]
Median DoR [95% CI][c], months	NE[d]	—	—	—	NE[d]

Abbreviations: CTA, clinical trial assay; DoR, duration of response; *HER2*m, human epidermal growth factor receptor 2 mutant; NE, not evaluable; ODxT, Oncomine Dx Target; ORR, objective response rate.

[a] Insufficient material, or samples that generated a no-call or invalid result in the ODxT Test.
[b] By blinded independent central review based on Response Evaluation Criteria in Solid Tumors, version 1.1.
[c] Two-sided 95% CI values are based on the exact (Clopper-Pearson) method for binomial distribution.
[d] DoR results were immature at this early cohort analysis data cutoff.

In the DESTINY-Lung02 study, 2 samples were identified as *HER2*m by CTAs used to enroll participants into the clinical trial that were subsequently determined to be *HER2*m-negative by the ODxT Test. Further evaluation of the raw sequencing data generated by the ODxT Test identified the variants as having a high probability of being novel variants not currently included in the ODxT Test hotspot file. This explains why these 2 samples were *HER2*m according to CTAs but not according to the ODxT Test.

Percent agreements between the ODxT Test and the CTAs used in the DESTINY-Lung01 and DESTINY-Lung02 trials as a measure of clinical accuracy also met the prespecified requirements of 90% or greater indicating high concordance of clinical accuracy between the ODxT Test and CTAs. The high concordance of clinical accuracy demonstrates that the ODxT Test is a safe and effective CDx test to identify NSCLC in patients with *HER2* mutations in FFPE tumor samples who are eligible for T-DXd treatment. Clinical efficacy for the ODxT Test was determined by comparing the ORR and DoR between the groups of patients in the DESTINY-Lung01 and DESTINY-Lung02 cohorts who were confirmed as *HER2*m by CTAs against those confirmed using the ODxT Test. The high levels of analytical and clinical accuracy and the clinical effectiveness of the ODxT Test demonstrated in this study align with a similar study that investigated its validity for identifying patients who have NSCLC with epidermal growth factor receptor (*EGFR*) exon 20 insertions and may be suitable for treatment with amivantamab.[19]

The development of CDx tests such as the ODxT Test can vastly increase the availability, accessibility, and reliability of testing tumor samples from patients with *HER2*m NSCLC to identify the most suitable treatment method. It was previously reported that inefficiencies of market development planning for CDx tests prevents 50% of patients from receiving the most appropriate test to advise on the most suitable biomarker-guided treatment.[22] The implementation of an approved NGS CDx method such as the ODxT Test in local laboratories offers benefits, including fast diagnosis times, identification of multiple specific mutations for each gene, versatility to add emerging biomarkers, and the ability to use a small sample size of FFPE tissue. As an approved NGS method in the United States and Japan, the ODxT Test can be adopted in local hospitals, allowing for faster biomarker identification and broader access to a wide range of diagnostic variables used to optimize targeted therapy decisions.[15]

One limitation of this study was that only 52.7% of tumor samples from DESTINY-Lung01 and 59.8% of tumor samples from DESTINY-Lung02 were evaluable for validation of the ODxT Test. Of 91 tumor samples from DESTINY-Lung01, 5 had an inadequate amount of tissue provided, 11 did not meet tumor content criteria as defined by assay user guidelines and study protocols, 12 did not provide DNA in sufficient concentrations for quantification, 1 was not processed correctly (it was not macrodissected), 12 were deemed invalid as they did not meet DNA library quality control metric criteria for AQ20MRL and/or DNA percent reads, and 2 were classified as no-call results (DNA library coverage reads $\leq$347 reads). Of 102 tumor samples from DESTINY-Lung02, 8 had an inadequate amount of tissue available, 13 did not meet tumor content criteria as defined by assay user guidelines and study protocols, 8 did not provide DNA in sufficient concentrations for quantification, 10 were deemed invalid as they did not meet DNA library quality control metric criteria for AQ20MRL and/or DNA percent reads, and 2 were classified as no-call results (DNA library coverage reads $\leq$347). Previous studies that investigated sample conditions from FFPE cancerous tissues outlined how samples may be unevaluable due to poor fixation techniques.[23] The rate of unevaluable samples in this study is in line with other clinical studies for patients with lung cancer, including studies validating the use of the ODxT Test to identify whether patients were suitable for treatment with amivantamab.[19,24,25] In this study, available archival and/or freshly collected tumor tissue processed into FFPE samples were used for ODxT testing. Therefore, only some of these samples (mainly the archival samples) had been successfully tested by an alternative method (CTA) previously. Furthermore, these same samples had longer storage times after testing by a previous method, so the FFPE DNA quality could have deteriorated because

of the longer storage time. For freshly collected tumor tissue samples, some could have been affected by poor fixation technique. There were no data to suggest that the ODxT Test was more sensitive to small changes in sample quantity and quality. The high failure rate at extraction/quantitation could have been due to insufficient quantities of tumor tissue extracted, poor fixation technique, or long storage time causing the DNA quality to deteriorate.

This study showed that the ODxT Test has high analytical and clinical accuracy for detecting HER2 mutations in NSCLC, based on concordance and agreement analyses of tumor sample testing results from DESTINY-Lung01 and DESTINY-Lung02. Furthermore, it demonstrated the validity of the ODxT Test for identifying patients who are likely to benefit from treatment with T-DXd.

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Data availability statement: Anonymized individual participant data on completed studies and applicable supporting study documents may be available upon request at https://vivli.org/. In cases where clinical study data and supporting documents are provided pursuant to our company policies and procedures, Daiichi Sankyo Companies will continue to protect the privacy of company and our clinical study subjects. Details on data-sharing criteria and the procedure for requesting access can be found at this web address: https://vivli.org/ourmember/daiichi-sankyo/.

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