SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next Generation Sequencing oncology panel, somatic or germline variant detection system
Device Trade Name:	Oncomine^{TM} Dx Target Test
Device Procode:	PQP
Applicant's Name and Address:	Life Technologies Corporation 7305 Executive Way Frederick, MD 21704
Date(s) of Panel Recommendation:	None

Premarket Approval Application (PMA) Number: P160045/S035

Date of FDA Approval:	August 11, 2022

The original PMA (P160045) Oncomine™ Dx Target (ODxT) Test was approved on June 22, 2017, for the detection of genetic alterations in patients who may benefit from one of three FDA-approved therapies for non-small cell lung cancer (NSCLC).

Subsequently, additional PMA supplements were approved for expanding the indications for use of ODxT Test for detecting *RET* fusions and *EGFR* exon 20 insertions in tumors from NSCLC patients, and for the identification of *IDH1* single nucleotide variants (SNVs) in cholangiocarcinoma (CC) patients for treatment with the corresponding therapeutic products, since its original approval. The SSEDs to support the previously approved indications are available on the CDRH website.

The current panel track supplement was submitted to expand the indications for use of the ODxT Test to include a companion diagnostic indication for the identification of *ERBB2/HER2* mutations in NSCLC patients who may benefit from Daiichi Sankyo's therapeutic product, ENHERTU® (fam-trastuzumab deruxtecan-nxki).

II. INDICATIONS FOR USE

The Oncomine™ Dx Target Test is a qualitative *in vitro* diagnostic test that uses targeted high throughput, parallel-sequencing technology to detect single nucleotide variants (SNVs), insertions, and deletions in 23 genes from DNA and fusions in *ROS1* and in *RET* from RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor samples from patients with non-small cell lung cancer (NSCLC), and

IDH1 SNVs from FFPE tumor tissue samples from patients with cholangiocarcinoma (CC), using the Ion PGM™ Dx System.

The test is indicated as a companion diagnostic to aid in selecting NSCLC and CC patients for treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1. List of Variants for Therapeutic Use

Tissue Type	Gene	Variant	Targeted Therapy
NSCLC	*BRAF*	*BRAF* V600E mutation	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
	EGFR	*EGFR* L858R mutation, *EGFR exon* 19 deletions	IRESSA® (gefitinib)
	EGFR	*EGFR* exon 20 insertions	EXKIVITY™ (mobocertinib)
			RYBREVANT™ (amivantamab-vmjw)
	ERBB2/HER2	*ERBB2/HER2* activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
	RET	*RET* fusions	GAVRETO™ (pralsetinib)
	ROS1	*ROS1* fusions	XALKORI® (crizotinib)
CC	*IDH1*	*IDH1 R132C, IDH1 R132G, IDH1 R132H IDH1 R132L,* and *IDH1 R132S* mutations	TIBSOVO® (ivosidenib)

Safe and effective use has not been established for selecting therapies using this device for the variants other than those in Table 1.

Results other than those listed in Table 1 are indicated for use only in patients who have already been considered for all appropriate therapies (including those listed in Table 1).

Analytical performance using NSCLC specimens has been established for the variants listed in Table 2.

Table 2. List of Variants with Established Analytical Performance Only

Gene	Variant Type	Amino Acid Change	Nucleotide Change
KRAS	COSM512	p.Gly12Phe	c.34_35delGGinsTT
KRAS	COSM516	p.Gly12Cys	c.34G>T

MET	COSM707	p.Thr1010Ile	c.3029C>T
PIK3CA	COSM754	p.Asn345Lys	c.1035T>A

The test is not indicated to be used for standalone diagnostic purposes, screening, monitoring, risk assessment, or prognosis.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the ODxT Test labeling.

V. DEVICE DESCRIPTION

The ODxT Test is an *in vitro* diagnostic test that provides primer panels, assay controls and interpretative software [an Assay Definition File (ADF)] designed for use with the Ion PGM Dx System and the Ion PGM Dx Reagents for detection of alterations in DNA [isolated from NSCLC and cholangiocarcinoma formalin-fixed, paraffin-embedded (FFPE) tumor specimens] and RNA isolated from NSCLC FFPE tumor specimens.

The ODxT Test consists of the following:

Oncomine™ Dx Target Test and Controls Kit (Combo Kit):
- Oncomine™ Dx Target Test DNA and RNA Panel Kit
- Oncomine™ Dx Target DNA Control Kit
- Oncomine™ Dx Target RNA Control Kit
- Ion Torrent™ Dx No Template Control Kit
- Oncomine™ Dx Target Test RNA Control Diluent Kit

Ion Torrent™ Dx FFPE Sample Preparation Kit:
- Ion Torrent™ Dx Total Nucleic Acid Isolation Kit
- Ion Torrent™ Dx cDNA Synthesis Kit
- Ion Torrent™ Dx DNA Quantification Kit
- Ion Torrent™ Dx RNA Quantification Kit
- Ion Torrent™ Dx Dilution Buffer Kit

Ion PGM™ Dx Reagents / Chips:
- Ion PGM™ Dx Library Kit
- Ion OneTouch™ Dx Template Kit
- Ion PGM™ Dx Sequencing Kit
- Ion 318™ Dx Chip Kit

Instrumentation and Software:

- The assay is run on the Ion PGM™ Dx System:
  - Ion OneTouch™ Dx System:
    - Ion OneTouch™ Dx Instrument
    - Ion OneTouch™ ES Dx Instrument
  - Ion PGM™ Dx Sequencer
  - Ion PGM™ Dx Chip Minifuge
  - Ion Torrent™ Server
  - Torrent Suite™ Dx
  - Other accessories:
    - Ion PGM™ Wireless Scanner
    - DynaMag™ Dx 16 2mL Magnet
    - DynaMag™ Dx 96-Well Plate Magnet

The system also utilizes specified accessories. The assay's definition files are provided on a USB memory device along with the ODxT Test User Guide:

- Oncomine™ Dx Target Assay Definition File (includes interpretive software)
- Oncomine™ Dx Target Test User Guide
- Veriti™ Dx Thermal Cycler Settings
- Electronic Document Instructions (provided to users both as a paper copy and a PDF document on the USB drive)

Nucleic Acid Extraction:
DNA and RNA extraction is performed using the proprietary Ion Torrent™ Dx FFPE Sample Preparation Kit. The deparaffinized sample is first subjected to protein digestion with Proteinase K at an elevated temperature in a guanidinium thiocyanate solution to facilitate release and protection of RNA and DNA by inhibiting nuclease activity. After a heating step to inactivate the Proteinase K enzyme, the digested sample is transferred into a spin column containing a silica-based filter membrane.

The RNA is selectively eluted and separated from DNA which is retained on the filter. The eluted RNA is mixed with ethanol and captured onto a second spin column containing a silica-based membrane filter. The RNA is retained, and cellular impurities are removed by a series of washes. The bound RNA is treated with DNase to reduce contaminating DNA. Following a series of washes to remove residual DNase and DNA degradation products, the purified RNA is eluted from the filter.

The DNA retained on the first filter is similarly subjected to a series of washes to remove cellular impurities and then purified DNA is eluted from the filter. The Elution Solution provided with the kit is a low ionic strength Tris-buffered solution containing EDTA that facilitates elution of nucleic acids from the silica filter. The solution provides appropriate pH for stability of RNA and DNA and inhibits nucleases by binding metal cofactors.

Quantification:
RNA and DNA quantification is performed using a fluorescence dye-binding assay and a qualified fluorometer/fluorescence reader capable of operating at the specific excitation and emission wavelengths. First, working solutions consisting of buffer and proprietary fluorophores are prepared for both DNA and RNA samples, as well as the DNA and RNA standards supplied at different concentrations in the kit (0 ng/µL to 10 ng/µL). Second, the DNA and RNA samples are incubated with their respective solutions at room temperature where the fluorophores bind to the target DNA and RNA molecules. When bound to the DNA and RNA, the fluorophores exhibit fluorescence enhancement at a specific excitation wavelength. The emitted fluorescent signals are captured and converted into signal fluorescence units. Third, the concentration (in ng/µL) of the DNA and RNA samples are determined by performing a linear regression with the values obtained from the DNA and RNA standards.

Sample Dilution Buffer is provided in the kit to dilute the DNA and RNA samples to a specific concentration required for cDNA synthesis and library preparation.

RT Step (RNA only):
RNA is enzymatically converted to cDNA using the Ion Torrent™ Dx cDNA Synthesis Kit. Ten nanograms (ng) of RNA is enzymatically converted to cDNA using an enzyme mix containing a proprietary engineered version of M-MLV reverse transcriptase (Superscript III RT), an RNase inhibitor, a proprietary helper protein, and a buffer containing random primers, dNTPs, and $MgCl_2$.

Library Preparation Workflow:
The process begins with polymerase chain reaction (PCR) and uses the ODxT Test DNA and RNA Panel and the Ion PGM™ Dx Library Kit to specifically amplify target regions of interest from cDNA (including cDNA from the RNA control) and DNA (including the DNA Control and No Template Control). For the detection of RNA fusions, the current device has optimization of the RNA workflow and has changes to the primer concentrations and the denaturation temperature used in PCR.

Two different libraries are generated and pooled for each sample: one for DNA targets and one for RNA targets. During library preparation for each sample, one of the 16 oligonucleotide barcodes in the Library Kit is used for the DNA-derived library and another oligonucleotide barcode is used for the RNA-derived library. This ensures the correct identification of each respective portion of the assay (DNA and RNA) from each patient sample. After library preparation, the DNA and RNA libraries for all samples and controls may be blended for the templating reaction.

Data Analysis:
This process is executed by the Torrent Suite™ Dx software, v. 5.12.5, which runs on the Ion Torrent™ Server. Together, these manage the complete end-to-end workflow from sample to variant call. The DNA reads are 'mapped' to the reference human genome (hg19) followed by detection of single nucleotide variants (SNV), insertions,

and deletions (del) using a reference hotspot file. The RNA reads are 'mapped' to a reference containing control sequences and candidate gene fusion sequences. Gene fusions are detected as present if they map to these reference sequences and pass certain filtering criteria provided by the ODxT Test ADF.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA-approved CDx alternatives for the detection of genetic alterations using FFPE tumor specimens, to those listed in Table 1 of the ODxT Test intended use statement. These approved alternative CDx tests are listed in Table 3 below. Each alternative has its own advantages and disadvantages. A patient should fully discuss any alternative with his/her physician to select the most appropriate method. For additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at:
https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm?source=govdelivery.

Table 3. List of FDA-Approved CDx Assays for Genes and Therapies Targeted by the ODxT Test

Gene and Variant	Therapy	Company and Device (PMA #)
BRAF V600E	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)	Foundation Medicine, Inc. – FoundationOne CDx™ (F1CDx) (P170019)
EGFR L858R and Exon 19 deletions	IRESSA® (gefitinib)	QIAGEN – *therascreen*® EGFR RGQ PCR Kit (P120022/S001)
		Foundation Medicine, Inc. – F1CDx (P170019)
		Roche Molecular Systems, Inc. – cobas® EGFR Mutation Test v2 (P120019/S019)

Note: There is no FDA approved CDx alternative using tumor tissue specimens for the detection of *EGFR* exon 20 insertions for identification of NSCLC patients eligible for treatment with EXKIVITY™ (mobocertinib) or RYBREVANT™ (amivantamab-vmjw). However, there is an FDA approved CDx alternative for the detection of *EGFR* exon 20 insertions in NSCLC patients using cfDNA isolated from plasma for treatment with RYBREVANT™ (amivantamab-vmjw) (See SSED for P200010/S001).

Similarly, there is no FDA approved CDx alternative using tumor tissue specimens for the detection of *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions) for identification of NSCLC patients eligible for treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki). However, there is an FDA approved CDx alternative for the detection of *ERBB2/HER2* activating mutations

(SNVs and exon 20 insertions) for identification of NSCLC patients using cfDNA isolated from plasma for treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki) (See SSED for P200010/S008).

VII. MARKETING HISTORY

The ODxT Test was introduced into interstate commerce in the United States on June 22, 2017, and is commercially available in the US, 12 countries in Europe (Austria, Belgium, Switzerland, Germany, Denmark, Spain, France, UK, Scotland, Italy, Netherlands, and Poland), Japan, Korea, Israel, and Saudi Arabia. The ODxT Test has not been withdrawn from the market for reasons related to safety and effectiveness.

The expansion of the indications for use of the ODxT Test described above in Section II are not currently approved and have not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Below is a list of the potential adverse effects (e.g., complications) associated with the use of the device.

- Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect ODxT Test results and subsequently improper patient management decisions in NSCLC and CC treatment.

- Patients with false positive results may undergo treatment with the therapy listed in the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy.

- There is also a risk of delayed results, which may lead to delay of treatment with the appropriate targeted therapy.

No adverse events were reported in connection with the clinical studies used to support this PMA as the studies were performed retrospectively using banked samples.

For the specific adverse events that occurred in the clinical studies, refer to the drug label (i.e., FDA approved package insert) available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Analytical validation studies demonstrating the performance of the ODxT Test in detecting the *ERBB2* SNV mutations and *ERBB2* exon 20 insertions (will be collectively called *ERBB2* activating mutations unless otherwise specified) are listed below. These studies were performed using intended use specimens and sample blends across all validation studies. Studies evaluating analytical accuracy/concordance, precision studies near the limit of detection (LoD), limit of blank (LoB), DNA input, interference, guardbanding, and stability of assay intermediates were conducted to support the indication for *ERBB2/HER2* activating mutations.

1. Analytical Accuracy/Concordance

An analytical accuracy study was performed using clinical specimens from 220 (101 positive for *ERBB2* SNV and *ERBB2* exon 20 insertion plus 119 negatives) formalin-fixed, paraffin-embedded (FFPE) NSCLC clinical tumor samples using an orthogonal externally validated next generation sequencing test method (referred to as Ev-NGS assay hereafter). The NSCLC clinical samples were obtained from the Daiichi Sankyo's clinical trial (DS8201-A-U204) and *ERBB2* SNV-negative and *ERBB2* exon 20 insertion-negative clinical samples were sourced from commercial vendors.

The 101 *ERBB2* activating mutation positive samples were derived from 3 cohorts, including 91 NSCLC samples positive for *ERBB2* activating mutations from the clinical study (cohort 1), 8 samples positive for *ERBB2* activating mutations from commercial biobank samples (cohort 2), 2 samples positive for *ERBB2* activating mutations from commercial samples (cohort 3), and 119 negative samples were commercially procured NSCLC – staged matched *ERBB2* mutation negatives screened by clinical trial assay (CTA). The concordance between the results from the ODxT Test and the Ev-NGS Assay were evaluated for positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) is provided in Table 4 and 5.

The point estimates of PPA, NPA, and OPA excluding unknown results were 100.0%, 99.1%, and 99.3%, respectively. Unknown is defined as insufficient samples and sample QC sequencing failures resulting in an invalid result or No Call for the variant. When including ODxT Test unknown results, the point estimates of PPA, NPA, and OPA were 97.4%, 92.4%, and 93.6%, respectively (Table 5). All 38 samples that were *ERBB2* activation mutation positive by both ODxT Test and the Ev-NGS Assay also agreed at the variant level.

Table 4. Concordance between ODxT Test and Ev-NGS Assay for *ERBB2* SNV and exon 20 Insertion Detection

ODxT Test	Ev-NGS Assay			
	Positive	**Negative**	**Unknown**	**Total**
Positive	38	1	17	56
Negative	0	108	1	109
Unknown*	1	8	46	55
Total	39	117	64	220

POS=Positive, NEG=Negative, *Unknown samples are defined as values due to insufficient sample, or sample QC sequencing failure resulting in an invalid result or No Call for the variant.

Table 5. Agreements between ODxT Test and Ev-NGS Assay for *ERBB2* SNV and exon 20 Insertion Detection

Agreement Parameter	Excluding Unknown*		Including Unknown*	
	Percent Agreement	**95% CI**	**Percent Agreement**	**95% CI**
PPA	100% (38/38)	90.8%, 100%	97.4% (38/39)	86.5%, 99.9%
NPA	99.1% (108/109)	95.0%, 100%	92.3% (108/117)	85.9%, 96.4%
OPA	99.3% (146/147)	96.3%, 100%	93.6% (146/156)	88.5%, 96.9%

*Unknown samples are defined as values due to insufficient sample, or sample QC sequencing failure resulting in an invalid result or No Call for the variant.

2. **Analytical Sensitivity**

   a. **Limit of Blank (LoB)**

ERBB2 exon 20 Insertions and ERBB2 SNVs

This study aimed to demonstrate assay specificity by verifying that the limit of blank (LoB) of the ODxT Test was equal to zero (i.e., no *ERBB2* activating mutation detection) when wild type (WT) NSCLC FFPE samples were evaluated.

To ensure that the ODxT Test does not generate a signal that might be classified as an *ERBB2* activation mutation positive result (false positive result), 4 WT NSCLC FFPE clinical samples for each of the *ERBB2* exon 20 insertions and *ERBB2* SNVs variants were included in this study and tested using 2 different lots of the ODxT Test reagents and 2 operators. For each sample, a total of 36 library replicates were made using 2 lots which is 18 library replicates per reagent lot. All sample replicates were sequenced. Results from this study showed that the false positive rate of the ODxT Test was zero (0) for both *ERBB2* exon 20 insertions and *ERBB2* SNVs variants since there were no positive calls at any of the variant locations analyzed by the test.

b. Limit of Detection (LoD)

ERBB2 exon 20 Insertions

The limit of detection (LoD) based on positive calls for the ODxT Test was estimated to determine the lowest allele frequency (AF) of *ERBB2* exon 20 insertions, at which 95% of the test replicates produced correct calls. Six (6) different AFs (dilutions) using 2 representative *ERBB2* exon 20 insertion clinical variants (see Table 6; COSM12552, a low prevalence bp insertion and COSM20959, a high prevalence12 bp insertion) were generated by combining nucleic acid extracted from variant positive clinical samples with gDNA extracted from wild type (WT) clinical samples (120 data points per *ERBB2* exon 20 insertion variant). A hybrid analysis approach was used. COSM20959 met the requirements for probit analysis (95% detection) and COSM12552 was calculated based on the empirical hit-rate approach (EHR). The claimed LoD based on probit (for 12 bp insertions) and empirical hit rate (for 3 bp insertion) approach for *ERBB2* exon 20 insertions at 10 ng DNA input are noted in Table 6 below.

Table 6. LoD for *ERBB2* exon 20 Insertions in NSCLC Clinical Samples

COSMIC ID	Insertion Size	Insertion Type	LoD Estimate (%AF)
COSM20959	12 bp	p.A775_G776insYVMA	5.0
COSM12552	3 bp	p.G776delinsVC	4.8

ERBB2 SNVs

The limit of detection (LoD) based on positive calls for the ODxT Test was estimated to determine the lowest allele frequency (AF) of *ERBB2* SNVs, at which 95% of the test replicates produced correct calls. Six (6) different AFs (dilutions) using 3 representative clinical samples containing 4 different variants [exon 8 (COSM48358 (S310F)), exon 17 (COSM436498 (R678Q)), exon 19 (COSM14060 (L755S)) and exon 20 (COSM18609 (G776V))] were generated by combining nucleic acid extracted from variant positive clinical samples with gDNA extracted from WT clinical samples (120 data points per *ERBB2* SNV variant). For COSM14060 and COSM48358 Probit model was used to estimate the LoD which generated LoD estimates of 4.6% and 5.8%, respectively. The empirical hit rate method was used to estimate the LoD for COSM18609 and COSM436498; reported as the mean AF of 5.2% and 4.9%, respectively at 10 ng DNA input (Table 7). Overall, the LoD for *ERBB*2 SNVs ranged from 4.6% to 5.8% AF for 4 clinical variants.

Table 7. LoD for ERBB2 SNVs in NSCLC Clinical Samples

COSMIC ID	Variant Type	LoD Estimate (%AF)
COSM14060	L755S	4.6
COSM48358	S310F	5.8

COSM18609	G776V	5.2
COSM436498	R678Q	4.9

c. DNA Input Study

To define the tolerance around the amount of input DNA required for the ODxT Test to accurately detect *ERBB*2 activating mutation (SNVs and *ERBB2* exon 20 insertion) variants, five (5) DNA input levels were tested: 3 levels below the standard input of 10 ng (5, 6.5, and 8.5 ng), one at 10 ng (standard input), and one above 10 ng input (15 ng).

***ERBB2* exon 20 Insertions**

In this study, two FFPE clinical samples harboring *ERBB2* variant COSM20959 (p.A775_G776insYVMA) were blended with gDNA from WT FFPE samples to create sample blends at LoD levels close to 2x LoD. Each sample blend was diluted to 5 ng/µl and inputs were adjusted to the appropriate levels for use in each testing reaction. A total of 96 DNA libraries, including the controls, were made, and a single reagent lot was used for this study. Positive call rates were determined at different input levels of DNA tested for each sample.

The data generated from the study were analyzed using an analysis of variance (ANOVA) to determine the relationship of input level to allele frequency. The study showed only one statistically significant difference in AF of COSM120959 for Blend 1, for which the %AF was slightly lower at DNA input level 8.5 ng compared to the rest of the input levels. Although statistically significant, the difference was not large enough to affect variant calling. No other AF differences between DNA input levels were statistically significant.

The data from this study demonstrated that the input range of 5 ng to 15 ng consistently detected *ERBB2* exon 20 insertions.

***ERBB2* SNVs**

Two (2) dual variant blends were prepared at a target AF level of 1.5x – 3x LoD. DNA Blend 1 contained *ERBB2* variants COSM48358 (S310F) and COSM14060 (L755S) and DNA Blend 2 contained *ERBB2* variants COSM436498 (R678Q) and COSM18609 (G776V). gDNA from *ERBB2* SNV-positive samples was blended with gDNA from WT FFPE samples and tested as described above.

The ANOVA analysis results for the DNA input study showed no statistically significant difference in the AFs among input levels tested.

The data analysis from this study demonstrated that the input range of 5 ng to 15 ng consistently detected *ERBB2* SNVs.

3. Analytical Specificity

Interference

To evaluate the potential impact of endogenous (necrotic tissue and hemoglobin) and exogenous interferents (paraffin, xylene, ethanol, Proteinase K, and wash buffer) on the performance of the ODxT Test in detecting *ERBB2* activating mutations (SNVs and exon 20 insertions), this study evaluated 4 clinical FFPE samples [one *ERBB2* exon 20 insertion (12 bp) and 3 *ERBB2* SNVs with 4 unique variants (S310F, L755S, R678Q and G776V)] in 2 replicates for 7 different conditions (6 with interfering substances and one control) taken through the entire test workflow, in the presence of endogenous or exogenous potential interferents. The interferent concentration tested was determined based on the CLSI EP07-3rd edition.

The study evaluated concordance of ODxT Test results. Concordance refers to agreement in ODxT Test variant call (positive, negative or no calls result), between experimental samples (samples with interferents) and control samples (samples without interferent).

a. Endogenous Interference

ERBB2 exon 20 Insertions and SNVs

Potential interference of necrotic tissue was evaluated in clinical study DS8201-A-U204 (subject of this PMA). To evaluate the potential impact of tumor necrosis on *ERBB2* SNVs and exon 20 insertion mutation variant calling, analysis based on tumor necrosis (0%, 0.1 – 5%, 5.1 – 10%, and 10.1 – 70%) was performed using ODxT Test. The analysis of the clinical study showed that among the range of tumor necrosis (0 to 70%) observed for the 38 clinical samples tested in this study, there was only one sample with an incorrect call at the 5.1-10% level. All other samples were accurately called 100% of the time.

Hemoglobin was evaluated at 4 mg/ml. The positive concordance with the control condition (with no calls being excluded) across all samples, and the overall concordance with the control condition across all samples were calculated. Although the concordance with the control condition across all samples was 100%, since challenging samples (i.e., samples close to 1x LoD) were not used in this study, a post-market study using NSCLC samples harboring insertion mutations near LoD, is planned (see section XIII).

b. **Exogenous Interference**

ERBB2 exon 20 Insertions and ERBB2 SNVs

Results from this study show that acceptance criteria of ≥95% with respect to PPA (no calls excluded), NPA (no calls excluded), and OPA (no calls excluded) across all samples were achieved as results were reported at 100%. These data demonstrate that the presence of potentially interfering substances does not impact the performance of the assay to detect *ERBB2* activating mutations in FFPE samples.

4. **Precision and Reproducibility**

Two reproducibility studies, an external sample processing reproducibility study and an external panel reproducibility study, were performed to evaluate precision and reproducibility of ODxT Test. The external sample reproducibility study starts from the nucleic acid extraction step to determine the reproducibility and repeatability of sample processing.

a. **External Sample Processing Reproducibility Study**

ERBB2 exon 20 Insertions

The purpose of this study was to demonstrate that processing of NSCLC FFPE samples as part of the ODxT Test workflow generated repeatable and reproducible results for the *ERBB2* exon 20 insertion variants. Multiple sample replicates, operators, reagent lots, and days were included in the test design to evaluate within run repeatability and assay reproducibility with a 95% confidence interval (CI). Four FFPE samples were tested [2 different *ERBB2* exon 20 insertion positives (COSM20959 (p.A775_G776insYVMA) and COSM85995 (p.G776delinsVC) and 2 WT]. Each sample was tested at 3 sites, with the operators using 3 lots of the Ion Torrent Dx FFPE Sample Preparation Kit, and generating 2 replicates for each lot, for a total of 18 replicate data points across 3 sites per each sample, 36 total across the 2 *ERBB2* exon 20 insertion variants tested.

The within run repeatability was 100% for *ERBB2* exon 20 insertion variants. Positive and negative call rates at the sample and variant level were 100% for *ERBB2* exon 20 insertion-positive and negative samples (Table 8). The sample level analysis demonstrated 100% positive agreement for *ERBB2* exon 20 insertion variants across all 3 operators.

Table 8. Reproducibility and Call Rates

Sample	Variant Type	Valid Sample Results (N)	Positive Calls A (A)	Negative Calls B (B)	No Calls (C)	Call Rate (A+B)/N %	No Calls Rate C/N %	Positive Call Rate 95% CI		Negative Call Rate 95% CI		Within-run Repeatability 95% CI	
								Including No Calls (A/N)	Excluding No Calls A/(A+B)	Including No Calls (B/N)	Excluding No Calls B/(A+B)	Including No Calls (A/N)	Excluding No Calls A/(A+B)
COSM 20959	p.A775_G776 insYVMA	18	18	0	0	100	0	100% (81.5%, 100%)	100% (81.5%, 100%)	0% (0%, 18.5%)	0% (0%, 18.5%)	100% (66.4%, 100%)	100% (66.4%, 100%)
COSM 85995	p.G776delins VC	18	18	0	0	100	0	100% (95%, 100%)	100% (94.9%, 100%)	0% (0%, 5%)	0% (0%, 5%)	100% (73.5%, 100%)	100% (73.5%, 100%

The PPA and NPA were determined for the *ERBB2* exon 20 insertion variants at the sample level. The sample level analysis demonstrated 100% positive agreement for the *ERBB2* exon 20 insertion variants across all 3 operators (Table 9). In addition, the sample level analysis demonstrated 100% negative agreement at all *ERBB2* insertion variant locations between the 3 operators who tested the same *ERBB2* exon 20 insertion negative samples.

Table 9. Positive Agreement Estimate at the Sample Level

Sample	**Variant Type**	**Replicates**	**Positive Percent Agreement, No Calls Included**	**Positive Percent Agreement, No Calls Excluded**
COSM 20959	p.A775_G776insYVMA	18	100%	100%
COSM 85995	p.G776delinsVC	18	100%	100%

Since the external sample reproducibility and within-run repeatability studies did not include sufficient replicates, a post-market study is planned with additional replicates (see section XIII).

ERBB2 SNVs
Refer to Summary of Safety and Effectiveness Data PMA P160045 (Section 5c) as these studies were previously performed, and the report submitted and approved under PMA P160045, and no changes have been implemented to the ODxT Test reagent formulations, packaging, assay workflow, QC method workflow, or the instruments used, since approval of the original PMA.

b. **External Panel Reproducibility Study (Assay Reproducibility)**

The external reproducibility study was conducted across 3 sites to demonstrate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility).

ERBB2 exon 20 Insertions

Two WT and 2 *ERBB2* exon 20 insertion-positive (COSM20959 (p.A775_G776insYVMA) and COSM12552 (p.G776delinsVC)) NSCLC FFPE clinical samples were tested across 3 sites and with 3 different reagent lots to demonstrate the ability of the ODxT Test to generate reproducible results in the presence of variability across sites, operators, and reagent lots. The DNA extracted from 2 *ERBB2* variant positive samples was blended with WT sample gDNA to a target AF of 0.9x – 1.3x and 1.5x – 3x of the estimated LoD.

The study included 2 instrument systems and 2 operators per site. At each site, each operator was assigned to 2 instrument systems, and each tested all 6 samples on both instrument systems using 2 different lots of reagents. Testing was conducted over 82 days with target amplification on non-consecutive days.

The number of valid results, number of positive calls, positive call rate, number of negative calls, negative call rate, number of no calls, no call rate, and the within-run repeatability for the *ERBB2* exon 20 insertion variants were calculated for all samples tested. The 95% two-sided exact CIs were calculated for the positive call rate, negative call rate, and within-run repeatability.

For the *ERBB2* exon 20 variant positive samples, COSM20959 (p.A775_G776insYVMA) and COSM12552 (p.G776delinsVC) were tested at 0.9x-1.3x LoD and 1.5x-3x LoD). The positive and negative call rates for the expected variant excluding no calls was 100% and 0%, respectively; while the

positive and negative call rates for the expected variant including no calls was 98.6% to 100% and 0%, respectively (Table 10).

Table 10. Call Rates: Reproducibility and Repeatability

Sample	LoD	Valid sample N results (N)	Positive calls A (A)	Negative calls B (B)	No calls (C)	Call rate (A+B)/N %	No calls rate C/N %	Positive call rate 95% CI		Negative call rate 95% CI		Within-run repeatability 95% CI	
								Including No Calls (A/N)	Excluding No Calls A/(A+B)	Including No Calls (B/N)	Excluding No Calls B/(A+B)	Including No Calls (A/N)	Excluding No Calls A/(A+B)
p.A775_G776insYVMA	0.9x-1.3x	72	71	0	1	98.6	1.4	98.6% (92.5%, 100%)	100% (94.9%, 100%)	0% (0%, 5%)	0% (0%, 5.1%)	100% (73.5%, 100%)	100% (73.5%, 100%)
p.G776delinsVC	0.9x-1.3x	72	72	0	0	100	0	100% (95%, 100%)	100% (94.9%, 100%)	0% (0%, 5%)	0% (0%, 5%)	100% (73.5%, 100%)	100% (73.5%, 100%
p.A775_G776insYVMA	1.5x-3x	72	72	0	0	100	0	100% (95%, 100%)	100% (95%, 100%)	0% (0%, 5%)	0% (0%, 5%)	100% (73.5%, 100%	100% (73.5%, 100%
p.G776delinsVC	1.5x-3x	72	72	0	0	100	0	100% (95%, 100%)	100% (95%, 100%)	0% (0%, 5%)	0% (0%, 5%)	100% (73.5%, 100%	100% (73.5%, 100%

For the *ERBB2* exon 20 variant positive samples, the negative call rate was 0% for the expected variant and 100% for all other *ERBB2* exon 20 variant locations (both including and excluding no calls). For the two WT samples, the negative call rate with 95% CI, including or excluding no calls, was 100% (94.9%, 100%).

For sample variants p.G776delinsVC (at 0.9x-1.3x LoD) as well as p.A775_G776insYVMA and p.G776delinsVC (at 1.5x-3x LoD), the within-run repeatability was 100% at all expected *ERBB2* exon 20 variant locations

when excluding or including no calls. For sample p.A775_G776insYVMA at 0.9x – 1.5x LoD, the within-run repeatability at the expected *ERBB2* exon 20 locations was 100% when excluding no calls and 98.6% when including no calls.

The PPA was determined for the positive expected *ERBB2* exon 20 variant locations at both the variant level (2 samples with the same *ERBB2* variant) as well as at the sample level. For p.A775_G776insYVMA, the variant level analysis demonstrated 98.6% positive agreement in the variant positive sample p.A775_G776insYVMA tested (N = 72 pairs). For p.G776delinsVC , the variant level analysis demonstrated 100% positive agreement in the 2 variant positive samples at 0.9x – 1.5x LoD and 1.5x-3x LoD.

For samples p.G776>VC (at 0.9x – 1.5x LoD), p.A775_G776insYVMA and p.G776>VC (at 1.5x-3x LoD), the sample level analysis demonstrated 100% positive agreement testing the same positive samples (N = 36 measurements, two replicates per sample). For sample p.A775_G776insYVMA at 1.5x-3x LoD, the sample level analysis demonstrated 97.2% (35/36) positive agreement with no calls included and 100% with no calls excluded.

***ERBB2 SNV*s**

A total of 7 FFPE clinical samples, including 3 *ERBB2* SNV samples containing 4 unique variants (COSM48358 (S310F), COSM14060 (L755S), COSM436498 (R678Q) and COSM18609 (G776V)) and 4 WT samples were tested. The DNA extracted from 3 *ERBB2* positive samples was blended with WT sample gDNA to generate 3 blends to targets AF of 0.9x – 1.3x, 1.3x – 1.8x, and 1.8x – 2.5x of the estimated LoD to demonstrate performance for detection at defined AF levels.

Testing was performed at 3 external sites. Each site had 2 instrument systems, 2 lots of reagents, and 2 operators. The reproducibility samples were provided as blends of *ERBB2* SNV variants or as WT samples. Repeatability (including within run variability) and reproducibility (variability across sites, operators, and instrument platforms) were evaluated using 3 lots of the ODxT Test and Controls and Kits for library preparation, templating, and sequencing. For each run, each operator took the DNA samples through library preparation, templating, and sequencing.

For the *ERBB2* SNV-positive samples (D1, D2, D3, D4, and D5), the positive and negative call rates for the expected variants, excluding and including no calls, were 100% and 0%, respectively.

The positive and negative call rates, with no calls included and excluded, for the 9 DNA sample blends tested in the study are shown in Table 11, along with the call rate and no call rate of each *ERBB2* SNV tested in each sample.

Table 11. Call Rates – *ERBB2* SNVs (Sample Blend Level)

				Positive Call Rate (95%CI)		Negative Call Rate (95%CI)	
Blend	**Sample and Variant Type**	**Call Rate %**	**No Calls Rate %**	**No Calls Included %**	**No Calls Excluded %**	**No Calls Included %**	**No Calls Excluded %**
D1	COSM 14060 L755S	100	0	100 (95.0-100.0)	100 (95.0-100.0)	0 (0.0-5.0)	0 (0.0-5.0)
	COSM 48358 S310F	100	0	100 (95.0-100.0)	100 (95.0-100.0)	0 (0.0-5.0)	0 (0.0-5.0)
D2	COSM 436498 R768Q	100	0	100 (94.9-100.0)	100 (94.9-100.0)	0.0% (0.0-5.1)	0.0% (0.0-5.1)
D3	COSM 18609 G776V	100	0	100 (95.0-100.0)	100 (95.0-100.0)	0 (0.0-5.0)	0 (0.0-5.0)
D4	COSM 14060 L755S	100	0	100 (94.9-100.0)	100 (94.9-100.0)	0.0% (0.0-5.1)	0.0% (0.0-5.1)
	COSM 48358 S310F	100	0	100 (94.9-100.0)	100 (94.9-100.0)	0.0% (0.0-5.1)	0.0% (0.0-5.1)
D5	COSM 436498 R768Q	100	0	100 (95.0-100.0)	100 (95.0-100.0)	0 (0.0-5.0)	0 (0.0-5.0)
	COSM 18609 G776V	100	0	100 (95.0-100.0)	100 (95.0-100.0)	0 (0.0-5.0)	0 (0.0-5.0)
D6*	*ERBB2* WT	98.2	1.8	0 (0.0-1.3)	0 (0.0-1.3)	98.2 (95.9-99.4)	100.0 (98.7-100.0)
D7	*ERBB2* WT	99.6	0.4	0 (0.0-1.3)	0 (0.0-1.3)	99.6 (98.1-100.0)	100 (98.7-100.0)
D8	*ERBB2* WT	96.8	3.2	0 (0.0-1.3)	0 (0.0-1.4)	96 (94.0-98.5)	100 (98.6-100.0)
D9	*ERBB2* WT	98.6	1.4	0 (0.0-1.3)	0 (0.0-1.3)	98.6 (96.5-99.6)	100 (98.7-100.0)

* D6 is the filler WT sample was used as a place holder to simplify experiment design.

For samples D1, D2, D3, D4 and D5, the within-run repeatability was 100% at all expected *ERBB2* SNVs variant when excluding or including no calls. The average positive percent agreement (APA) and average negative percent agreement (ANA) were determined for each of the *ERBB2* SNV positive sample blends and wild type sample blends for all *ERBB2* SNVs, respectively. The results suggest for both APA and ANA ranged from 97.1% to 100%.

5. Guardbanding Studies

ERBB2 exon 20 Insertions

The purpose of the guardbanding study was to evaluate tolerability of ODxT Test workflow to detect *ERBB2* exon 20 insertions for 11 critical assay steps that include DNA control volume, DNA panel volume, HiFi mix volume, FuPa reagent volume, switch solution volume, barcode adapter volume, incubation time, bubble formation after adding AMPure, residual ethanol, thermal cycling temperature offset, and Elution Solution (ES) final volume titration. The study was conducted as previously described in Section IX. A.9.a. of the P160045 SSED.

To evaluate the workflow tolerance, a single variant blend from FFPE clinical specimens containing COSM20959 (p.A775_G776insYVMA) was prepared at 2-3x LoD levels. Additional test conditions were added to narrow the acceptable tolerance range based on results. The ODxT Test did not tolerate a 2.5 µL residual ethanol volume, therefore, a narrower residual ethanol volume of 1.75 µL was tested for acceptability.

An analysis of variance (ANOVA) was conducted to analyze the results. No significant differences between the high and low conditions, relative to the standard operating procedure (SOP), were observed. The AF was not significantly different from the AF observed when testing using the SOP condition, and no statistically significant difference in percent AF was observed in any resulting *ERBB2* exon 20 insertion data.

ERBB2 SNVs

From the library preparation portion of the workflow, 4 conditions were assessed using gDNA from three clinical samples bearing *ERBB2* variants: the volume of DNA Panel, the volume of HiFi, the residual volume of ethanol and the temperature offset for the thermal cycler. These 4 conditions were assessed using 2 blends composed of clinical FFPE samples prepared at target AF level of 1x-3x LoD.

Eighteen (18) libraries were made for each blend for conditions 1, 2 and 4 (HiFi, DNA Panel volume and thermal cycler temperature, respectively). Twelve (12) libraries per blend were made for condition 3 (residual EtOH) due to the highest

levels of ethanol tested causing library and run failures. The carryover residual ethanol volume had to be lowered from 2.5 µL to 1.75 µL as the assay did not tolerate 2.5 µL carryover of 70% ethanol volume. Ultimately, the 2 residual ethanol conditions that were included for data analysis for both blends were the SOP (0 µL ethanol) and the High 1 (1 µL ethanol) conditions. Despite a narrower acceptable range established for residual EtOH, no significant differences between the high and low conditions, relative to the SOP, were observed when detecting *ERBB2* SNVs.

6. **Stability of Assay Intermediates Studies**

The purpose of the study was to find out if the ODxT Test workflow allows for partially completed reactions (or intermediates) to be held at defined storage conditions prior to proceeding to the next step in the workflow. The stability of the intermediate products was evaluated by incorporating all of the pre-defined hold times specified in the User Guide.

ERBB2 exon 20 Insertions

The hold time studies were conducted with 2 *ERBB2* exon 20 insertion variants [COSM20959 (p.A775_G776insYVMA) and COSM85995 (p.G776>VC)]. Each sample was tested under three different test conditions (Table 12 below).

This study used one DNA blend sample for all conditions. The DNA blend was composed of DNA extracted from FFPE clinical samples contained WT and clinical *ERBB2* exon 20 insertion variants. The blend used had an observed allelic frequency of 1.2x LoD for the 12 bp *ERBB2* exon 20 insertion variant and 2x – 3x LoD for the 3 bp *ERBB2* exon 20 insertion variant.

Table 12. Designated Hold Time Test Conditions

Condition	Eluted Library Hold Time
A) Nominal hold	No hold
B) Library hold	30 days hold
C) Combo hold[1]	No hold
[1] Includes steps 1-2, and steps 4-9 (refer to the ODxT Test user guide)	

For each hold condition investigated in this study, the relative percentage change in mean DNA variant AF from the corresponding mean AF at the nominal condition was used as metrics to evaluate stability. These results demonstrated that assay workflow intermediates are stable for the periods tested when held at their defined storage conditions, with respect to the assay's ability to report insertion variants.

ERBB2 SNVs

This study tested 2 clinical sample *ERBB2* blends. DNA Blend 1 was composed of COSM48358 (S310F) and COSM14060 (L755S) and DNA Blend 2 was composed of COSM436498 (R678Q) and COSM18609 (G776V). Blend 1 and Blend 2 were prepared at a target AF level of 1.5x – 3x LoD. gDNA from *ERBB2* SNV-positive samples was blended with gDNA from WT FFPE samples. Each sample blend was tested under three different test conditions (Table 10 above).

For each hold condition investigated in this study, the relative percentage change in mean DNA variant AF from the corresponding mean AF at the nominal condition. This study demonstrated that assay intermediates are stable for maximum 30-day library hold and combination hold of 17.5 days defined in the User Guide.

7. Stability Studies

The reagent shelf-life stability, tissue block/cut slide studies, and the freeze-thaw stability of DNA from formalin-fixed paraffin-embedded (FFPE) clinical samples and FFPE cell line samples were either ongoing studies whose interim reports were evaluated, or not performed, and therefore, post-market stability studies are planned with NSCLC samples harboring insertion mutations (see section XIII).

8. Cross-Contamination

Please refer to the Summary of Safety and Effectiveness Data of P160045 (Section IX.C) for platform-level carryover/cross-contamination data for ODxT Test.

9. Reagent Lot Interchangeability

Please refer to the Summary of Safety and Effectiveness Data of P160045 (Section X.D) for platform-level reagent lot interchangeability data for ODxT Test.

B. Animal Studies

No animal studies were conducted using ODxT Test.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The safety and effectiveness of the ODxT Test for selecting NSCLC subjects who may benefit from treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki) was demonstrated through testing of DNA in tissue specimens from patients enrolled into one of two Daiichi Sankyo Studies DS8201-A-U204 (DESTINY Lung 01; NCT03505710) used to support the efficacy of ENHERTU® (fam-trastuzumab

deruxtecan-nxki). A clinical bridging study was conducted to assess clinical agreement between samples with *ERBB2* activating mutations (SNVs and exon 20 insertions) status tested with the clinical trial assay (CTA) and the ODxT Test in the intent-to-test population. A summary of the ODxT Test clinical validation study is presented below.

A. ODxT Test Clinical Bridging Study for *ERBB2* Activating Mutations (SNVs and exon 20 Insertions)

1. Clinical Study Design

Daiichi Sankyo clinical trial DS8201-A-U204 (NCT03505710) is a Phase 2, multicenter, open-label, 3-cohort study of intravenously administered (ENHERTU® (trastuzumab deruxtecan-nxki) in subjects with unresectable and/or metastatic NSCLC. The primary ENHERTU® population comprises *ERBB2/HER2* activating mutation-positive subjects (exon 20 insertions and or SNVs) from the Daiichi Sankyo clinical trial DS8201-A-U204 (Cohort 2) who had relapsed from or is refractory to standard therapy or for whom no standard treatment is available. Ninety-one (91) patients were enrolled based on the presence of *ERBB2/HER2* activating mutations (SNVs and Exon 20 insertions) in their tumor tissue sample analyzed by clinical trial assays (CTAs).

a. Key Inclusion Criteria:

- Must have provided informed consent for study participation before performance of any study-specific procedure or test.
- Age ≥20 y old in Japan, ≥18 y old in other countries.
- Pathologically documented unresectable and/or metastatic non-squamous NSCLC.
- Has relapsed from or is refractory to standard treatment or for which no standard treatment is available.
- Subject has any known documented activating *HER2* mutation from an archival tumor tissue sample analyzed by CLIA laboratory or equivalent, specifically exon 20 insYVMA (Y772_A775dup), insGSP (G778_P780dup), insTGT (G776delinsVC), single base pair substitutions L755S, V777L, or S310F or another *HER2* mutation listed in the appendix 6 (Clinical Study Protocol).

b. Key Exclusion Criteria

- Previously treated with *ERBB2/HER2*-targeted therapies, except for pan-HER class tyrosine kinase inhibitors.

2. Follow-up Schedule

The ODxT Test bridging study involved retrospective testing of samples; as such, no additional patient follow-up was conducted in regard to the clinical bridging study.

3. Clinical Endpoints

The clinical endpoint used to assess ENHERTU® efficacy in the DS8201-A-U204 clinical study was objective response rate (ORR) by RECIST version 1.1 as assessed by independent central review (ICR).

4. Diagnostic Objective and Endpoints

The primary objective of the clinical bridging study is to demonstrate the safety and effectiveness of the ODxT Test for the selection of NSCLC patients with *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions) for treatment with ENHERTU®. The primary endpoint is ORR by RECIST version 1.1 as assessed by ICR and compared to the benchmark ORR of the DS8201-A-U204 clinical study.

A sensitivity analysis was conducted to model the impact of the ODxT Test(+) CTA(-) population on the efficacy in the intended use population.

5. <u>ODxT Test Bridging Study</u>

Clinical validation of the ODxT Test was performed by retrospectively testing FFPE NSCLC samples obtained from subjects enrolled in the Daiichi Sankyo clinical trial DS8201-A-U204. Stage-matched NSCLC FFPE tissue samples procured from commercial vendors were also used. Test samples included *ERBB2* activating mutation (SNVs and exon 20 insertions) positive samples detected by CTAs and enrolled in the clinical trial, as well as NSCLC samples from commercial vendors that were tested with the representative CTAs that were used to enroll subjects into the clinical trial DS8201-A-U204.

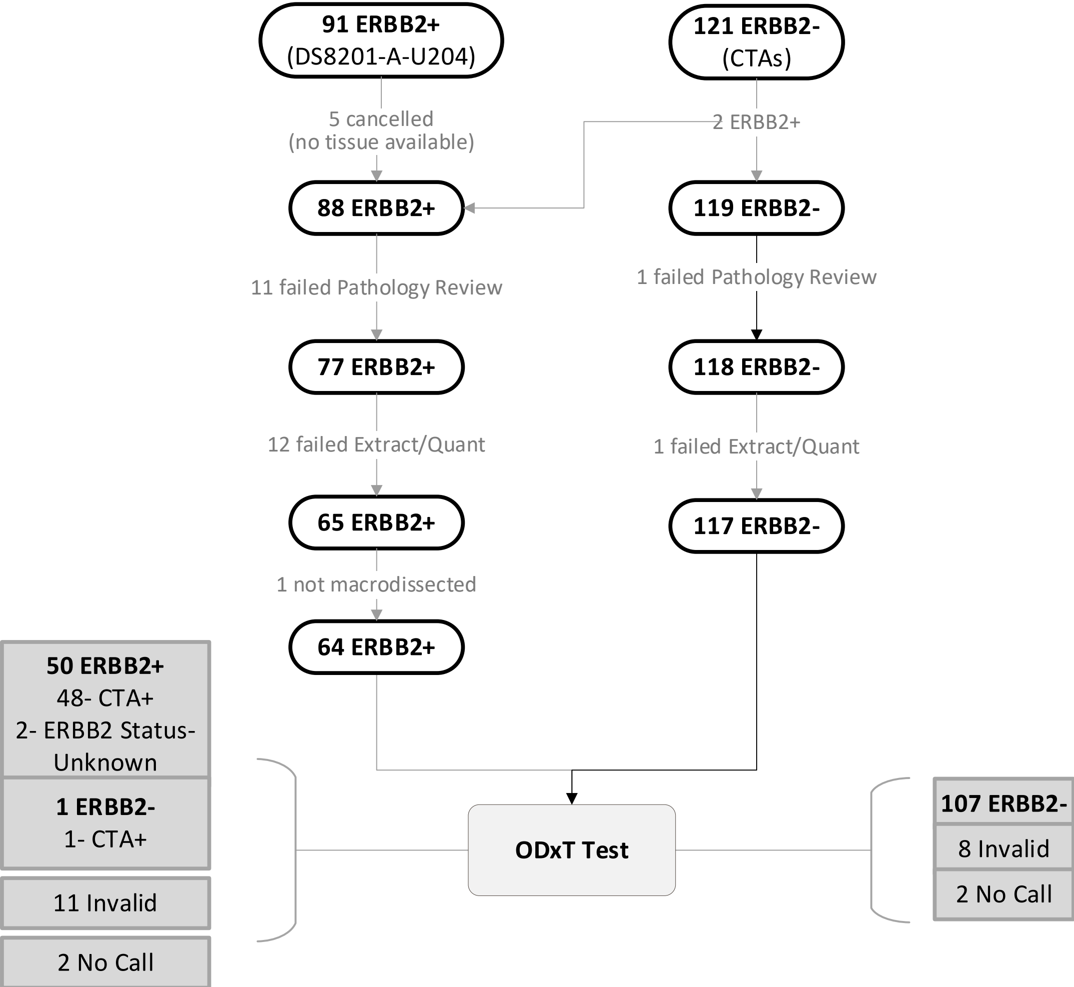
For this clinical validation study, *ERBB2* activating mutation (SNVs and exon 20 insertions) positive and negative samples were tested by the ODxT Test, and the agreements (PPA and NPA) between the ODxT Test and the CTAs were evaluated. Also, a sensitivity analysis was conducted to model the impact of the ODxT Test (+) CTA (-) population on efficacy in the intended use population.

B. <u>Accountability of PMA Cohort</u>

Sample accountability and the ODxT test results are presented in Figure 1. The sample were composed of 91 patients (cohort 2) from Phase 2 DS8201-A-U204

clinical trial enrolled based on *ERBB2* SNV-positive (n=13) or *ERBB2* exon 20 insertion-positive (n=78) CTA results and 121 samples that were stage-matched commercially sourced NSCLC samples, screened by the enrolling CTAs. Of the 121 commercial samples that generated results, 119 were identified as *ERBB2* SNV-negative and *ERBB2* exon 20 insertion-negative (*ERBB2*-negative), and 2 were identified as *ERBB2* SNV-positive.

Figure 1. Sample Accountability Clinical Accuracy Analysis Population



Of the 93 CTA-positive (91 from DS8201-A-U204 trial and 2 from commercial sources) samples, 88 were available for testing by the ODxT Test, 86 were obtained from the DS8201-A-U204 clinical trial, and 2 were from the screening of stage-matched commercially sourced NSCLC samples.

Prior to sequencing, 24 of the 88 samples were cancelled due to failure to meet test input requirements: 11 samples did not meet the minimum tumor content requirement, 12 samples failed the DNA concentration requirements, and 1 sample was not processed correctly (not macro-dissected). In 64 (62 from trial

and 2 from commercial source) of the 88 samples, 50 were *ERBB2* mutation-positive for both SNVs and exon 20 insertions by the ODxT Test (48 positives were from clinical trial and 2 from commercial source), 1 sample was *ERBB*2-negative (total CDx-evaluable *from trial* were n=49; 48 positive by ODxT Test and 1 negative by ODxT Test), 2 samples reported No Call results, and 11 samples reported an invalid result (failed DNA library QC metric).

Of the 121 NSCLC *ERBB2* status-unknown samples tested by the screening CTAs, 2 samples were *ERBB2*-positive, 1 sample was cancelled due to insufficient tumor content, and 1 sample failed the DNA concentration cutoff. For 117 (out of 121 samples) *ERBB2*-negative samples, 107 were ERBB2-negative by ODxT Test, 8 samples were reported as an invalid result, and 2 samples were reported as “No Call” result.

C. Patient Demographics, Disease and Sample Characteristics

A comparison of DS8201-A-U204 study subject demographics, disease, and sample characteristics for the primary efficacy population between the ODxT Test evaluable and unevaluable groups was performed (Table 13). Differences between the ODxT Test evaluable and unevaluable groups were compared for the following variables: age, gender, ethnicity, race, specimen type and ECOG.

Table 13. Demographic and Clinical Characteristics with ODxT Test Evaluable vs Unevaluable Status in CTA+ Subjects

Characteristics		CDx Evaluable (N=49)	CDx Unevaluable (N=42)	p-values	Total (N=91)
Age Years	Mean SD	62.4 (1.43)	57.8 (2.09)	0.065	60.3 (1.25)
Gender	Female	30	30	0.377	60
	Male	19	12		31
Race	Asian	14	17	0.330	31
	Black	1	0		1
	White	25	15		40
	Other	9	10		19
Ethnicity	Hispanic	2	0	0.657	2
	Non-Hispanic	36	32		68
	Other	11	10		21
ECOG	0	15	9	0.345	24
	1	33	33		66
	Missing	1	0		1
Specimen Type	Archived	44	31	0.056	75
	Fresh	5	11		16

Tumor content	Mean SD	49.4 (3.15)	24 (3.29)	<0.001	38.9 (2.66)
Necrosis Percent	Mean SD	4.4 (1.65)	2.1 (0.99)	0.284	3.5 (1.05)
Continuous variables summarized: Mean +/- SD (N). p-value for continuous variables from t-test. Categorical variables summarized: Proportion (n, %). p-value for categorical variables from Fisher's exact test. Subjects with a given characteristic that is completely missing are displayed as “Other”. All other data displayed as per the database.					

Sample characteristics for the status-unknown, commercially sourced NSCLC samples that were screened to confirm CTA- status are presented in Table 14. These samples served as the negative sample set for this study.

Table 14. Demographic and Disease Characteristics for CTA- Subjects: ODxT Test Evaluable vs ODxT Test Unevaluable

Characteristics		CDx Evaluable (N=107)	CDx Unevaluable (N=12)	p-vales	Total (N=119)
Age Years	Mean SD	61.4 (0.82)	60.5 (1.43)	0.751	61.3 (0.75)
Gender	Female	22	1	0.446	23
	Male	79	10		89
	Unknown	6	1		7
Race	Asian	2	0	0.520	2
	Hispanic	2	1		3
	White	73	8		81
	Other	30	3		33
Ethnicity	Asian	1	0	0.120	1
	Hispanic	5	2		2
	Slavic	72	7		79
	White	17	0		21
	Other	12	3		15
CTA Tests	BWH	43	12	<0.001	55
	OCA	64	0		64
Tumor content	Mean SD	55.4 (1.74)	42.1 (6.50)	0.020	54 (1.72)
Necrosis Percent	Mean SD	17.1 (1.75)	6.3 (3.08)	0.044	16 (1.63)
Continuous variables summarized: Mean +/- SD (N). p-value for continuous variables from t-test. Categorical variables summarized: Proportion (n, %). p-value for categorical variables from Fisher's exact test. Subjects with a given characteristic that is completely missing are displayed as “Other”. All other data displayed as per the database.					

D. Safety and Effectiveness Results

1. Safety Results

The safety of ENHERTU® was evaluated at two dose levels: 6.4 mg/kg DESTINY-Lung 01 DS8201-A-U204 and 5.4 mg/kg DESTINY-Lung 02 DS8201-A-U206. ENHERTU is being approved at the lower dose (5.4 mg/kg) due to increased rates of Interstitial Lung Disease/pneumonitis at the higher dose. Adverse events observed with the higher dose are unrelated to the ODxT Test.

Data regarding the safety of ENHERTU® (fam-trastuzumab deruxtecan-nxki) therapy are presented in the original drug approval. Refer to the ENHERTU® (fam-trastuzumab deruxtecan-nxki) label for more information. No adverse events were reported in the conduct of the diagnostic studies used to support this sPMA as these involved retrospective testing of tissue specimens only.

2. Effectiveness Results

a. Concordance Results

To evaluate the clinical accuracy of the ODxT Test, the concordance between the CTA results and the ODxT Test results for all *ERBB2* activating mutation positive and *ERBB*2-negative samples was assessed (with the exception of the 8 commercially procured samples that were included for the analytical accuracy only). In total, 50 (48 were from clinical trial and 2 were commercially sourced) *ERBB2* activating mutation positive samples and 107 *ERBB2*-negative samples had concordant results between CTA and ODxT Test (Table 15).

Table 15. Concordance between ODxT Test and CTA Results

ODxT Test	CTA Test		
	Positive	Negative	Total
Positive	50	0	50
Negative	1	107	108
Unknown	42	12	54
Total	93	119	212

PPA, NPA, and OPA are shown in Table 16. Agreement between ODxT Test and CTA were calculated using the CTA results as the reference. There were 51 CTA positives that gave valid ODxT Test results. Of the 51 (48 positive by ODxT Test from efficacy, 2 positive by ODxT from the commercial samples (total of 50), and 1 negative by ODxT Test from efficacy (total of 51)). The point estimates of PPA, NPA, and OPA excluding samples with unknown status were 98.0% (50/51), 100% (107/107), and 99.4% (157/158), respectively. When including ODxT Test

unknown results, the point estimates of PPA, NPA, and OPA were 78.1%, 91.5% and 86.7%, respectively (Table 16).

Table 16. Agreement between ODxT Test and CTA

Agreement Parameter	Excluding Unknowns*		Including Unknowns*	
	Percent Agreement	95% CI	Percent Agreement	95% CI
PPA	98% (50/51)	89.6%, 100%	78.1% (50/64)	66%, 87.5%
NPA	100 (107/107)	96.6%, 100%	91.5% (107/117)	84.8%, 95.8%
OPA	99.4% (157/158)	96.5%, 100%	87.6% (157/181)	80.9%, 91.3%

*Unknown samples are defined as values due to insufficient sample, or sample QC sequencing failure resulting in an invalid result or No Call for the variant.

b. Clinical Efficacy Based on ODxT (CDx) Test Results

The efficacy of fam-trastuzumab deruxtecan-nxki (ENHERTU®) was evaluated in Daiichi Sankyo DS8201-A-U204 (DESTINY Lung 01, n=91) and DS8201-A-U206 (DESTINY Lung 02, n=52) studies. Demographic and baseline disease characteristics were similar for patients in both the DESTINY-Lung 01 and DESTINY-Lung 02 studies. Also, the response rates were consistent across the evaluated dose levels (5.4 mg/kg and 6.4 mg/kg). The efficacy of ENHERTU® (fam-trastuzumab deruxtecan-nxki) in both study populations (DESTINY Lung 01 and DESTINY Lung 02) and in those subjects positive for *ERBB2* activating mutations (SNVs and Exon 20 insertion) by the ODxT Test as shown in Table 17 are comparable.

Of the 91 ERBB2 subjects in the full analysis set of the DS8201-A-U204 clinical trial (Cohort 2), 49 samples had valid CDx Test results (48 samples were CTA+, CDx+ while one sample was CTA+, CDx+ negative result) available, and 42 samples were unevaluable, which included 42 samples with unknown status. Thus, the analysis of effectiveness was based on a bridging study using 49 patient samples that were evaluable by the CDx Test.

Based on objective response rate (ORR) being the primary efficacy endpoint for the DS8201-A-U204 trial, a comparison of responders and non-responders in CDx evaluable and unevaluable categories, was performed. Key efficacy results from the DS8201-A-U204 trial, assessed by independent review committee from the May 3, 2021, data cut is shown in Table 17 below.

In patients with valid CDx Test results (n=49), the ORR was 57.1%, (95% CI: 42.2%, 71.2%) and 28 patients responded. The ORRs are presented in Table 17, overall and stratified by CDx Test result, in the primary efficacy analysis population (CTA+, Cohort 2 subjects). The ORR was 58.3%

(95% CI: 43.2%, 72.4%) in CDx Test-positive samples (n=48), 0.0% (95% CI: 0.0%, 97.5%) in CDx Test-negative samples (n=1), and 52.4% (95% CI: 36.4%, 68.0%) in CDx Test unevaluable samples (n=22).

Table 17. Efficacy Results of ENHERTU® Clinical Study and ORR Stratified by ODxT Test Results

	DESTINY Lung 01- 6.4 mg/kg					DESTINY Lung 02 - 5.4 mg/kg*
Clinical Efficacy	CDx+ (n=48)	CDx- (n=1)	CDx Evaluable (n=49)	CDx Unevaluable (n=42)	Total FAS (n =91)	Total FAS N =52
ORR	58.3% (28/48)	0.0%	57.1% (28/49)	52.4% (22/42)	54.9% (50/91)	57.7% (30/52)
95% CI	43.2%, 72.4%	0%, 97.5%	42.2%, 71.2%	36.4%, 68%	44.2%, 65.4%	43.2%, 71.3%
CR	0	0	0	1	1	1
PR	28	0	28	21	49	29
DOR Median, months, 95%CI	12 5.5%, 18.2%	-	12 5.5%, 18.2%	8.5 4.3%, NE	9.3 5.7%, 14.7%	8.7 7.1%, NE

* This is the primary efficacy population for the approval of fam-trastuzumab deruxtecan-nxki (ENHERTU®) ORR = Overall Response Rate; 95% CI, calculated using Clopper-Pearson method, CR = complete response, PR= partial response, DOR = Duration of Response; 95% CI calculated Brookmeyer-Crowley method. NE= Not estimable

The duration of response (DOR) in subjects that were ODxT Test+/CTA+ was 12 months, compared to 9.3 months for the CTA+, Cohort 2 population. The DOR for DESTINY Lung 01 was 9.3 months which is similar to DOR for DESTINY Lung 02 of 8.7 months.

c. Sensitivity Analysis

The primary objective analysis described above demonstrated ENHERTU® efficacy in the CDx-positive, and CTA-positive subset of the intended use population. In the clinical bridging study, 49 out of 91 (54%) samples from patients enrolled in the study using clinical trial assay positive (CTA+) patients were tested with the CDx (ODxT) Test. Thus, 46% of the CTA+ samples in the clinical bridging study lack CDx results due to invalid results, insufficient tumor tissue, unacceptable tumor content, or insufficient DNA concentration. As missing data can potentially impact concordance estimates and drug efficacy, sensitivity analyses were conducted to evaluate whether missing data may have had an impact on CDx test results, concordance, and/or clinical outcome. In addition, the sensitivity analysis evaluated the impact on drug efficacy for

the ODxT Test+ ∩ CTA- subgroup, which was not enrolled into the clinical trial due to negative CTA results.

A comparison of DS8201-A-U204 study subject demographics, disease, and sample characteristics for the primary efficacy population between the ODxT Test evaluable and unevaluable groups is presented in Table 18. Differences between ODxT Test evaluable and unevaluable groups were compared for the following variables: age, gender, ethnicity, race, specimen type, CTA test site, CTA test format, CTA test method and Eastern Cooperative Oncology Group (ECOG). If the results demonstrated that a characteristic was related to sample missingness, then that characteristic was considered for inclusion in the Likely Case imputation model.

Table 18. Associations of Demographic and Clinical Characteristics with ODxT Test Evaluable vs Unevaluable Status in CTA+ Subjects

Characteristic	P-value
Age (Years)	0.0687
Gender	0.3073
Ethnicity	0.9987
Race	0.4883
ECOG	0.5774
Specimen Type	0.0529
Sample Test Site*	1.0000
CLIA/CLIA-like Certification*	1.0000
CTA Assessment Method*	1.0000
CTA Assay Format*	0.5824
Sample Collection Site*	0.9509
CTA Test Name	1.0000
Tumor Content	<.0001
Percent Necrosis	0.3004

p-value from logistic regression models (dependent variables=ODxT evaluable vs unevaluable status). *Validity of the model fit is questionable possibly due to quasi-complete separation of data points.

The characteristics of age, specimen type and tumor content were identified as predictive for ODxT Test evaluable status using a significance level of 0.1 and these covariates were included in the imputation model. There were a total of 42 missing ODxT Test results and the missing ODxT Test results were imputed by multiple imputation method using Logistic Regression with a Fully Conditional Specification with age, specimen type, tumor content and clinical outcome (ORR). The imputation was performed 50 times. The overall ORR is 55.6 which is similar to ORR for both CDx+ and clinical trial cohorts demonstrating robustness of the drug efficacy results.

3. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included one investigator who was full-time of the sponsor and had disclosable financial interests/arrangements as defined in 21 CFR 54.2(a), (b), (c) and (f) and described below:

- Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: [0]
- Significant payment of other sorts: [0]
- Proprietary interest in the product tested held by the investigator: [0]
- Significant equity interest held by investigator in sponsor of covered study: [0]

The applicant has adequately disclosed the financial interest/arrangements with clinical investigators. Statistical analyses were conducted by FDA to determine whether the financial interests/arrangements had any impact on the clinical study outcome. The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel, an FDA advisory committee, for review and recommendation.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

For the intended use to identify the *ERBB2* activating mutations (SNVs and *ERBB2* exon 20 insertions) in NSCLC patients to be treated with ENHERTU® (fam-trastuzumab deruxtecan-nxki), the effectiveness of the ODxT Test was demonstrated through analytical studies using patient samples from the intended use population and a clinical bridging study using specimens from cohort 2 of the

DS8201-A-U204 clinical study. The data from the analytical validation and clinical studies support the reasonable assurance of safety and effectiveness of the ODxT Test when used in accordance with the indications for use. Data show that patients who had qualifying *ERBB2* activating mutations (SNVs and *ERBB2* exon 20 insertions) received benefit from treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki), and support the addition of the CDx indication to the ODxT Test.

B. <u>Safety Conclusions</u>

The risks of the device are based on data collected in the analytical studies conducted to support sPMA approval as described above. The ODxT Test is an *in vitro* diagnostic test, which involves testing of DNA and RNA extracted from FFPE tumor tissue.

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions in cancer treatment. Patients with false positive results may undergo treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki) therapy of the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with the indicated therapy.

C. <u>Benefit-Risk Determination</u>

Treatment with fam-trastuzumab deruxtecan-nxki provides a meaningful clinical benefit to NSCLC patients with *ERBB2* activating mutations (SNVs and *ERBB2* exon 20 insertion mutations), as demonstrated in the DS8201-A-U204 trial and the DS8201-A-U206.

For the intended use of identifying *ERBB2* activating mutations (SNVs and *ERBB2* exon 20 insertions) in NSCLC patients to be treated with fam-trastuzumab deruxtecan-nxki, the probable benefit of the ODxT Test was demonstrated through a clinical bridging study and an analytical accuracy study to the Ev-NGS assay: this was achieved by retrospectively testing clinical specimens from patients enrolled the DS8201-A-U204 trial. Agreements between ODxT Test and CTA were calculated using the CTA results as the reference. The point estimates of PPA, NPA, and OPA excluding unknown (i.e., invalid/No Call ODxT Test results and samples with insufficient material) were 98.0%, 100%, and 99.4%, respectively. In addition, key clinical efficacy endpoints of Phase II study DS8201-A-U204 were reported in the population positive for *ERBB2* activating mutations (SNVs and *ERBB2* exon 20 insertions) by the ODxT Test (clinical efficacy). The efficacy in ODxT Test CDx cohort (ORR 58.3%; 95% CI 43.2% – 72.4%) was clinically meaningful, given the patient population, and supported the

efficacy observed as reported in the drug label (ORR 57.7%, 95% CI 43.2%, 71.3%) trial (DS8201-A-U206).

Given the available information and the analytical and clinical data provided in the submission, the data supports the conclusion that the ODxT Test has probable benefit in selecting NSCLC patients with *ERBB2* activating mutations (SNVs and *ERBB2* exon 20 insertions), for treatment with fam-trastuzumab deruxtecan-nxki.

There is potential risk associated with the use of this device, mainly due to 1) false positives, false negatives, and failure to provide a result and 2) incorrect interpretation of test results by the user.

The risks of the ODxT Test for selection of NSCLC patients with *ERBB2* activating mutations (SNVs and exon 20 insertions) are associated with the potential mismanagement of patient's treatment resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial and may lead to adverse events or may have delayed access to other treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial therapeutic regimen. The risks of erroneous results are partially mitigated by the analytical performance of the device.

The likelihood of false results was assessed by an analytical and clinical validation studies, which partially mitigate the probable risk of the ODxT Test device. A key clinical study that mitigates the risk of the device was the analytical accuracy study, which showed in comparison to the Ev-NGS assay, point estimates of PPA, NPA, and OPA excluding unknown results, of 100.0%, 99.1%, and 99.3%, respectively. This, along with the clinical bridging concordance data to the CTA, demonstrates robust performance of this device for detection of *ERBB2* activating mutations (SNVs and exon 20 insertions). Additional factors, including the clinical and analytical performance of the device included in this submission, have been taken into account and demonstrated that the assay is expected to have acceptable performance.

1. Patient Perspectives
   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for the indications of the ODxT device the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the clinical validation study support the performance of the ODxT Test

as an aid for the identification of *ERBB2* activating mutations (SNV mutations and *ERBB2* exon 20 insertions) in NSCLC patients for whom fam-trastuzumab deruxtecan-nxki may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order for the PMA (P160045/S035) on 08/11/2022. Additional non-clinical study is requested as conditions of approval cited in the approval order are described below.

The following data should be provided as separate reports, which may be followed by a PMA supplement, where applicable. The study data and conclusions should be submitted within 1 year of the PMA approval date, unless otherwise specified.

1. Thermo Fisher Scientific/Life Technologies Corp. must provide data from well-designed and well-controlled precision studies evaluating external sample processing reproducibility and within-run repeatability with an adequate number of replicates using intended use clinical specimen carrying *ERBB2* exon 20 insertions. The data from this study must be adequate to support precision (starting from sample processing) for *ERBB2* mutations (specifically *ERBB2* exon 20 insertions) in the intended use population.

2. Thermo Fisher Scientific/Life Technologies Corp. must provide data from a well-designed and well-controlled interference substances study evaluating hemoglobin as an interferent for the *ERBB2* exon 20 insertion variant calling using intended use specimens near 1-1.5x LoD. The data from this study must be adequate to support the finding that the potential endogenous interfering substance hemoglobin in NSCLC do not adversely impact ERBB2 exon 20 insertion mutations calling.

3. Thermo Fisher Scientific/Life Technologies Corp. must provide data from well-designed and well-controlled stability studies as follows:

   a. To demonstrate robust *ERBB2* exon 20 insertion calling within the ODxT Test stability claims for reagent shelf-life stability study using intended use specimens. The data from this study must be adequate to support stability claims for insertions in the intended use population.

   b. To demonstrate robust *ERBB2* exon 20 insertion calling within the ODxT Test when using NSCLC formalin-fixed paraffin embedded (FFPE) tissue blocks and cut slides derived from clinical trial samples at or near LoD (1 – 1.5x LoD). The study should demonstrate that prolonged storage of FFPE blocks carrying *ERBB2* exon 20 insertion mutations has no effect on the outcome of the ODxT Test.

c. To demonstrate stability of freeze-thaw of DNA derived from FFPE clinical samples and FFPE cell line samples containing *ERBB2* exon 20 insertion mutations. The data from this study must be adequate to support the freeze-thaw stability claims of DNA for robust detection of insertion mutations in the intended use population.

The study protocols for 3(b) and 3(c) should be submitted within 60 days of the PMA approval date, and the study data and conclusions should be submitted within 2 years of the PMA approval date.

4. Thermo Fisher Scientific/Life Technologies Corp. will provide validation data for the merging of multiple assay definition files (ADF) associated with approved companion diagnostic indications. The final data and the associated updates to the Torrent Suite Dx software for the merged ADF and Torrent Suite Dx versions to be commercialized to support new approved indications based upon the final aggregation protocol will be submitted within 6 months of approval of this PMA supplement.

5. Thermo Fisher Scientific/Life Technologies Corp. will provide results and software validation documentation from regression testing on the commercial release configuration to confirm there are no defects for the merged ADF based on the approved aggregation validation protocol and no new defects other than those listed in the approved Torrent Suite Dx versions. This information should be submitted within 6 months of approval of this PMA supplement.

The study data and conclusions for # 4 and # 5 should be submitted within 6 months of the PMA approval date.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

XV. REFERENCES

None.