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 Torrent PGM Dx System and the Ion Torrent PGM Dx Reagents for detection of alterations in DNA [isolated from NSCLC, CC, and MTC formalin-fixed, paraffin-embedded (FFPE) tumor specimens] and RNA isolated from NSCLC and TC 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 Torrent™ 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 Torrent™ 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 Guides:

- 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 inactive 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 (SNVs), multi-nucleotide variants (MNVs), 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

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, CC, MTC, and TC 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

To support the NSCLC indication, non-clinical studies were leveraged using approved non-clinical data from P160045/S019. A summary of the studies utilized to support the NSCLC and TC indications is provided below stratified by tissue type. Please note, the thyroid cancer indication is split into two tissue subsets –MTC to evaluate *RET* mutations (SNVs, MNVs, and deletions) and TC to evaluate *RET* fusions.

A. NSCLC Studies

1. Laboratory Studies

The evidence in support of the performance of the ODxT Test in detecting RET fusions in NSCLC was from the data presented using intended use specimens and sample blends across all validation studies. Studies evaluating analytical accuracy/concordance, precision studies at the limit of detection (LoD), limit of blank (LoB), tissue input, tissue content, interferents, and stability were conducted to support the indication for RET fusions in NSCLC.

a. Analytical Accuracy/Concordance

An analytical accuracy study was performed to demonstrate the concordance between the ODxT Test and an externally validated next generation sequencing test method

The tumor cell content in FFPE samples used as input material was evaluated using FFPE NSCLC samples included in the clinical validation study to determine whether tumor content affected the performance of the ODxT Test. A total of 91 RET fusion-positive and 125 RET fusion-negative samples were included in the study analysis. The tumor cell content of each specimen and region of interest was estimated before the study by an external pathology lab. All samples yielded valid results for both the ODxT Test (Passing Run, RNA Control, and RNA Sample QC criteria) and the Ev-NGS assay. The PPA, NPA, and OPA agreement between the ODxT Test and the Ev-NGS assay was 100% across all tumor content ranges (<30%, ≥30-40%, ≥40-60%, ≥60%). The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels, demonstrating that the RET fusion detection performance of the ODxT Test was equivalent at all ranges of tumor content level; and the tumor content level of the clinical samples had no impact on the performance of the ODxT Test.

c. Interference

To evaluate the potential impact of endogenous (necrotic tissue and hemoglobin) and exogenous interferents (paraffin, xylene, ethanol, proteinase K, and wash buffer), this study evaluated clinical FFPE samples in 6 replicates for RNA for every combination of sample and condition taken through the entire test workflow. The impact of potentially interfering substances on assay performance was evaluated, and the results were compared to the control (no interferents) condition.

i. Endogenous Interference

A review of clinical samples harboring variants detected by the ODxT Test, including ROS1 in the presence of varying levels of tissue necrosis. Hemoglobin was evaluated at 4 mg/mL. The concordance with the control condition (with no calls being excluded) across all samples, for all CDx RNA fusions tested were calculated to be 100%. The data demonstrate that hemoglobin does not adversely impact the performance of the assay.

ii. Exogenous Interference

For the study with exogenous interferents, the concordance with the control condition across all samples and interferents, for all CDx RNA fusions tested were calculated to be 100%. The data support that these interfering substances can be tolerated by the assay at the levels tested.

d. Precision and Reproducibility

i. External Panel Reproducibility Study (Assay Reproducibility)

An external reproducibility study was conducted across 3 sites with 2 operators per site, 3 lots of the ODxT Test Controls, NTC Kits, IVD Ion PGM Dx Library Kits, OneTouch Dx Template Kits, Ion PGM Dx Sequencing Kits and Dx Chip Kits used at each site using samples that included RET fusions. All samples and replicates generated a PPA of 100% (90.3%, 100%) and a NPA of 100% (81.5%, 100%), with the exception of two replicates of one RET fusion positive sample, which generated a false negative result across 2 runs, yielding a PPA of 94.4% (81.3%, 99.3%). An investigation into the false negative result determined that operator error resulted in the wrong barcode being added to the RNA libraries during the workflow. As mentioned above, an additional single-site reproducibility study will be performed post-market to confirm the LoD of RET fusions using samples near 1X LoD (see Section XIII of Summary of Safety and Effectiveness Data for P160045/S019).

ii. Precision

Precision for the RNA fusion variants was estimated with respect to positive variant locations for within-run, between-system, between-operator, between-site, between-lot and total variability. When including or excluding No Calls from the assay reproducibility study data, the within-run repeatability was 100%, with the exception of one RET fusion positive sample, which had a within run repeatability of 98.1%, with a lower limit of the corresponding 95% CI of ≥ 81.5%, at each of the RNA fusion variant locations.

e. Guard Banding

No new guard banding studies were conducted, please refer to P160045/S019 and P160045 for guard banding studies.

f. Stability Studies

Please refer to the Summary of Safety and Effectiveness Data P160045 and P160045/S019 for platform validation of reagent, DNA, and FFPE slide stability.

The stability of FFPE cut slides and FFPE blocks used in the clinical study showed a minimum stability of 5 months. Additional stability studies to demonstrate shelf-life, in-use, extracted RNA, stored FFPE block, and stored FFPE slide stability will be completed as conditions of approval (see Section XIII of Summary of Safety and Effectiveness Data for P160045/S019).

g. Kit Lot Interchangeability

There were no changes to the reagents and specifications therefore, for reagent lot interchangeability results, please see Summary of Safety and Effectiveness Data for P160045.

2. Animal Studies

Not Applicable.

B. Thyroid Cancer Studies

1. Laboratory Studies

The evidence in support of the performance of the ODxT Test in detecting RET fusions and mutations in thyroid cancer (TC) was from the data presented using intended use specimens and sample blends across all validation studies. Studies evaluating analytical accuracy/concordance, limit of blank (LoB), tissue input, RNA input, sample processing reproducibility, interferents, limit of detection (LoD), tumor content, assay reproducibility, guardbanding, and stability of FFPE tissue slides were conducted to support the indication for RET fusions and mutations in thyroid cancer. Concordance was evaluated for both MTC and TC datasets.

a. Analytical Accuracy/Concordance (MTC)

An analytical accuracy study was performed to demonstrate the concordance between the ODxT Test and an Ev-NGS for the detection of RET DNA variants in MTC using FFPE MTC tumor specimens. This study evaluated 46 RET DNA variant-positive specimens from patients enrolled in the LIBRETTO-001 clinical trial. RET DNA variant negative samples consisted of 81 commercially procured TC samples screened by a representative LLT using validated NGS test.

Of the 46 RET DNA variant-positive samples tested by the ODxT Test, 36 were positive, 6 samples were negative, 3 samples yielded an invalid result, and 1 sample was excluded due to insufficient DNA quantity. For the Ev-NGS assay, 35 samples were positive, 7 samples were negative, and 1 sample yielded an invalid result.

Of the 81 LLT-negative samples tested by the ODxT Test, 54 were negative, 1 sample was positive, 25 samples yielded an invalid result, 1 sample was not tested due to insufficient DNA quantity. For the Ev-NGS assay, 59 samples were negative, 1 sample was positive, 18 yielded invalid results, 1 sample did not meet the required DNA input quantity and 2 samples were excluded due to insufficient amount of DNA. In all, 102 samples were used to evaluate concordance between the ODxT Test as an investigational method and the Ev-NGS assay.

The concordance between the ODxT Test and the Ev-NGS assay was calculated (Table 7). A summary of PPA, NPA, and OPA in reference to Ev-NGS assay and corresponding 95% two-sided exact CIs is provided in Table 8. The point estimates of PPA, NPA, and OPA excluding unknown results were 100.0%, 98.3%, and 98.9%, 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 100.0%, 86.4%, and 91.2%, respectively (Table 8).

Table 7. Contingency Table of ODxT Test and Ev-NGS Assay Results for RET DNA Variants Detection (MTC)

ODxT Test	Ev-NGS Assay			
Frequency	**POS**	**NEG**	**UNK**	**Total**
POS	36	1	0	37
NEG	0	57	3	60
UNK	0	8	22	30
Total	36	66	25	127

Table 8. Concordance between ODxT Test and the Ev-NGS assay – RET DNA Variants (MTC)

Agreement Measure	Excluding Unknowns		Including Unknowns	
	Percent Agreement	**95% CI**	**Percent Agreement**	**95% CI**
PPA	100.0% (36/36)	(90.3%, 100.0%)	100.0% (36/36)	(90.3%, 100.0%)
NPA	98.3% (57/58)	(90.8%, 100.0%)	86.4% (57/66)	(75.7%, 93.6%)
OPA	98.9% (93/94)	(94.2%, 100.0%)	91.2% (93/102)	(83.9%, 95.9%)

b. Analytical Accuracy/Concordance (TC)

An analytical accuracy study was performed to demonstrate the concordance between the ODxT Test and an Ev-NGS for the detection of RET RNA fusions in TC using FFPE TC tumor specimens. This study evaluated 32 RET DNA variant-positive specimens from patients enrolled in the LIBRETTO-001 clinical trial. Of these, one sample failed the pathology review and was excluded. RET DNA variant negative samples consisted of 68 commercially procured TC samples screened by a representative LLT using validated NGS test.

Of the 31 RET fusion-positive samples, 25 were positive for the ODxT Test, 2 samples were negative, 2 samples yielded an invalid result, and 2 samples were not tested due to insufficient RNA quantity. For the Ev-NGS assay, 25 samples were positive, 2 samples were negative, 2 samples yielded an invalid result and 2 samples were not tested due to insufficient RNA quantity .

Of the 68 RET fusion-negative samples tested by the ODxT Test, 58 were negative, and 10 samples yielded an invalid result. For the Ev-NGS assay, 60 samples were negative, 7 yielded invalid results, and 1 sample was not tested due to insufficient RNA quantity. In all, 87 samples were used to evaluate concordance between the ODxT Test as an investigational method and the Ev-NGS assay.

Concordance between the ODxT Test and the Ev-NGS assay with respect to the PPA, NPA, and OPA is shown below (Table 9 and Table 10). The point estimates of PPA, NPA, and OPA excluding unknown results were all 100.0%. Unknown is defined as

insufficient samples and sample QC sequencing failures resulting in an invalid result for the variant. When including ODxT Test unknown results, the point estimates of PPA, NPA, and OPA were 100.0%, 91.9%, and 94.3%, respectively.

Table 9. Contingency Table of ODxT Test and Ev-NGS Assay Results for RET Fusions Detection (TC)

ODxT Test	Ev-NGS Assay			
Frequency	**POS**	**NEG**	**UNK**	**Total**
POS	25	0	0	25
NEG	0	57	3	60
UNK	0	5	10	15
Total	25	62	13	100

Table 10. Concordance between ODxT Test and the Ev-NGS assay – RET Fusions (TC)

Agreement Measure	Excluding Unknowns		Including Unknowns	
	Percent Agreement	**95% CI**	**Percent Agreement**	**95% CI**
PPA	100.0% (25/25)	(86.3%, 100.0%)	100.0% (25/25)	(86.3%, 100.0%)
NPA	100.0% (57/57)	(93.7%, 100.0%)	91.9% (57/62)	(82.2%, 97.3%)
OPA	100.0% (82/82)	(95.6%, 100.0%)	94.3% (82/87)	(87.1%, 98.1%)

c. Analytical Sensitivity

i. Limit of Blank (LoB)

This study was performed to test the frequency of false positive calls for RET DNA variants and RET RNA fusions detected by the ODxT Test in wild-type (WT) clinical samples. For DNA, a previously tested set of negative FFPE clinical NSCLC samples known to be WT for RET DNA variant locations was reanalyzed to evaluate the false positive rate and verify that the LoB was equal to 0. For RNA, a set of negative FFPE clinical TC samples known to be WT for RET fusion isoforms was tested to evaluate the false positive rate and similarly verify that the LoB was equal to 0.

To ensure that the ODxT Test does not generate a signal that might be classified as an RET mutation positive result or RET fusion positive result (false positive result), 4 WT NSCLC FFPE and 4 WT TC clinical samples 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. The updated ODxT Test Kit RNA workflow was used for RNA library preparation. The result showed that the false positive rate of the ODxT Test was zero (0) for both RET DNA mutations and RET RNA fusions since there were no positive calls at any of the variant locations analyzed by the test for all 8 samples.

ii. Limit of Detection (LoD)

The limit of detection (LoD) based on positive calls for the ODxT Test was estimated to determine the lowest allele frequency (AF) of RET DNA mutations and the lowest fusion reads of RET RNA fusions, at which 95% of the test replicates produced correct calls. The LoD was evaluated for 4 representative RET DNA variants and 2 RET RNA fusion isoforms detected by the ODxT Test in clinical TC samples. For RET DNA variants, the LoD is the lowest allelic frequency (AF) of SNV, MNV, or deletion variants that can be detected at least 95% of the time. For RET RNA fusions, the LoD is the lowest fusion reads that can be detected at least 95% of the time. Two DNA sample blends (each with 2 RET DNA variants) were generated by blending RET variant-positive DNA with RET WT DNA. Two RNA sample blends (each with one RET RNA fusion) were created by blending RET fusion-positive RNA with RET WT RNA. Six different dilution levels (AFs for DNA and fusion reads for RNA) per sample blend were tested. Each level was tested with 10 replicates per sample blend for each of the two reagent lots for a total of 20 replicates per level.

The claimed LoD based on the empirical hit-rate approach for 4 representative RET DNA variants were determined to have allelic frequencies ranging from 4.9% to 5.5% as shown in Table 11 below. The claimed LoD based on the empirical hit-rate approach for 2 representative RET RNA fusion isoforms were determined to be 210 for CCDC6-RET and 236 for NCOA4-RET, respectively as shown in Table 12 below.

Table 11. Estimated LoD for RET DNA Mutations

Target	Variant Type	Variant Amino Acid Change	Est. LoD (Allelic Frequency)
COSM965	SNV	p.M918T	0.049
COSM977	MNV	p.A883F	0.055
COSM962	Deletion	p.D898_E901del	0.051
COSM1738369	SNV	p.C634G	0.051

Table 12. Estimated LoD for RET RNA Fusions

Fusion Isoform	Est. LoD (Fusion Reads)
CCDC6-RET.C1R12.COSF1271	210
NCOA4-RET.N7R12.COSF1491	236

iii. Tissue Input

This study was performed to verify that the number of slides used for extraction of TC FFPE tissue samples provide adequate nucleic acid concentrations to meet the DNA and RNA input criteria for the ODxT Test. The test requires DNA at a concentration of ≥0.83 ng/µL and RNA at a concentration of ≥1.43 ng/µL. A total

of 25 FFPE thyroid samples were analyzed, including 15 resections, 5 core needle biopsy (CNB), and 5 fine needle aspirate (FNA) samples.

Fourteen resection samples with ≥20% tumor content were prepared without macrodissection, one resection sample with <20% to ≥10% tumor cell content was macrodissected, and the 5 CNB and 5 FNA samples were prepared without macrodissection. For resection samples with ≥20% tumor cell content, 1–2 × 5 µm sections were used per extraction. For the resection sample with <20% tumor cell content and ≥10% tumor cell content that was macrodissected, 2 × 5 µm sections were used in the extraction. For CNBs, 9 × 5 µm sections were used per extraction. For FNAs, 7 × 5 µm sections were used per extraction. DNA and RNA concentrations were determined using the Ion Torrent Dx DNA Quantification Kit and Ion Torrent Dx RNA Quantification Kit, respectively.

Of all samples tested, 100% (25/25) yielded a DNA concentration of ≥0.83 ng/µL and an RNA concentration of ≥1.43 ng/µL, meeting the minimum concentration requirements. In addition, all 25 samples were carried through library preparation to sequencing and passed DNA and RNA sample library quality metrics.

iv. RNA Input

This study was performed to compare RET fusion reads over a range of RNA:DNA input ratios to define the tolerance around the amount of input RNA required for the ODxT Test to accurately detect RET fusions. RNA from RET fusion-positive and wild-type (RET fusion-negative) TC FFPE clinical samples were blended to create sample blends at fusion read levels of approximately 1–1.5X LoD. A DNA blend composed of two common RET DNA variants was used to prepare a DNA library to function as a filler library in ODxT Test runs. Sample RNA and DNA libraries were prepared with input ratios corresponding to the range of levels shown in Table 13. Six replicates of each input ratio were run, and the RET fusion reads were tabulated.

Table 13. RNA:DNA Input Ratio

RNA:DNA Input (ng)	Average Log Fusion Reads
10:10	2.39
5:15	2.63
6.5:15	2.62
8.5:15	2.60
10:15	2.39
15:15	2.53

The results showed 100% call rates for RET fusions across the RNA:DNA input ratios tested, and further showed that mapped reads and log-transformed fusion reads were not impacted by varying the input ratio from the standard RNA:DNA ratio of 10 ng:10 ng. This data demonstrated that the input range of 5 ng to 15 ng of RNA can consistently detect the RET fusions.

v. Tumor Content

The tumor cell content in FFPE samples used as input material was evaluated using FFPE TC samples included in the clinical validation study to determine whether tumor content affected the performance of the ODxT Test. In total, 133 specimens were included in the study analysis. Of these samples, 68 were FFPE MTC samples including 15 RET mutation-positive and 53 RET mutation-negative samples, and 65 were FFPE TC samples including 9 RET fusion-positive and 56 RET mutation-negative samples.

The tumor cell content of each specimen and region of interest was estimated before the study by an external pathology lab. All samples yielded valid results for both the ODxT Test (Passing Run, DNA/RNA Control, and DNA/RNA Sample QC criteria) and the Ev-NGS assay. The PPA, NPA, and OPA agreement between the ODxT Test and the Ev-NGS assay was 100% across all tumor content ranges (<30%, ≥30-40%, ≥40-60%, ≥60%). The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels, demonstrating that the RET fusion detection performance of the ODxT Test was equivalent at all ranges of tumor content level; and the tumor content level of the clinical samples had no impact on the performance of the ODxT Test.

d. Interference

To evaluate the potential impact of endogenous (hemoglobin and colloid) interferents on the performance of the ODxT Test in detecting RET DNA variants and RET RNA fusions in TC FFPE samples, a total of 4 TC FFPE clinical samples (2 RET DNA variant-positive and 2 RET RNA fusion-positive) were tested in 6 replicates. Two wild-type TC samples with high colloid content were used for blending with RET DNA variant and RET RNA fusion samples to achieve a higher colloid content. Hemoglobin was evaluated at 4 mg/ml and colloid were evaluated at >40%. For these 2 interferents tested, the positive concordance with control condition across all samples were calculated to be 100%. The data demonstrate that hemoglobin and colloid do not affect assay performance at the level tested in detection of the RET DNA variants and RET RNA fusions.

e. Precision and Reproducibility

i. Internal Sample Processing Reproducibility

The purpose of this study was to demonstrate that the processing of TC FFPE samples as part of the ODxT Test workflow generated repeatable and reproducible results for the RET variants. Four TC FFPE samples (2 RET DNA variant-positive MTC samples and 2 RET fusion-positive TC samples) and 2 WT TC FFPE samples were evaluated in this study. Each sample was extracted 12 times (3 FFPE extraction kit lots × 4 replicates per kit lot) at one internal test site

with 2 operators, for a total of 12 replicates per sample. The testing site used 2 Ion PGM™ Dx instrument systems.

The call rate, no call rate, positive call rate, negative call rate, and within-run repeatability were computed for each RET variant and WT sample (Table 14). The positive call rates for both the RET mutation positive samples and RET fusion positive samples are 100%, and the negative call rate at each clinical variant location for the two WT samples was 100% and 95.8%, respectively. The within run repeatability was 100% for all samples at all RET variant loci when excluding no calls. The sample level analysis demonstrated 100% positive agreement for RET DNA variants and RET RNA fusions across all 2 operators.

Table 14. Call Rates at Positive Variant Locations

Sample	Variant identification (Variant Type)	# valid sample results (N)	# positive calls (A)	# negative calls (B)	# no calls (C)	Positive call rate (95% C.I.)		Negative call rate (95% C.I.)		Within-run repeatability (95% C.I.)	
						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	Excluding no calls
1	COSM965 p.Met918Thr (SNV)	12	12	0	0	100% (73.5%, 100%)	100% (73.5%, 100%)	0% (0%, 26.5%)	0% (0%, 26.5%)	100% (54.1%, 100%)	100% (54.1%, 100%)
2	COSM962 p.Asp898_Glu9 01del (Deletion)	12	12	0	0	100% (73.5%, 100%)	100% (73.5%, 100%)	0% (0%, 26.5%)	0% (0%, 26.5%)	100% (54.1%, 100%)	100% (54.1%, 100%)
3	CCDC6-RET.C1R12.COSF1271 (Fusion)	12	12	0	N/A	N/A	100% (73.5%, 100%)	N/A	0% (0%, 26.5%)	N/A	100% (54.1%, 100%)
4	NCOA4-RET.N7R12 (Fusion)	12	12	0	N/A	N/A	100% (73.5%, 100%)	N/A	0% (0%, 26.5%)	N/A	100% (54.1%, 100%)
5	N/A (Wild type)	48	0	48	0	0% (0%, 7.4%)	0% (0%, 7.4%)	100% (92.6%, 100%)	100% (92.6%, 100%)	100% (85.8%, 100%)	100% (85.8%, 100%)
6	N/A (Wild type)	48	0	46	2	0% (0%, 7.4%)	0% (0%, 7.7%)	95.8% (85.7%, 99.5%)	100% (92.3%, 100%)	95.8% (79.9%, 99.9%)	100% (85.2%, 100%

ii. External Panel Reproducibility

The purpose of the external panel reproducibility study was to evaluate the within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility) of the ODxT Test, independent of sample processing steps, for detection of RET DNA variants and RET RNA fusions. This study was conducted across 3 sites with 2 operators/instruments per site, 2 lots at each site using RET DNA variant-positive MTC sample blends (4 RET DNA variant-positive MTC samples were blend with

WT samples) and RET RNA fusion-positive TC samples blends (2 RET RNA fusion-positive TC samples were blended with WT samples).

DNA extracted from RET DNA variant-positive MTC samples were blended with WT DNA to a target AF of 0.9x-1.5x and 2-3x LoD of the estimated LoD. RNA extracted from RET RNA fusion-positive TC samples were blended with WT RNA to a target fusion reads of 0.9x-1.5x and 2-3x LoD of the estimated LoD. In initial studies, 6 DNA sample blends (4 variant-positive blends and 2 WT blends) and 6 RNA sample blends (4 fusion-positive blends and 2 WT blends) were evaluated and each sample blend was tested in 3 replicates across 3 sites by 2 operators using 2 instrument systems with 2 lots of reagents, yielding 72 total sequencing results per sample blend. Three additional DNA sample blends (2 variant-positive blends and 1 WT blend) and 3 additional RNA sample blends (2 fusion-positive blends and 1 WT blend) were subsequently prepared and tested to more closely approach the two LoD levels targeted in the study (0.9x-1.5x and 2-3x LoD).

The positive call rates, negative call rates and within-run repeatability, both including and excluding no calls, at RET variant locations for all samples are outlined in Table 15 and Table 16. For all RET DNA mutation-positive sample blends, the positive call rate (No Calls excluded) for each RET mutant of interest at all AF target levels tested was 100.0%. For all RNA RET fusion-positive sample blends, the positive call rate for each RET fusion isoform of interest ranged from 94.4% (at the lowest fusion read target levels tested) to 100.0% (at the highest fusion read target levels tested). The within-run repeatability were 100% for the RET DNA variants tested, with one WT blend showing a 97.9% repeatability with no calls included. The within-run repeatability for the RET RNA fusion blends tested ranged from 88.9% to 100%.

Table 15. Call Rates for RET DNA mutations: Reproducibility and Repeatability

Sample	RET Variant ID	Total calls	Positive Calls	Negative Calls	No Calls	Call Rate	No Call Rate	Positive Call Rate (95%CI)		Negative Call Rate (95%CI)		With-In Run Repeatability (95%CI)	
								No Calls included	No Calls excluded	No Calls included	No Calls excluded	No Calls included	No Calls excluded
D1	COSM965	72	72	0	0	100.0%	0.0%	100.0% (95.0%, 100.0%)	100.0% (95.0%, 100.0%)	0.0% (0.0%, 0.5%)	0.0% (0.0%, 0.5%)	100.0% (90.3%, 100.0%)	100.0% (90.3%, 100.0%)
	COSM977	72	72	0	0	100.0%	0.0%	100.0% (95.0%, 100.0%)	100.0% (95.0%, 100.0%)	0.0% (0.0%, 0.5%)	0.0% (0.0%, 0.5%)	100.0% (90.3%, 100.0%)	100.0% (90.3%, 100.0%)
D2	COSM965	72	72	0	0	100.0%	0.0%	100.0% (95.0%, 100.0%)	100.0% (95.0%, 100.0%)	0.0% (0.0%, 0.5%)	0.0% (0.0%, 0.5%)	100.0% (90.3%, 100.0%)	100.0% (90.3%, 100.0%)
	COSM977	72	72	0	0	100.0%	0.0%	100.0% (95.0%, 100.0%)	100.0% (95.0%, 100.0%)	0.0% (0.0%, 0.5%)	0.0% (0.0%, 0.5%)	100.0% (90.3%, 100.0%)	100.0% (90.3%, 100.0%)

Sample	RET Variant ID	Total calls	Positive Calls	Negative Calls	No Calls	Call Rate	No Call Rate	Positive Call Rate (95%CI)		Negative Call Rate (95%CI)		With-In Run Repeatability (95%CI)	
								No Calls included	No Calls excluded	No Calls included	No Calls excluded	No Calls included	No Calls excluded
D3	COSM 1738369	72	72	0	0	100.0%	0.0%	100.0% (95.0%, 100.0%)	100.0% (95.0%, 100.0%)	0.0% (0.0%, 0.5%)	0.0% (0.0%, 0.5%)	100.0% (90.3%, 100.0%)	100.0% (90.3%, 100.0%)
	COSM962	72	72	0	0	100.0%	0.0%	100.0% (95.0%, 100.0%)	100.0% (95.0%, 100.0%)	0.0% (0.0%, 0.5%)	0.0% (0.0%, 0.5%)	100.0% (90.3%, 100.0%)	100.0% (90.3%, 100.0%)
D4	COSM 1738369	72	72	0	0	100.0%	0.0%	100.0% (95.0%, 100.0%)	100.0% (95.0%, 100.0%)	0.0% (0.0%, 0.5%)	0.0% (0.0%, 0.5%)	100.0% (90.3%, 100.0%)	100.0% (90.3%, 100.0%)
	COSM962	72	72	0	0	100.0%	0.0%	100.0% (95.0%, 100.0%)	100.0% (95.0%, 100.0%)	0.0% (0.0%, 0.5%)	0.0% (0.0%, 0.5%)	100.0% (90.3%, 100.0%)	100.0% (90.3%, 100.0%)
D5	RET WT	288	0	282	6	97.9%	2.1%	0.0% (0.0%, 1.3%)	0.0% (0.0%, 1.3%)	97.9% (95.5%, 99.2%)	100.0% (98.7%, 100.0%)	97.9%[1] (94.0%, 99.6%)	100.0% (97.4%, 100.0%)
D6	RET WT	288	0	288	0	100.0%	0.0%	0.0% (0.0%, 1.3%)	0.0% (0.0%, 1.3%)	100.0% (98.7%, 100.0%)	100.0% (98.7%, 100.0%)	100.0% (97.5%, 100.0%)	100.0% (97.5%, 100.0%)
D7	COSM 1738369	72	72	0	0	100.0%	0.0%	100.0% (95.0%, 100.0%)	100.0% (95.0%, 100.0%)	0.0% (0.0%, 0.5%)	0.0% (0.0%, 0.5%)	100.0% (90.3%, 100.0%)	100.0% (90.3%, 100.0%)
	COSM962	72	72	0	0	100.0%	0.0%	100.0% (95.0%, 100.0%)	100.0% (95.0%, 100.0%)	0.0% (0.0%, 0.5%)	0.0% (0.0%, 0.5%)	100.0% (90.3%, 100.0%)	100.0% (90.3%, 100.0%)
D8	COSM965	72	72	0	0	100.0%	0.0%	100.0% (95.0%, 100.0%)	100.0% (95.0%, 100.0%)	0.0% (0.0%, 0.5%)	0.0% (0.0%, 0.5%)	100.0% (90.3%, 100.0%)	100.0% (90.3%, 100.0%)
D9	RET WT	288	0	288	0	100.0%	0.0%	0.0% (0.0%, 1.3%)	0.0% (0.0%, 1.3%)	100.0% (98.7%, 100.0%)	100.0% (98.7%, 100.0%)	100.0% (97.5%, 100.0%)	100.0% (97.5%, 100.0%)

Table 16. Call Rates for RET RNA fusions: Reproducibility and Repeatability

Sample Blend ID	RET Variant ID	Total Calls	Positive Calls	No Calls	Positive Call Rate	Obs. Mean Fusion Reads	Obs. Variant Level (xLoD)	Positive Call Rate (95%CI)	Negative Call Rate (95%CI)	With-In Run Repeatability (95%CI)
R1	CCDC6-RET.C1R12.COSF1271	72	70	2	100.0%	376	1.8x	97.2% (90.3%, 99.7%)	2.8% (0.3%, 9.7%)	94.4% (81.3%, 99.3%)
R2	CCDC6-RET.C1R12.COSF1271	72	72	0	100.0%	697	3.3x	100.0% (95.0%, 100.0%)	0.0% (0.0%, 5.0%)	100.0% (90.3%, 100.0%)
R3	NCOA4-RET.N7R12	72	71	1	100.0%	406	1.7x	98.6% (92.5%, 100.0%)	1.4% (0.0%, 7.5%)	97.2% (85.5%, 99.9%)
R4	NCOA4-RET.N7R12	72	72	0	100.0%	585	2.5x	100.0% (95.0%, 100.0%)	0.0% (0.0%, 5.0%)	100.0% (90.3%, 100.0%)

f. Guardbanding Studies

The purpose of the guardbanding study was to evaluate tolerability of ODxT Test workflow to detect RET DNA mutations and RET RNA fusions in TC for 7 critical assay steps. For RET DNA mutations, this study included 1 condition to test the tolerance of the Proteinase K enzyme volume, and for RET RNA fusions, this study included 7 conditions to test the tolerance of Proteinase K volume, DNase volume, DNase incubation time, cDNA synthesis Enzyme Mix volume and Reaction Mix volume, cDNA Target Amplification panel volume and HiFi mix volume. To evaluate the workflow tolerances, a single DNA blend from FFPE clinical specimens containing RET DNA mutation and a single RNA blend from FFPE clinical specimens containing RET RNA Fusion were used as the input material.

No significant differences between the high and low conditions, relative to the standard operating procedure (SOP), were observed. For RET DNA mutations, 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 RET DNA mutation data. For RET RNA fusions, although high variation in fusion reads was observed, the difference in fusion read counts observed in the study is consistent with the assay measurement variability observed in the reproducibility studies and the percent positive calls are 100% across the tolerance level for all the critical components.

g. FFPE Tissue Slide Stability

This study was performed to evaluate the stability of FFPE slide sections as a tissue source for the detection of RET variants in MTC and TC with the ODxT Test. FFPE sections from 2 clinical MTC samples and 2 TC samples, each containing a unique prevalent RET DNA variant or RNA fusion, were tested with the ODxT Test workflow at baseline (T0) and 4 time points after slide preparation: 3 months, 6 months, 9 months, and 12 months. Slide-mounted 5-µM tissue sections (non-paraffin dipped) from each sample were prepared from FFPE tissue blocks at the start of the study and stored at room temperature (15°C to 30°C) during the study. At each time point, 2 replicate nucleic acid extractions were performed for all clinical samples using the Ion Torrent Dx Total Nucleic Acid Isolation Kit. Each extraction used 1–2 slides per sample. Samples were quantified, carried through the library and template preparation workflow steps, then sequenced using ODxT Test kit components.

Overall, 100% of both DNA and RNA samples yielded positive calls as outlined in Table 19 and Table 20 below. For RET DNA mutations, the AF was not significantly different for every time point up to and including 12 months, and no statistically significant difference in percent AF was observed in any resulting RET DNA mutation data. For RET RNA fusions, while the percent positive calls are 100% across the tolerance level for all the critical components, a significant decrease in actual fusion reads (>50%) for samples with both CCDC6-RET and NCOA4 -RET variants were observed after three

months and the trend is maintained for all the later timepoints. Since the RNA Control QC metrics displayed a similar trend in both total mappable read and control variant fusion reads as seen with the clinical samples, the decrease in fusion reads can be traced to amplifiability differences and higher performing replicates in the run conducted at baseline (T0) relative to each subsequent timepoint through 12 months. Potential factors that may have contributed to the higher baseline performance include but are not limited to the quality of library preparation and recovery, variance in library pooling, templating and sequencing efficiency disparity, and variance in chip loading. This data indicated that the observed difference between baseline and subsequent timepoints is not correlated with RET fusions, TC tissue samples, or TC FFPE slide samples stored for up to 12 months. The results show that MTC and TC FFPE slides stored at 15°C to 30°C are stable for 12 months.

Table 19. DNA Variants

RET Variant ID	Mean Allelic Frequency					
	T0 Baseline	3 mo.	6 mo.	9 mo.	12 mo.	Lower threshold (0.7 x T0 baseline)
COSM965	0.467	0.463	0.435	0.440	0.449	0.327
COSM962	0.713	0.652	0.710	0.656	0.680	0.499

Table 20. RNA Fusions

RET Variant ID	Mean log10 Fusion Reads					
	T0 Baseline	3 mo.	6 mo.	9 mo.	12 mo.	Lower threshold (0.6 x T0 baseline)
CCDC6-RET.C1R2.COSF1271	3.298	2.903	2.488	2.873	2.865	1.979
NCOA4-RET.N7R12	3.664	3.348	3.149	3.258	3.309	2.198

h. RNA Stability

Storage and freeze-thaw (FT) stability of RNA extracted from FFPE TC clinical samples were assessed at baseline, 3 months + 1 week, 6 months + 1 week, 9 months + 1 week and 12 months + 1 week to support the stability of the extracted RNA at 12 months using the Ion Torrent™ Dx FFPE Sample Preparation Kit. RNA extracted from two RET fusion-positive TC clinical FFPE samples were blended with WT RNA to create RNA sample blends with fusion reads at 1-1.5xLoD. Each sample blend was aliquoted and stored at -90°C to -60°C. For baseline, the samples were sequenced within one week of aliquoting. For the purpose of this study, the date of library preparation target amplification is considered to be start of the time point. At each time point, RNA aliquots were frozen and thawed once or 3 times and then sequenced using the ODxT Test. The data supported stability of extracted RNA stored at -90°C to -60°C for 12 months after 3 FT cycles.

2. Animal Studies

Not Applicable.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

Life Technologies conducted three clinical bridging studies to establish the reasonable assurance of safety and effectiveness of the ODxT Test for selecting NSCLC and thyroid cancer (MTC and TC) subjects who may benefit from treatment with RETEVMO® (selpercatinib) in the US. Data from these clinical studies were the basis for the PMA approval decision. Summaries of the clinical studies are presented below, stratified by tissue type.

A. ODxT Test Clinical Bridging Study for RET Fusions in NSCLC

The safety and effectiveness of the ODxT Test for detecting RET fusions in NSCLC patients who may benefit from treatment with RETEVMO was demonstrated in a retrospective analysis of samples from patients enrolled in the LIBRETTO-001 trial (NCT03157128). A bridging study was conducted to assess the clinical efficacy of the ODxT Test in identifying patients positive for RET fusions for treatment with RETEVMO and the concordance between RET fusions tested with local laboratory tests (LLT) and the ODxT Test in the intent-to-test population. Retrospective testing with the ODxT Test was done for a subset of LIBRETTO-001 patients from the NSCLC drug efficacy population Primary Analysis Set (PAS) and Supplemental Analysis Set 1 (SAS1), and stage-matched commercially sourced RET fusion negative NSCLC samples screened with a representative LLT.

For the bridging study analysis, the retrospective testing population consisted of 144 samples positive for RET fusions originally tested by LLTs, and 136 samples negative for RET fusions.

1. Clinical Study Design

LIBRETTO-001 is an ongoing multicenter, open-label, multi-cohort Phase 1/2 study in patients with advanced solid tumors, including RET fusion-positive solid tumors (e.g. NSCLC, thyroid, pancreas, colorectal), RET-mutant MTC, and other tumors with RET activation (e.g., mutations in other tumor types or other evidence of RET activation). Study objectives include determination of the recommended Phase 2 dose, characterization of safety and pharmacokinetic properties and assessment of anti-tumor activity of RETEVMO. LIBRETTO-001 was initiated on May 2, 2017. Phase 1 dose levels ranged from 20 mg once daily (QD) to 200 mg twice daily (BID). Following the dose escalation portion of the study, a Phase 2 dose expansion was initiated for patients with advanced solid tumors harboring a RET gene alteration. The recommended phase 2 dose was determined to be 160 mg orally twice daily. Adult patients received RETEVMO until unacceptable toxicity or disease progression. The major efficacy outcome measures were confirmed overall response rate (ORR) and

duration of response as determined by a blinded independent central review (BICR) assessment according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. RETEVMO was approved by FDA for RET fusion positive NSCLC, RET fusion positive TC and RET mutation positive MTC in May 2020 based on safety and efficacy data from patients in LIBRETTO-001 with RET alterations.

Specific to NSCLC, the first 105 patients with advanced or metastatic *RET* fusion-positive NSCLC who had progressed on platinum-based chemotherapy were designated as the Primary Analysis Set (PAS). Patients with advanced or metastatic NSCLC without prior systemic therapy were assigned to the Supplemental Analysis Set (SAS1). Primary bridging study samples were from PAS and supplemental bridging study samples were from SAS1.

a. Key Inclusion Criteria (Phase 1)

- Patients with a locally advanced or metastatic solid tumor who:
    - have progressed on or are intolerant to standard therapy, or
    - no standard therapy exists, or
    - in the opinion of the Investigator, are not candidates for or would be unlikely to tolerate or derive significant clinical benefit from standard therapy, or
    - decline standard therapy.

Prior MKI(s) with anti-RET activity are allowed.

b. Key Inclusion Criteria (Phase 2)

- Cohorts 1 and 3 (NSCLC, MTC or TC patients with prior therapy): failed or intolerant to standard of care
- Cohorts 1-4 (NSCLC, MTC or TC patients with or without prior therapy): enrollment will be restricted to patients with evidence of a RET gene alteration in tumor (i.e., not just blood). However, a positive germline DNA test for a RET gene mutation is acceptable in the absence of tumor tissue testing for patients with MTC.

c. Key Exclusion Criteria (Phases 1 and 2)

- Phase 2, Cohorts 1-4, an additional validated oncogenic driver that could cause resistance to LOXO-292 treatment.
- Symptomatic primary CNS tumor, metastases, leptomeningeal carcinomatosis, or untreated spinal cord compression.
- Clinically significant active cardiovascular disease or history of myocardial infarction within 6 months prior to planned start of LOXO-292 or prolongation of the QT interval corrected for heart rate using Fridericia's formula (QTcF) > 470 msec on at least 2/3 consecutive electrocardiograms (ECGs) and mean QTcF > 470 msec on all 3 ECGs during Screening. Correction of suspected

drug-induced QTcF prolongation may be attempted at the Investigator's discretion if clinically safe to do so.
- Uncontrolled symptomatic hyperthyroidism or hypothyroidism.
- Uncontrolled symptomatic hypercalcemia or hypocalcemia.
- Current treatment with certain strong cytochrome P450 3A4 (CYP3A4) inhibitors or inducers.
- Current treatment with proton pump inhibitors (PPIs).
- Pregnancy or lactation.
- Active second malignancy other than minor treatment of indolent cancers.

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 primary clinical efficacy endpoint utilized for the LIBRETTO-001 trial was objective response rate (ORR) based on RECIST 1.1 or RANO, as appropriate to tumor type as assessed by independent central review (ICR).

4. Diagnostic Objective and Endpoints

The primary objective of this clinical bridging study is to demonstrate the safety and effectiveness of the ODxT Test for the selection of NSCLC patients with *RET* fusions for treatment with RETEVMO®. The primary endpoint is ORR by RECIST version 1.1 as assessed by ICR and compared to the benchmark ORR of the LIBRETTO-001 clinical study.

5. ODxT Test Bridging Study

The bridging portion of the study was used to establish concordance (agreement) between enrollment LLTs and ODxT Test results (Positive, Negative, and Unknown) and to determine the clinical outcomes (specifically with respect to the primary efficacy endpoint of overall response rate (ORR)) based on ODxT Test results from LIBRETTO-001 NSCLC specimens. Sensitivity analyses for the clinical concordance and clinical outcomes (ORR) were performed including analyses that evaluated the impact of unknown/unevaluable ODxT Test results.

6. Accountability of PMA Cohort

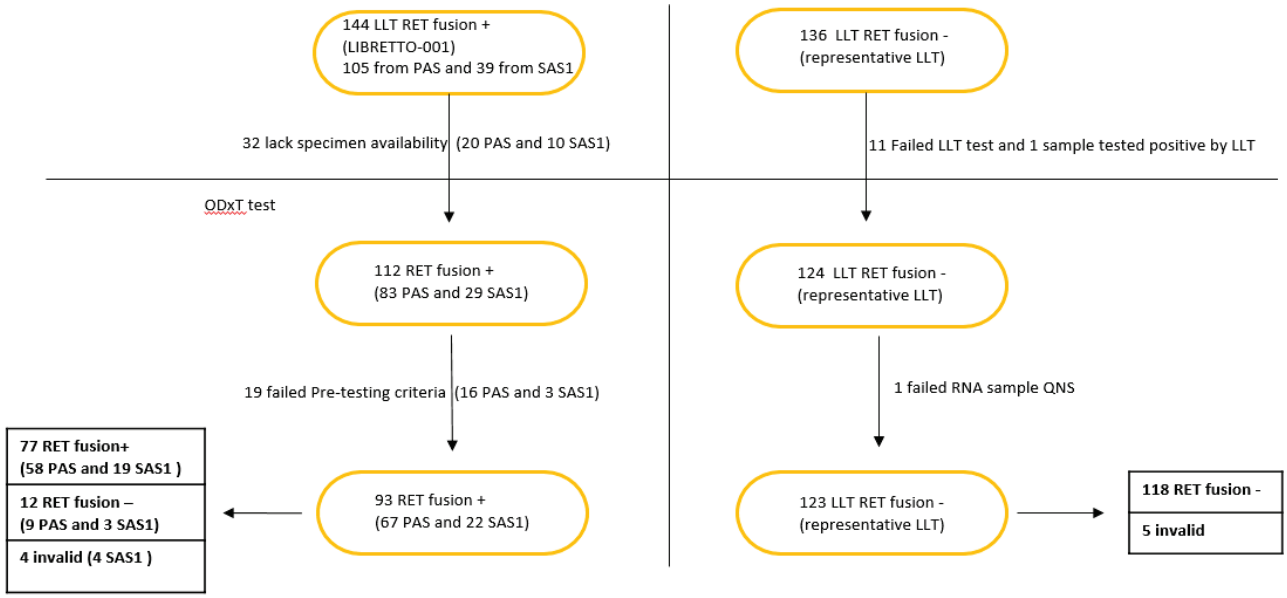
For the bridging study analysis, the retrospective testing population consisted of 144 samples positive for RET fusions originally tested by LLTs. As shown in **Figure 1** below, of the 144 RET fusion-positive samples, 32 samples were not tested by the ODxT test due to lack of specimen availability (20 samples from PAS and 10 samples from SAS1). Additionally, 136 stage-matched commercially sourced RET fusion-negative NSCLC samples were screened with a NGS-based assay as the

representative LLT. During screening of the procured samples, 1 sample was identified as RET fusion-positive, and 11 samples failed testing with the representative LLT. In total, 112 RET fusion-positive NSCLC samples from patients enrolled by LLTs in the LIBRETTO-001 trial and 124 commercially sourced RET fusion-negative NSCLC samples were evaluated in the bridging study.

Of the 112 RET fusion-positive samples (83 from PAS and 29 from SAS1), 19 samples (16 from PAS and 3 from SAS1) failed the pre-testing criteria (input requirement) for the ODxT Test. Of the remaining 93 RET fusion-positive samples, 77 samples (58 from PAS and 19 from SAS1) were called positive by the ODxT Test, 12 samples (9 from PAS and 3 from SAS1) were called negative by the ODxT Test, and 4 samples from SAS1 failed the ODxT Testing.

Of the remaining 124 RET fusion-negative samples, one sample failed the RNA concentration cutoff, five were invalid by the ODxT Test and 118 were called negative by the ODxT Test.

Figure 1: Sample Accountability Chart for Study Samples



7. Patient Demographics, Disease and Sample Characteristics

The demographics, disease characteristics, and specimen characteristics for the key patient subgroups were similar (Table 21) between ODxT Test evaluable, ODxT Test unknown/unevaluable, and LLT+ patients [LIBRETTO-001 (PAS, SAS1)].

Table 21. Patient Demographic and Disease Characteristics between ODxT Test Evaluable, ODxT Test Unknown/ Unevaluable, and LLT+ Patients in LIBRETTO-001 – PAS and SAS1

Characteristic		LIBRETTO-001 (PAS, SAS1)	ODxTT Evaluable	ODxTT UNK/Unevaluable
Subject	N	144	89	55
Age	Mean (SD)	59.1 (12.2)	60.7 (11.3)	56.5 (13.2)
Gender	Female	84 (58.3%)	56 (62.9%)	28 (50.9%)
	Male	60 (41.7%)	33 (37.1%)	27 (49.1%)
Race	White	83 (57.6%)	50 (56.2%)	33 (60.0%)
	Asian	47 (32.6%)	29 (32.6%)	18 (32.7%)
	Other	13 (9.0%)	9 (10.1%)	4 (7.3%)
	Missing	1 (0.7%)	1 (1.1%)	0 (0.0%)
ECOG*	0	50 (34.7%)	26 (29.2%)	24 (43.6%)
	1	92 (63.9%)	62 (69.7%)	30 (54.5%)
	2	2 (1.4%)	1 (1.1%)	1 (1.8%)
LLT* Assay Type	NGS* on Tumor	113 (78.5%)	70 (78.7%)	43 (78.2%)
	NGS* on Blood	17 (11.8%)	10 (11.2%)	7 (12.7%)
	PCR	2 (1.4%)	1 (1.1%)	1 (1.8%)
	FISH*	12 (8.3%)	8 (9.0%)	4 (7.3%)

* ECOG = Eastern Cooperative Oncology Group; NGS = next generation sequencing; LLT = local laboratory test; FISH = fluorescence in situ hybridization.

8. Safety and Effectiveness Results

a. Safety Results

The safety with respect to treatment with selpercatinib was addressed during the review of the NDA and is not addressed in detail in this Summary of Safety and Effectiveness Data. The evaluation of safety was based on the analysis of adverse events (AEs), clinical laboratory evaluations, physical examinations, and vital signs. Please refer to Drugs@FDA for complete safety information on RETEVMO® (selpercatinib).

No adverse events were reported in connection with the bridging study used to support this PMA supplement, as the study was performed retrospectively using banked samples.

b. Effectiveness Results

i. Concordance Results

To evaluate the clinical accuracy of the ODxT Test, the concordance analysis between the ODxT Test and LLT results was conducted on 112 RET fusion positive patients (83 from PAS and 29 from SAS1), and 124 RET fusion negative stage-matched commercially sourced NSCLC samples (Table 22). The

PPA, NPA and OPA shown in Table 23 were calculated using the LLTs as the reference method. Notably, there were no cases identified as ODxT Test RET fusion-positive among the LLT screen-negative samples. The point estimates of PPA, NPA, and OPA were 86.5%, 100.0%, and 94.2% respectively, when excluding ODxT Test UNK (invalid/insufficient material) results. When including ODxT Test unknown results, the point estimates of PPA, NPA, and OPA were 68.8%, 95.2% and 82.6%, respectively.

Table 22. Contingency Table of ODxT Test and LLTs (Reference) Results for RET fusion detection in NSCLC

ODxTT	LLTs			
	POS		NEG	Total
	PAS	SAS1		
POS	58	19	0	77
NEG	9	3	118	130
UNK	16	7	6	29
Total	83	29	124	236

Table 23. Agreement between ODxT Test and LLTs (Reference) Results for RET fusion detection in NSCLC

Parameter		Agreed	Total	Agreement	95% CI
PPA	Exclude UNK	77	89	86.5%	(77.6%, 92.8%)
NPA		118	118	100.0%	(96.9%, 100.0%)
OPA		195	207	94.2%	(90.1%, 97.0%)
PPA	Include UNK	77	112	68.8%	(59.3%, 77.2%)
NPA		118	124	95.2%	(89.8%, 98.2%)
OPA		195	236	82.6%	(77.2%, 87.2%)

The PPA of 86.5% can potentially be attributed to variability in LLT platform technologies and associated variable performance across the LLTs used to enroll. In addition, most of the LLTs evaluated DNA, while the ODxT Test evaluated RNA; changes at the DNA level do not always result in corresponding detectable transcriptional changes.

The PPA and OPA estimates separately within PAS and SAS1 are shown in Table 24 (NPA is the same as that shown in Table 23). These estimates show that there is no statistically significant difference in the agreement metrics (when excluding ODxTT UNK results) between these two Cohorts.

Table 24. Agreement between ODxT Test and LLTs (Reference) Results by NSCLC Analysis Set

Subgroup	Parameter		Agreed	Total	Agreement	95% CI
PAS	PPA	Exclude UNK	58	67	86.6%	(76.0%, 93.7%)
	OPA		176	185	95.1%	(91.0%, 97.8%)
	PPA	Include UNK	58	83	69.9%	(58.8%, 79.5%)
	OPA		176	207	85.0%	(79.4%, 89.6%)

Subgroup	Parameter		Agreed	Total	Agreement	95% CI
SAS1	PPA	Exclude UNK	19	22	86.4%	(65.1%, 97.1%)
	OPA		137	140	97.9%	(93.9%, 99.6%)
	PPA	Include UNK	19	29	65.5%	(45.7%, 82.1%)
	OPA		137	153	89.5%	(83.6%, 93.9%)

ii. Primary Clinical Efficacy Analysis

In total, 77 ODxT Test RET fusion-positive NSCLC patients from the LIBRETTO-001 trial were included in the drug efficacy evaluation (Table 25). Two groups of patients: PAS (patients who received prior platinum chemotherapy), and SAS1 (patients who were treatment naïve) were evaluated in the following analyses. Reported overall response rates (ORR) are based on blinded independent central radiology review by RECIST v1.1.

Table 25. Summary of Best Response Rate by ODxT Test Assessment Status in the PAS and SAS1 of NSCLC Patients (Independent Central Radiology Assessment per RECIST v1.1)

ORR: CR + PR	ODxTT Positive | LLT positive		ODxTT Negative | LLT positive		ODxTT Unk/Unevaluable | LLT positive		LLT+ NDA Drug Efficacy Population	
	ORR% (n/N)	95% CIs	ORR% (n/N)	95% CIs	ORR% (n/N)	95% CIs	ORR% (n/N)	95% CIs
PAS	67.2% (39/58)	(53.7%, 79.0%)	55.6% (5/9)	(21.2%, 86.3%)	60.5% (23/38)	(43.4%, 76.0%)	63.8% (67/105)	(53.9%, 73.0%)
SAS1	79.0% (15/19)	(54.4%, 94.0%)	100.0% (3/3)	(29.2%, 100%)	88.2% (15/17)	(63.6%, 98.5%)	84.6% (33/39)	(69.5%, 94.1%)

- In the PAS, for the 58 patients who were ODxT Test-positive | LLT-positive, the ORR was 67.2% (39 patients with complete response (CR) or partial response (PR) out of 58); while for the 9 patients who were ODxT Test-negative | LLT-positive and the 38 patients who were ODxTT (Unk/Unevaluable) | LLT-positive, the ORR were 55.6% (5 patients with CR or PR out of 9) and 60.5% (23 patients with CR or PR out of 38), respectively. This suggests that, allowing for variability due to different sample sizes, the ORR for ODxT Test-positive is maintained relative to what was observed in the NDA drug efficacy analysis for PAS (63.8%, 67 patients with CR or PR out of 105).
- In the SAS1, for the 19 patients who were ODxT Test-positive | LLT-positive, the ORR was 79.0% (15 patients with CR or PR out of 19); while for the 3 patients who were ODxT Test-negative | LLT-positive and the 17 patients who were ODxTT (Unk/Unevaluable) | LLT-positive, the ORR were 100.0% (3 patients with CR or PR out of 3) and 88.2% (15 patients with CR or PR out of 17), respectively. This suggests that, allowing for variability due to different sample sizes, the ORR for ODxT Test-positive is maintained relative to what was observed in the NDA drug efficacy analysis

10. 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.

B. ODxT Test Clinical Bridging Study for RET Mutations and Fusions in Thyroid Cancer

The safety and effectiveness of the ODxT Test for detecting RET mutations and fusions in TC patients who may benefit from treatment with RETEVMO was demonstrated in a retrospective analysis of samples from patients enrolled in the LIBRETTO-001 trial (NCT03157128). A bridging study was conducted to assess the clinical efficacy of the ODxT Test in identifying patients positive for RET mutations and fusions for treatment with RETEVMO and the concordance between RET mutations and fusions tested with local laboratory tests (LLT) and the ODxT Test in the intent-to-test population. Retrospective testing with the ODxT Test was done for a subset of LIBRETTO-001 patients from the MTC drug efficacy population Primary Analysis Set (PAS) and Supplemental Analysis Set (SAS1) and from the TC drug efficacy population Cohorts 1 and 2. Stage-matched commercially sourced RET mutation-fusion negative TC samples screened with the representative LLT were utilized.

For the bridging study analysis, the retrospective testing population consisted of 153 samples positive for RET mutations/fusions originally tested by LLTs, and 84 samples negative for RET fusions.

1. Clinical Study Design

The LIBRETTO-001 study is described in Section X.A.1.

Specific to TC, the first 55 patients with advanced or metastatic *RET* mutant MTC who had been previously treated with cabozantinib or vandetanib (or both) were designated as the Primary Analysis Set (PAS). Patients with advanced or metastatic *RET*-mutant MTC who were naïve to cabozantinib and vandetanib were assigned to the Supplemental Analysis Set 1 (SAS1).

This clinical study also enrolled patients with *RET* fusion-positive thyroid cancer who were RAI-refractory and had received sorafenib, lenvatinib, or both (Cohort 1), and patients with *RET* fusion-positive thyroid cancer who were radioactive iodine (RAI)-refractory (if RAI was an appropriate treatment option) and were systemic therapy naïve (Cohort 2).

Importantly, TC is split into two main tissue subsets – medullary thyroid cancer (MTC) to evaluate RET mutations (SNVs, MNVs, and deletions) and thyroid cancer (TC) to evaluate RET fusions.

Key inclusion and exclusion criteria are discussed in Section X.A.1.

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

As discussed in Section X.A.3, the primary clinical efficacy endpoint of the LIBRETTO-001 trial was objective response rate (ORR) based on RECIST 1.1 or RANO, as appropriate to tumor type as assessed by ICR.

4. Diagnostic Objective and Endpoints

The primary objective of this clinical bridging study is to demonstrate the safety and effectiveness of the ODxT Test for the selection of thyroid cancer patients with *RET* mutations (MTC) and *RET* fusions (TC) for treatment with RETEVMO®. The primary endpoint is ORR by RECIST version 1.1 as assessed by ICR and compared to the benchmark ORR of the LIBRETTO-001 clinical study.

5. ODxT Test Bridging Study

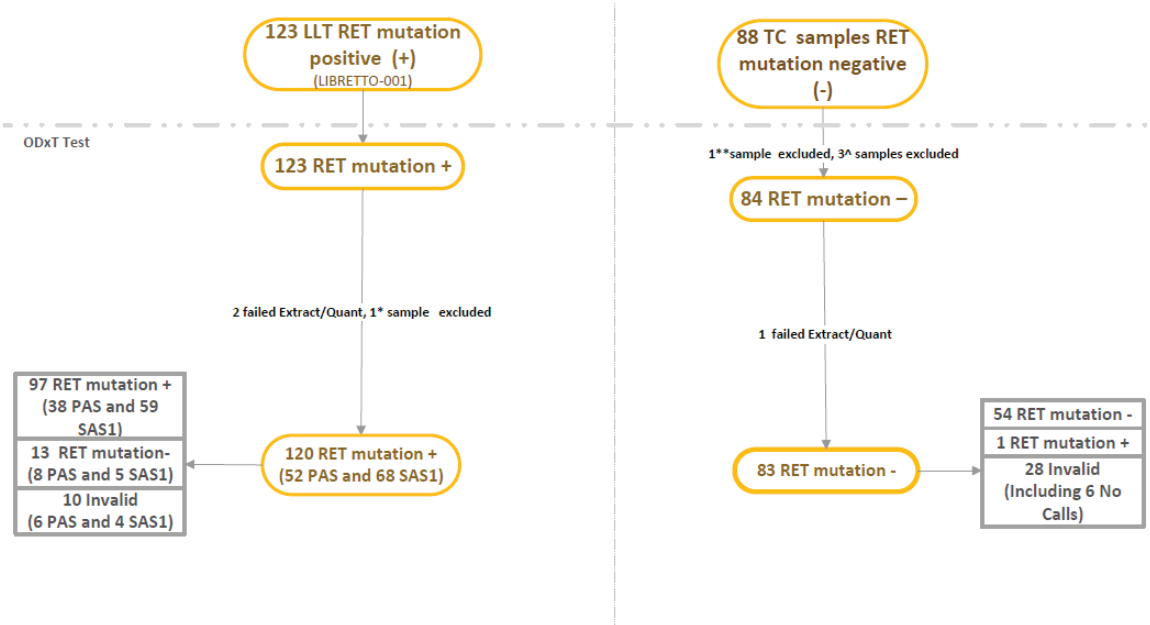
The bridging portion of the study was used to establish concordance (agreement) between the LIBRETTO-001 enrollment LLTs and ODxT Test results (Positive, Negative, and Unknown) to determine the clinical outcomes (specifically, Overall Response Rate (ORR)) based on ODxT Test results from LIBRETTO-001 TC specimens. MTC bridging study samples were from the MTC PAS and MTC SAS1. Additionally, available specimens collected from LIBRETTO-001 patients with RET

fusion-positive TC that either had prior therapy (Cohort 1) or were systemic therapy naïve (Cohort 2) were evaluated. Sensitivity analysis for the clinical concordance and clinical outcomes (ORR) were performed including analyses that evaluated the impact of missing ODxT Test results.

6. Accountability of PMA Cohort

In total, 123 RET mutation-positive MTC samples and 30 RET fusion-positive TC samples from patients enrolled by LLTs in the LIBRETTO-001 trial were evaluated in the bridging study. Additionally, 84 RET mutation and RET fusion-negative commercially sourced TC samples (16 MTC and 68 TC) were identified after screening with a representative LLT. Due to the limited availability of RET mutation negative MTC samples, the MTC studies utilized both MTC and TC commercially sourced samples; however, the TC studies, utilized only TC commercially sourced samples. **Figure 2** details the sample accountability for MTC bridging study samples while **Figure 3** details the sample accountability for TC bridging study samples.

Figure 2. ODxT Test sample accountability chart for MTC bridging study samples



*Sample WOL-03-046 excluded from analysis due to presence of RET insertion.
^Samples WOL-03-010, WOL-03-061 and WOL-03-127 excluded from analysis as samples were procured with unknown RET status and tested RET positive in LLT assay.
**Sample excluded from analysis due to same subject sample duplication. Sample is documented in the Note to File

Pr(LLT+|ODxTT+) respectively. Since the NPA (Pr(ODxTT-|LLT-)) is not 100% when excluding ODxT Test "Invalid" results (Table 31), the weight Pr(LLT+|ODxTT+) is not 100%. The bridging efficacy analysis to evaluate final drug efficacy in ODxT Test-positive intended use population was performed for PAS and SAS 1 cohorts separately.

The weighted overall ORR in ODxT Test-positive subjects within PAS were 68.4% (95% CI: 53.8% - 83.0%), 68.2% (95% CI: 53.6% - 82.8%), 68.0% (95% CI: 53.4% - 82.6%), 67.7% (95% CI: 53.1% - 82.4%) and 67.5% (95% CI: (52.8% - 82.2%) when a range of ORR values for the ODxT Test-positive | LLT-negative subjects were assumed as 100%, 75%, 50%, 25%, and 0% of the observed ORR in the ODxT Test-positive | LLT-positive subjects, assuming that the prevalence of LLT-positive subjects in the intended use population is 60%.

The weighted overall ORR in ODxT Test-positive subjects within SAS1 were 78.0% (95% CI: 67.5% - 88.4%), 77.7% (95% CI: 67.3% - 88.2%), 77.4% (95% CI: 67.0% - 87.9%), 77.2% (95% CI: 66.6% - 87.7%) and 76.9% (95% CI: (66.3% - 87.5%) when a range of ORR values for the ODxT Test-positive | LLT-negative subjects were assumed as 100%, 75%, 50%, 25%, and 0% of the observed ORR in the ODxT Test-positive | LLT-positive subjects, assuming that the prevalence of LLT-positive subjects in the intended use population is 60%.

The sensitivity analysis results demonstrate that the estimated drug efficacy in the ODxT Test-positive set of the primary bridging studies for PAS and SAS1 remain robust to missing ODxT Test results (data not shown).

This bridging drug efficacy analysis for separate dataset demonstrated the ability of the ODxT Test to correctly identify the MTC patients who are likely to respond to selpercatinib irrespective of whether they had received prior cab/van treatment or were treatment naïve.

iii. Concordance Results (TC)

The concordance analysis between the ODxT Test and LLT results was conducted on 30 RET fusion positive patients (19 from Cohort 1 and 11 from Cohort 2), and 68 RET fusion negative stage-matched commercially sourced TC samples (Table 35). The PPA, NPA and OPA shown in Table 36 were calculated using the LLTs as the reference method. Notably, there were no cases identified as ODxT Test RET fusion-positive among the LLT screen-negative samples. The point estimates of PPA, NPA, and OPA were 92.0%, 100.0%, and 97.6% respectively, when excluding ODxT Test UNK (invalid/insufficient material) results. When including ODxT Test unknown results, the point estimates of PPA, NPA, and OPA were 76.7%, 85.3% and 82.7%, respectively.

Table 35. Contingency Table of ODxT Test and LLT (Reference) Results – RET

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 RET fusions in NSCLC patients and RET fusions and mutations in TC patients to be treated with RETEVMO, the effectiveness of the ODxT Test was demonstrated through a clinical bridging study using specimens from patients screened for enrolled into the LIBRETTO-001 study. The data from the analytical validation and clinical bridging studies support the reasonable assurance of safety and effectiveness of the ODxT Test when used in accordance with the indications for use. Data from the LIBRETTO-001 study show that patients who had qualifying RET fusions and mutations received benefit from treatment with RETEVMO and support the addition of the CDx indication to the ODxT Test.

B. Safety Conclusions

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 one of the therapies listed in Table 1 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. Benefit-Risk Determination

Treatment with RETEVMO provides a meaningful clinical benefit to NSCLC and TC patients with RET fusions and RET fusions/mutations, respectively, as demonstrated in the LIBRETTO-001 trial. For the intended use of identifying RET fusions in NSCLC patients and RET fusions and mutations in TC patients to be treated with RETEVMO, the probable benefit of the ODxT Test was demonstrated through a clinical bridging study using specimens from patients screened for enrollment into the LIBRETTO-001 study. In the NSCLC clinical dataset, clinically meaningful ORR response was observed in patients irrespective of whether they had received prior platinum chemotherapy or were treatment-naïve patients with metastatic RET fusion-positive NSCLC detected by the ODxT Test. Within the TC dataset, results from the MTC and TC datasets demonstrated that the ODxT Test could be used safely and was effective as an aid to

identify TC patients eligible to receive treatment with RETEVMO. Given the available information and the analytical data provided in the submission, the data supports the conclusion that the ODxT Test has probable benefit in selecting NSCLC patients with RET fusions and TC patients with RET fusions and mutations for treatment with RETEVMO.

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 RET fusions or TC patients with RET fusions or mutations, 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. Additional factors, including the clinical and analytical performance of the device included in this submission, have been taken into account and demonstrate that the assay is expected to have acceptable performance.

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 Test 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 bridging study support the performance of the ODxT Test as an aid for the identification of RET fusions in NSCLC patients and RET fusions and mutations in TC patients for whom RETEVMO® (selpercatinib) may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order for the PMA (P160045/S031) on 09/21/2022. Additional clinical study is requested as conditions of approval cited in the approval order are described below.

The following data should be provided as a single report, 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 clinical outcome data as assessed by overall response rate from at least 17 additional thyroid cancer patients enrolled and treated with RETEVMO in the clinical study LIBRETTO-001 tested with the ODxT. This information must be provided to confirm the clinical effectiveness of the ODxT Test as a companion diagnostic (CDx) device for identification of patients with thyroid cancer with RET fusions who may benefit from treatment with RETEVMO.

2. Thermo Fisher Scientific/Life Technologies Corp. must provide data from additional RET fusion negative thyroid cancer samples screened by representative local laboratory tests (LLT) to supplement the existing 58 LLT- samples in order to obtain a total of ~100 LLT- thyroid cancer samples with valid ODxTT results. This information must be provided to confirm the clinical effectiveness of the ODxT Test as a companion diagnostic (CDx) device for identification of patients with thyroid cancer with RET fusions who may benefit from treatment with RETEVMO.

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.