

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:	TruSight™ Oncology Comprehensive
Device Procode:	PQP
Applicants' Name and Address:	Illumina Inc. 5200 Illumina Way, San Diego, CA 92122
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P230011/S006
Date of FDA Notice of Approval:	August 14, 2026

The original PMA (P230011) for TruSight Oncology Comprehensive (TSO Comp) was approved on August 21, 2024, for the detection of genetic alterations in patients who may benefit from an FDA-approved therapy (larotrectinib) for solid tumors and an FDA-approved therapy (selpercatinib) for non-small cell lung cancer (NSCLC). The test also was approved to provide tumor profiling information for use by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. The SSED to support these indications is available on the CDRH website and is incorporated by reference here.

The current supplement was submitted to expand the indications for TSO Comp to include companion diagnostic (CDx) indications for the detection of RET fusions in thyroid cancer (TC) and RET small DNA variant mutations in medullary thyroid cancer (MTC) patients who may benefit from treatment with RETEVMO (selpercatinib).

II. INDICATIONS FOR USE

TruSight™ Oncology Comprehensive is a qualitative in vitro diagnostic test that uses targeted next-generation sequencing to detect variants in 517 genes using nucleic acids extracted from formalin-fixed, paraffin embedded (FFPE) tumor tissue samples from cancer patients with solid malignant neoplasms using the Illumina® NextSeq™ 550Dx instrument. The test can be used to detect single nucleotide variants, multi-nucleotide variants, insertions, and deletions from DNA, and fusions in 24 genes and splice variants in one gene from RNA. The test also reports a Tumor Mutational Burden (TMB) score and Microsatellite Instability (MSI) status.

The test is intended to be used as a companion diagnostic to identify cancer patients who may benefit from treatment with the targeted therapies listed in Table 1, in accordance with the approved therapeutic product labeling.

In addition, the test is intended to provide tumor profiling information for use by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. Genomic findings other than those listed in Table 1 of the intended use statement are not conclusive or prescriptive for labeled use of any specific therapeutic product.

Table 1: Companion Diagnostic Indications

Tumor Type	Biomarker(s) Detected	Therapy
Solid Tumors	<i>NTRK1/2/3</i> fusions	VITRAKVI [®] (larotrectinib)
Non-Small Cell Lung Cancer (NSCLC) or Thyroid Cancer (TC)	<i>RET</i> fusions	RETEVMO [®] (selpercatinib)
Medullary Thyroid Cancer (MTC)	<i>RET</i> SNVs, MNVs, insertions and deletions	RETEVMO [®] (selpercatinib)

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the TruSight Oncology Comprehensive labeling.

V. DEVICE DESCRIPTION

TruSight Oncology (TSO) Comprehensive is a distributed *in vitro* diagnostic (IVD) test. The assay is for use as part of a test system with the NextSeq 550Dx instrument and sequencing reagents. The TSO Comprehensive assay contains reagents, software, and procedures for testing DNA and RNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples.

The assay employs qualitative next-generation sequencing (NGS) comprehensive genomic profiling (CGP) that assesses genomic variants in a large panel of cancer-related genes. It is an enrichment based targeted NGS test comprised of library preparation and enrichment reagents to enable DNA and/or RNA sequencing from FFPE tissue from solid tumors encompassing multiple cancer types. DNA and/or RNA extracted from FFPE tissue is used to prepare libraries, which are then enriched for cancer-related genes and sequenced on the NextSeq 550Dx instrument. TSO Comprehensive user-facing software allows sequencing run set up, secondary analysis, and annotation of detected variants from the sequencing results generated on the NextSeq 550Dx instrument.

TSO Comprehensive can be run with DNA and RNA, DNA only, or RNA only samples. The reported results will be reflective of the input nucleic acid.

The test detects single nucleotide variants (SNVs), multi-nucleotide variants (MNVs) and insertions and deletions from DNA, and gene fusions and a splice variant from RNA. The assay detects small DNA variants in 517 genes, RNA fusions in 24 genes and RNA splice variants in one gene. The TSO Comprehensive assay also reports a Tumor Mutational Burden (TMB) score and Microsatellite Instability (MSI) status.

Test Output

The output of the test includes results from the following levels:

- Level 1: Companion Diagnostic (CDx) Claims noted in Table 1 of the Intended Use
- Level 2: Cancer Mutations with Evidence of Clinical Significance
- Level 3: Cancer Mutations with Evidence of Potential Clinical Significance

Genomic findings other than those listed in Table 1 of the Companion diagnostic indications table of the intended use statement (i.e., Levels 2 and 3) are not prescriptive or conclusive for labeled use of any specific therapeutic product.

Test Kit Contents

The TSO Comprehensive assay is for use as part of a test system with the NextSeq 550Dx instrument and associated sequencing reagents. Components of the TSO Comprehensive assay are listed in Table 2. Illumina provides reagent kits and software (Local Run Manager TSO Comprehensive (US) analysis module), which supports both tumor profiling and companion diagnostic (CDx) claims (please refer to the “Software” section below for additional details). The Knowledge Base (updated monthly) is also required to be used with the assay for tumor profiling and is available for download on the Illumina Lighthouse Portal. The assay contains reagents with sufficient volume to generate 24 DNA and 24 RNA libraries inclusive of patient samples and controls (sold separately).

Materials required but not provided are described in the text below Table 2. A detailed list of required instruments, software, reagents, consumables, and storage conditions is further described in the product labeling (TSO Comprehensive Package Insert).

Table 2. Reagent Components of the TSO Comprehensive Assay

Storage Temp	Component Name	Volume (µL)	Quantity
TSO Comprehensive RNA Library Prep			
-25 to -15	First Strand Synthesis Mix (FSM)	260	1
-25 to -15	Second Strand Mix (SSM)	720	1
-25 to -15	Elution Primer Frag Mix (EPH3)	250	1
-25 to -15	Reverse Transcriptase (RVT)	70	1
TSO Comprehensive Library Prep			
-25 to -15	End Repair A-tailing A (ERA1-A)	85	2
-25 to -15	End Repair A-tailing B (ERA1-B)	210	2
-25 to -15	Adapter Ligation Buffer 1 (ALB1)	1730	2
-25 to -15	DNA Ligase 3 (LIG3)	190	2
-25 to -15	Short Universal Adapters 1 (SUA1)	290	1
-25 to -15	UMI Adapters v1 (UMI)	290	1
-25 to -15	Stop Ligation Buffer (STL)	480	2
-25 to -15	Enhanced PCR Mix (EPM)	550	2
2 to 8	Resuspension Buffer (RSB)	12400	1
2 to 8	Sample Purification Beads (SPB)	6110	2
2 to 8	TE Buffer (TEB)	10000	1
TSO Comprehensive Index Primers			

Storage Temp	Component Name	Volume (μL)	Quantity
-25 to -15	UP Index Primers	24	16
-25 to -15	CP Index Primers	20	16
TSO Comprehensive Enrichment			
2 to 8	Target Capture Buffer 1 (TCB1)	870	2
2 to 8	Streptavidin Mag Beads (SMB)	7780	2
2 to 8	2N NaOH (HP3)	400	2
2 to 8	Elute Target Buffer 2 (ET2)	290	2
2 to 8	Library Normalization Beads 1 (LNB1)	1040	1
2 to 8	Library Normalization Wash 1 (LNBW1)	4800	2
2 to 8	Library Normalization Storage Buffer 1 (LNS1)	3500	2
2 to 8	Resuspension Buffer (RSB)	12400	1
2 to 8	Sample Purification Beads (SPB)	6110	2
-25 to -15	Target Capture Additives 1 (TCA1)	521	2
-25 to -15	Enhanced Enrichment Wash (EEW)	50400	1
-25 to -15	Enrichment Elution 2 (EE2)	1650	3
-25 to -15	Enhanced PCR Mix (EPM)	550	2
-25 to -15	PCR Primer Cocktail 3 (PPC3)	150	2
-25 to -15	Library Normalization Additives 1 (LNA1)	4600	1
-25 to -15	PhiX Internal Control (PX3 or PhiX)	10	1
TSO Comprehensive Content Set			
-25 to -15	Oncology RNA Probe Pool (OPR1)	290	1
-25 to -15	Oncology DNA Probe Pool 2 (OPD2)	290	1

Reagents/Consumables Required but not Provided

For a detailed list of required, but not provided reagents and consumables, please refer to the product labeling (TSO Comprehensive Package Insert).

- DNA/RNA Extraction and Purification Reagents
- DNA/RNA Quantification Reagents
- TruSight Oncology DNA Control v2
- TruSight Oncology RNA Control v2
- Ethanol (EtOH) 100% (200 proof), molecular biology grade
- RNase/DNase-free water
- NextSeq 550Dx High-Output Reagent Kit v2.5 (300 cycles)

Required Equipment/Materials, Not Provided

For a detailed list of required, but not provided equipment, please refer to the product labeling (TSO Comprehensive Package Insert).

- NextSeq 550Dx Instrument
- Ultrasonicator
- Thermal cycler
- Vortexer
- Microsample incubators

- Microcentrifuge
- Plate Centrifuge
- Plate shaker
- Sealing wedge or roller
- Magnetic Stand
- Precision pipettes
- Pipette-aid
- Ice or cold block
- 10 mL serological pipettes
- Adhesive seals for 96-well plates
- Microcentrifuge tubes (1.7mL and 2mL), nuclease-free
- Nuclease-free reagent reservoirs
- 15mL and 50mL conical tubes
- Aerosol-resistant pipette tips
- 96-well storage plates, 0.8 mL
- 96-well PCR plates compatible with thermal cycler, 0.2 ml (polypropylene wells)
- Dry heat block

Software

The Local Run Manager TSO Comprehensive (US) analysis module resides on the NextSeq 550Dx instrument as part of the Local Run Manager software to facilitate TSO Comprehensive assay run setup and to perform the secondary analysis of sequencing results. It contains the following components:

- TSO Comprehensive Claims Packages
- TSO Comprehensive Software Suite
- TSO Comprehensive USB Kit

Knowledge Base: Updated regularly and available for download on the Illumina Lighthouse Portal.

Instruments

TSO Comprehensive assay is validated for use on the Illumina NextSeq 550Dx instrument as part of a test system. An Illumina service representative installs the appropriate version of the TSO Comprehensive (US) analysis module on the Local Run Manager NextSeq 550Dx instrument, including the Knowledge Base, prior to use of the TSO Comprehensive assay and conducts training for the end user. Other required equipment and the specifications for the specific equipment for use with the TSO Comprehensive assay are described in the “Required Equipment/Materials, Not Provided” section.

Principles of Operation

The TSO Comprehensive assay involves the processes described below.

Specimen Requirements, Collection, and Preparation

The TSO Comprehensive assay requires nucleic acid (DNA and/or RNA) isolated from FFPE tissue specimens. Before performing the TSO Comprehensive assay, tissue samples should be examined by a pathologist to ensure that it is appropriate for this test. Tissue cannot be decalcified and should be fixed using formalin fixative suitable for molecular analyses (for example, 10% neutral-buffered

formalin). Recommended tissue volume is $\geq 1.0 \text{ mm}^3$, which is equivalent to a cumulative viable tissue area (the sum of the viable tissue area in all sections submitted for extraction) of $\geq 200 \text{ mm}^2$ using $5 \text{ }\mu\text{m}$ thick sections, or $\geq 100 \text{ mm}^2$ using $10 \text{ }\mu\text{m}$ thick sections. Tumor content for RNA variants depends on the extent of expression. A minimum of 20% tumor content (by area) is required to detect somatic driver mutations. A minimum of 40% tumor content is necessary for robust reporting of MSI status. An outcome of MS-Stable may not be reliable if tumor content is less than 40%. A high amount of necrotic tissue ($\geq 25\%$) can interfere with the TSO Comprehensive assay's ability to detect RNA fusions. If sample sections contain more than 25% necrosis in total tissue area, then the necrotic tissue must be macrodissected.

Nucleic Acid Extraction

The TSO Comprehensive assay requires nucleic acid (DNA and/or RNA) isolated from FFPE tissue using appropriate extraction methods. The required input for the TSO Comprehensive assay is 40 ng RNA and/or 40 ng DNA. For DNA, extraction kits should be able to yield this input amount at a minimum concentration of 3.33 ng/uL . Shearing requires a final volume of $52 \text{ }\mu\text{L}$ (0.77 ng/uL) with a minimum of $40 \text{ }\mu\text{L}$ TEB (provided) used as the diluent. For RNA, extraction kits should be able to yield this input amount in a final volume of 8.5 uL (minimum concentration of 4.7 ng/uL). Proteinase K or equivalent enzyme should not be increased during extraction from the standard concentration provided (interference was tested using 0.04 mg/mL , as well as 2.6 mg/mL and 5.2 mg/mL , 2 times and 4 times the excess from a concentration of 1.3 mg/mL per assay reaction, respectively). This assay has been validated with extracted DNA stored at -25°C to -15°C for up to 28 days, and extracted RNA stored at -85°C to -65°C for up to 28 days.

Library Preparation

Library preparation is performed using the TSO Comprehensive Library Prep Kit (PN 20031118, PN 20031119) for DNA and the RNA Library Prep Kit (PN 20031127) for RNA. For RNA, 40 ng total is converted to double-stranded complementary DNA (cDNA). For genomic DNA (gDNA), 40 ng of gDNA is sheared into small fragments. Universal adapters for sequencing are ligated onto the cDNA and gDNA fragments. P5 and P7 adapter sequences are incorporated into each library to enable the capture of library fragments onto the surface of the flow cell during sequencing. The adapters include i5 and i7 index sequences to identify each individual sample and, in the case of libraries from gDNA samples, individual molecules with the use of Unique Molecular Identifiers (UMIs).

Enrichment

Resultant libraries are enriched for specific genes of interest using a capture-based method. Biotinylated probe sequences that span gene regions of interest targeted by the assay are hybridized to the libraries. The probes and hybridized targeted libraries are isolated from non-targeted libraries by capture with streptavidin magnetic particles. The targeted enriched libraries are washed and amplified. The quantity of each enriched library is normalized using a bead-based method to ensure equal representation in the pooled libraries for sequencing.

Sequencing

Normalized, enriched libraries are pooled and clustered onto a flow cell and then sequenced using sequencing by synthesis (SBS) chemistry on the NextSeq 550Dx instrument. SBS chemistry uses a reversible terminator method to detect single, fluorescently labeled deoxynucleotide triphosphate (dNTP) bases as they are incorporated into growing DNA strands. During each sequencing cycle, a

single dNTP is added to the nucleic acid chain. The dNTP label serves as a terminator for polymerization. After each dNTP incorporation, the fluorescent dye is imaged to identify the base and then cleaved to allow incorporation of the next nucleotide. Four reversible terminator-bound dNTPs (A, G, T, and C) are present as single, separate molecules. As a result, natural competition minimizes incorporation bias.

Data Analysis

Primary Analysis

During the primary analysis, base calls are made directly from signal intensity measurements during each sequencing cycle, resulting in base-by-base sequencing. A quality score is assigned to each base call, and the sequencing data is stored in binary base call (BCL) format.

Secondary Analysis

Secondary analysis includes the following sequential steps:

- Validation of run processing and quality control
Sequencing run quality metrics are evaluated to determine if they are within an acceptable range. Please refer to Table 3 for additional information about run-level quality metrics.
- FASTQ File Generation
Sequencing data stored in BCL format is demultiplexed using index sequences unique to each sample added during the library preparation step to assign clusters to the library from which they originated. Each cluster contains two indexes (i5 and i7 sequences, one at each end of the library fragment). The combination of those index sequences is used to demultiplex the pooled libraries. After demultiplexing, FASTQ files are generated, which are files that contain the sequencing reads for each individual sample library and the associated quality scores for each base call, excluding reads from any clusters that did not pass filter.
- Alignment and Error Correction
Sequencing reads derived from DNA sample libraries are aligned to a reference genome using the Burrows-Wheeler Aligner (BWA-MEM) to align DNA sequences to the hg19 reference genome, generating BAM files (*.bam), and BAM index files (*.bam.bai). These files are further processed to remove errors (including errors introduced during PCR amplification or sequencing). Reads derived from the same unique DNA molecule are collapsed into a single representative sequence using their unique molecular identifier (UMI). Indel realignment is performed to recover signal for insertions and deletions that may have been lost during initial alignment. Simultaneously, overlapping read pairs are bioinformatically combined into a single consensus read. All reads are then output as a final set of BAM files with corresponding BAM index files.

Sequencing reads derived from RNA sample libraries are downsampled to approximately 30 million reads per RNA sample library by randomly selecting reads from the input FASTQ files following a probability distribution. The ends of the RNA sequences are then trimmed to a maximum length of 76 base pairs and are aligned to the hg19 reference genome. Candidate splice junctions are identified, generating BAM files and BAM index files for aligned reads,

and a tab-delimited text file for candidate splice junctions, using STAR aligner. Duplicate reads are then marked in the BAM files so they can be excluded from downstream steps.

- Variant Calling

Small variant calling is performed for DNA sample libraries to detect small variants [single-nucleotide variants (SNVs), multi-nucleotide variants (MNVs) up to 3 base pairs (bp) in length, insertions, and deletions. TSO Comprehensive performance has been demonstrated for insertions up to 24 base pairs and deletions up to 25 base pairs. The error-corrected BAM files (collapsed and insertions and deletions realigned) are used as input by an initial variant calling algorithm to detect small variants. This creates an unfiltered genome Variant Call Format (gVCF) file, which contain reference or variant calls for each targeted locus. Candidate variants are then filtered for assay- specific and sample processing artifacts by using adjusted quality scores calculated by comparing the observed variant frequency against a baseline noise distribution for the same site (assay-specific artifacts) and by comparing the evidence for the candidate variant against the baseline level of noise associated with that base change in the sample (sample-specific artifacts). This results in filtered gVCF files, which then uses a phased variant caller to identify MNVs, indels, and deletions in the EGFR and RET genes that are candidates for phasing. Overlapping reads are clustered in the neighborhood into a minimal set of clusters that contain the same variants. Variants are detected by examining the Concise Idiosyncratic Gapped Alignment Report (CIGAR) strings in the BAM file and comparing read sequences to the reference genome sequence. Finally, MNVs, indels, and deletions detected by the phased variant caller are merged into the filtered gVCF files resulting in merged gVCF files.

Fusion calling and splice variant calling are performed for RNA sample libraries. For fusion calling, candidate fusions are identified from supplemental alignments and anomalous read pairs (reads aligning to different chromosomes or in unexpected orientations) in the BAM files for the fusion genes targeted by the TSO Comprehensive assay. These reads are assembled into candidate fusion contigs, which are then aligned back to the reference genome (hg19). These candidate fusion contigs are then evaluated against various filters before being reported as detected (please refer to ‘RNARNA Fusion Calling’ in the Local Run Manager TruSight Oncology Comprehensive (US) analysis module Workflow Guide for additional information).

For splice variant calling, candidate splice variants (junctions) from the alignment stage are compared against a database of known transcripts and a splice variant baseline of non-tumor junctions. Any splice variants that match the database or baseline are filtered out unless they are in a set of junctions with known oncological function. If there is sufficient read support, the candidate splice variant is kept.

Tertiary Analysis

Tertiary analysis, performed by the Local Run Manager TSO Comprehensive (US) analysis module, consists of annotation of the different variant types followed by the calling/score calculation specific to the intended variants.

- Annotation

Detected small DNA variants are annotated using the Nirvana annotation engine with information from the RefSeq database and various population databases (COSMIC, ClinVar, dbSNP, 1000 Genomes, and gnomAD). For TMB, using the gVCF from the small variant

phasing step as input, Nirvana annotates filtered small variant calls with static (not updatable) annotation databases for use by the downstream TMB calculation. For tumor profiling variants, Nirvana annotates filtered small variant calls with an updatable RefSeq database (included as part of the KB and may be updated periodically).

RNA fusions identified during RNA fusion calling are merged with fusions from proximal genes identified during RNA splice variant calling. The merged fusions are then annotated with gene symbols or names corresponding to a static database of transcripts (GENCODE Release 19).

Detected RNA splice variants are annotated using the Nirvana annotation engine with information from the RefSeq database. Nirvana annotates detected RNA splice variant calls with a static (not updatable) RefSeq database for use by downstream Companion Diagnostic calling. Splice variants are annotated with transcript-level changes (affected exons in the gene transcript) with respect to RefSeq, the same as the static RefSeq database used by the small variant annotation process. For tumor profiling variants, Nirvana annotates detected RNA splice variant calls with an updatable RefSeq database (included as part of the KB and may be updated periodically). Splice variants are annotated with transcript-level changes (affected exons in the gene transcript) with respect to RefSeq.

- TMB Score Calculation

Calculation of the TMB score [which includes SNVs, insertion, and deletion variants and is derived from the count of non-driver somatic variants per megabase (evaluable region)] is generated from the gVCF file generated by the Small Variant Filter step and the annotations generated during small variant Annotation by calculating the number of somatic non-hotspot variants with variant allele frequency (VAF) $\geq 5\%$ divided by the evaluable region size. Driver mutations are identified and filtered based on COSMIC count.

Variants are flagged as likely germline for calculating the TMB score, applying a combination of population database and post-database filtering strategies. After database filtering, the proximity filter labels variants as germline if they are surrounded by database-labeled germline variants, and variants identified as likely germline are excluded from the TMB score calculation. The evaluable region is the dynamically adjusted per sample based on sequencing depth, and genomic regions with a high background noise level are excluded from the TMB calculation.

- MSI Score Calculation

To determine the MSI status of a sample, a total of 130 predefined MSI homopolymer sites are evaluated. Sites with sufficient coverage are considered usable, and TSO Comp needs at least 40 sites to be usable for an MSI score to be reported. For each site, the repeat length distribution is compared against the baseline database samples to determine if the repeat distribution is significantly shifted. The final MSI score is calculated as the number of unstable microsatellite sites divided by the total number of usable microsatellite sites assessed. A sample is considered MSI-High if its MSI score is $\geq 20.00\%$.

- Companion Diagnostic (CDx) Calling

For each installed companion diagnostic (CDx) intended use, the TSO Comprehensive (US)

analysis module determines the applicability of the CDx intended use for each patient sample based on the tumor type of the patient sample. If the tumor type of the patient is an exact match or a descendant of the tumor type for a CDx intended use, it is considered applicable to that CDx intended use. If the tumor type of the patient is not applicable to a CDx intended use, then the CDx intended use is not evaluated for that sample.

If a required nucleic acid type for a CDx intended use is not sequenced or fails QC, or required controls in the run fail, then the patient sample is not evaluated for that CDx intended use. If a variant type or biomarker required for a CDx intended use fails QC, then the patient sample is not evaluated for that CDx intended use. When it is determined that a CDx intended use is applicable for a patient sample, the required libraries are sequenced, required QC measures pass, and required controls pass, the companion diagnostic intended use is evaluated for the patient sample. Detected variants and/or biomarkers in the patient sample are evaluated to determine the result for the CDx intended use. The evaluation is done through an algorithm specific to the CDx intended use, which assesses the presence and/or absence of variants/biomarkers that match the CDx intended use.

- **Tumor Profiling Variant Calling**

After companion diagnostic results are determined, all passing, detected variants in a patient sample are matched against the installed Knowledge Base to determine the cancer mutations that have evidence of clinical significance (Level 2) or have potential clinical significance (Level 3). A genomic finding is either a single variant with evidence of clinical significance or potential clinical significance, or a grouping of variants that, when detected together, have evidence of clinical significance or potential clinical significance.

- When multiple variants are listed together as a genomic finding, it means that there is evidence for clinical significance or potential clinical significance for those variants together, in at least one of the sources listed in the Informatics Details of the report. If there are multiple genomic findings, and a variant is included in more than one of these findings, then that variant may be listed more than one time on a report. A genomic finding with a single variant will only be listed at the highest level, including the “Companion Diagnostics Results” section, where it meets criteria for reporting. Refer to “Positive CDx Results” section in the Local Run Manager TruSight Oncology Comprehensive (US) analysis module Workflow Guide for additional details.

Results Report Generation

The interpreted variant results, the TMB score, and the MSI Status are summarized in the TSO Comprehensive assay results report generated for the testing laboratory, which consists of a “Companion Diagnostics Results” section, an “Other Alterations and Biomarkers Identified” section for reporting “Cancer Mutations with Evidence of Clinical Significance” (Level 2) or “Cancer Mutations with Potential Clinical Significance” (Level 3), a “Companion Diagnostics QC” section, and a “Companion Diagnostics Intended Use Evaluated” section. Please refer to the LRM TSO Comp Workflow Guide for additional information about the test reports.

Controls

Positive Controls

TruSight Oncology DNA Control v2 and TruSight Oncology RNA Control v2 are required for use as positive controls. These controls consist of a multiplexed blend of biosynthetic DNA/RNA with multiple verified sequence mutations in a background of genomic DNA/RNA. The controls should be included for each DNA and RNA sequencing run (including combined runs) within a given library preparation event. The controls will be checked for quality of library preparation after sequencing. Failure of the control to meet the pre-defined quality metrics will result in all test samples on the run related to that specific control (DNA Control for DNA samples, RNA Control for RNA samples) to be invalidated.

No Template Control (NTC)

A no template control (NTC) consists of Tris-EDTA Buffer solution (provided in TSO Comprehensive kit as reagent “TEB”) for DNA samples, and DNase/RNase-free water (not included in the TSO Comprehensive kit) for RNA samples to determine if the library preparation is free of contamination by evaluating for the presence of sequencing reads (median exon coverage for DNA, number of genes with median deduplicated coverage for RNA) that map to the genes targeted by the assay. The NTC should be included for each DNA and RNA sequencing run (including combined runs) within a given library preparation event. If a NTC control fails, the assay instructions for use will require that the entire library preparation event is repeated for all samples.

Quality Metrics

Quality metrics (Table 3) are assessed across the following categories:

- Run-level: Metrics that are quantified per sequencing run; if external control fails these criteria, no results are reported for the entire batch of samples.
- Sample-level: Metrics that are quantified per sample; no device results are generated for samples failing these metrics.
- Analyte-level: Metrics that are quantified for individual alteration types and positions. Variants passing analyte-level metrics are reported.

Table 3. Summary of TSO Comprehensive post-sequencing library quality control metrics

Variant Type	Metric	Run/ Sample/ Analyte	Required Value	Description	Impact of Failure
All variants	PCT_RF_READ S (%)	Run-Level	≥ 80.0	Percentage of reads passing filter (PF).	Sequencing run invalidated. No results reported for any sample in the run.
	PCT_Q30_R1 (%)	Run-Level	≥ 80.0	Average percent of base calls with quality score of Q30 or higher for Read 1.	
	PCT_Q30_R2 (%)	Run-Level	≥ 80.0	Average percent of base calls with quality score of Q30 or higher for Read 2.	

Variant Type	Metric	Run/ Sample/ Analyte	Required Value	Description	Impact of Failure
DNA variants	CONTAMINATION_SCORE	Sample-Level	≤ 3106 OR (> 3106 and P VALUE ≤ 0.049)	A metric assessing the likelihood of contamination using the VAF of common variants. The contamination score is based on VAF distribution of SNPs. The contamination P value is used to assess highly rearranged genomes. It is only applicable when contamination score is above Upper Spec Limit.	No TMB or small variant results called
	MEDIAN_INSERT_SIZE (bp)	Sample-Level	≥ 70	The median fragment length in the sample.	
	MEDIAN_EXON_COVERAGE	Sample-Level	≥ 150	Median exon fragment coverage across all exon bases.	
	PCT_EXON_50X (%)	Sample-Level	≥ 90.0	Percent exon bases with 50X fragment coverage.	
	Small DNA Variants ⁶	Analyte-Level	AQ ≥ 20 for Frequent COSMIC mutations ¹ AQ ≥ 60 for other mutations ¹	Metric to determine whether a small DNA variant is called	Small DNA variant not called
	Small DNA Variants ⁶	Analyte-Level	LQ ≥ 20 for Frequent COSMIC mutations ² LQ ≥ 60 for other mutations ²	Metric to determine whether a small DNA variant is called	
	TMB	Analyte-Level	N/A ³	N/A	N/A
	MSI	Analyte Level	≥ 40 evaluable sites	Minimum number of sites evaluable for MSI score calculation	MSI status not determined

Variant Type	Metric	Run/ Sample/ Analyte	Required Value	Description	Impact of Failure
RNA variants	Median_CV_Gene_500X	Sample-Level	≤ 0.93	For each gene with at least 500x coverage, the coefficient of variation in coverage across the gene body is computed. This metric is the median of these values. A high value indicates a high level of variation and indicates a problem in library preparation such as low sample input and/or probe pulldown issues. This metric is computed using all reads (including reads marked as duplicates).	No fusions or splice variant results called.
	Total_On_Target_Reads	Sample-Level	$\geq 9,000,000$	The total number of reads that map to the target regions. This metric is computed using all reads (including reads marked as duplicates).	
	MEDIAN_INSERT_SIZE (bp)	Sample-Level	≥ 80	The median fragment length in the sample.	
	RNA Fusions	Analyte-Level	Fusion score $\geq 0.45^4$ Deduped supporting reads ≥ 5	Metric to determine whether a RNA fusion gene is called	RNA fusion not called
	RNA Splice Variants	Analyte-Level	Splice score = 1 ⁵	Metric to determine whether a RNA splice variant is called	RNA splice variant not called

¹Variant quality score (AQ) is calculated as $-10 \cdot \log_{10}(\text{p-value})$, where p-value is the binomial test by comparing the observed VAF and depth to a baseline of normal FFPE samples.

²Likelihood ratio (LQ) is calculated as $-10 \log_{10}(\text{Likelihood (observed variant is an error)} / \text{DP error rate} / \text{Likelihood (observed variant is a mutation)} / \text{DP, VAF})$

³Evaluation of TMB does not have a reporting threshold, as the value is quantitative (score), not qualitative (classification)

⁴Fusion score is a weighted sum that takes into account the number of unique supporting reads, fusion contig length, breakpoint homology, and other associated metrics.

⁵Splice score increments by 0.1 for each supporting reads for the splice junction and is capped at 1.

⁶Accuracy of small DNA tumor profiling variants below 5% variant allele frequency (VAF) has not been established.

Table 4. TruSight Oncology Control Metrics

Output Type	Metric	Specification	Impact of Specification Failure
Positive Control	DNA External Control	51 of 54 specified variants detected	No small DNA variant, TMB, or MSI results reported.
		MSI-High (MSI score $\geq 20\%$ unstable sites) and Usable MSI Sites ≥ 30	No MSI results reported.
	RNA External Control	20 of 22 specified variants detected	No fusions or splice variant results reported.
Non-Template Control (NTC)	DNA Median Exon Coverage	≤ 8	No small DNA variant, TMB, or MSI results reported
	RNA Gene above Median Cutoff	≤ 1	No fusions or splice variant results reported.
DNA or RNA Positive Control	CDx Control Requirements	CDx control validation requires detection of at least one representative variant for a biomarker	No CDx results reported for a biomarker.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA approved companion diagnostic (CDx) alternatives for the detection of genetic alterations using FFPE tumor specimens as listed in Table 1 of the TSO Comprehensive assay intended use statement (Section II). The approved CDx tests are listed in Table 5 below.

Table 5. Alternative FDA-approved CDx assays for CDx biomarkers identified by the TSO Comprehensive Assay

Indication	Gene	Device (PMA)	Company	Technology	Therapy
Solid Tumors	<i>NTRK1/2/3</i> Fusions	FoundationOne® CDx (P170019/S017)	Foundation Medicine, Inc.	NGS	VITRAKVI® (larotrectinib)

Indication	Gene	Device (PMA)	Company	Technology	Therapy
Non-Small Cell Lung Cancer (NSCLC)	RET Fusions	OncomineDx Target Test (P160045/S019 and P160045/S031)	Life Technologies Corporation	NGS	RETEVMO® (selpercatinib)
Solid Tumors	RET Fusions	FoundationOne® CDx (P170019/S043)	Foundation Medicine, Inc.	NGS	RETEVMO® (selpercatinib)
Medullary Thyroid Cancer (MTC)	RET mutations (SNVs, MNVs, and deletions)	OncomineDx Target Test (P160045/S031)	Life Technologies Corporation	NGS	RETEVMO® (selpercatinib)
Thyroid Cancer (TC)	RET Fusions	OncomineDx Target Test (P160045/S031)	Life Technologies Corporation	NGS	RETEVMO® (selpercatinib)

For additional details see the FDA List of Cleared or Approved Companion Diagnostic Devices at: <https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools>

VII. **MARKETING HISTORY**

The TSO Comprehensive Premarket Approval (P230011) was originally approved on August 21, 2024, by FDA and has been commercially available in the U.S. since November 2024.

VIII. **POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH**

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect TSO Comprehensive assay results and subsequently improper patient management decisions. 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 indicated 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 related to the approved therapeutics, please see approved FDA therapeutic product labeling.

IX. **SUMMARY OF NONCLINICAL STUDIES**

A. **Laboratory Studies**

A summary of the evidence in support of the performance of the TSO Comprehensive assay in

detecting *RET* fusions in thyroid cancer (TC) and *RET* small DNA variant mutations in medullary thyroid cancer (MTC) is provided below. Studies evaluating analytical accuracy/concordance, precision, reproducibility, and limit of detection (LoD) were conducted to support the indication for *RET* fusions in TC and *RET* mutations in MTC.

1. Analytical Accuracy/Concordance

RET Fusion Detection in Thyroid Cancer (TC)

An accuracy study was performed to evaluate the concordance between TSO Comprehensive assay and an externally validated NGS assay (comparator method) for detection of *RET* fusions in TC.

The accuracy study was conducted with TC and MTC samples obtained from the LIBRETTO-001 trial and commercial sources. Across both sets of samples, 272 samples were tested using both the TSO Comprehensive assay and the comparator method. Of the 272 samples, 86 samples returned invalid results with the comparator method (out of these, 35 were also invalid by TSO Comp) and 47 samples returned no calls with comparator (out of these, 1 was invalid by TSO Comp); while 18 samples had invalid results by TSO Comprehensive assay only (invalid rate of 6.6%). Thus, 121 of the 272 samples had valid results by both methods. The agreement analysis is shown in Table 6. Based on valid test results, the PPA and NPA between the TSO Comprehensive assay and comparator method were 81.8% (18/22; 95% CI: 59.7%, 94.8%), and 100% (99/99; 95% CI: 96.3%, 100%), respectively.

There were four samples that were positive by the comparator method but negative by the TSO Comprehensive assay. Three of these samples had KIF5B-*RET* fusions called by the comparator method with 117, 4472, and 6913 supporting reads. These were all supplemental TC samples that were negative by the TSO Comprehensive assay and the representative local test used in the *RET* clinical performance bridging study. KIF5B-*RET* fusions are the most prevalent *RET* fusion species seen in lung adenocarcinoma and are not observed in TC. The other sample had a CUX1-*RET* fusion called by the comparator method with number of supporting reads close to its LoD. This was a LIBRETTO-001 sample from an MTC patient who was *RET* mutation positive by the local test used for LIBRETTO-001 enrollment but whose *RET* fusion status is unknown. The most common oncogenic drivers of MTC are *RET* DNA mutations, while MTC tumors harboring *RET* fusions are extremely rare.

Table 6. Concordance Between the TSO Comprehensive Assay and Comparator Method for Detection of *RET* Fusions in Thyroid Cancer

		Comparator Method Result		
		RET Fusion Positive	RET Fusion Negative	Total
TSO Comprehensive Assay Result	RET Fusion Positive	18	0	18
	RET Fusion Negative	4 ²	99	103
	Invalid	0	18	18
	Total	22	117	139

		Comparator Method Result		
		RET Fusion Positive	RET Fusion Negative	Total
Agreement Statistics	PPA% (n/N; 95% CI ¹)	81.8% (18/22; 59.7% -94.8%)		
	NPA% (n/N; 95% CI ¹)	100.0% (99/99; 96.3% - 100%)		
Agreement Statistics Including Invalids	PPA% (n/N; 95% CI ¹)	81.8% (18/22; 59.7% -94.8%)		
	NPA% (n/N; 95% CI ¹)	84.6% (99/117; 76.8% - 90.6%)		

¹CI based on Clopper-Pearson (exact) method

RET Small DNA Variant Mutations Detection in Medullary Thyroid Cancer (MTC)

An accuracy study was performed to evaluate the concordance between TSO Comprehensive assay and externally validated NGS assays (comparator methods) for detection of RET small DNA variant mutations in MTC.

The accuracy study was conducted using MTC samples obtained from the LIBRETTO-001 trial, and supplemental MTC and TC samples obtained from commercial sources. Combining LIBRETTO-001 and supplemental samples, 283 samples were tested using the TSO Comprehensive assay and a comparator method. Of the 283 samples, 22 had invalid comparator method results, and 42 samples had invalid results by TSO Comprehensive assay only (invalid rate of 14.8%). Thus, 219 of the 283 samples had valid results by the TSO Comprehensive assay and a comparator method.

There were 21 SNVs, 2 MNVs, and 4 deletions represented in the samples that were positive by TSO Comprehensive and the comparator method. The agreement analysis is shown in Table 7. For the primary analysis, concordance was assessed by analyzing the CDx RET variants that are detected by both the TSO Comprehensive assay and comparator method. Based on valid test results, the PPA and NPA between the TSO Comprehensive assay and comparator method were 81.8% (27/33;95% CI: 64.5, 93.0) and 99.5% (185/186; 97.0, >99.9), respectively.

Table 7. Concordance Between the TSO Comprehensive Assay and Comparator Method for Detection of RET Small DNA Variants Mutations in Medullary Thyroid Cancer

		Comparator Method Result		
		RET Mutation Positive	RET Mutation Negative	Total
TSO Comprehensive Assay Result	RET Mutation Positive	27	1	28
	RET Mutation Negative	6 ²	185	191
	Invalid	6	36	42
	Total	39	222	261
Agreement Statistics	PPA% (n/N; 95% CI ¹)	81.8% (27/33; 64.5% -93.0%)		
	NPA% (n/N; 95% CI ¹)	99.5% (185/186; 97.0% - >99.9%)		

Agreement Statistics Including Invalids	PPA% (n/N; 95% CI ¹)	69.2% (27/39; 52.4% -83.0%)
	NPA% (n/N; 95% CI ¹)	83.3% (185/222; 77.8% - 88.0%)

¹CI based on Clopper-Pearson (exact) method.

There were six samples that were comparator method positive (all with a RET Val804Met mutation with VAFs ranging from 0.02 to 0.09) but negative by the TSO Comprehensive assay. Five of these 6 samples had VAFs below the TSO Comprehensive assay's average LoD of 0.049 for SNVs. All six samples were supplemental TC samples (classified as Papillary Thyroid Cancer, PTC) that were also RET mutation negative by the representative local test used in the RET clinical performance bridging study. The RET Val804Met variant is most frequently of germline origin and is found in MTC, not PTC, which has not been reported to harbor activating RET DNA mutations. The VAFs reported by the comparator method are characteristic of somatic variants and are inconsistent with the typical range for germline variants. The range of VAF values reported in multiple samples is consistent with previously reported deamination-related artifacts for the comparator method.

There was one sample that was comparator method negative but positive by the TSO Comprehensive assay. The TSO Comprehensive called a RET Met918Thr variant with a VAF of 0.35. The RET Met918Thr variant is the most common RET mutation in acquired MTC. The local test used in the LIBRETTO-001 study also called this mutation a RET Met918Thr variant.

2. Precision and Reproducibility

a. Precision

Within-laboratory precision was evaluated for RET fusions in 4 samples from 3 tumor types (non-small cell lung cancer, thyroid cancer, and atypical Spitz tumor from skin tissue specimen), and RET small DNA variants (SNV, MNV, deletion) from five FFPE samples of medullary thyroid cancer. One RET insertion and three RET delins (at the protein level, the variants are net deletions or an MNV at the DNA level) were tested in the FFPE-treated cell lines. Each sample was tested at two variant levels: ~1x LoD (low variant level) and ~2–3x LoD (high variant level) with the exception of the sample harboring CCDC6-RET, which was only tested at the low variant level. Each of the samples at each test level was run in duplicates in each library preparation event across three (3) operators. Each operator started library preparation on three (3) non-consecutive start days and sequenced on three (3) designated NextSeq 550Dx instruments. Three (3) reagents lots were tested, generating 54 observations per sample per level. Three variant/levels had fewer than 54 observations due to invalid libraries.

Variant calling was evaluated separately for the two variant levels for a given variant from pooled observations across all variables (operators, reagent lots, instruments, days, and replicates). The PPC, PNC, and associated two-sided 95% confidence interval (Wilson score) are summarized in the following tables (Table 8 and Table 9). It should be noted that some levels had fewer than 54 observations; these were due to invalid libraries that did not meet the necessary QC metrics (4 out of 1096 libraries failed, 0.4% invalid rate).

For RET small DNA variants at the high variant level (~2–3x LoD), the TSO Comprehensive

assay demonstrated 100% Percent positive calls (PPC) and percent negative calls (PNC) for each variant except one RET delins variant (E632_V637delinsAA, net nucleotide deletion) which could not be consistently detected by TSO Comprehensive assay (Table 8). Refer to Limit of Detection for CDx study results.

For RET small DNA variants at the low variant level (~1x LoD), PPCs ranged from 83.3% to 100.0%. For variants with PPC < 95%, the mean VAFs (RET C634Y and RET D898_E901del) were below the respective limits of detection. At the low variant level, 100% PNC was achieved for all variants.

Table 8. Summary Results for Targeted RET Small DNA Variants

Variant Level	Variant	Variant Type	Mean VAF	PPC (95% CI)	PNC (95% CI)	LoD Level
Low (~1x LoD)	RET p.(M918T)	SNV	0.042	96.2 (51/53) (87.2, 99.0)	100 (483/483) (99.2, 100)	0.9x
	RET p.(D898_E901del)	Deletion	0.048	87.0 (47/54) (75.6, 93.6)	100 (482/482) (99.2, 100)	0.9x
	RET p.(D631_L633delins E*)	Deletion	0.056	98.1 (53/54) (90.2, 99.7)	100 (482/482) (99.2, 100)	1.0x
	RET p.(C634Y)	MNV	0.028	83.3 (45/54) (71.3, 91.0)	100 (482/482) (99.2, 100)	0.6x
	RET p.(C618R)	SNV	0.046	94.4 (51/54) (84.9, 98.1)	100 (482/482) (99.2, 100)	0.9x
	RET p.(C634_R635delins WG)	MNV	0.014	98.1 (53/54) (90.2, 99.7)	100 (482/482) (99.2, 100)	0.5x
	RET p.(C634_T636dup)	Insertion	0.034	98.1 (51/52) (89.9, 99.7)	100 (484/484) (99.2, 100)	0.5x
	RET p.(L629_I638delinsC D)	Deletion	0.021	100 (54/54) (93.4, 100)	100 (482/482) (99.2, 100)	1.0x
	RET p.(E632_V637delins AA)	Deletion	N/A	Not tested	100 (536/536) (99.3, 100)	N/A
High (~3x LoD)	RET p.(M918T)	SNV	0.078	100 (52/52) (93.1, 100)	100 (464/464) (99.2, 100)	1.7x

Variant Level	Variant	Variant Type	Mean VAF	PPC (95% CI)	PNC (95% CI)	LoD Level
	RET p.(D898_E901del)	Deletion	0.088	100 (54/54) (93.4, 100)	100 (462/462) (99.2, 100)	1.6x
	RET p.(D631_L633delins E*)	Deletion	0.161	100 (32/32) (89.3, 100)	100 (484/484) (99.2, 100)	2.9x
	RET p.(C634Y)	MNV	0.095	100 (54/54) (93.4, 100)	100 (462/462) (99.2, 100)	2.1x
	RET p.(C618R)	SNV	0.146	100 (54/54) (93.4, 100)	100 (462/462) (99.2, 100)	2.8x
	RET p.(C634_R635delins WG)	MNV	0.030	100 (54/54) (93.4, 100)	100 (462/462) (99.2, 100)	1.0x
	RET p.(C634_T636dup)	Insertion	0.071	100 (54/54) (93.4, 100)	100 (462/462) (99.2, 100)	1.0x
	RET p.(E632_V637delins AA)	Deletion	0.127	77.8 (42/54) (65.1, 86.8)	100 (462/462) (99.2, 100)	N/A
	RET p.(L629_I638delinsC D)	Deletion	0.055	100 (54/54) (93.4, 100)	100 (462/462) (99.2, 100)	2.5x

For RET fusions, at the higher variant level (~2 – 3x LoD), the TSO Comprehensive assay demonstrated 100% PPC for all fusions (Table 9). It should be noted that KIF5B-RET at the low level had more than 54 observations; this was due to the preparation of two dilution mixtures targeting the same variant level. Therefore, the observations were combined. At the low variant level (~1x LoD), the PPC for RET fusions ranged from 90.7% to 98.1%. For variants with PPC < 95% (NCOA4-RET), the supporting reads were below the respective Limits of Detection (15.8 supporting reads for NCOA4-RET). 100% PNC was achieved for all variants at both levels.

Table 9. Summary Results for RET Fusions

Variant Level	Targeted Fusions	Mean Supporting Reads	PPC (n/N) (95% CI)	PNC (n/N) (95% CI)
~1x LoD	NCOA4-RET ¹	13.3	90.7 (49/54) (80.1, 96.0)	100.0 (537/537) (99.3, 100.0)

Variant Level	Targeted Fusions	Mean Supporting Reads	PPC (n/N) (95% CI)	PNC (n/N) (95% CI)
	CCDC6-RET ²	18.7	98.1 (53/54) (90.2, 99.7)	100.0 (591/591) (99.4, 100.0)
	KIF5B-RET (sample 1) ³	17.3	95.4 (103/108) (89.6, 98.0)	100.0 (430/430) (99.1, 100.0)
	KIF5B-RET (sample 2) ³	17.3	96.2 (51/53) (87.2, 99.0)	
~3x LoD	NCOA4-RET ¹	24.8	100.0 (54/54) (93.4, 100.0)	100.0 (481/481) (99.2, 100.0)
	CCDC6-RET ²	N/A	Not tested ⁴	100.0 (589/589) (99.4, 100.0)
	KIF5B-RET (sample 1) ³	43.8	100.0 (54/54) (93.4, 100.0)	100.0 (428/428) (99.1, 100.0)
	KIF5B-RET (sample 2) ³	44.6	100.0 (53/53) (93.2, 100.0)	

¹Thyroid tissue

²Atypical Spitz tumor

³Lung Tissue from NSCLC

⁴CCDC6-RET was tested at one level only (i.e., ~1x LoD due to insufficient materials).

Table 10. Results for RET Fusions by Operator

Variant Level	Targeted Fusions	Operator ²	N	PPC (n/N)	95% CI ¹
~1x LoD	NCOA4-RET	1	18	94.4 (17/18)	(74.2, 99.0)
		2	18	83.3 (15/18)	(60.8, 94.2)
		3	18	94.4 (17/18)	(74.2, 99.0)
	CCDC6-RET ³	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	94.4 (17/18)	(74.2, 99.0)
	KIF5B-RET ⁴ (Sample ID 10755)	1	36	91.7 (33/36)	(78.2, 97.1)
		2	16	93.8 (15/16)	(71.7, 98.9)
		3	36	97.2 (35/36)	(85.8, 99.5)
		4	20	100.0 (20/20)	(83.9, 100.0)
	KIF5B-RET (Sample ID 10756)	1	18	94.4 (17/18)	(74.2, 99.0)
		2	7	100.0 (7/7)	(64.6, 100.0)
		3	18	94.4 (17/18)	(74.2, 99.0)
		4	10	100.0 (10/10)	(72.2, 100.0)
	Aggregate RET fusions (low level)		269	95.2 (256/269)	(91.9, 97.2)
~3x LoD		1	18	100.0 (18/18)	(82.4, 100.0)

	NCOA4-RET	2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	KIF5B-RET (Sample ID 10755)	1	18	100.0 (18/18)	(82.4, 100.0)
		2	8	100.0 (8/8)	(67.6, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
		4	10	100.0 (10/10)	(72.2, 100.0)
	KIF5B-RET (Sample ID 10756)	1	18	100.0 (18/18)	(82.4, 100.0)
		2	7	100.0 (7/7)	(64.6, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
		4	10	100.0 (10/10)	(72.2, 100.0)
	Aggregate RET Fusions (high level)		161	100.0 (161/161)	(97.7, 100.0)

¹ 2-sided 95% CI with Wilson Score test.

² For the RNA fusions KIF5B-RET (Sample ID 10755) and KIF5B-RET (Sample ID 10756) operator 4 replaced operator 2 with 10 of the 18 replicates and 20 of the 36 replicates for high and low levels, respectively for testing with lots IUO6 and IUO6.5.

³ CCDC6-RET was tested at one level only (i.e., ~1x LoD due to insufficient materials).

⁴ Two dilution mixtures for KIF5B-RET (sample 10755) “low” level and LMNA-*NTRK1* (sample 19219) “high” level were tested. The two dilution mixtures having slightly different dilution-fold in the normal diluent were intended to target to the same variant level. Since the observed mean supporting reads for both mixtures were comparable, the observations from both mixtures were combined for analysis.

Table 11. Results for RET Fusions by Lot

Variant Level	Targeted Fusions	Lot ²	N	PPC (n/N)	95% CI ¹
~1x LoD	NCOA4-RET	M	18	83.3 (15/18)	(60.8, 94.2)
		PQ2	18	94.4 (17/18)	(74.2, 99.0)
		PQ3	18	94.4 (17/18)	(74.2, 99.0)
	CCDC6-RET ³	M	18	94.4 (17/18)	(74.2, 99.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	KIF5B-RET ⁴ (Sample ID 10755)	IUO5	36	91.7 (33/36)	(78.2, 97.1)
		IUO6	36	97.2 (35/36)	(85.8, 99.5)
		IUO6.5	36	97.2 (35/36)	(85.8, 99.5)
	KIF5B-RET (Sample ID 10756)	IUO5	17	94.1 (16/17)	(73.0, 99.0)
		IUO6	18	94.4 (17/18)	(74.2, 99.0)
		IUO6.5	18	100.0 (18/18)	(82.4, 100.0)
Aggregate RET fusions (low level)			269	95.2 (256/269)	(91.9, 97.2)
~3x LoD	NCOA4-RET	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
		IUO5	18	100.0 (18/18)	(82.4, 100.0)

	KIF5B-RET (Sample ID 10755)	IUO6	18	100.0 (18/18)	(82.4, 100.0)
		IUO6.5	18	100.0 (18/18)	(82.4, 100.0)
	KIF5B-RET (Sample ID 10756)	IUO5	17	100.0 (17/17)	(81.6, 100.0)
		IUO6	18	100.0 (18/18)	(82.4, 100.0)
		IUO6.5	18	100.0 (18/18)	(82.4, 100.0)
Aggregate RET fusions (low level)			161	100.0 (161/161)	(97.7, 100.0)

¹ 2-sided 95% CI with Wilson Score test

² 3 waves of testing took place over course of study. 3 sets of unique lots were tested per wave (i.e. Lots M, PQ2, and PQ3 for wave 1, Lots PQ1, PQ2 and PQ3 for wave 2 and Lots IUO5, IUO6 and IUO 6.5 for wave 3)

³ CCDC6-RET was tested at one level only (i.e., ~1x LoD) due to insufficient materials.

⁴ Two dilution mixtures for KIF5B-RET (sample 10755) “low” level and LMNA-*NTRK1* (sample 19219) “high” level were tested. The two dilution mixtures having slightly different dilution-fold in the normal diluent were intended to target to the same variant level. Since the observed mean supporting reads for both mixtures were comparable, the observations from both mixtures were combined for analysis.

Restricted maximum likelihood (REML) variance components analysis was performed to evaluate total variation of the underlying continuous variable (supporting reads for RNA fusions) and estimate the components of precision [standard deviation (SD), coefficient of variation (CV)] for each source of variation [operators, instruments, days, reagent lots, residual and total]. The results are presented in Table 12 for RET small DNA variants, and Table 13 for RET fusions. The residual component was the largest contributor to total variance for both small DNA variants and RNA fusions at both levels, supporting the conclusion that detection of these variants by TSO Comprehensive assay is robust to operators, lots, instruments, and days.

Table 12. Variance Components Analysis of VAF for Targeted RET Small DNA Variants

VAF Level	Variant	Variant Type	N Valid Attempts	Mean VAF	Operator SD (%CV)	Instrument SD (%CV)	Lot SD (%CV)	Day SD (%CV)	Residual SD (%CV)	Total SD (%CV)
Low (~1x LoD)	RET p.(M918T)	SNV	53	0.042	0.000 (0.0)	0.001 (3.0)	0.000 (0.0)	0.000 (0.0)	0.011 (25.6)	0.011 (25.7)
	RET p.(D898_E901del)	Deletion	54	0.048	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.004 (8.7)	0.014 (30.0)	0.015 (31.2)
	RET p.(D631_L633delinsE)	Deletion	54	0.056	0.000 (0.0)	0.002 (3.0)	0.006 (11.6)	0.000 (0.0)	0.010 (18.5)	0.012 (22.0)
	RET p.(C634Y)	MNV	54	0.028	0.000 (0.0)	0.000 (0.0)	0.001 (3.3)	0.000 (0.0)	0.009 (30.7)	0.009 (30.9)
	RET p.(C618R)	SNV	54	0.046	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.014 (31.3)	0.014 (31.3)

VAF Level	Variant	Variant Type	N Valid Attempts	Mean VAF	Operator SD (%CV)	Instrument SD (%CV)	Lot SD (%CV)	Day SD (%CV)	Residual SD (%CV)	Total SD (%CV)
	RET p.(C634_R635delinsWG)	MNV	54	0.014	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.014 (29.9)	0.014 (29.9)
	RET p.(C634_T636dup)	Insertion	52	0.034	0.000 (0.0)	0.000 (1.4)	0.002 (7.0)	0.000 (0.0)	0.007 (20.6)	0.007 (21.8)
	RET p.(L629_I638delinsCD)	Deletion	54	0.021	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.007 (32.6)	0.007 (32.6)
High (~3x LoD)	RET p.(M918T)	SNV	52	0.078	0.002 (3.1)	0.000 (0.0)	0.000 (0.0)	0.007 (9.1)	0.018 (23.1)	0.020 (25.0)
	RET p.(D898_E901del)	Deletion	54	0.088	0.000 (0.0)	0.000 (0.0)	0.001 (1.4)	0.006 (7.0)	0.017 (19.2)	0.018 (20.5)
	RET p.(D631_L633delinsE)	Deletion	52*	0.164	0.000 (0.0)	0.000 (0.0)	0.005 (3.0)	0.000 (0.0)	0.020 (12.1)	0.020 (12.4)
	RET p.(C634Y)	MNV	54	0.095	0.000 (0.0)	0.002 (2.5)	0.002 (2.1)	0.000 (0.0)	0.014 (15.0)	0.015 (15.3)
	RET p.(C618R)	SNV	54	0.146	0.003 (1.7)	0.000 (0.0)	0.020 (13.7)	0.002 (1.1)	0.018 (12.6)	0.027 (18.7)
	RET p.(C634_R635delinsWG)	MNV	54	0.030	0.001 (4.8)	0.000 (0.0)	0.000 (0.0)	0.001 (3.4)	0.006 (19.5)	0.006 (20.4)
	RET p.(C634_T636dup)	Insertion	54	0.071	0.001 (2.1)	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.009 (13.4)	0.010 (13.6)
	RET p.(E632_V637delinsAA)	Deletion	54	0.127	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.072 (56.2)	0.072 (56.2)
	RET p.(L629_I638delinsCD)	Deletion	54	0.055	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.001 (2.2)	0.013 (23.8)	0.013 (23.9)

* Includes 20 observations that failed QC for Median Exon Coverage but have coverage at the RET variant position well above the low depth cutoff of 100 (Mean Depth = 374).

Table 13. Variance Components Analysis of Supporting Reads for Targeted RET RNA Fusions

Supporting Reads Level	Fusion	N Valid Attempts	Mean Supporting	Operator SD (%CV)	Instrument SD (%CV)	Lot SD (%CV)	Day SD (%CV)	Residual SD (%CV)	Total SD (%CV)
~1x LoD	NCOA4-RET	54	13.3	1.67 (13)	0.00 (0)	0.00 (0)	1.67 (13)	5.09 (38)	5.61 (42)
	CCDC6-RET	54	18.7	0.00 (0)	1.14 (6)	5.44 (29)	0.00 (0)	6.17 (33)	8.30 (44)
	KIF5B-RET (Sample 1)	108	17.3	2.11 (12)	2.50 (14)	2.89 (17)	3.52 (20)	7.09 (41)	9.04 (52)
	KIF5B-RET (Sample 2)	53	17.3	2.05 (12)	3.72 (22)	3.65 (21)	2.41 (14)	5.95 (34)	8.52 (49)
~2-3x LoD	NCOA4-RET ¹	54	24.8	3.05 (12)	0.00 (0)	5.92 (24)	0.00 (0)	6.78 (27)	9.50 (38)
	KIF5B-RET (Sample 1)	54	43.8	4.15 (9)	0.96 (2)	12.57 (29)	6.52 (15)	15.23 (35)	21.23 (48)
	KIF5B-RET (Sample 2)	53	44.6	5.37 (12)	4.97 (11)	13.73 (31)	0.00 (0)	12.41 (28)	19.90 (45)

%CV: Percent coefficient of variation.
SD: Standard deviation.

b. Reproducibility

Reproducibility of the TSO Comprehensive assay across 3 testing sites (1 internal, 2 external). At each site, each operator (2) tested the panel members in duplicate over the course of 3 non-consecutive testing days, generating 6 observations per target per panel member. In total, 36 observations were generated per panel member (3 sites/instruments × 2 operators × 3 start days × 2 within-run replicates).

Testing was conducted with a DNA panel that included nine panel members from FFPE samples (from medullary thyroid cancer tissue), and three from a DNA cell line, that contained RET small DNA variants. There were four panel members from FFPE samples (from papillary thyroid, unclassified thyroid, and lung tissue) that contained RET fusions.

Panel members were tested at both low (~1x LoD) and high (2-3x LoD) variant levels.

Percent positive calls (PPC) and percent negative calls (PNC) for targeted small DNA variants and RNA fusion variants, at both the high and low levels, were determined. Two-sided 95% confidence intervals (CIs) associated were also calculated using the Wilson score method. Analyses were performed to estimate PPC and PNC (with associated 95% CIs) in the targeted high- level and low-level panel members by combining TSO Comprehensive assay observations for a given target in a group of panel members representing the applicable variant type (for example, small DNA variants and RNA fusions) across sites/instruments, operators, and runs. For each targeted variant, TSO Comprehensive assay observations in other panel members at the high level targeted for the same variant type but not containing the same variant as determined by the majority rule, were combined to calculate PNC. It should be noted that some levels had fewer than 36 observations; these were due to invalid libraries that did not meet the necessary QC metrics (4 out of 576 libraries failed, invalid rate of 0.7%).

For the high-level small DNA variant panel members, the overall PPC was 100% (207/207; 95% CI: 98.2%, 100%) (Table 14). The overall PNC for the high-level small DNA variant panel members was 100% (1035/1035; 95% CI: 99.6%, 100%) (Table 15). For low-level targeted small DNA variant panel members, the overall PPC for the low-level targeted small DNA variant panel members was 99.1% (210/212; 95% CI: 96.6%, 99.7%) (Table 14), and the overall PNC was 100% (1026/1026; 95% CI: 99.6%, 100%) (Table 15).

Table 14. PPC of Targeted RET Small DNA Variants

Variant Level	Variant Type	Targeted Variant (Nucleotide)	Targeted Variant (Amino Acid)	N	Mean VAF ¹	Percent Positive Calls (%)	95% CI ²
High	SNV	chr10_4361741 6_T_C	RET p.(M918T)	34	0.156	100 (34/34)	(89.8, 100)
	SNV	chr10_4360994 9_G_C	RET p.(C634S)	36	0.140	100 (36/36)	(90.4, 100)
	SNV	chr10_4361499 6_G_A	RET p.(V804M)	33	0.116	100 (33/33)	(89.6, 100)
	MNV	chr10_4360994 9_GC_AT	RET p.(C634Y)	35	0.195	100 (35/35)	(90.1, 100)
	Deletion	chr10_4361561 1_GAGATGTT TATGA_G	RET p.(D898_E901del)	33	0.199	100 (33/33)	(89.6, 100)
	Insertion	chr10_4360994 6_T_TGTGCC GCAC	RET p.(C634_T636dup)	36	0.095	100 (36/36)	(90.4, 100)
	All small DNA	All small DNA variants high	All small DNA variants high	207	N/A ¹	100 (207/207)	(98.2, 100)

Variant Level	Variant Type	Targeted Variant (Nucleotide)	Targeted Variant (Amino Acid)	N	Mean VAF ¹	Percent Positive Calls (%)	95% CI ²
	variants high						
Low	SNV	chr10_43617416_T_C	RET p.(M918T)	35	0.042	100 (35/35)	(90.1, 100)
	SNV	chr10_43601830_G_A	RET p.(V292M)	35	0.033	94.3 (33/35)	(81.4, 98.4)
	SNV	chr10_43613840_G_C	RET p.(E768D)	36	0.044	100 (36/36)	(90.4, 100)
	MNV	chr10_43609949_GC_AT	RET p.(C634Y)	36	0.071	100 (36/36)	(90.4, 100)
	Deletion	chr10_43615611_GAGATGTT TATGA_G	RET p.(D898_E901del)	34	0.065	100 (34/34)	(89.8, 100)
	Insertion	chr10_43609946_T_TGTGCC GCAC	RET p.(C634_T636dup)	36	0.037	100 (36/36)	(90.4, 100)
	All small DNA variants low	All small DNA variants low	All small DNA variants low	212	N/A ¹	99.1 (210/212)	(96.6, 99.7)

N/A, not applicable.

¹ VAF, variant allele frequency.

² 95% two-sided confidence interval calculated via the Wilson score method.

Table 15 PNC of Targeted RET Small DNA Variants

Variant Level	Variant Type	Targeted Variant (Nucleotide)	Targeted Variant (Amino Acid)	N ¹	Percent Negative Calls (%)	95% CI ²
High	SNV	chr10_43617416_T_C	RET p.(M918T)	173	100 (173/173)	(97.8, 100)
	SNV	chr10_43609949_G_C	RET p.(C634S)	171	100 (171/171)	(97.8, 100)
	SNV	chr10_43614996_G_A	RET p.(V804M)	174	100 (174/174)	(97.8, 100)

Variant Level	Variant Type	Targeted Variant (Nucleotide)	Targeted Variant (Amino Acid)	N ¹	Percent Negative Calls (%)	95% CI ²
	MNV	chr10_43609949_GC_AT	RET p.(C634Y)	172	100 (172/172)	(97.8, 100)
	Deletion	chr10_43615611_GA GATGTTTATGAG	RET p.(D898_E901del)	174	100 (174/174)	(97.8, 100)
	Insertion	chr10_43609946_T_TGTGCCGCAC	RET p.(C634_T636dup)	171	100 (171/171)	(97.8, 100)
	All small DNA variants high	All small DNA variants high	All small DNA variants high	1035	100 (1035/1035)	(99.6, 100)
Low	SNV	chr10_43617416_T_C	RET p.(M918T)	177	100 (177/177)	(97.9, 100)
	SNV	chr10_43601830_G_A	RET p.(V292M)	143	100 (143/143)	(97.4, 100)
	SNV	chr10_43613840_G_C	RET p.(E768D)	176	100 (176/176)	(97.9, 100)
	MNV	chr10_43609949_GC_AT	RET p.(C634Y)	176	100 (176/176)	(97.9, 100)
	Deletion	chr10_43615611_GA GATGTTTATGA_G	RET p.(D898_E901del)	178	100 (178/178)	(97.9, 100)
	Insertion	chr10_43609946_T_TGTGCCGCAC	RET p.(C634_T636dup)	176	100 (176/176)	(97.9, 100)
	All small DNA variants low	All small DNA variants low	All small DNA variants low	1026	100 (1026/1026)	(99.6, 100)

¹ All observations pooled from panel member-variant combinations for which the majority call is negative (targeted variants harboring fusions with less than 50% calls positive).

² 95% two-sided confidence interval calculated via the Wilson score method.

Table 16 shows the variance components analysis of variant allele frequencies (VAFs) across the approximately 36 observations for each panel member. The standard deviation (SD) and percent coefficient of variation (%CV; total and for each source) were calculated and presented for each targeted RET small DNA variant.

Table 16. Variance Components Analysis of VAF in Targeted Small DNA Variants Panel Members

Variant Level	Variant Type	Targeted Variant (Nucleotide)	Targeted Variant (Amino Acid)	N	Mean VAF	Site SD (%CV)	Operator SD (%CV)	Day SD (%CV)	Replicate SD (%CV)	Total SD (%CV)
High	SNV	chr10_43617_416_T_C	RET p.(M918T)	34	0.156	0.011 (7.2)	0.000 (0.0)	0.000 (0.0)	0.017 (10.8)	0.020 (13.0)
	SNV	chr10_43609_949_G_C	RET p.(C634S)	36	0.140	0.006 (4.6)	0.000 (0.0)	0.005 (3.7)	0.014 (10.2)	0.017 (11.8)
	SNV	chr10_43614_996_G_A	RET p.(V804M)	33	0.116	0.005 (4.1)	0.000 (0.0)	0.002 (1.7)	0.012 (10.7)	0.013 (11.6)
	MNV	chr10_43609_949_GC_AT	RET p.(C634Y)	35	0.195	0.000 (0.0)	0.000 (0.0)	0.009 (4.4)	0.012 (6.0)	0.015 (7.5)
	Deletion	chr10_43615_611_GAGA_TGTTTATG_A_G	RET p.(D898_E901 del)	33	0.199	0.000 (0.0)	0.000 (0.0)	0.011 (5.5)	0.017 (8.6)	0.020 (10.2)
	Insertion	chr10_43609_946_T_TGT_GCCGCAC	RET p.(C634_T636 dup)	36	0.095	0.003 (3.0)	0.000 (0.0)	0.000 (0.0)	0.009 (9.6)	0.010 (10.1)
Low	SNV	chr10_43617_416_T_C	RET p.(M918T)	35	0.042	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.009 (22.2)	0.009 (22.2)
	SNV	chr10_43601_830_G_A	RET p.(V292M)	35	0.033	0.000 (0.0)	0.003 (9.8)	0.002 (6.2)	0.007 (21.7)	0.008 (24.6)
	SNV	chr10_43613_840_G_C	RET p.(E768D)	36	0.044	0.003 (6.0)	0.000 (0.0)	0.000 (0.0)	0.008 (17.5)	0.008 (18.5)
	MNV	chr10_43609_949_GC_AT	RET p.(C634Y)	36	0.071	0.000 (0.0)	0.008 (10.7)	0.000 (0.0)	0.011 (14.9)	0.013 (18.4)
	Deletion	chr10_43615_611_GAGA_TGTTTATG_A_G	RET p.(D898_E901 del)	34	0.065	0.002 (2.5)	0.006 (9.9)	0.004 (6.4)	0.010 (16.2)	0.013 (20.2)
	Insertion	chr10_43609_946_T_TGT_GCCGCAC	RET p.(C634_T636 dup)	36	0.037	0.005 (13.8)	0.000 (0.0)	0.003 (9.1)	0.006 (15.9)	0.008 (22.9)

For the RET fusion panel members at 2-3x LoD, PPC and PNC values were 100.0%. For the low-level RET fusion panel members, PPC values were 97.2% and 97.1%, and PNC values were both 100.0% (Table 17 and Table 18). Further, none of the individual sites showed any additional discordance (Table 19).

Table 17. PPC of TSO Comprehensive Assay for Detection of RET Fusions in High- and Low-Level Targeted Panel Members

Variant Level	Targeted Fusion	n	Mean Supporting Reads	PPC (%) (n/N)	95% CI ¹
~1x LoD	NCOA4-RET ³	36	15.8	97.2 (35/36)	(85.8, 99.5)
	KIF5B-RET ^{2,4}	34	16.6	97.1 (33/34)	(85.1, 99.5)
~2-3x LoD	NCOA4-RET ³	36	36.7	100.0 (36/36)	(90.4, 100.0)
	CCDC6-RET ^{2,3}	36	33.4	100.0 (36/36)	(90.4, 100.0)

¹95% 2-sided confidence interval (CI) calculated via the Wilson Score method.

²2 different fusions (KIF5B-RET and CCDC6-RET) were used for low- and high-level panel members due to sample insufficiency.

³Thyroid tissue

⁴Lung tissue from NSCLC

Table 18. PNC of TSO Comprehensive Assay for Detection of RET Fusions in High- and Low-Level Targeted Panel Members

Variant Level	Targeted Fusions	n ¹	PNC (%) (n/N)	95% CI ²
~1x LoD	NCOA4-RET	213	100.0% (213/213)	(98.2%, 100.0%)
	KIF5B-RET	251	100.0% (251/251)	(98.5%, 100.0%)
~2-3x LoD	NCOA4-RET	215	100.0% (215/215)	(98.2%, 100.0%)
	CCDC6-RET	251	100.0% (251/251)	(98.5%, 100.0%)

¹ All observations pooled from panel member-variant combinations for which the majority call is negative, i.e., targeted variants harboring fusions with less than 50% calls positive.

² 95% 2-sided confidence interval (CI) calculated via the Wilson Score method.

Table 19. PPC of TSO Comprehensive Assay for Detection of RET Fusions in High- and Low-Level Targeted Panel Members by Site

Variant Level	Targeted Fusions	Site	n	PPC (%) (n/N)	95% CI ¹
~1x LoD	NCOA4-RET	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	91.7 (11/12)	(64.6, 98.5)
	KIF5B-RET	S1	11	100.0 (11/11)	(74.1, 100.0)
		S2	11	90.9 (10/11)	(62.3, 98.4)
		S3	12	100.0 (12/12)	(75.8, 100.0)
~2-3x LoD	NCOA4-RET	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)

	CCDC6-RET	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)

¹ 95% 2-sided confidence interval (CI) calculated via the Wilson Score method.

Table 20 (RET Fusions) shows the variance components analysis of supporting reads across the approximately 36 observations within each targeted fusion. The SD and %CV (total and for each source) were calculated and presented for each targeted fusion.

Table 20. Variance Components Analysis of Supporting Reads in Targeted RET Fusion Panel Members

Variant Level	Fusion	n	Mean Supporting Reads	Site SD (%CV)	Operator SD (%CV)	Day SD (%CV)	Replicate SD (%CV)	Total SD (%CV)
~1x LoD	NCOA4-RET	36	15.8	2.08 (13)	1.03 (7)	0.00 (0)	5.11 (32)	5.61 (36)
	KIF5B-RET	34	16.6	2.07 (12)	0.00 (0)	1.58 (10)	5.83 (35)	6.39 (39)
~2-3x LoD	NCOA4-RET	36	36.7	4.64 (13)	4.09 (11)	6.17 (17)	5.20 (14)	10.17 (28)
	CCDC6-RET	36	33.4	7.25 (22)	2.56 (8)	6.53 (20)	5.51 (16)	11.49 (34)

%CV: Percent coefficient of variation.

SD: Standard deviation.

Controls Reproducibility

The reproducibility of TruSight Oncology DNA Control v2 and TruSight Oncology RNA Control v2 was evaluated with the TSO Comprehensive assay at three external sites. At each site, two operators/instruments using three reagent lots over three non-consecutive days, tested three lots in duplicates of the TruSight Oncology DNA Control v2 and three lots in duplicates of the TruSight Oncology RNA Control v2 with three lots of the TSO Comprehensive assay. A total of 102 valid observations for each of the DNA and RNA controls v2 were assessed for library validity based on the QC metric used by the TSO Comprehensive assay. For small DNA variants, 51 out of 54 variant calls need to be detected to pass. For fusion and splice variants, 20 out of 22 variants need to be detected to pass.

In summary, the percentage of TruSight Oncology DNA control v2 libraries that passed was 98.0% (100/102; 95% CI: 93.1%, 99.5%) for small DNA variants. The percentage of TruSight Oncology RNA control v2 libraries that passed was 96.1% (98/102; 95% CI: 90.3%, 98.5%).

3. Analytical Sensitivity: Limit of Blank (LoB)

Please refer to the Summary of Safety and Effectiveness Data (SSED) for P230011 for Analytical Sensitivity: Limit of Blank (LoB).

4. Analytical Sensitivity: Limit of Detection (LoD)

The Limit of Detection (LoD) of RET small DNA variants was evaluated using four medullary thyroid cancer FFPE tissue samples, which included RET SNVs, deletion, and MNV, as well as four FFPE-treated cell lines containing RET insertion and delins (at the protein level, the variants are net deletions or an MNV at the DNA level) variants. Each sample was diluted to at least five distinct test levels. For each test level, 18 observations were generated per reagent lot and per variant. These observations were produced by three operators using three different sequencing instruments, initiating library preparation on three non-consecutive days, with two replicates per sample test level. Two reagent lots were tested independently. The LoD for each variant was determined using either probit regression or the hit rate approach, defined as the lowest test level with a hit rate (point estimate) $\geq 95\%$. The higher LoD value between the two reagent lots was reported as the final LoD for each variant (Table 21).

Four FFPE samples in three different tissue types (papillary thyroid, skin, and lung) and one FFPE-treated cell line were tested for RET fusions. FFPE cell lines were first used to estimate the LoD values for each fusion gene, then verified with clinical FFPE samples. For verification, there were 18 observations for each test level per lot per variant generated by three operators and three sequencing instruments initiating library preparation on three non-consecutive days with two replicates of each sample per test level, and three reagent lots (54 total observations per variant). The claimed limits of detection for each fusion (Table 22) are the lowest mean supporting reads that reached a hit rate (point estimate) $\geq 95\%$.

Table 21. Limit of Detection for RET Small DNA Variants

Variant	Variant Type	Chr ¹	Position	Reference	Alternative	LoD (VAF)
RET p.(C618R)	SNV	Chr10	43609096	T	C	0.053
RET p.(M918T)	SNV	Chr10	43617416	T	C	0.045
RET p.(C634Y)	MNV	Chr10	43609949	GC	AT	0.045
RET p.(D898_E901del ²)	Deletion	Chr10	43615611	GAGATGTTTATGA	G	0.055
RET p.(C634_T636dup)	Insertion	Chr10	43609946	T	TGTGCCGCAC	0.071 (0.036) ³
RET p.(L629_I638delinsCD)	Deletion	Chr10	43609933	CTGTGCGACG AGCTGTGCCG CACGGTGATC	TGTGAT	0.022
RET p.(C634_R635delinsWG)	MNV	Chr10	43609950	CCGC	GGGT	0.030 (0.015) ³
RET p.(E632_V637delinsAA)	Deletion	Chr10	43609943	AGCTGTGCCGCA CGGTG	CTGCT	Undetermined ⁴

¹ Chr = Chromosome

² This DNA variant was analyzed by probit regression; the other DNA variants were analyzed by the hit rate approach.

³ Two C95 values are presented—the first number is the determined C95 based on the two lots tested in this study, which is potentially overestimated. The second number presented in the parenthesis is the suggested alternative lower and closer to the “true” C95 value based on combining three lots tested in the Within-Laboratory Precision for CDx markers study.

⁴ This variant requires phasing and never achieved $\geq 95\%$ hit rate. All individual components making up this delins variant were detected, but they were not always able to be correctly phased and merged into a single variant. The hit rate did not increase with increasing VAF.

Table 22. Limit of Detection for RET Fusions

Gene	Fusion	Tumor Type	Limit of Detection (Supporting Reads)
RET	NCOA4-RET	Papillary Thyroid	15.8
	KIF5B-RET	Non-Small Cell Lung Cancer	16.6
	CCDC6-RET	Atypical Spitz Tumor	18.7

5. Reagent Kit Lot Interchangeability

A reagent kit lot interchangeability study was not performed because assay components from different reagent kit master lots are not interchangeable. Reagent kit lots are identified on the reagent kit box label and master lot sheet.

6. Tissue Comparability

Please refer to the Summary of SSED for P230011 for Tissue Comparability.

7. Interference

Please refer to the P230011 SSED for Interference studies.

8. Guardbanding

Please refer to the P230011 SSED for Guardbanding studies.

9. Cross Contamination

Please refer to the P230011 SSED for Cross-Contamination studies.

10. Extraction Method Equivalency

Please refer to the P230011 SSED for Extraction Method Equivalency study.

11. Specimen Stability Studies

Please refer to the P230011 SSED for Specimen Stability Studies.

12. Reagent Stability Studies

Please refer to the P230011 SSED for Reagent Stability Studies.

B. Animal Studies

No animal studies were conducted using TSO Comprehensive assay.

C. Additional Studies

No additional studies were conducted using TSO Comprehensive assay.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

Illumina, Inc. conducted 2 separate clinical bridging studies to establish reasonable assurance of safety and effectiveness of the TSO Comprehensive assay for detection of RET fusions in thyroid cancer (TC) patients who may benefit from treatment with selpercatinib, and RET mutations in medullary thyroid cancer (MTC) patients who may benefit from treatment with selpercatinib. Data from these clinical studies were the basis for the PMA approval decision. A summary of each of the clinical studies is presented below.

1. Clinical Bridging Study to Identify RET Fusions in Patients with TC

A. Study Design

To evaluate the TSO Comprehensive assay as a companion diagnostic (CDx) for the selection of TC subjects for treatment with RETEVMO (selpercatinib), RET fusion-positive samples from subjects enrolled in the LIBRETTO- 001 clinical trial using a data cutoff of Dec 2019, and supplemental TC samples from commercial sources were tested in a Clinical Bridging Study.

The study was conducted to assess the concordance between the TSO Comprehensive assay and local tests (LTs, used to determine RET fusion status for enrollment in the LIBRETTO-001 clinical trial), and to assess the clinical efficacy of the selpercatinib in the RET fusion-positive TC subjects identified by the TSO Comprehensive assay. RET fusion status of the subjects was originally determined by testing tissue or blood using various LTs (including NGS).

Study Inclusion Criteria – LIBRETTO-001 Subjects

- Subject was enrolled in LIBRETTO-001.
- Subject had RET fusion positive TC or RET mutation positive MTC.
- Subject had available residual FFPE tissue (in block or slide form) and/or sufficient purified RNA extracted from the FFPE tissue that was collected/retained under informed consent.

Study Exclusion Criteria – LIBRETTO-001 Samples

- Subject had withdrawn from LIBRETTO-001 or did not have appropriate consent.

LIBRETTO-001 Sample Inclusion Criteria

- Sample was collected/retained under informed consent and was available.
- Sample could be FFPE tissue (in block or slide form) and/or purified RNA extracted from the FFPE tissue.

LIBRETTO-001 Sample Exclusion Criteria

- Sample did not meet FFPE tumor content and/or RNA requirements outlined in the TSO Comp assay package insert.
- Sample was withdrawn/rejected in LIBRETTO-001 or did not have appropriate consent.

Follow-up Schedule

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

Primary Objective and Endpoints

The primary objective for the clinical bridging study was to demonstrate safety and effectiveness of the TSO Comprehensive assay for the selection of TC patients with RET fusions for treatment with RETEVMO.

The primary endpoint for the LIBRETTO-001 trial was the objective response rate (ORR), which is the proportion of patients with a best overall response of confirmed complete response (CR) or confirmed partial response (PR) based on IRC assessment using Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 criteria.

The bridging study was used to establish concordance between enrollment LTs and TSO Comprehensive results (Positive, Negative, and Unknown) and to determine the clinical outcomes (specifically with respect to the primary efficacy endpoint of ORR) based on TSO Comprehensive assay results from LIBRETTO-001 TC specimens. Sensitivity analyses for clinical concordance and clinical outcomes (ORR) were performed to evaluate the impact of missing TSO Comprehensive results.

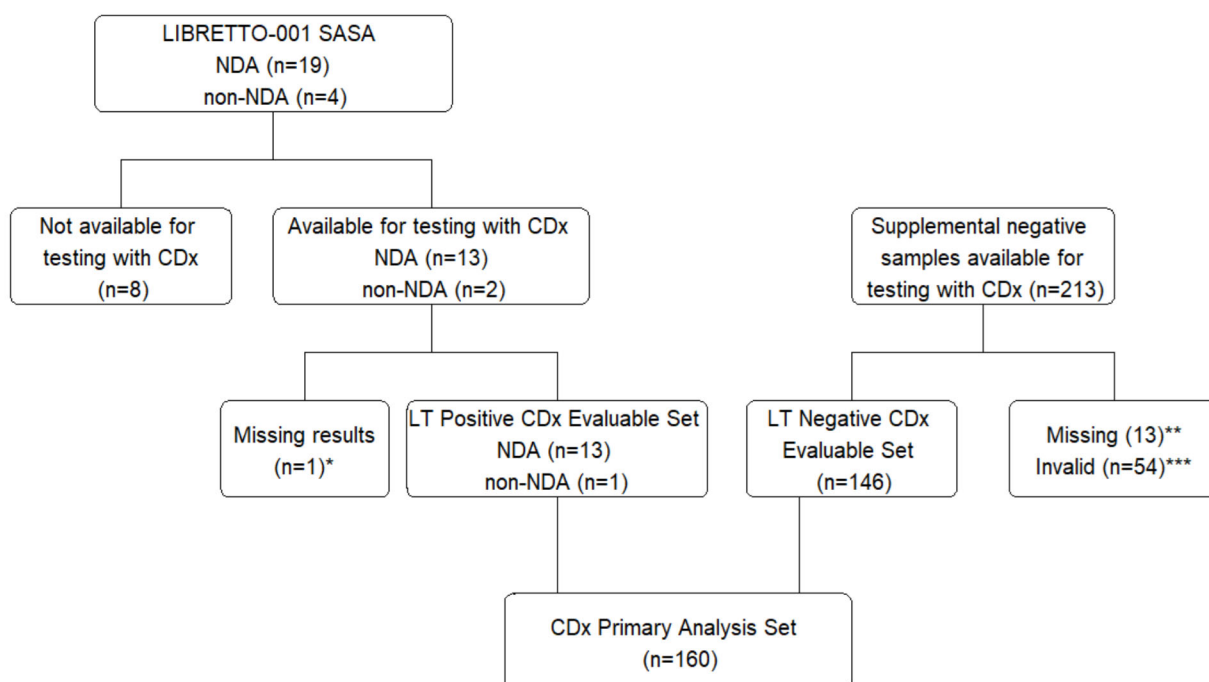
B. Accountability of PMA Cohort

The primary analysis was performed using 14 samples (10 of 14 with clinical outcome data) from the LIBRETTO-001 clinical trial from TC subjects who had received prior systemic therapy and were LT positive and enrolled in the study. A secondary analysis set (n=13, 8 of 13 subjects with clinical outcome data) of LT positive samples from TC subjects who were treatment-naïve and enrolled in the clinical trial were also evaluated. The combined analysis set consisting of both the previously treated and treatment naïve patient subpopulations was evaluated as well. There were 213 LT negative samples supplemented in the bridging study as there were no screen failed samples collected in the LIBRETTO-001 clinical trial. Of these supplemental samples, 146 samples had valid TSO Comprehensive assay results and were included in the analysis. These supplemental samples were negative as determined by previous testing with a representative LT assay.

CDx Primary Analysis Set

There were 23 LIBRETTO-001 Supportive Analysis Set (SAS-A) samples available; 19 samples were from the NDA efficacy set and 4 samples were from the non-NDA efficacy set. Of these samples, 8 samples were not available for testing (LT Positive CDx Unevaluable Set) and 1 was available for testing but was not tested due to failed pathology review (LT Positive CDx Unevaluable Set). 14 samples (13 NDA and 1 non-NDA) were tested with the TSO Comp assay, had valid results (LT Positive CDx Evaluable Set) and were included in the CDx Primary Analysis Set. Additionally, 146 samples were tested with the TSO Comp assay and had valid results (LT Negative CDx Evaluable Set) and were included in the CDx Primary Analysis Set as shown in Figure 1.

Figure 1. RET Fusions in Thyroid Cancer CDx Bridging Study: Sample Accounting for the Primary Analysis Set



LT: local tests or representative LT; NDA: new drug application; SASA: supportive analysis set A, CDx: TSO Comp, Missing: samples available for testing but not meeting tissue or RNA requirements. Invalid: Samples failed sequencing run QC metrics, library QC, or required control didn't pass.

* One sample failed pathology assessment.

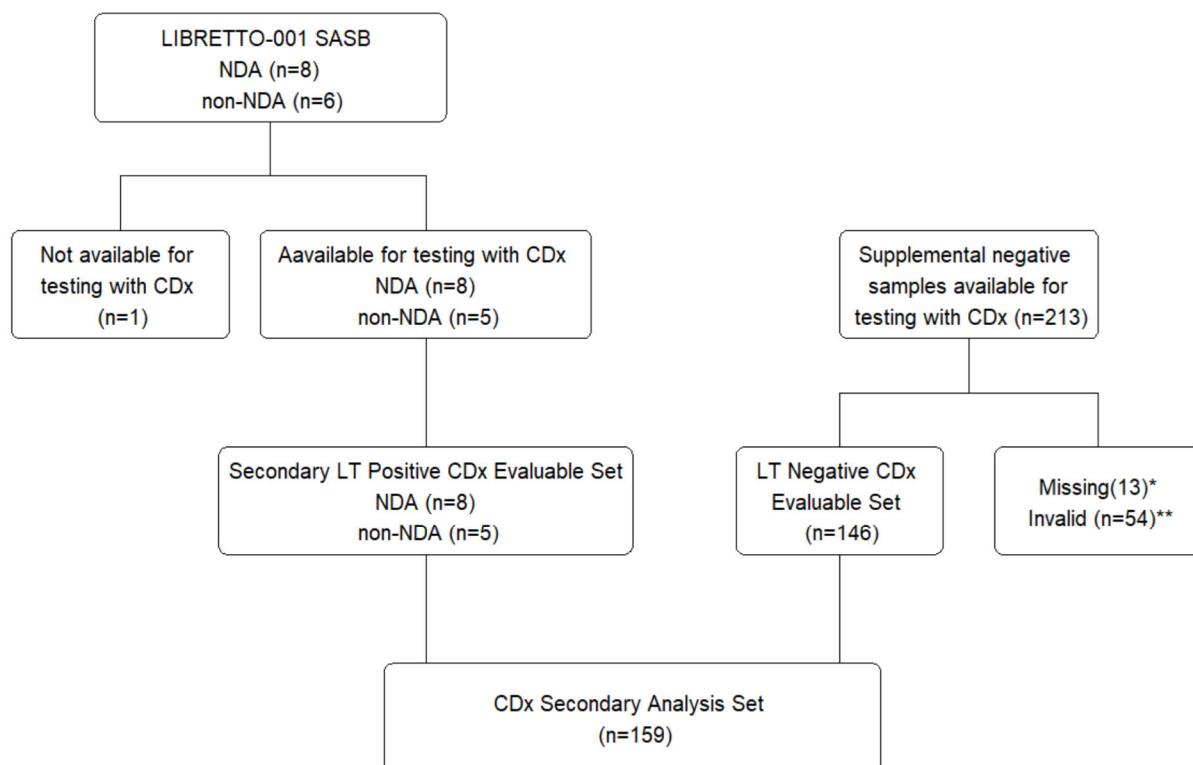
** 13 samples failed RNA assessment.

*** 15 samples failed due to software error, 39 samples failed RNA library QC metrics.

CDx Secondary Analysis Set

There were 14 LIBRETTO-001 SAS-B samples; 8 samples were from the NDA efficacy set and 6 samples were from the non-NDA efficacy set. Of these, 1 sample was not available for testing with the TSO Comp assay. 13 samples (8 NDA and 5 non-NDA) were tested and had valid results (Secondary LT Positive CDx Evaluable Set) and were included in the CDx Secondary Analysis Set as shown in Figure 2. The 146 supplemental negative samples included in the LT Negative CDx Evaluable Set were included in the CDx Secondary Analysis Set.

Figure 2. RET Fusions in Thyroid Cancer CDx Bridging Study: Sample Accounting for the Secondary Analysis Set



LT: local tests or representative LT; NDA: new drug application; SASB: supportive analysis set B. CDx: TSO Comp, Missing: samples available for testing but not meeting tissue or RNA requirements or meeting inclusion criteria but not tested due to operator error, Invalid: Samples failed sequencing run QC metrics, library QC, or required control didn't pass.

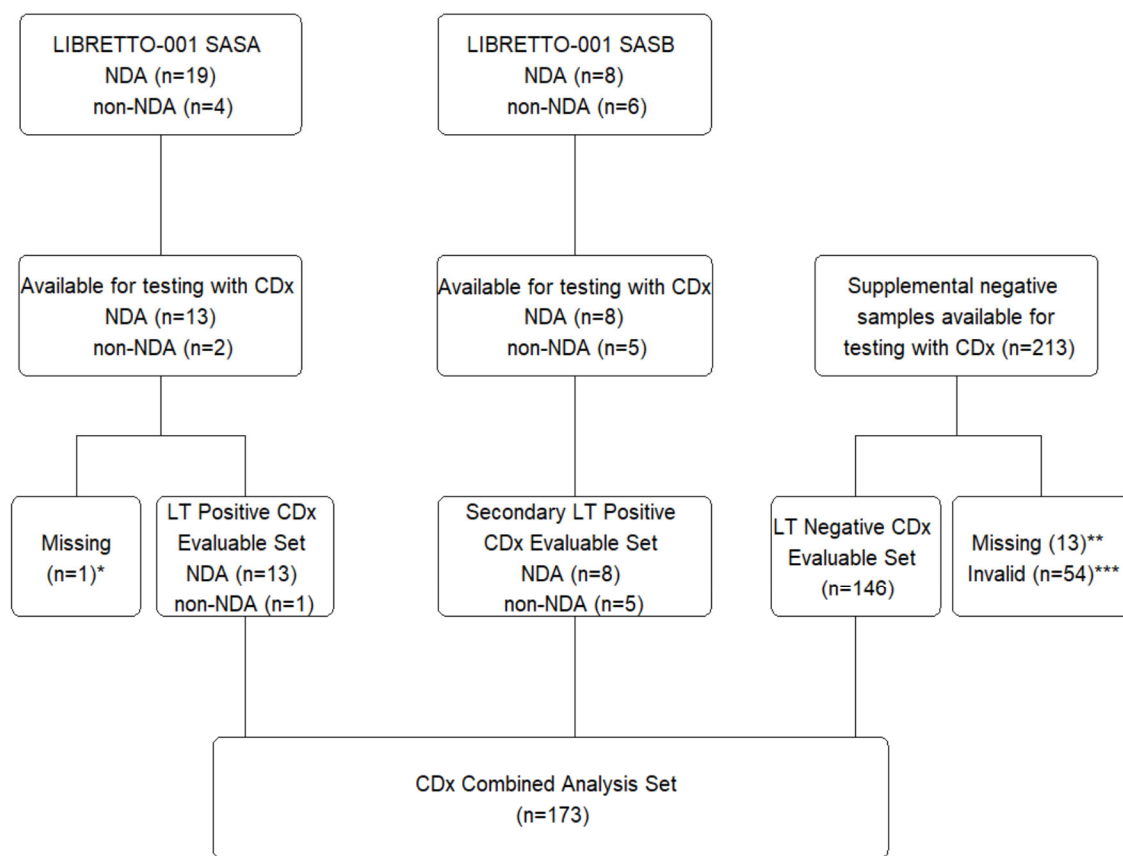
* 13 samples failed RNA assessment.

** 15 samples failed due to software error, 39 samples failed RNA library QC metrics.

CDx Combined Analysis Set

The 14 samples in the LT Positive CDx Evaluable Set, the 13 samples in the Secondary LT Positive CDx Evaluable Set, and the 146 samples included in the LT Negative CDx Evaluable Set were included in the CDx Combined Analysis Set as shown in Figure 3.

Figure 3. RET Fusions in Thyroid Cancer CDx Bridging Study: Sample Accounting for the Combined Analysis Set



LT: local tests or representative LT; NDA: new drug application, SASA: supportive analysis set A; SASB: supportive analysis set B, CDx: TSO Comp, Missing: samples available for testing but not meeting tissue or RNA requirements or meeting inclusion criteria but not tested due to operator error, Invalid: Samples failed sequencing run QC metrics, library QC, or required control didn't pass.

* One sample failed pathology assessment.

** 13 samples failed RNA assessment.

*** 15 samples failed due to software error, 39 samples failed RNA library QC metrics.

C. **Study Population Demographics and Baseline Parameters**

Table 23 summarizes demographic information for the LT Positive CDx Evaluable (n = 14) and Unevaluable (n = 9) subsets of the LIBRETTO-001 Supportive Analysis Set-A (SAS-A). Race categories are reported based on categories recorded from the specimen collection protocols. Table 24 summarizes demographic information for the LT Positive CDx Evaluable (n = 27) and Unevaluable (n = 10) subsets of the LIBRETTO-001 SAS-A and Supportive Analysis Set-B (SAS-B). Table 25 summarizes baseline disease characteristics information for the LT Positive CDx Evaluable and Unevaluable subsets of the LIBRETTO-001 SAS-A. Table 26 summarizes baseline disease characteristics information for the LT Positive CDx Evaluable and Unevaluable subsets of the LIBRETTO-001 SAS-A and SAS-B. The demographic and baseline clinical characteristics were comparable between the evaluable and unevaluable sets. SAS-B included only 1 LT+ CDx unevaluable sample and thus, statistical analysis to compare evaluable vs unevaluable samples was not performed.

Table 23. RET Fusions in Thyroid Cancer CDx Bridging Study: Baseline Demographic Characteristics for Thyroid Cancer Samples From LIBRETTO-001 SAS-A

	LT +	LT+ CDx Evaluable Set	LT+ CDx Unevaluable Set	P-value
Number of Patients	23	14	9	
Age (years)				0.36
Mean (SD)	56.0 (17.8)	53.5 (19.6)	59.8 (14.9)	
Median (Min, Max)	54.0 (25.0, 88.0)	51.0 (25.0, 88.0)	60.0 (38.0, 81.0)	
Gender, n (%)				1.00
Female	13 (56.5%)	8 (57.1%)	5 (55.6%)	
Male	10 (43.5%)	6 (42.9%)	4 (44.4%)	
Race, n (%)				0.39
White	16 (69.6%)	11 (78.6%)	5 (55.6%)	
Asian	3 (13.0%)	2 (14.3%)	1 (11.1%)	
Black Or African American	2 (8.7%)	0 (0.0%)	2 (22.2%)	
Other	2 (8.7%)	1 (7.1%)	1 (11.1%)	
Ethnicity, n (%)				0.16
Hispanic Or Latino	2 (8.7%)	0 (0.0%)	2 (22.2%)	
Not Hispanic Or Latino	20 (87.0%)	13 (92.9%)	7 (77.8%)	
Missing	1 (4.3%)	1 (7.1%)	0 (0.0%)	
Country, n (%)				0.50
USA	21 (91.3%)	12 (85.7%)	9 (100%)	
Other ^a	2 (8.7%)	2 (14.3%)	0 (0.0%)	
Weight (kg)				0.73
Mean (SD)	73.9 (22.2)	75.9 (25.1)	70.7 (17.8)	

Median (Min, Max)	69.9 (41.3, 121.4)	71.9 (41.3, 121.4)	69.0 (49.4, 99.2)	
Height (cm) Mean (SD)	168.8 (12.1)	169.1 (13.9)	168.4 (9.8)	0.95
Median (Min, Max)	168.5 (148.0, 197.0)	167.0 (148.0, 197.0)	169.0 (153.0, 182.0)	
BMI (kg/m²) Mean (SD)	25.5 (5.8)	26.0 (6.5)	24.7 (4.7)	0.65
Median (Min, Max)	24.6 (17.3, 35.6)	24.8 (17.3, 35.6)	24.4 (19.1, 30.9)	
Smoking Status, n (%) Former Smoker	2 (8.7%)	0 (0.0%)	2 (22.2%)	0.14
Never A Smoker	21 (91.3%)	14 (100%)	7 (77.8%)	

CDx: companion diagnostic; LT: local tests or representative LT; LT+: LT positive.

^a Includes Hong Kong and Japan

Table 24. RET Fusions in Thyroid Cancer CDx Bridging Study: Baseline Demographic Characteristics for Thyroid Cancer Samples From LIBRETTO-001 SAS-A and SAS-B

	LT +	LT+ CDx Evaluable Set	LT+ CDx Unevaluable Set	P-value
Number of Patients	37	27	10	
Age (years)				0.49
Mean (SD)	55.2 (17.7)	53.9 (18.9)	58.8 (14.4)	
Median (Min, Max)	54.0 (20.0, 88.0)	54.0 (20.0, 88.0)	59.0 (38.0, 81.0)	
Gender, n (%)				0.48
Female	18 (48.6%)	12 (44.4%)	6 (60.0%)	
Male	19 (51.4%)	15 (55.6%)	4 (40.0%)	
Race, n (%)				0.04
White	27 (73.0%)	22 (81.5%)	5 (50.0%)	
Asian	3 (8.1%)	2 (7.4%)	1 (10.0%)	
Black Or African American	2 (5.4%)	0 (0.0%)	2 (20.0%)	
Other	4 (10.8%)	2 (7.4%)	2 (20.0%)	
Missing	1 (2.7%)	1 (3.7%)	0 (0.0%)	
Ethnicity, n (%)				0.64
Hispanic Or Latino	6 (16.2%)	4 (14.8%)	2 (20.0%)	
Not Hispanic Or Latino	28 (75.7%)	21 (77.8%)	7 (70.0%)	
Missing	3 (8.1%)	2 (7.4%)	1 (10.0%)	
Country, n (%)				0.55
USA	34 (91.9%)	24 (88.9%)	10 (100%)	
Other ^a	3 (8.1%)	3 (11.1%)	0 (0.0%)	

Weight (kg)				0.18
Mean (SD)	79.2 (22.7)	82.4 (24.0)	70.7 (16.8)	
Median (Min, Max)	78.3 (41.3, 121.4)	80.2 (41.3, 121.4)	70.0 (49.4, 99.2)	
Height (cm)	169.6 (11.4)	170.6 (11.9)	167.2 (10.0)	0.43
Mean (SD)				
Median (Min, Max)	170.0 (148.0, 197.0)	172.0 (148.0, 197.0)	168.5 (153.0, 182.0)	
BMI (kg/m²)	27.0 (7.0)	27.8 (7.7)	25.1 (4.7)	0.36
Mean (SD)				
Median (Min, Max)	26.7 (17.3, 51.8)	26.7 (17.3, 51.8)	25.6 (19.1, 30.9)	
Smoking Status, n (%)	8 (21.6%)	6 (22.2%)	2 (20.0%)	1.00
Former Smoker				
Never A Smoker	29 (78.4%)	21 (77.8%)	8 (80.0%)	

CDx: companion diagnostic; LT: local tests or representative LT; LT+: LT positive.

^a Includes Australia, Hong Kong and Japan

Table 25. RET Fusions in Thyroid Cancer CDx Bridging Study: Baseline Disease Characteristics for Thyroid Cancer Samples From LIBRETTO-001 SAS-A

	LT+	LT+ CDx Evaluable Set	LT+ CDx Unevaluable Set	P-value
Number of Patients	23	14	9	
ECOG, n (%)				1.00
0	8 (34.8%)	5 (35.7%)	3 (33.3%)	
1	13 (56.5%)	8 (57.1%)	5 (55.6%)	
2	2 (8.7%)	1 (7.1%)	1 (11.1%)	
Tumor Stage at Diagnosis, n (%)				1.00
Stage II	1 (4.3%)	1 (7.1%)	0 (0.0%)	
Stage IV/IVB/IVC	21 (91.3%)	12 (85.7%)	9 (100%)	
Missing	1 (4.3%)	1 (7.1%)	0 (0.0%)	
Tumor Grade, n (%)				0.78
Moderately Differentiated	1 (4.3%)	1 (7.1%)	0 (0.0%)	

	LT+	LT+ CDx Evaluable Set	LT+ CDx Unevaluable Set	P-value
Poorly Differentiated	6 (26.1%)	4 (28.6%)	2 (22.2%)	
Undifferentiated	1 (4.3%)	1 (7.1%)	0 (0.0%)	
Not Applicable	4 (17.4%)	3 (21.4%)	1 (11.1%)	
Unknown	11 (47.8%)	5 (35.7%)	6 (66.7%)	
Tumor Subtype, n (%)				0.59
Anaplastic Thyroid Cancer	2 (8.7%)	2 (14.3%)	0 (0.0%)	
Hurthle Cell Thyroid Cancer	1 (4.3%)	1 (7.1%)	0 (0.0%)	
Papillary Thyroid Cancer	17 (73.9%)	10 (71.4%)	7 (77.8%)	
Poorly Differentiated Thyroid Cancer	3 (13.0%)	1 (7.1%)	2 (22.2%)	
Metastasis Status, n (%)				NA
Yes	23 (100%)	14 (100%)	9 (100%)	

CDx: companion diagnostic; LT: local tests or representative LT; LT+: LT positive.

Note: Statistical test is based on non-missing values. Mann-Whitney test was used for comparing continuous variables, Fisher-Freeman-Halton test was used for comparing categorical variables.

Table 26. RET Fusions in Thyroid Cancer CDx Bridging Study: Baseline Disease Characteristics for Thyroid Cancer Samples Combined From LIBRETTO-001 SAS-A and SAS-B

	LT+	LT+CDx Evaluable Set	LT+CDx Unevaluable Set	P-value
Number of Patients	37	27	10	
ECOG, n (%)				1.00
0	15 (40.5%)	11 (40.7%)	4 (40.0%)	
1	19 (51.4%)	14 (51.9%)	5 (50.0%)	
2	3 (8.1%)	2 (7.4%)	1 (10.0%)	
Tumor Stage at Diagnosis, n (%)				1.00
Stage II	1 (2.7%)	1 (3.7%)	0 (0.0%)	
Stage IIIA/IIIB	1 (2.7%)	1 (3.7%)	0 (0.0%)	
Stage IV/IVB/IVC	34 (91.9%)	24 (88.9%)	10 (100%)	
Missing	1 (2.7%)	1 (3.7%)	0 (0.0%)	

Tumor Grade, n (%)				0.39
Well Differentiated	2 (5.4%)	2 (7.4%)	0 (0.0%)	
Moderately Differentiated	1 (2.7%)	1 (3.7%)	0 (0.0%)	
Poorly Differentiated	14 (37.8%)	12 (44.4%)	2 (20.0%)	
Undifferentiated	1 (2.7%)	1 (3.7%)	0 (0.0%)	
Not Applicable	4 (10.8%)	3 (11.1%)	1 (10.0%)	
Unknown	15 (40.5%)	8 (29.6%)	7 (70.0%)	
Tumor Subtype, n (%)				0.45
Anaplastic Thyroid Cancer	2 (5.4%)	2 (7.4%)	0 (0.0%)	
Hurthle Cell Thyroid Cancer	1 (2.7%)	1 (3.7%)	0 (0.0%)	
Papillary Thyroid Cancer	31 (83.8%)	23 (85.2%)	8 (80.0%)	
Poorly Differentiated Thyroid Cancer	3 (8.1%)	1 (3.7%)	2 (20.0%)	
Metastasis Status, n (%)				NA
Yes	37 (100%)	27 (100%)	10 (100%)	

CDx: companion diagnostic; LT: local tests or representative LT; LT+: LT positive. Note: Statistical test is based on non-missing values. Mann-Whitney test was used for comparing continuous variables, Fisher-Freeman-Halton test was used for comparing categorical variables.

D. Safety and Effectiveness Results

Safety Results

The safety with respect to treatment with selpercatinib was addressed during the review of the NDA. Testing for the TSO Comprehensive assay bridging study was conducted retrospectively on banked study specimens and the test results were not reported to patients. No patient treatment decisions were made as a result of any of the TSO Comprehensive assay test results; therefore, as expected, there were no Unanticipated Adverse Device Effect (UADE) events related to the study products or procedures to patients or operators. Please refer to [fda.gov/drugs](https://www.fda.gov/drugs) for complete safety information on RETEVMO (selpercatinib).

Effectiveness Results – Concordance

The results for the primary, secondary, and combined analysis groups are detailed below. These analysis sets are not mutually exclusive as they all include the same set of supplemental negative samples. Additionally, the combined analysis set includes the LT positive samples from the primary and secondary analysis sets.

Concordance Results:

To evaluate the clinical accuracy of the TSO Comprehensive assay, the concordance analysis between the TSO Comprehensive assay and LT results was conducted on 27 RET fusion positive patients (14 from Primary Analysis Set consisting of previously treated TC subjects and 13 from Secondary Analysis Set consisting of treatment-naïve subjects), and 200 RET fusion negative supplemental samples (Table 27). The PPA and NPA shown in Table 28 were calculated using the LTs as the reference method. The point estimates of PPA and NPA were 88.9% and 100% respectively, when excluding TSO Comprehensive invalid results. When including TSO Comprehensive invalid results, the point estimates of PPA and NPA were 88.9% and 73.0%, respectively.

PPA excluding and including invalids separately for the primary and secondary analysis groups are shown in Table 29. For the primary analysis group, there were 3 samples that were LT positive but negative by the TSO Comprehensive assay. All 3 samples were from subjects who had a partial response to selpercatinib. Additional analysis of the raw TSO Comprehensive data revealed that all 3 discordant samples did have RET fusion read counts (3 to 4 reads) that fell just under the valid reportable fusion read count cutoff of 5 reads, suggesting that the amount of RET fusion analyte in these samples was just below the assay's reportable range (5 to undefined reads) and close to the assay's C95 for RET fusions (~16 supporting reads).

Table 27. Concordance Between TSO Comprehensive Assay and Local Test (LT) Results for RET Fusions in TC

TSO Comprehensive Assay	LT			
	POS		NEG	Total
	Primary Analysis Set	Secondary Analysis Set		
POS	11	13	0	24
NEG	3*	0	146	149
Invalids	0	0	54	54
Total	14	13	200	227

*All 3 samples were from subjects who had a partial response to selpercatinib that had RET fusion read counts (3 to 4 reads) that fell just under the valid reportable fusion read count cutoff of 5 reads and thus, were negative by TSO Comprehensive.

Table 28. Agreement Between TSO Comprehensive Assay and Local Test Results for RET Fusions in TC

Parameter	Agreed	Total	Agreement	95% CI*
PPA Exclude Invalids	24	27	88.9%	70.8%-97.6%
NPA Exclude Invalids	146	146	100%	97.5%-100%
PPA Include Invalids	24	27	88.9%	70.8%-97.6%
NPA Include Invalids	146	200	73.0%	66.3%-79.0%

* CI based on Clopper-Pearson (exact) method.

Table 29. Agreement Between TSO Comprehensive Assay and Local Test Results for RET Fusions in TC by Cohort

Subgroup	Parameter	Agreed	Total	Agreement	95% CI*
Primary Analysis Set – Prior Therapy	PPA Exclude Invalids	11	14	78.6%	49.2%-95.3%
	PPA Include Invalids	11	14	78.6%	49.2%-95.3%
Secondary Analysis Set – Systemic Therapy Naïve	PPA Exclude Invalids	13	13	100%	75.3%-100%
	PPA Include Invalids	13	13	100%	75.3%-100%

* CI based on Clopper-Pearson (exact) method.

Sensitivity Analysis for Concordance

Missing TSO Comp results for the LT Positive CDx Unevaluable Set (SAS-A dataset from previously treated subjects) were imputed 500 times using a logistic regression model including race and age, ECOG score, and tumor subtype as these covariates may be associated with clinical outcome. Table 30 shows the PPA and OPA using the imputed TSO Comp results. Note: No sensitivity analysis was performed on the LT Negative CDx Unevaluable Set because the observed NPA was 100% and very limited demographic and disease characteristics are available for supplemental negative samples; NPA is assumed to 100% for the evaluation of OPA. The PPA and OPA estimates including imputed results were 81.3% (95% CI: 64.3% to 98.2%) and 97.4% (95% CI: 94.8% to 100%), respectively.

Table 30. CDx Bridging Study: Concordance Between the TSO Comp Assay and the LTs with Imputed TSO Comp Assay Results

Agreement	Minimum	Maximum	Median	Estimate	95% CI ^a
PPA	69.6%	87.0%	82.6%	81.3%	64.3%-98.2%
OPA	95.7%	98.2%	97.5%	97.4%	94.8%-100%

LTs: local tests; PPA: Positive percent agreement; OPA: Overall Percent Agreement.

^a CI was calculated based on method described by Gelman et al (Gelman, et al. 2013).

Efficacy Results in the RET Fusion Positive Population

Table 31 shows the objective response rate (ORR) for selpercatinib in the RET fusion-positive subject populations as detected by the TSO Comprehensive assay. The percentage of TSO Comp positive / LT positive subjects with a response in the primary analysis set, which included previously treated subjects, was 70.0% (7/10; 95% CI: 34.8%, 93.3%). While the concordance analysis included 11 TSO Comp positive subjects in the primary analysis set, one sample was from outside of the NDA efficacy analysis set and did not have six-month follow-up data at the time of the data cut off, and therefore, was not assessed for efficacy. The ORR in the secondary analysis set, which included the treatment-naïve subjects, was 100% (8/8; 95% CI: 63.1%, 100%). While the concordance analysis included 13 TSO Comp positive subjects in the secondary analysis set, five samples were from outside of the NDA efficacy analysis set and did not have six-month follow-up data at the time of the data cut off, and therefore, these 5 samples were not assessed for efficacy. The ORR in the combined analysis set (previously treated and treatment naïve subjects) was 83.3% (15/18; 95% CI: 58.6%, 96.4%).

Table 31. Summary of Best Response Rate by TSO Comprehensive Assay Assessment Status in Cohorts 1 and 2 of TC Patients (Independent Central Radiology Assessment per RECIST v1.1)

ORR: CR + PR	TSO Comprehensive Positive, LT Positive		TSO Comprehensive Negative, LT Positive		TSO Comprehensive Unevaluable, LT Positive		LT Positive NDA Drug Efficacy Population	
	ORR% (n/N)	95% CIs ¹	ORR% (n/N)	95% CIs ¹	ORR% (n/N)	95% CIs ¹	ORR% (n/N)	95% CIs ¹
Primary Analysis Set ²	70.0% (7/10)	34.8%-93.3%	100% (3/3)	29.2%-100%	83.3% (5/6)	35.9%-99.6%	78.9% (15/19)	54.4%-93.9%
Secondary Analysis Set ³	100% (8/8)	63.1%-100%	N/A	N/A	N/A	N/A	100% (8/8)	63.1%-100%

¹ CI based on Clopper-Pearson (exact) method.

² Subjects previously treated with systemic therapy.

³ Treatment-naïve subjects.

Sensitivity Analysis for Efficacy Results

A sensitivity analysis was performed to evaluate the impact of missing TSO Comp assay results on the ORR estimate. Using the 500 imputed sets of TSO Comp assay results from the LT Positive CDx Unevaluable Set (SAS-A dataset from previously treated subjects), ORRs were calculated for the LT+CDx+ population (Table 32). The median ORR, including the imputed TSO Comp results, was 73.3% (95% CI: 50.1% to 95.7%), which was comparable to the observed ORR in the CDx Primary Analysis Set (ORR = 70.0% [7/10]; 95% CI: 34.8% to 93.3%) and the ORR of the LIBRETTO-001 SAS-A NDA drug efficacy population (ORR = 78.9% [15/19]; 95% CI: 54.4% to 93.9%).

Table 32. CDx Bridging Study: Clinical Efficacy (ORR) in TSO Comp Positive Patient Population with Imputed TSO Comp Assay Results

Subgroup	Estimate	Minimum	Maximum	Median	95% CI ^a
LT+ TSO Comp+	72.9%	69.2%	75.0%	73.3%	50.1%-95.7%
LT+ TSO Comp-	100%	100%	100%	100%	29.24%-100%

ORR: objective response rate.

^a CI was calculated based on method described by Gelman et al (Gelman, et al. 2013).

E. Pediatric Extrapolation

RETEVMO® (selpercatinib) received approval in the United States for adults and pediatric patients 2 years of age and older with advanced or metastatic *RET* fusion-positive thyroid cancer who require systemic therapy and who are radioactive iodine-refractory (if radioactive iodine is appropriate). Under FDA's pediatric classification category, RETEVMO is approved for use under the aforementioned indications in the "children and adolescents" pediatric grouping. The youngest patient enrolled in the study was 20 years of age. For the LIBRETTO-001 trial, data from adult patients aged 20 years and older are considered generalizable to the pediatric population aged 18-21 years and can be relied on to establish safety and effectiveness within the 18-21 years age group.

F. 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. This clinical investigator did not have disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

2. Clinical Bridging Study to Identify RET Mutations in patients with MTC

A. Study Design

To validate the TSO Comprehensive assay as a companion diagnostic (CDx) for the selection of MTC subjects for treatment with RETEVMO (selpercatinib), RET mutation-positive MTC samples from subjects enrolled in the LIBRETTO- 001 clinical trial using a data cutoff of Dec 2019, and supplemental MTC and TC samples from commercial sources were used in a Clinical Bridging Study.

The study was conducted to assess the concordance between the TSO Comprehensive assay and LTs (used to determine RET mutation status for enrollment in the LIBRETTO-001 clinical trial), and to assess the clinical efficacy of selpercatinib in the RET mutation-positive MTC subjects identified by the TSO Comprehensive assay. RET mutation status of the subjects was originally determined by testing tissue or blood using various LTs (including NGS and PCR).

Study Inclusion Criteria – LIBRETTO-001 Subjects

- Subject was enrolled in LIBRETTO-001.
- Subject had RET mutation positive MTC.
- Subject had available residual FFPE tissue (in block or slide form) and/or sufficient purified DNA extracted from the FFPE tissue that was collected/retained under informed consent.

Study Exclusion Criteria – LIBRETTO-001 Samples

- Subject had withdrawn from LIBRETTO-001 or did not have appropriate consent.

LIBRETTO-001 Sample Inclusion Criteria

- Sample was collected/retained under informed consent and was available.
- Sample could be FFPE tissue (in block or slide form) and/or purified DNA extracted from the FFPE tissue.

LIBRETTO-001 Sample Exclusion Criteria

- Sample did not meet FFPE tumor and/or DNA requirements outlined in the TSO Comp assay package insert.
- Sample was withdrawn/rejected in LIBRETTO-001 or did not have appropriate consent.

Follow-up Schedule

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

Primary Objective and Endpoints

The primary objective for the clinical bridging study was to demonstrate safety and effectiveness of the TSO Comprehensive assay for the selection of MTC patients with RET mutations for treatment with RETEVMO.

The primary endpoint for the LIBRETTO-001 trial was the objective response rate (ORR), which is the proportion of patients with a best overall response of confirmed complete response (CR) or confirmed partial response (PR) based on IRC assessment using Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 criteria.

The bridging study was used to establish concordance between enrollment LTs and TSO Comprehensive results (Positive, Negative, and Unknown) and to determine the clinical outcomes (specifically with respect to the primary efficacy endpoint of ORR) based on TSO Comprehensive assay results from LIBRETTO-001 MTC specimens. Sensitivity analyses for the clinical concordance and clinical outcomes (ORR) were performed.

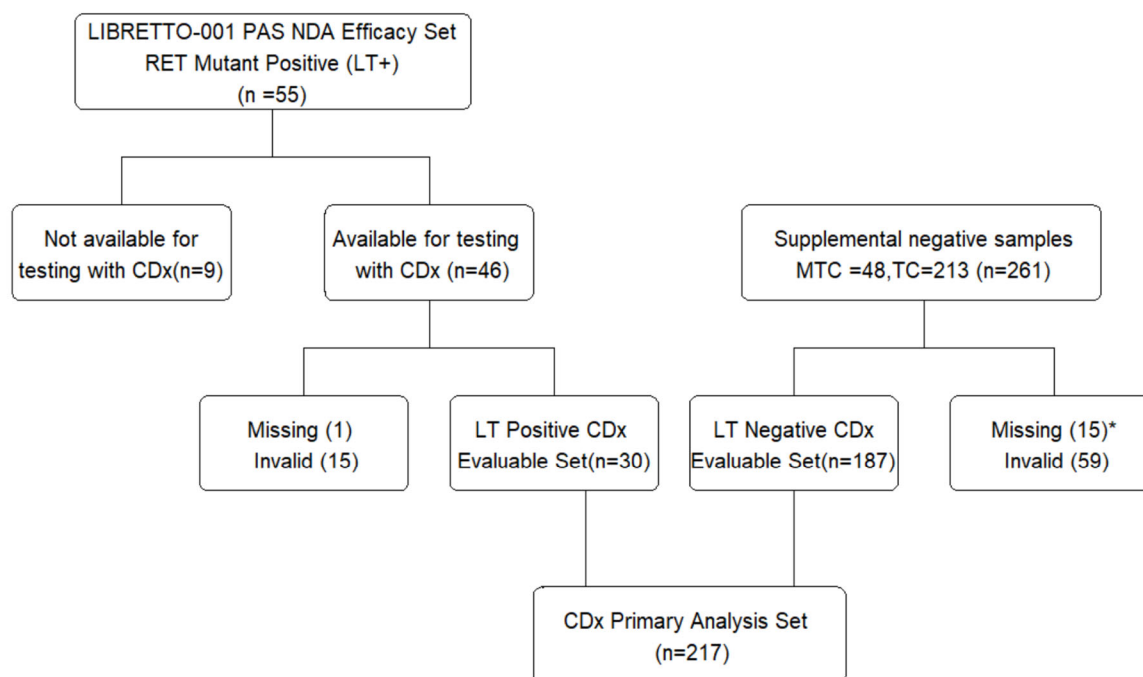
B. Accountability of the PMA Cohort

The primary analysis was performed using 30 samples from the LIBRETTO-001 clinical trial from MTC subjects who had received prior systemic therapy and were LT positive and enrolled in the study. A secondary analysis set (n=39, 33 of 39 subjects with clinical outcome data) of LT positive samples from MTC subjects who were treatment-naïve and enrolled in the clinical trial were also evaluated. There were 261 LT negative samples supplemented in the bridging study as there were no screen failed samples collected in the LIBRETTO-001 clinical trial. Of these supplemental samples, 113 samples had valid TSO Comprehensive assay results and were included in the analysis. These supplemental samples were negative as determined by previous testing with a representative LT assay.

CDx Primary Analysis Set

There were 55 LIBRETTO-001 Primary Analysis Set (PAS) MTC samples available; all were from the LIBRETTO-001 NDA efficacy set. Of these samples, 9 samples were not available for testing (LT Positive CDx Unevaluable Set) and 46 were available for testing with the TSO Comp assay. Out of these 46 samples, 1 was not tested due to failed pathology review (LT Positive CDx Unevaluable Set), 15 had final invalid results (tested in a failed sequencing run and never retested, or failed DNA small variant and TMB QC in a valid run) (LT Positive CDx Unevaluable Set), and 30 samples had valid results (LT Positive CDx Evaluable Set) and were included in the CDx Primary Analysis Set as shown in Figure 4. Additionally, there were 261 supplemental negative MTC (n = 48) and TC (n = 213) samples available for testing with the TSO Comp assay; of these, 187 had valid results (LT Negative CDx Evaluable Set) and were included in the CDx Primary Analysis Set.

Figure 4: RET Mutations in Medullary Thyroid Cancer Bridging Study: Sample Accounting for the Primary Analysis Set



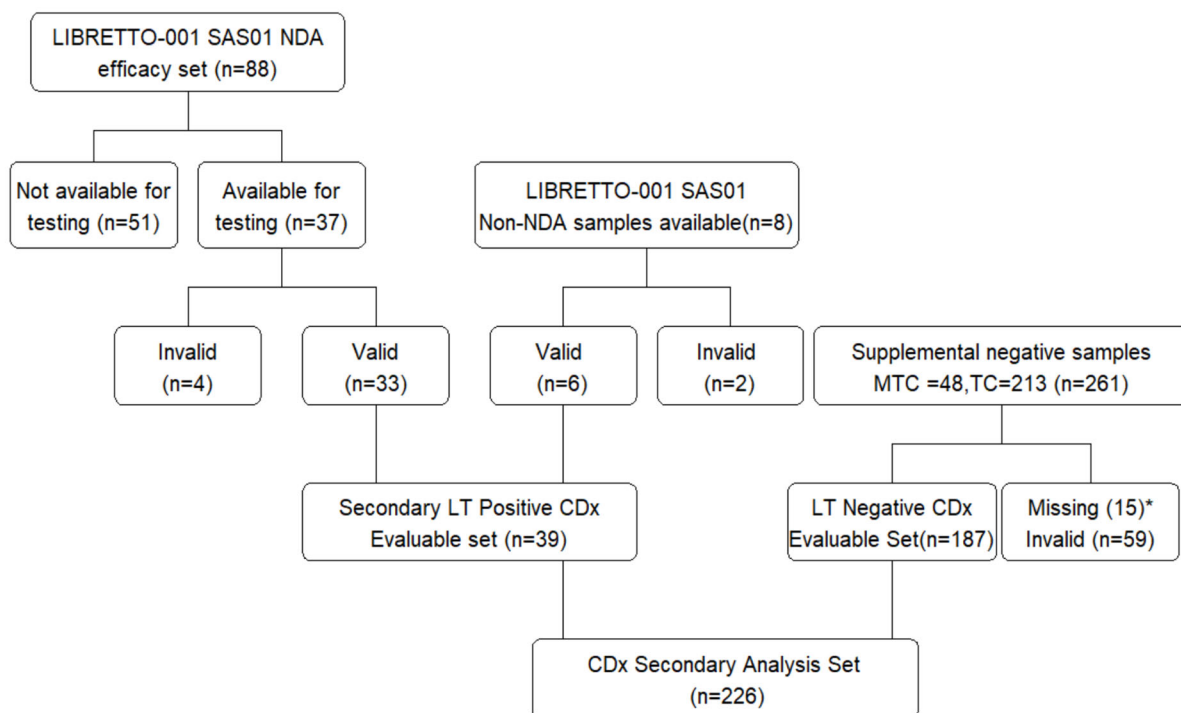
LT: local tests or representative LT, NDA: new drug application, CDx: TSO Comp, Missing: samples available for testing but not meeting tissue or DNA requirements or samples met testing requirement but not tested, or sample ID undetermined, Invalid: Samples failed sequencing run QC metrics, library QC or failed DNA Small Variant and TMB QC, or required control didn't pass.

*Two samples failed pathology assessment; 10 failed DNA requirement; 2 not tested; 1 sample ID undetermined.

CDx Secondary Analysis Set

There were 88 samples from the NDA efficacy set; 51 samples were not available for testing with the TSO Comp assay (Secondary LT Positive CDx Unevaluable Set). There were 40 non-NDA samples; 32 were not available for testing (Secondary LT Positive CDx Unevaluable Set). Of the 45 LIBRETTO-001 SAS1 (NDA plus non-NDA) samples available for testing with the TSO Comp assay, 6 had final invalid results (failed DNA small variant and TMB QC or failed library QC) (Secondary LT Positive CDx Unevaluable Set); 39 samples had valid results (Secondary LT Positive CDx Evaluable Set) and were included in the CDx Secondary Analysis Set as shown in Figure 5. The 187 supplemental negative samples included in the LT Negative CDx Evaluable Set were included in the CDx Secondary Analysis Set.

Figure 5. RET Mutations in Medullary Thyroid Cancer Bridging Study: Sample Accounting for the Secondary Analysis Set



LT: local tests or representative LT, NDA: new drug application, CDx: TSO Comp. Missing: samples available for testing but not meeting tissue or DNA requirements or meeting inclusion criteria but not tested due to operator error, or sample ID undetermined. Invalid: Samples failed library QC or failed DNA Small Variant and TMB QC.

*Two samples failed pathology assessment; 10 failed DNA requirement; 2 not tested; 1 sample ID undetermined.

CDx Combined Analysis Set

The 30 samples in the LT Positive CDx Evaluable Set, the 39 samples in the Secondary LT Positive CDx Evaluable Set, and the 187 supplemental negative samples included in the LT Negative CDx Evaluable Set were included in the CDx Combined Analysis Set as shown in Figure 6.

```
graph TD; A[LIBRETTO-001 PAS and SAS01  
NDA efficacy set (n=55+88)] --> B[Not available for testing (n=9+51)]; A --> C[Available for testing (n=46+37)]; C --> D[Missing (n=1)  
invalid (n=19)]; C --> E[Valid (n=30+33)]; E --> F[Combined LT Positive CDx  
Evaluable set(n=63+6)]; G[LIBRETTO-001 SAS01  
Non-NDA samples available  
for testing (n=8)] --> H[Valid (n=6)]; G --> I[Invalid (n=2)]; H --> F; I --> J[Supplemental negative samples  
MTC=48, TC=213 (n=261)]; J --> K[LT Negative CDx  
Evaluable Set(n=187)]; J --> L[Missing (15)*  
invalid (n=59)]; K --> M[CDx Combined Analysis Set  
(n=256)]; F --> M;
```

LIBRETTO-001 PAS and SAS01
NDA efficacy set (n=55+88)

Not available for testing (n=9+51)

Available for testing (n=46+37)

Missing (n=1)
invalid (n=19)

Valid (n=30+33)

Combined LT Positive CDx
Evaluable set(n=63+6)

LIBRETTO-001 SAS01
Non-NDA samples available
for testing (n=8)

Valid (n=6)

Invalid (n=2)

Supplemental negative samples
MTC=48, TC=213 (n=261)

LT Negative CDx
Evaluable Set(n=187)

Missing (15)*
invalid (n=59)

CDx Combined Analysis Set
(n=256)

*Two samples failed pathology assessment; 10 failed DNA requirement; 2 not tested; 1 sample ID undetermined.

Table 33 summarizes demographic information for the LT Positive CDx Evaluable (n = 30) and Unevaluable (n = 25) subsets of the LIBRETTO-001 primary analysis set (PAS) subjects. Race categories are reported based on categories recorded from the specimen collection protocols. Table 34 summarizes demographic information for the LT Positive CDx Evaluable (n = 39) and Unevaluable (n = 89) subsets of the LIBRETTO-001 supplemental analysis set 1 (SAS01). Table 35 summarizes baseline disease characteristics information for the LT Positive CDx Evaluable and Unevaluable subsets of the LIBRETTO-001 PAS. Table 36 summarizes baseline disease characteristics information for the LT Positive CDx Evaluable and Unevaluable subsets of the LIBRETTO-001 SAS01. The demographic and baseline clinical characteristics were comparable between the evaluable and unevaluable sets.

Table 33. RET Mutations in Medullary Thyroid Cancer Bridging Study: Baseline Demographic Characteristics for Medullary Thyroid Cancer Samples From LIBRETTO-001 PAS

	LT +	LT + CDx Evaluable Set	LT + CDx Unevaluable Set	P-value
Number of Patients	55	30	25	
Age (years)				0.16
Mean (SD)	56.2 (14.8)	53.6 (15.3)	59.3 (13.8)	
Median (Min, Max)	57.0 (17.0, 84.0)	55.5 (17.0, 75.0)	63.0 (22.0, 84.0)	
Gender, n (%)				0.78
Female	19 (34.5%)	11 (36.7%)	8 (32.0%)	
Male	36 (65.5%)	19 (63.3%)	17 (68.0%)	
Race, n (%)				0.49
White	49 (89.1%)	28 (93.3%)	21 (84.0%)	
Black Or African American	1 (1.8%)	0 (0.0%)	1 (4.0%)	
Other	5 (9.1%)	2 (6.7%)	3 (12.0%)	
Ethnicity, n (%)				0.31
Hispanic Or Latino	4 (7.3%)	1 (3.3%)	3 (12.0%)	
Not Hispanic Or Latino	50 (90.9%)	29 (96.7%)	21 (84.0%)	
Missing	1 (1.8%)	0 (0.0%)	1 (4.0%)	
Country, n (%)				0.16
USA	45 (81.8%)	27 (90.0%)	18 (72.0%)	
Other ^a	10 (18.2%)	3 (10.0%)	7 (28.0%)	
Weight (kg)				0.94
Mean (SD)	76.6 (24.3)	76.6 (26.3)	76.6 (22.1)	
Median (Min, Max)	74.0 (42.3, 176.8)	73.8 (42.3, 176.8)	74.0 (49.6, 132.3)	
Height (cm)				0.49
Mean (SD)	171.1 (9.4)	170.2 (8.6)	172.2 (10.4)	
Median (Min, Max)	173.0 (150.0, 196.0)	171.0 (154.0, 185.0)	174.0 (150.0, 196.0)	
BMI (kg/m²)				0.83
Mean (SD)	26.2 (8.2)	26.3 (8.4)	26.0 (8.3)	
Median (Min, Max)	24.2 (16.4, 59.1)	26.2 (17.4, 59.1)	23.2 (16.4, 51.0)	
Smoking Status, n (%)				0.63

	LT +	LT + CDx Evaluable Set	LT + CDx Unevaluable Set	P-value
Former Smoker	19 (34.5%)	9 (30.0%)	10 (40.0%)	
Current Smoker	4 (7.3%)	2 (6.7%)	2 (8.0%)	
Never A Smoker	31 (56.4%)	19 (63.3%)	12 (48.0%)	
Missing	1 (1.8%)	0 (0.0%)	1 (4.0%)	

LT: local tests or representative LT, LT+: LT positive.

^a Spain, Switzerland, Australia, France.

Table 34: RET Mutations in Medullary Thyroid Cancer Bridging Study: Baseline Demographic Characteristics for Medullary Thyroid Cancer Samples From LIBRETTO-001 SAS01

	LT+	Secondary LT+ CDx Evaluable Set	Secondary LT+ CDx Unevaluable Set	P-value
Number of Patients	128	39	89	
Age (years) Mean (SD)	55.2 (15.0)	54.8 (15.4)	55.3 (15.0)	0.95
Median (Min, Max)	57.0 (15.0, 87.0)	57.0 (16.0, 82.0)	56.0 (15.0, 87.0)	
Gender, n (%) Female	51 (39.8%)	13 (33.3%)	38 (42.7%)	0.34
Male	77 (60.2%)	26 (66.7%)	51 (57.3%)	
Race, n (%) White	110 (85.9%)	35 (89.7%)	75 (84.3%)	1.00
Asian	7 (5.5%)	2 (5.1%)	5 (5.6%)	
Black Or African American	2 (1.6%)	0 (0.0%)	2 (2.2%)	
Native Hawaiian Or Other Pacific Islander	1 (0.8%)	0 (0.0%)	1 (1.1%)	
Other	7 (5.5%)	2 (5.1%)	5 (5.6%)	
Missing	1 (0.8%)	0 (0.0%)	1 (1.1%)	
Ethnicity, n (%) Hispanic Or Latino	5 (3.9%)	1 (2.6%)	4 (4.5%)	1.00
Not Hispanic Or Latino	120 (93.8%)	38 (97.4%)	82 (92.1%)	
Missing	3 (2.3%)	0 (0.0%)	3 (3.4%)	
Country, n (%) USA	89 (69.5%)	30 (76.9%)	59 (66.3%)	0.30
Other ^a	39 (30.5%)	9 (23.1%)	30 (33.7%)	
Weight (kg) Mean (SD)	74.4 (19.8)	76.3 (22.0)	73.6 (18.9)	0.44
Median (Min, Max)	72.7 (26.8, 148.3)	77.5 (26.8, 148.3)	71.2 (42.6, 123.1)	
Height (cm) Mean (SD)	171.1 (9.2)	171.1 (10.4)	171.1 (8.7)	0.77
Median (Min, Max)	171.0 (150.0, 199.0)	173.0 (150.0, 192.0)	170.0 (152.0, 199.0)	
BMI (kg/m²) Mean (SD)	25.3 (5.9)	26.0 (6.7)	25.1 (5.5)	0.46

Median (Min, Max)	24.9 (11.6, 49.6)	25.8 (11.6, 49.6)	24.7 (15.6, 40.2)	
Smoking Status, n (%)				0.86
Former Smoker	46 (35.9%)	14 (35.9%)	32 (36.0%)	
Current Smoker	8 (6.2%)	3 (7.7%)	5 (5.6%)	
Never A Smoker	74 (57.8%)	22 (56.4%)	52 (58.4%)	

LT: local tests or representative LT; LT+: LT positive.

^a Includes Canada, Singapore, Australia, France, Italy, Spain and United Kingdom.

Table 35. RET Mutations in Medullary Thyroid Cancer Bridging Study: Baseline Disease Characteristics for Medullary Thyroid Cancer Samples From LIBRETTO-001 PAS

	LT+	LT+ CDx Evaluable Set	LT+ CDx Unevaluable Set	P-value
Number of Patients	55	30	25	
ECOG, n (%)				0.62
0	11 (20.0%)	7 (23.3%)	4 (16.0%)	
1	41 (74.5%)	22 (73.3%)	19 (76.0%)	
2	3 (5.5%)	1 (3.3%)	2 (8.0%)	
Tumor Stage at Diagnosis, n (%)				0.20
Stage III/IIIA	2 (3.6%)	0 (0.0%)	2 (8.0%)	
Stage IV/IVA/IVC	51 (92.7%)	29 (96.7%)	22 (88.0%)	
Missing	2 (3.6%)	1 (3.3%)	1 (4.0%)	
Tumor Grade, n (%)				0.70
Well Differentiated	2 (3.6%)	1 (3.3%)	1 (4.0%)	
Moderately Differentiated	1 (1.8%)	0 (0.0%)	1 (4.0%)	
Poorly Differentiated	5 (9.1%)	4 (13.3%)	1 (4.0%)	
Undifferentiated	1 (1.8%)	1 (3.3%)	0 (0.0%)	
Not Applicable	15 (27.3%)	7 (23.3%)	8 (32.0%)	
Unknown	31 (56.4%)	17 (56.7%)	14 (56.0%)	
Metastasis Status, n (%)				0.45
Yes	54 (98.2%)	30 (100%)	24 (96.0%)	

No	1 (1.8%)	0 (0.0%)	1 (4.0%)	
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LT: local tests or representative LT; LT+: LT positive.

Note: Statistical comparison is based on non-missing values.

Table 36. RET Mutations in Medullary Thyroid Cancer Bridging Study: Baseline Disease Characteristics for Medullary Thyroid Cancer Samples From LIBRETTO-001 SAS01

	LT+	Secondary LT+ CDx Evaluable Set	Secondary LT+ CDx Unevaluable Set	P-value
Number of Patients	128	39	89	
ECOG, n (%)				0.66
0	60 (46.9%)	20 (51.3%)	40 (44.9%)	
1	63 (49.2%)	17 (43.6%)	46 (51.7%)	
2	5 (3.9%)	2 (5.1%)	3 (3.4%)	
Tumor Stage at Diagnosis, n (%)				0.46
Stage II/IIB	3 (2.3%)	0 (0.0%)	3 (3.4%)	
Stage III	2 (1.6%)	1 (2.6%)	1 (1.1%)	
Stage IV/IVA/IVB/IVC	119 (93.0%)	36 (92.3%)	83 (93.3%)	
Missing	4 (3.1%)	2 (5.1%)	2 (2.2%)	
Tumor Grade, n (%)				0.37
Well Differentiated	9 (7.0%)	2 (5.1%)	7 (7.9%)	
Moderately Differentiated	1 (0.8%)	1 (2.6%)	0 (0.0%)	
Poorly Differentiated	7 (5.5%)	1 (2.6%)	6 (6.7%)	
Undifferentiated	1 (0.8%)	0 (0.0%)	1 (1.1%)	
Not Applicable	15 (11.7%)	7 (17.9%)	8 (9.0%)	
Unknown	94 (73.4%)	28 (71.8%)	66 (74.2%)	
Missing	1 (0.8%)	0 (0.0%)	1 (1.1%)	
Metastasis Status, n (%)				1.00
Yes	126 (98.4%)	39 (100%)	87 (97.8%)	

No	2 (1.6%)	0 (0.0%)	2 (2.2%)	
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LT: local tests or representative LT; LT+: LT positive.

Note: Statistical comparison is based on non-missing values.

D. **Safety and Effectiveness Results**

Safety Results

The safety with respect to treatment with selpercatinib was addressed during the review of the NDA. Testing for the TSO Comprehensive assay bridging study was conducted retrospectively on banked study specimens, and the test results were not reported to patients. No patient treatment decisions were made as a result of any of the TSO Comprehensive assay test results; therefore, as expected, there were no Unanticipated Adverse Device Effect (UADE) events related to the study products or procedures to patients or operators. Please refer to fda.gov/drugs for complete safety information on RETEVMO (selpercatinib).

Effectiveness Results

Concordance

The results for the primary, secondary, and combined analysis groups are detailed below. These analysis sets are not mutually exclusive as they all include the same set of supplemental negative samples. Additionally, the combined analysis set includes the LT positive samples from the primary and secondary analysis sets.

Concordance Results:

To evaluate the clinical accuracy of the TSO Comprehensive assay, the concordance analysis between the TSO Comprehensive assay and LT results was conducted on 90 RET mutation positive patients (45 from Primary Analysis Set consisting of previously treated MTC subjects and 45 from Secondary Analysis Set consisting of treatment-naïve subjects), and 246 RET mutation negative supplemental samples (Table 37). The PPA and NPA shown in Table 38 were calculated using the LTs as the reference method. The point estimates of PPA and NPA were 97.1% and 99.5% respectively, when excluding TSO Comprehensive invalid results. When including TSO Comprehensive invalid results, the point estimates of PPA and NPA were 74.4% and 75.6%, respectively.

PPA excluding and including invalids separately for the primary and secondary analysis groups are shown in Table 39. For the primary analysis group, there were 2 LT-positive samples that were negative by TSO Comprehensive assay. Of these 2 samples with discordant results, one was from a subject who had a complete response to selpercatinib and LT method detected a RET Met918Thr variant in this subject. The other sample was from a subject who had stable disease after 6 months of follow-up and LT method detected a mutation affecting exon 6 extracellular cysteine. RET variants affecting extracellular cysteine residues are well-established drivers of MTC and are commonly found in MEN2A and familial MTC.

Table 37. Concordance Between TSO Comprehensive Assay and Local Test (LT) Results for RET Mutations in MTC

TSO Comprehensive Assay	LT			
	POS		NEG	Total
	Cohort 1	Cohort 2		
POS	28	39	1*	68
NEG	2**	0	186	188
Invalids	15	6	59	80
Total	45	45	246	336

* This subject was from the supplemental negative sample cohort (negative by LT) and does not have treatment response data. TSO Comprehensive assay detected a RET Cys620Phe variant with a VAF of 0.3807.

**Of these 2 subjects, one had a complete response to selpercatinib and LT method detected a RET Met918Thr variant in this subject. The other subject had stable disease after 6 months of follow-up and LT method detected a mutation affecting exon 6 extracellular cysteine.

Table 38. Agreement Between TSO Comprehensive Assay and Local Test Results for RET Mutations in MTC

Parameter	Agreed	Total	Agreement	95% CI
PPA Exclude Invalids	67	69	97.1%	89.9%-99.6%
NPA Exclude Invalids	186	187	99.5%	97.1%-100%
PPA Include Invalids	67	90	74.4%	64.2%-83.1%
NPA Include Invalids	186	246	75.6%	69.7%-80.8%

Table 39. Agreement Between TSO Comprehensive Assay and Local Test Results for RET Mutations in MTC by Cohort

Subgroup	Parameter	Agreed	Total	Agreement	95% CI
Primary Analysis Set – Prior Therapy	PPA Exclude Invalids	28	30	93.3%	77.9%-99.2%
	PPA Include Invalids	28	45	62.2%	46.5%-76.2%
Secondary Analysis Set – Systemic Therapy Naive	PPA Exclude Invalids	39	39	100%	91.0%-100%
	PPA Include Invalids	39	45	86.7%	73.2%-94.9%

Sensitivity Analysis for Concordance

Missing TSO Comp results for the LT Positive CDx Unevaluable Set were imputed 500 times using a logistic regression model including age, race, BMI, and country. Table 40 shows the PPA and OPA using the imputed TSO Comp results. No sensitivity analysis was performed on the LT Negative CDx Unevaluable Set because the supplemental samples do not have all the baseline characteristics available; the NPA is assumed to be 99.5% (from the primary analysis) for the evaluation of OPA. The PPA and OPA estimates including imputed results were 93.8% (95% CI: 86.4% to 100%) and 98.1% (95% CI: 96.2% to 100%), respectively.

Table 40. CDx Bridging Study: Concordance Between the TSO Comp Assay and the LTs with Imputed CDx Assay Results

Agreement	Minimum	Maximum	Median	Estimate	95% CI ^a
PPA	87.3%	96.4%	94.5%	93.8%	86.4%-100%
OPA	96.6%	98.7%	98.3%	98.1%	96.2%-100%

PPA: Positive percent agreement; OPA: Overall Percent Agreement; CI: confidence interval.

^a CI was calculated based on method described by Gelman et al (Gelman, et al. 2013).

Efficacy Results in the RET Mutation Positive Population

Table 41 shows the objective response rate (ORR) for selpercatinib in the RET mutation-positive subject populations as detected by the TSO Comprehensive assay. The percentage of subjects with a response in the primary analysis set, which included previously treated subjects, was 71.4% (20/28; 95% CI: 51.3%-86.8%). The ORR in the secondary analysis set, which included the treatment-naïve subjects, was 72.7% (24/33; 95% CI: 54.5%, 86.7%). The ORR in the combined analysis set (previously treated and treatment naïve subjects) was 72.1% (44/61; 95% CI: 59.2%, 82.9%).

Table 41. Summary of Best Response Rate by TSO Comprehensive Assay Assessment Status in Primary and Secondary Analysis Sets of MTC Patients (Independent Central Radiology Assessment per RECIST v1.1)

ORR: CR + PR	TSO Comprehensive Positive, LT Positive		TSO Comprehensive Negative, LT Positive		TSO Comprehensive Unevaluable, LT Positive		LT Positive NDA Drug Efficacy Population	
	ORR% (n/N)	95% CIs ¹	ORR% (n/N)	95% CIs ¹	ORR% (n/N)	95% CIs ¹	ORR% (n/N)	95% CIs ¹
Primary Analysis Set ²	71.4% (20/28)	51.3%- 86.8%	50.0% (1/2)	1.3%- 98.7%	68.0% (17/25)	46.5%- 85.1%	69.1% (38/55)	55.2%- 80.9%
Secondary Analysis Set ³	72.7% (24/33)	54.5%- 86.7%	N/A	N/A	72.7% (40/55)	59.0%- 83.9%	72.7% (64/88)	62.2%- 81.7%

¹ CI based on Clopper-Pearson (exact) method.

² Subjects previously treated with systemic therapy.

³ Treatment-naïve subjects.

Sensitivity Analysis for Efficacy Results

Sensitivity analysis was performed to estimate the objective response rate (ORR) of selpercatinib in the entire TSO Comprehensive positive population (TSO Comprehensive+) consisting of the TSO Comprehensive+, LT+ and TSO Comprehensive+, LT- subpopulations. ORR was estimated by the method proposed by Li, including consideration of the frequency of RET mutations in the population (assumed to be 55%), and PPA and NPA as determined from the concordance analysis. The ORR for the missing TSO Comprehensive+, LT- population (ORR [TSO Comprehensive+, LT-] = $c * \text{ORR [TSO Comprehensive+, LT+]}$) varied from 0 to 100% ($c = 0$ to 1), where ORR (TSO Comprehensive+, LT+) was the observed ORR.

ORRs were calculated for the LT+TSO Comp+ and LT+TSO Comp- subpopulations (Table 42). The median ORR including the imputed TSO Comp results in the LT+TSO Comp+ population was

69.8% (95% CI: 57.2% to 82.5%), which was comparable to the observed ORR (LT+TSO Comp+) in the CDx Primary Analysis Set (ORR = 71.4% [20/28]; 95% CI: 51.3% to 86.8%) and the ORR of the LIBRETTO-001 PAS NDA drug efficacy population (ORR = 69.1% [38/55]; 95% CI: 55.2% to 80.9%). The median ORR including the imputed TSO Comp results in the LT+TSO Comp– population was 60.0% (95% CI: 0% to 100%).

Table 42. MTC Bridging Study: Clinical Efficacy (ORR) in Subpopulations with Imputed CDx Assay Results

Subgroup	Estimate	Minimum	Maximum	Median	95% CI ^a
LT+TSO Comp +	69.8%	67.3%	74.0%	69.8%	57.2%-82.5%
LT+TSO Comp -	56.6%	20.0%	83.3%	60.0%	0%-100%

E. Pediatric Extrapolation

RETEVMO (selpercatinib) received approval in the United States for adults and pediatric patients 2 years of age and older with advanced or metastatic medullary thyroid cancer (MTC) with a *RET* mutation, as detected by an FDA-approved test, who require systemic therapy. Under FDA’s pediatric classification category, RETEVMO is approved for use under the aforementioned indications in the “children and adolescents” pediatric grouping. The youngest patient enrolled in the study was 15 years of age.

F. 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. This clinical investigator did not have disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

XII. 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 of Medical Devices, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

For the intended use to identify RET fusions in tumors from TC patients for therapy with selpercatinib, the effectiveness of the TSO Comprehensive assay is supported by the results of a clinical bridging study using specimens from the LIBRETTO-001 clinical study. The data from analytical validation and clinical studies support the reasonable assurance of safety and effectiveness of the TSO Comprehensive assay when used in accordance with the indications for use. Data shows that TC patients who had qualifying RET fusions received benefit from treatment with selpercatinib and support this CDx indication to the TSO Comprehensive assay.

For the intended use to identify RET mutations in tumors from MTC patients for therapy with selpercatinib, the effectiveness of the TSO Comprehensive assay is supported by the results of a clinical bridging study using specimens from the LIBRETTO-001 clinical study. The data from analytical validation and clinical studies support the reasonable assurance of safety and effectiveness of the TSO Comprehensive assay when used in accordance with the indications for use. Data shows that MTC patients who had qualifying RET mutations received benefit from treatment with selpercatinib and support this CDx indication to the TSO Comprehensive assay.

B. Safety Conclusions

The risks of the device are based on data collected in the analytical studies conducted to support PMA approval as described above. The TSO Comprehensive assay is an in vitro diagnostic test, which involves testing of DNA and/or 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 selpercatinib therapy, as per the intended use statement, without clinical benefit, and may experience adverse reactions associated with these therapies, or a delay in administration of more appropriate therapy. Patients with false negative results may not be considered for treatment with the indicated therapy, denying them the potential clinical benefit from treatment with these therapies. There is also a risk of delayed results, which may lead to delay of treatment with the indicated therapy.

C. Benefit-Risk Determination

The probable benefit of the TSO Comprehensive assay, for the selection of RET fusion positive thyroid cancer (TC) patients, for treatment with selpercatinib was demonstrated through a clinical bridging study using specimens from patients screened for enrollment into the LIBRETTO-001 clinical trial. The clinical bridging concordance study showed a PPA of 88.9% (24/27) and an NPA of 100.0%, excluding unknown results, in the combined analysis set. In addition, this bridging study showed clinically meaningful ORR in patients with RET fusions-positive TC detected by TSO Comprehensive assay. The clinical bridging efficacy data, indicated that the RET fusion positive population by the TSO Comprehensive assay had clinically meaningful response in the primary analysis set (pre-treated), with an ORR of 70.0% (7/10; 95% CI: 34.8%, 93.3%), as well as in the secondary analysis set (treatment naïve), with an ORR of 100% (8/8; 95% CI: 63.1%, 100%); notably, the efficacies observed in the NDA primary and secondary analysis sets were maintained. Additional factors, including the analytical accuracy study and analytical performance of the device for RET fusions, included in this submission, have been considered and have demonstrated acceptable performance, further supporting the probable clinical benefit demonstrated by the bridging study; however, due to the small number of subjects in this study, additional clinical data for RET-fusion positive patients is sought in the

post-market space, to further confirm the benefit that has thus far been observed.. In addition, the totality of the analytical and clinical data indicates that the TSO Comprehensive assay has probable benefit in selecting patients with TC with RET fusions for treatment with selpercatinib.

The probable benefit of the TSO Comprehensive assay, for the selection of RET mutation positive advanced/metastatic medullary thyroid cancer (MTC) patients, for treatment with selpercatinib was demonstrated through a clinical bridging study using specimens from patients screened for enrollment into the LIBRETTO-001 clinical trial. The concordance study, to enrolling tests, showed a PPA of 97.1% (67/69) and an NPA of 99.5%, excluding unknown results, which indicates robust performance for this variant type. The percentage of subjects with a response in the primary analysis set, which included previously treated subjects, was 71.4% (20/28; 95% CI: 51.3%-86.8%). The ORR in the secondary analysis set, which included the treatment-naïve subjects, was 72.7% (24/33; 95% CI: 54.5%, 86.7%). Overall, the data was robust, to sensitivity analysis, indicating that this device has acceptable performance in detecting RET small variants (SNVs, MNVs, insertions, deletions), to select MTC patients for treatment with selpercatinib.

Additional factors, in particular analytical accuracy study, in addition to analytical performance of the device for RET mutations in other studies included in this submission, have been considered and have demonstrated acceptable performance, further supporting the clinical bridging study. Thus, the totality of the analytical and clinical data indicates that the TSO Comprehensive assay has probable benefit in selecting patients with MTC with RET mutations for treatment with selpercatinib, also considering the representation of the analytes across analytical and clinical studies.

There is also 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 TSO Comprehensive assay for selection of TC patients with RET fusions, and MTC patients with RET 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. In addition, there could be a delay in the generation of results, which also presents a probable risk to the patient. The risks of erroneous results are partially mitigated by the analytical and clinical performance of the device. The likelihood of false results was assessed by analytical and clinical validation studies, which partially mitigate the probable risk of the TSO Comprehensive assay.

1. Patient Perspective

This submission either did not include specific information on patient perspectives, or the information did not serve as part of the basis of the decision to approve or deny the PMA for this device.

In conclusion, given the available information above, the data support that for the TSO Comprehensive assay, and the indications noted in the intended use statement, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application supports the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the analytical and clinical validation studies support the performance of TSO Comprehensive assay as an aid for identification of *RET* fusions in patients with TC who may benefit from RETEVMO (selpercatinib) treatment, and *RET* mutations in patients with MTC who may benefit from RETEVMO (selpercatinib) treatment.

XIV. CDRH DECISION

CDRH issued an approval order on August 14, 2026.

The final non-clinical condition of approval cited in the approval letter is described below.

1. Illumina, Inc. must provide supplementary clinical data as assessed by comparison to overall response rate from up to 21 additional thyroid cancer patients enrolled and treated with RETEVMO in the clinical study LIBRETTO-001 tested with the TruSight Oncology Comprehensive in the post-market setting. Any reduction in the sample size must be justified in the final study report. This information must be provided to confirm the clinical effectiveness of the TruSight Oncology Comprehensive as a companion diagnostic (CDx) device for identification of patients with thyroid cancer with *RET* fusions who may benefit from treatment with RETEVMO. The final data, conclusions, study reports, and any necessary labeling revisions should be submitted within 1 year of the approval date of this PMA supplement.

XV. 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.

XVI. REFERENCES

1. Gelman, Andrew. Bayesian Data Analysis, Third Edition. United Kingdom, Taylor & Francis, 2014.
2. Li M. Statistical consideration and challenges in bridging study of personalized medicine. J Biopharm Stat. 2015;25(3):397-407. doi: 10.1080/10543406.2014.920340. PMID: 24897254.