SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next generation sequencing oncology panel, somatic variant detection system
Device Trade Name:	PGDx elio tissue complete CDx
Device Procode:	PQP
Applicant's Name and Address:	Personal Genome Diagnostics Inc. (hereafter "PGDx") 3600 Boston St. Suite 10 Baltimore, MD 21224 USA
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P250036
Date of FDA Notice of Approval:	July 30, 2026

II. INDICATIONS FOR USE

PGDx elio tissue complete CDx is a qualitative next-generation sequencing-based in vitro diagnostic device that uses a high-throughput hybridization-based capture technology utilizing DNA isolated from formalin-fixed paraffin embedded tumor tissue. The PGDx elio tissue complete CDx targeted panel can detect single nucleotide variants, small insertions and deletions, copy number amplifications, and translocations. It is intended to be used as a companion diagnostic to identify melanoma patients who may benefit from treatment with the targeted therapies listed in Table 1.1 below in accordance with the approved therapeutic product labeling.

Table 1.1 PGDx elio tissue complete CDx Companion Diagnostic Indications

Indication	Biomarker	Therapy
Melanoma	BRAF V600E	BRAF inhibitors approved by FDA or BRAF/MEK inhibitor combinations approved by FDA
Melanoma	BRAF V600K	BRAF inhibitors approved by FDA or BRAF/MEK inhibitor combinations approved by FDA

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the PGDx elio Tissue Complete CDx Test labeling.

V. DEVICE DESCRIPTION

PGDx elio tissue complete CDx (PGDx ETC CDx) 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 PGDx elio tissue complete CDx assay contains reagents, software, and procedures for testing genomic DNA isolated 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 genomic DNA sequencing from FFPE tissue. Genomic DNA extracted from FFPE tissue is used to prepare libraries, which are then enriched for cancer-related genes and sequenced on the NextSeq 550Dx instrument. PGDx elio tissue complete CDx 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.

The assay detects DNA variants, including BRAF V600E/K (Companion Diagnostic claim listed in Table 1.1 of the Intended Use).

This test is intended to be run exclusively on the Illumina NextSeq 550Dx instrument through the PGDx elio Local Run Manager (LRM) Module.

Test Output
The output of the test includes: Companion Diagnostic (CDx) claims noted in Table 1 of the Intended Use.

Test Kit Contents
PGDx elio tissue complete CDx is for use as part of a test system with the NextSeq 550Dx instrument and associated sequencing reagents. Components of the PGDx elio tissue complete CDx assay are listed in Table 2. PGDx provides reagent kits and software (PGDx elio platform using the LRM Module), which supports both tumor profiling and companion diagnostic (CDx) claims (please refer to the "Software" section below for additional details). The assay contains reagents with sufficient volume to generate 34 DNA libraries inclusive of patient samples and controls. 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 (PGDx elio tissue complete CDx Manual, Part 2 – User Guide).

Table 2. Reagent Components of PGDx elio tissue complete CDx Assay

Component Name (P/N)	Description	PGDx elio tissue complete CDx Box
ER/AT Buffer (20201)	End-repair/A-tailing	Library Preparation Kit, Box 1 of 2
ER/AT Enzyme (20202)	End-repair/A-tailing	Library Preparation Kit, Box 1 of 2
Ligation Buffer (20203)	Ligation	Library Preparation Kit, Box 1 of 2
DNA Ligase (20204)	Ligation	Library Preparation Kit, Box 1 of 2
HotStart PCR Mix (2x) (20205)	Index PCR	Library Preparation Kit, Box 1 of 2
Primer Mix (10x) (20206)	Index PCR	Library Preparation Kit, Box 1 of 2
Nuclease-Free Water (20207)	Index and capture PCR	Library Preparation Kit, Box 1 of 2
MB_01 (20209)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_02 (20210)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_03 (20211)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_04 (20212)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_05 (20213)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_06 (20214)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_07 (20215)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_08 (20216)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_09 (20217)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_10 (20218)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_11 (20219)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_12 (20220)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_13 (20221)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_14 (20222)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_15 (20223)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_16 (20224)	Index PCR	Library Preparation Kit, Box 1 of 2
MB_17 (20225)	Index PCR	Library Preparation Kit, Box 1 of 2

Component Name (P/N)	Description	PGDx elio tissue complete CDx Box
Pre-PCR Beads (20301)	Purification	Library Preparation Kit, Box 2 of 2
Hyb Blocker 1 (20401)	Target capture	Capture Kit, Box 1 of 4
100 µM Primer 1 (20402)	Capture PCR	Capture Kit, Box 1 of 4
100 µM Primer 2 (20403)	Capture PCR	Capture Kit, Box 1 of 4
Hyb Blocker 2 (20404)	Target capture	Capture Kit, Box 1 of 4
RNase Block (20405)	Target capture	Capture Kit, Box 1 of 4
Hybridization Buffer (20406)	Target capture	Capture Kit, Box 1 of 4
DNA Pol Buffer (20407)	Capture PCR	Capture Kit, Box 1 of 4
DNA Pol Enzyme (20408)	Capture PCR	Capture Kit, Box 1 of 4
dNTP Mix 20409)	Capture PCR	Capture Kit, Box 1 of 4
Nuclease- Free Water (20207)	Capture PCR	Capture Kit, Box 1 of 4
Binding Buffer (20501)	Target capture	Capture Kit, Box 2 of 4
Wash Buffer 1 (20502)	Target capture	Capture Kit, Box 2 of 4
Wash Buffer 2 (20503)	Target capture	Capture Kit, Box 2 of 4
Post-PCR Beads (20601)	Purification	Capture Kit, Box 3 of 4
Capture Beads (20602)	Purification	Capture Kit, Box 3 of 4
Capture Bait (20701)	Target capture	Capture Kit, Box 4 of 4
External Control (20802)	Control	External Control Box

Reagents/Consumables Required, but not Provided

- Disposable reservoirs, sterile
- DNA fragment analyzer consumables
- DNA mechanical shearing consumables
- Standard, thin walled 96-well PCR plates
- 96-well deep well plates
- 0.5 mL microtubes (compatible with fluorometer)

- 1.5 or 2 mL LoBind microtubes
- Conical tubes (5 mL, 15 mL, and/or 50 mL)
- 96-well plate adhesive seals Ice or cold block
- 20 µL tips, filtered, low retention
- 200 µL tips, filtered, low retention
- 1000 µL tips, filtered, low retention
- 8-strip domed caps
- 8-strip tubes
- Lint free tissues
- Alcohol wipes
- Appropriate lab Personal Protective Equipment (PPE)

Reagents, Not Provided

- Molecular Biology Grade (MBG) water – Non DEPC-treated
- Tris- ethylenediaminetetraacetic acid (EDTA) Buffer, pH 7.6
- Molecular Biology Grade (MBG) 100% ethanol
- Tween 20
- 10 mM Tris-HCl, pH 8.5
- 1 M Tris-HCl, pH 7.0
- 1 N NaOH
- DNA fragment analyzer reagents
- Fluorometer reagents
- Decontamination spray or wipes adequate for work with DNA Sequencing
- Reagent Kit: NextSeq 550Dx High Output Reagent Kit (300 Cycle)

Table 3: Required Equipment/Materials, Not Provided

Equipment	Notes
NextSeq 550Dx	Genome sequencer
DNA Shearing Instrument	Mechanically shears DNA to the appropriate size.
DNA fragment analyzer	Automated sample processing; determines size, quantity and purity for quick library QC.
Fluorometer	Uses detection of target-specific fluorescence to provide fast and sensitive quantitation of samples prior to library preparation and sequencing. Separate fluorometers are required in pre-PCR and post-PCR areas.
Magnetic stand	Designed for paramagnetic bead purification from standard and deep 96-well microplates. Separate magnetic stands are required in pre-PCR and post-PCR areas.

Equipment	Notes
Mini-centrifuge or micro-centrifuge	Tabletop micro-centrifuge or mini-centrifuge capable of holding 0.5 mL to 2.0 mL tubes. Separate micro- or mini-centrifuge units are required in pre-PCR and post-PCR areas.
Thermal cycler	One 96-well dual-block thermal cycler (or two 96-well single block thermal cyclers) is required in the post-PCR area.
Tabletop 96-well plate centrifuge	Any plate centrifuge capable of maintaining 280 x g for at least 1 minute is sufficient. Separate plate centrifuges are required for pre-PCR and post-PCR areas.
Thermomixer	Thermomixer capable of temperatures ranging from 20 °C to 70 °C and shaking at 1700 RPM. Two thermomixers (or thermal cycler positions) are required in the pre-PCR area and one thermomixer is required in the post-PCR area.
Tabletop vortex mixer	Separate vortex mixers are required in pre-PCR and post-PCR areas.
Single-channel pipettor	Separate sets of pipettors are required in pre-PCR and post-PCR areas. Pipettors should be calibrated regularly and verified accurate within 5% of stated volume.
Multi-channel pipettor (P-20, P200)	Separate sets of pipettors are required in pre-PCR and post-PCR areas. Pipettors should be calibrated regularly and verified accurate within 5% of stated volume.

Software

The proprietary PGDx elio server contains analysis and reporting software necessary for the PGDx elio tissue complete CDx assay. The software is only compatible with NextSeq 550Dx instruments with the PGDx elio LRM Module installed. The PGDx elio server saves reports only and does not provide storage or backup of raw sequencing data. The PGDx elio server should be placed in accordance with consensus standards. It is recommended that the PGDx elio server be placed in a server room.

Sequence data is processed using the PGDx elio platform software. The software contains a user interface that tracks samples and status from sequencing through analysis and reporting. Prior to analysis, users configure analysis plans, including sample names and sequencing index, in the user interface. An automated pipeline of software for bioinformatic analysis is used to identify and report genomic alterations. Reports of identified alterations are available in the user interface for download. Sequencing and sample metrics are available in run and case reports, including sequencing yield and quality.

Instruments
PGDx elio tissue complete CDx assay is validated for use on the Illumina NextSeq 550Dx instrument as part of a test system. PGDx will complete server installation configurations in the lab, including installation qualification (IQ) and operational qualification (OQ), to ensure that the server performs to specifications. Upon installation of Illumina Nextseq 550Dx by an Illumina service representative, PGDx personnel performs the installation of the PGDx elio LRM Module on the NextSeq 550Dx instrument. The Installation of the PGDx elio LRM Module makes the PGDx elio tissue complete CDx assay available as a dropdown selection in the NextSeq 550Dx software with setting up a sequencing run, ensuring that the run configuration conforms to IVD parameters. Other required equipment and the specifications for the specific equipment for use with the PGDx elio tissue complete CDx assay are described in the "Required Equipment/ Materials, Not Provided" section.

Principles of Operation
The PGDx elio tissue complete CDx assay workflow is described below.

Sample Requirements, Collection, and Preparation
The PGDx elio tissue complete CDx assay requires genomic DNA isolated from FFPE tissue specimens. 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 analysis. The assay is validated for use with DNA recovered from tissue with a minimum of 20% viable tumor nuclei. If less than 100% of the tissue section contains ≥ 20% tumor purity, the tissue should be macro-dissected to select as much viable tumor as possible and minimize the amount of adjacent non-tumor tissue.

DNA Extraction
PGDx elio tissue complete CDx requires DNA isolated from FFPE tissue using appropriate extraction methods. The recommended DNA input for the assay is 100 ng at a minimum concentration of 1 ng/µL; however, results can be obtained with a minimum genomic DNA input of 50 ng. Extraction kits should be able to yield this 50 ng input amount at a minimum concentration of 1 ng/uL. Shearing requires 100 ng DNA in a final volume of 130 µl (0.77 ng/µl) in TE buffer (not provided) used as the diluent. This assay has been validated with extracted DNA stored at -20 ◦C for up to 9 months

Library Preparation
Library preparation is performed using the Library Prep Kit (B12119, B12120). Genomic DNA is quantified using a fluorometer. Genomic DNA molecules are then mechanically sheared to a target size of 200 bp and subjected to a magnetic bead purification step to remove smaller fragments and perform an exchange of buffer. Fragmented DNA is end-repaired, phosphorylated, and A-tailed. Indexed adapters are then ligated to the A-tailed DNA molecules. Unincorporated adapters and reagents are removed by magnetic bead purification. Adapter-ligated DNA is enriched by PCR amplification. Primer dimers and residual reagents are removed by magnetic bead

purification. Library quality is assessed using a DNA fragment analyzer prior to hybrid capture.

Hybrid Capture and Enrichment
The adapter-ligated library is hybridized with biotinylated RNA library baits, and targeted regions are captured using magnetic streptavidin coated beads. Captured libraries are purified to remove baits and incompletely hybridized DNA fragments. Captured libraries are enriched by PCR amplification. Primer dimers and residual reagents are removed by magnetic bead purification. Final library quality is assessed using a DNA fragment analyzer prior to sequencing. Sample libraries are then normalized into a sequencing pool of up to 15 samples and the external control.

Sequencing
Pooled sample libraries are fluorometrically quantified, loaded on a sequencing flow cell and 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
Sequence data is processed using the PGDx elio platform software. The software contains a user interface that tracks sample status from sequencing through analysis and reporting. Users configure sequencing runs, and an automated pipeline of software for bioinformatic analysis identifies and reports genomic alterations. After processing, the software generates FASTQ files containing sequences and quality scores for each sample. The FASTQ files are then aligned to a reference genome to generate BAM files, which are processed for variant calling.

Alignment
Genome alignment is performed to map sequence reads for each sample to the human reference genome (version GRCh37/hg19) using BWA-MEM (Li 2013) and Bowtie2 (Langmead 2012) software. Alignments are saved as Binary Alignment Map (BAM) formatted files, which contain read placement information relative to the reference genome with quality scores. Aligned BAM files are further processed in the pipeline to identify genomic alterations.

Sequence Coverage
For a sample to pass quality metrics and report alterations, sequence coverage is assessed across the PGDx elio tissue complete CDx panel, requiring 90% of targeted regions with >100x median coverage. A subset of designed regions in the PGDx elio tissue complete region of interest (ROI) experiences variable coverage and is excluded from reporting.

Additional sequence coverage assessments are performed to report specific variants in select genes, where failure to meet thresholds results in an indeterminate status.

Sequence Mutations
The PGDx elio tissue complete CDx pipeline identifies sequence mutations, including SNVs. Candidate mutations are evaluated for characteristics of high confidence somatic variants, including sequence coverage and quality, genomic context, functional annotation, germline status, and prevalence in a database of normal controls.

Report Generation
PGDx elio tissue complete CDx generates a report for the Companion Diagnostic (CDx) claims noted in Table 1.1 of the Intended Use. Variants are categorized into Companion Diagnostic Findings. The Companion Diagnostic Findings are limited to biomarkers approved by the FDA as a CDx in specific indications.

Reporting software was designed to mask results that have low confidence allele frequencies levels near the calling threshold. The PGDx elio tissue complete analytical pipeline calculates a 95% CI around the estimated MAF for all sequence mutations.

Indeterminates*: Q*uality metrics are assessed to check for low coverage or incomplete data needed to identify an alteration. Indeterminate status is reported when 1) no evidence of the alteration was found, but minimum coverage was not met to support the verified limit of detection, or 2) insufficient evidence of the alteration was observed, but minimum coverage thresholds were not met to report the variant. Supporting evidence of detected alterations and coverage in read data is available in the Complete Case Record (CCR). Indeterminate status is reported when evidence of a sequence mutation is observed in regions of low coverage below < 80x. Indeterminate status is also reported for select genes and codons when low coverage is observed and there is no evidence of an alteration. The minimum coverage threshold range is 116x - 248x in cases where select genes and codons are called negative.

Controls

Negative Control: A no template control (NTC) can be processed to serve as a negative control to validate the acceptability of all the test samples processed through library preparation and capture steps by testing for sample or reagent contamination. The NTC is not included on the sequencing run.

Positive Control: An external control that is provided in the PGDx elio tissue complete assay reagent kit consists of cell line derived-DNA with multiple verified sequence mutations. The external control is processed from library preparation through sequencing to serve as an end-to-end control to demonstrate assay performance. The external control is checked for quality during library preparation and after sequencing. Failure of the external control to meet the pre-defined quality metrics will result in all test samples on the run being reported as “No result.”

Quality Metrics

Reporting takes in account the quality metrics outlined in Table 4. Quality metrics are assessed across the following categories: • Batch-level: Metrics that are quantified per sequencing run; failing batch-level metrics generates "No result" reports samples failing these criteria. If the external control fails these criteria, "No result" is reported for the entire batch of samples. • Sample-level: Metrics that are quantified per sample; generates "No result" report for a sample failing QC. • Analyte-level: Metrics that are quantified for individual alteration types and positions, such as sequence coverage. Variants passing analyte-level QC are reported.

Table 4. Summary of PGDx elio tissue complete CDx Post-Sequencing Quality Control Metrics

Quality Metric	Level of Qualification	Qualification Step	Passing Criteria
DNA Input	Sample-level	DNA extraction, Fluorometer	DNA amount: ≥ 100 ng recommended ≥ 50 ng minimum
Library Prep	Sample-level	Library prep, DNA fragment analyzer	Pre-capture library yield ≥ 15 ng/µL within 180-800 bp with an average size ≥ 250 bp
Target Enrichment Yield	Sample-level	Hybrid Capture, DNA fragment analyzer	Final library yield ≥ 10 nM within 180-800 bp with an average size ≥ 250 bp
Primer Dimer Check	Sample-level	Hybrid Capture, DNA fragment Analyzer	Final library contains < 5% primer dimer in 100-180 bp region settings
NTC Check	Batch-level	Hybrid Capture, DNA fragment Analyzer	Concentration ≤ 5 ng/µL within 180-800 bp region settings for an average size > 250bp
Cluster Density	Batch-level	Post-sequencing, automated in software	Sequencer Cluster Density ≥ 130
Q30 Reads	Batch-level	Post-sequencing, automated in software	%Q30 (Read1 and Read4) ≥ 80% %Q30 (Read2 and Read3) ≥ 85%
External Control	Batch-level	Post-sequencing, automated in software	All expected sequence mutations are detected

Quality Metric	Level of Qualification	Qualification Step	Passing Criteria
Percent Regions Covered	Sample-level	Post-sequencing, automated in software	≥ 90% exons with > 100x Median Distinct Coverage
Percent Reads Identified	Sample-level	Post-sequencing, automated in software	Percent Reads Identified 15%-35%

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several other FDA approved CDx alternatives for the detection of BRAF V600 mutations as shown in Table 5. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.
For additional details, see the list of FDA Cleared or Approved Companion Diagnostic Devices at List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools) | FDA.

Table 5. List of other available FDA approved CDx alternatives for BRAF V600 E/K mutations in melanoma

Diagnostic Name (Manufacturers)	Sample Type	Drug Trade name (Generic) NDA/BLA	Biomarker(s)	Biomarker(s) (Details)	PMA/510(k)/513(f)(2)/ HDE (Approval/ Clearance/ Grant Date
Cobas 4800 BRAF v600 Mutation Test (Roche Molecular Systems, Inc)	Melanoma Tissue	Zelboraf (vemurafenib) NDA 202429	BRAF	V600E	P110020 (08/17/2011)
Cobas 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc)	Melanoma Tissue	Cotellic (cobimetinib) NDA 206192 in combination with Zelboraf (vemurafenib) NDA 202429	BRAF	V600E or V600K	P110020/S016 (11/07/2016)
FoundationOne CDx (Foundation Medicine, Inc)	Melanoma Tissue	Mekinist (trametinib) NDA 204114	BRAF	V600E and V600K	P170019 (11/30/2017)

Diagnostic Name (Manufacturers)	Sample Type	Drug Trade name (Generic) NDA/BLA	Biomarker(s)	Biomarker(s) (Details)	PMA/510(k)/513(f)(2)/ HDE (Approval/ Clearance/ Grant Date
FoundationOne CDx (Foundation Medicine, Inc)	Melanoma Tissue	Tecentriq (atezolizumab) BLA 761034 in combination with Cotellic (cobimetinib) NDA 206192 and zelboraf (vemurafenib) NDA 202429	BRAF	V600 mutations	P170019/S030 (01/19/2022)
MI Cancer Seek (MCS) (Caris Life Sciences)	Melanoma Tissue	Mekinist (trametinib) NDA 204114	BRAF	V600E or V600K	P240010 (11/05/2024)
THXID BRAF Kit (bioMerieux Inc.)	Melanoma Tissue	Mekinist (trametinib) NDA 2014114	BRAF	V600E or V600K	P120014 (05/29/2013)
THXID BRAF Kit (bioMerieux Inc.)	Melanoma Tissue	Tafinlar (dabrafenib) NDA 202806	BRAF	V600E	P120014 (05/29/2013)
THXID BRAF Kit (bioMerieux Inc.)	Melanoma Tissue	Braftovi (encorafenib) NDA 210496 in combination with Mektovi (binimetinib) NDA 210498	BRAF	V600E or V600K	P120014/S008 (06/27/2018)
MI Cancer Seek (MCS) (Caris Life Sciences)	Melanoma Tissue	Mekinist (trametinib) NDA 204114	BRAF	V600E or V600K	P240010 (11/05/2024)
THXID BRAF Kit (bioMerieux Inc.)	Melanoma Tissue	Mekinist (trametinib) NDA 2014114	BRAF	V600E or V600K	P120014 (05/29/2013)

VII. MARKETING HISTORY

PGDx elio tissue complete CDx has not been marketed as an IVD in the United States or any foreign country.

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 PGDx elio tissue complete CDx results and subsequently improper patient management decisions and treatment decisions for patients with melanoma carrying BRAF V600 E/K mutations. 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 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 NON-CLINICAL STUDIES

1. Laboratory Studies

The performance of PGDx elio tissue complete CDx test was established using DNA isolated from FFPE tissue in melanoma patients. The FFPE samples were analyzed for BRAF V600E or V600K mutations, which were utilized to determine the eligibility for the appropriate treatment.

Accuracy

BRAF V600E/K accuracy in melanoma was assessed in the clinical validation study using 379 intended use samples where PGDx elio tissue complete CDx BRAF V600E/K results were compared to results of an FDA-approved RT-PCR assay (see "Clinical" section).

In addition, analytical accuracy for BRAF V600E/K was assessed using a dataset consisting of 146 pan-cancer FFPE samples made up of 20 BRAF-positive samples (10 melanoma samples and 10 non-melanoma samples) and 126 BRAF-negative samples. PGDx performance was compared to 2 orthogonal methods: 1 NGS-based assay and 1 PCR-based assay. Samples were blinded to lab operator or data analyst until after the study was completed. Variant concordance was assessed between PGDx ETC CDx variant calls and the associated orthogonal method, which showed 100% PPA in 2 orthogonal methods for both variants (Table 6).

Table 6. The total number and concordance of BRAF V600E and V600K samples used in the CDx accuracy in melanoma

Test	Variant	No. Samples	True Positives	False Negatives	False Positives	True Negatives	PPA	NPA
NGS Targeted Panel	V600E	70	7	0	0	63	100%	100 %
NGS Targeted Panel	V600K	70	1	0	0	69	100%	100 %
PCR	V600E	34	12	0	0	22	100%	100 %
PCR	V600K	34	0	0	0	22	n/a	100 %

Assay Acceptance Rates

Multiple factors can influence overall robustness and performance of complex molecular tests, including pre-analytical factors and overall sample quality. Data aggregated for melanoma clinical FFPE samples (excluding the clinical validation study) showed an invalid rate of 5.6%. However, in the clinical validation study 379 samples (of 389) were successfully tested giving an invalid rate of 2.6% (assay success rate of 97.4%).

Precision

Four studies were conducted to assess precision: single site reproducibility, 2 multi-site reproducibility studies (MSR1 and MSR 2), and a single site precision study used to confirm the established LoD for BRAF V600K.

Single Site Reproducibility (BRAF V600E)

Single-site reproducibility was assessed by detecting variant levels across clinical and cell line blends; one case included a cell line blend targeting BRAF V600E at an expected VAF of 4.74%. DNA was sequenced across two different NextSeq instruments, and results were compared within runs, between operators, and between non-consecutive days. Reproducibility was evaluated using PPA and NPA, with expected variant status as the reference (Table 7). BRAF V600E demonstrated 100% PPA (16/16) and 100% NPA (32/32) across all conditions tested, with an average VAF of 5.08% (range 3.90% to 5.40% VAF).

Table 7. Single-site Reproducibility BRAF V600E Performance Summary

Variant	Within Run		Between Operator		Between Day		Between Instrument	
	PPA (n/N) (CI95)	NPA (n/N) (CI95)	PPA (n/N) (CI95)	NPA (n/N) (CI95)	PPA (n/N) (CI95)	NPA (n/N) (CI95)	PPA (n/N) (CI95)	NPA (n/N) (CI95)
BRAF V600E	100% (16/16) (80.6%, 100%)	100% (32/32) (89.3%, 100%)	100% (16/16) (80.6%, 100%)	100% (32/32) (89.3%, 100%)	100% (16/16) (80.6%, 100%)	100% (32/32) (89.3%, 100%)	100% (16/16) (80.6%, 100%)	100% (32/32) (89.3%, 100%)

CI95 refers to the 95% Confidence Intervals

Single Site Reproducibility (BRAF V600K)

To assess the precision of PGDx ETC CDx for BRAF V600K, samples from the LoD confirmation study were assessed for within run repeatability, inter-operator precision, and inter-lot precision at a single site. FFPE sample DNA from an advanced melanoma patient was blended with normal FFPE DNA to target the established LoD of 4.42%. Data from 20 variant observations across two reagent kit lots, comparing a total of 4 batches, were analyzed to assess the within-run repeatability, inter-operator precision, and inter-lot precision of PGDx ETC CDx. The average positive agreement (APA) for BRAF V600K was calculated across lots, operators, and within runs (Table 8). The results demonstrate 100% concordance/call rate for BRAF V600K across all replicates, including between lots and operators, with 100% APA (380/380) in an aggregate pairwise comparison. Hit rate per lot is also provided (Table 9).

Table 8. BRAF V600K Primary lot to lot pairwise replicate analysis

Comparison Type	Xpp	Xpn	Xnp	Xnn	APA (%) n/N (CI95)	ANA (%) n/N (CI95)
Overall	190	0	0	0	100% 380/380 (99.0%, 100%)	n/a
Between Operator	150	0	0	0	100% 300/300 (98.7%, 100%)	n/a
Within Operator	40	0	0	0	100% 80/80 (95.4%, 100%)	n/a
Between Lot	100	0	0	0	100% 200/200 (98.1%, 100%)	n/a
Within Lot	90	0	0	0	100% 180/180 (97.9%, 100%)	n/a

CI95 refers to the 95% Confidence Intervals

Table 9. BRAF V600K Hit Rates per Lot

Lot	Variant	Positive (n)	Negative (n)	Total (n)	Median VAF (%)	Mean VAF (%)	Hit Rate % n/N (CI95%)
Lot 1	BRAF V600K	10	0	10	4.55	4.75	100% 10/10 (72.2%, 100.0%)
Lot 2	BRAF V600K	10	0	10	4.75	5.14	100% 10/10 (72.2%, 100.0%)

CI95 refers to the 95% Confidence Intervals

Multi-site Reproducibility (MSR1)

Multi-site reproducibility of the PGDx elio tissue complete CDx assay was assessed across 3 different sites with DNA extracted from 6 blended cell line samples and a single colorectal FFPE sample. These samples represented a variety of DNA alterations, including BRAF V600 Each of the samples was tested in duplicate by 2 operators across 6 distinct sequencing runs at each of the 3 independent laboratory sites using a single kit lot. BRAF V600E reproducibility from MSR1 demonstrated 100% PPA (72/72) and 100% NPA (180/180) (Table 10) across all sites and conditions. BRAF V600K was not observed in the MSR1 study; however, no false positive observations were identified, indicating 100% NPA (252/252) across all conditions.

Table 10: MSR1 Analytical Reproducibility of BRAF V600E/K

Study	Sample Type	BRAF Variant Assessed	Performance
Multi-site Reproducibility 1	Cell line blends	V600E	100% PPA (72/72) (94.9%, 100%) 100% NPA (180/180) (97.9%, 100%)
Multi-site Reproducibility 1	Cell line blends	V600K	PPA: N/A no observations 100% NPA (252/252) (98.5%, 100%)

Multi-site Reproducibility (MSR2)

Multi-site reproducibility of the PGDx elio tissue complete CDx assay was assessed across 3 different sites, using DNA extracted from 3 BRAF V600E-positive non-melanoma samples and 1 BRAF V600K-positive melanoma sample. Each of the 4 samples was tested in duplicate by 2 different operators on 12 sequencing runs across 3 non-consecutive days at each of the 3 independent laboratory sites using a single kit lot. Reproducibility was assessed using 3 ways; (1) agreement for each positive variant detected across all replicates is reported (Positive call rate), and (2) modal analysis was

used for per specimen reproducibility, and (3) Average Positive Agreement (APA) and Average Negative Agreement (ANA). Reproducibility for BRAF V600E (3 non-melanoma samples) from MSR2 was 100% PPA (107/107) and 100% NPA (388/388) (Table 11) for all samples with a passing status across all sites and other conditions. Similarly, reproducibility for BRAF V600K (1 melanoma sample) from MSR2 was 100% PPA (36/36) and 100% NPA (459/459) for all samples with a passing status across all sites and conditions tested.

Table 11: MSR2 Analytical Reproducibility of BRAF V600E/K

Study	Sample Type	BRAF Variant Assessed	Performance
Multi-site Reproducibility 2	Clinical FFPE + 1 cell line	V600E	100% PPA (107/107) (96.5%, 100%) 100% NPA (388/388) (99.0%, 100%)
Multi-site Reproducibility 2	Clinical FFPE	V600K	100% PPA (36/36) (90.4%, 100%) 100% NPA (459/459) (99.2%, 100%)

An additional multi-site reproducibility study to evaluate detection of BRAF V600E/K mutations was performed using two clinical FFPE melanoma samples (1 positive for V600E and 1 positive for V600K) diluted with normal FFPE DNA to 1-1.5x the confirmed limit of detection. These 2 blended clinical samples were then assessed across 2 sites, by 2 operators per site, and with 2 reagent lots, in triplicate, for a total of 24 replicates per variant (2 sites x 2 operators x 2 lots x 3 replicates = 24 replicates). The outcome of the study resulted in 100% concordance across all sample replicates for the CDx variants (Table 12). APA and ANA was also evaluated to assess the imprecision caused by different sources of variance across both sites (Table 13).

Table 12. Multi Site reproducibility BRAF V600E/K overall concordance

Variant	Assessment	All Observations	Average Between Runs	Average Between Operators	Average Between Sites
BRAF V600E	PPA (n/N), CI95	100% 24/24, (86.2%, 100.0%)	100% 3/3, (43.85%, 100.0%)	100% 6/6, (60.96%, 100.0%)	100% 12/12, (75.75%, 100.0%)
	NPA (n/N), CI95	100% 24/24, (86.2%, 100.0%)	100% 3/3, (43.85%, 100.0%)	100% 6/6, (60.96%, 100.0%)	100% 12/12, (75.75%, 100.0%)

Variant	Assessment	All Observations	Average Between Runs	Average Between Operators	Average Between Sites
BRAF V600K	PPA (n/N), CI95	100% 24/24, (86.2%, 100.0%)	100% 3/3, (43.85%, 100.0%)	100% 6/6, (60.96%, 100.0%)	100% 12/12, (75.75%, 100.0%)
	NPA (n/N), CI95	100% 24/24, (86.2%, 100.0%)	100% 3/3, (43.85%, 100.0%)	100% 6/6, (60.96%, 100.0%)	100% 12/12, (75.75%, 100.0%)

CI95 refers to the 95% Confidence Intervals

Table 13. BRAF V600E and V600K APA and ANA Summary

Comparison Type	V600E APA (%) n/N, (CI95)	V600K ANA (%) n/N, (CI95)	APA (%) n/N, (CI95)	ANA (%) n/N, (CI95)
Overall	100.0% 120/120, (96.9%, 100.0%)	100.0% 120/120, (96.9%, 100.0%)	100.0% 120/120, (96.9%, 100.0%)	100.0% 120/120, (96.9%, 100.0%)
Between Site	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)
Between Operator	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)
Between Lot	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)
Within Run	100.0% 48/48, (92.6%, 100.0%)	100.0% 48/48, (92.6%, 100.0%)	100.0% 48/48, (92.6%, 100.0%)	100.0% 48/48, (92.6%, 100.0%)

CI95 refers to the 95% Confidence Intervals

Additionally, an aggregate assessment of assay performance near LoD, assessing both the multi-site reproducibility study and the LoD confirmation study using clinical samples, resulted in 100% (24 replicates in the multi-site study + 20 replicates in the LoD confirmation study = 44/44) call rate for both V600E and V600K variants in melanoma clinical samples. The aggregate mean VAF for BRAF V600E and V600K across the 44 replicates assessed were 3.46% and 5.35%, respectively, near the clinical samples confirmed LoDs of 3.1% VAF for V600E and 4.7% VAF for V600K.

Lot to Lot Precision

Performance of PGDx elio tissue complete CDx was assessed across 3 unique kit lots by determining concordance of variant calls in FFPE tissue samples. The 3 unique kit lots were utilized to process 2 test cases (1 BRAF V600E-positive cell line, 1 BRAF V600K-positive cell line blend) in triplicate. All samples were sequenced on the same instrument. BRAF V600E APA was 100% and the ANA was 100% for each of the three lots (Table 14). The overall performance is consistent with that of the reproducibility study.

Table 14. Lot to Lot Precision

Gene and Variant	Lot Comparison	True Positive	False Negative	False Positive	True Negative	APA (%) n/N (CI95)	ANA (%) n/N (CI95)
BRAF V600E	L1 and L2	3	0	0	12	100% (3/3) (61%, 100%)	100% (12/12) (86.2%, 100%)
BRAF V600E	L1 and L3	3	0	0	12	100% (3/3) (61%, 100%)	100% (12/12) (86.2%, 100%)
BRAF V600E	L2 and L3	3	0	0	12	100% (3/3) (61%, 100%)	100% (12/12) (86.2%, 100%)

CI95 refers to the 95% Confidence Intervals

Limit of Blank (LoB)

Non-cancerous FFPE tissues (n=42) and reference materials from National Institute of Standards and Technology (n=2, NA24531 and NA24385) were evaluated for analytical specificity to assess the risk of false positives in normal tissues when detecting BRAF V600E/K using PGDx elio tissue complete CDx assay. Reference standards were processed in 5 replicates each with 10 total replicates at 100ng input, and FFPE samples were tested at two DNA input levels (50 ng and 100 ng) across two reagent lots, resulting 89 total replicates. Specificity for the reference standards was 100% with no unverified BRAF V600E/K mutations detected across 5 replicates for each standard. Similarly, no BRAF V600E/K variants were observed in any FFPE sample at either DNA input level, resulting an overall specificity of 100% (89/89).

Limit of Detection (LoD)

LoD of the PGDx elio tissue complete CDx assay is defined as the mutant allele fraction (MAF) at which 95% of replicates for a variant type are reliably detected. The LoD study was comprised of two steps: LoD establishment using cell lines and LoD confirmation with FFPE clinical tumor samples from clinical melanoma cases. Details of the LoD confirmation results are discussed and shown below.

The LoD for BRAF V600E and V600K were established using a dilution series derived from a cell line (for V600E) and a melanoma FFPE sample (for V600K). The LoD was subsequently confirmed in one unique melanoma FFPE sample per CDx variant, diluted to the established LoD level in noncancerous DNA derived from FFPE tissue. Each clinical sample was tested in 10 replicates across 2 reagent kit lots, yielding 20 replicates per sample. The confirmed LoD was defined as the mean variant allele frequency (VAF) across the 2 lots (Table 15).

Table 15. Analytical Sensitivity (LoD VAF) for CDx Variants in FFPE Tumor Tissue

Mutation Type	Mutation	Tumor Type	Average VAF (%)
SNV	BRAF V600E	Melanoma	3.1
MNV	BRAF V600K	Melanoma	4.7

DNA Extraction

Compatibility was assessed for BRAF V600E/K in 2 studies. First, an FFPE tissue sample containing BRAF V600E was extracted with 3 extraction methods and processed in duplicate. Second, an FFPE tissue sample containing BRAF V600E was extracted in duplicate by 2 operators with 3 extraction methods and processed in duplicate. In both studies, Method 2 (bead-based) and Method 3 (automated) were compared to the reference Method 1 (column-based). In the first study, BRAF V600E PPA was 100% (2/2) and NPA was 100% (12/12) when comparing to the reference method. In the second study, BRAF V600E PPA was 100% (16/16), and NPA was 100% (55/55) when comparing to the reference method. PGDx elio tissue complete CDx is compatible with genomic DNA extracted from FFPE samples using any appropriate commercially available FFPE extraction method.

DNA Input

BRAF V600E/K mutations were evaluated using 61 clinical FFPE samples (in 17 tumor types) previously tested in the panel-wide analytical accuracy study. Each sample was blinded and tested at 50 ng DNA input with PGDx elio tissue complete CDx to test concordance (PPA and NPA) to the reference input of 100 ng. PGDx elio tissue complete CDx yielded concordant results for BRAF V600E with 100% PPA (7/7) and 100% NPA (49/49) observed in the 50 ng DNA input compared to the 100 ng reference. These data indicate PGDx elio tissue complete CDx is robust around the recommended 100 ng DNA input for BRAF V600E/K.

Analytical Specificity

Exogenous Interfering Substances

The impact of exogenous interfering substances on the performance of the PGDx elio tissue complete CDx assay was assessed in the presence of 4 interfering substances(Proteinase K, Indexed adapters, melanin, and ethanol) at varying amounts

(Table 16). The samples were evaluated for concordance of variant calls when compared to samples processed without the interfering substances. Replicates for 5 test cases were analyzed for 8 experimental and 2 baseline conditions. Analysis of BRAF V600E showed high PPA (100% 6/6) and NPA (100% 114/114) across Proteinase K conditions. Please refer to K192063 for additional data for other named interferents.

Endogenous Interfering Substances

The impact of necrosis and FFPE block age on the performance of PGDx elio tissue complete CDx was evaluated by assessing the first pass and overall pass rates of samples processed in the panel-wide accuracy study. Of 521 samples enrolled for accuracy, 448 were evaluated for necrosis over a range of 0-75%, and 378 were evaluated for age of block over a range of 0-253 months.

Of the 521 clinical samples in the study, 46 were positive for BRAF V600E, and 16 of them were melanoma samples. Percent necrosis of these samples ranged from 0% to 50%, and block age ranged from 0 (DNA Extracted same day as fixation) days to 189 days. A total of 19 of the BRAF V600E positive samples (10 were melanoma samples) had orthogonal data from the comparator assay with 100% PPA (19/19). There were 4 BRAF V600K positive samples, all of which were melanoma samples. One BRAF V600K positive sample had orthogonal data from the comparator assay with 100% PPA (1/1). Percent necrosis for this sample was 0% and FFPE block age was 21 days (Table 16).

Table 16: BRAF V600E/K in Performance Summary

Variant	% Necrosis Range	Block Age Range	PPA (n/N) (CI95)	Success Rate
BRAF V600E	0% - 50%	0 – 189 days	100% (19/19) (83.2%, 100%)	100%
BRAF V600K	0%	21 days	100% (1/1) (20.7%, 100%)	100%

CI95 refers to the 95% Confidence Intervals

Carryover/Cross contamination

Cross-contamination (contamination from one sample to another within the same batch) and sample carryover (contamination from a previous sequencing run when using the same instrument) were assessed by evaluating false positive and false negative variant calls in 29 FFPE samples. Seven of the 29 cases had known positive variants; the remaining samples were known negative samples. All FFPE samples were assessed across 2 batches to test for contamination within and between runs. In batch 1, a checkerboard pattern within a 96-well plate was created by alternating the samples with representative positive variants and known negative samples. Batch 2 contained known negative samples and was pooled and sequenced directly after completion of batch 1 sequencing, following standard instrument cleaning procedures. No positive

variant results were observed in known negative samples tested. Please refer to K192063 for additional information.

Guardbanding
Systematic variations of reagent volumes, enzyme concentrations, and bead drying times in the laboratory workflow were tested to verify the robustness of PGDx elio tissue complete CDx. A total of 14 guardbanding conditions were tested, which included variations to the ER/AT step, the ligation step, the post-ligation PCR and cleanup steps, capture baiting, and post-hybridization PCR. A BRAF V600E-positive melanoma clinical FFPE sample, and 1 BRAF V600K-positive clinical FFPE sample blend) were assessed in this study. The PPA and NPA for BRAF V600E and V600K variants were 100% across all evaluated conditions.

Stability

Sample Stability
The sample stability study tested the robustness of PGDx ETC CDx using 2 DNA sample types: genomic DNA stored after extraction and pooled libraries stored prior to sequencing. DNA stability was assessed for extracted DNA stored at ≤ -20 °C prior to processing using a total of 3 unique BRAF V600E/K melanoma samples (2 BRAF V600K-positive melanoma samples and 1 2 BRAF V600E-positive samples, one of which is in melanoma tissue). The duration of DNA storage at the time of the evaluation in this study ranged from 97 to 377 days, with a median of 330 days (~10.6 months) and a mean of 295 days (~9.5 months). DNA stability was assessed for pooled sequencing libraries stored at ≤ -20 °C prior to sequencing using a total of 2 BRAF V600E-positive melanoma samples. Samples were sequenced at the time of initial extraction (or prior to freezing of the pools) to determine a reference status of the variants, which was labeled as T0.

For the genomic DNA stability assessment, both BRAF V600E and V600K had a 100% PPA (2/2) and 100% NPA (42/42) (Table 17). The 2 cases that contained V600E were stored for 97 and 354 days, and the 2 cases that contained V600K were stored for 328 and 330 days before reprocessing

Table 17: BRAF V600 Sample Stability Performance (All Conditions)

Gene	Variant	PPA (n/N) (CI95)	NPA (n/N) (CI95)
BRAF	V600E	100% (2/2) (34.2%, 100%)	100% (42/42) (91.6%, 100%)
BRAF	V600K	100% (2/2) (34.2%, 100%)	100% (42/42) (91.6%, 100%)

CI95 refers to the 95% Confidence Intervals

The stability of sequencing pools for BRAF V600E PPA was 100% (3/3) and NPA was 100% (23/23) (Table 18). There were no observations of BRAF V600K in the sequencing pools, however 100% specificity (26/26) was observed.

Table 18. BRAF V600E sequencing pools stability

Gene	Variant	7-week PPA	7-week NPA	10-week PPA	10-week NPA
BRAF	V600E	100% (2/2) (34.2%, 100%)	100% (11/11) (74.1%, 100%)	100% (1/1) (20.7%, 100%)	100% (12/12) (75.8%, 100%)
BRAF	V600K	N/A	100% (13/13) (77.2%, 100%)	N/A	100% (13/13) (77.2%, 100%)

In the clinical cohort (n = 44 passing samples), BRAF V600E PPA was 100% (2/2) and NPA was 100% (42/42). BRAF V600K PPA was 100% (2/2), and NPA was 100% (42/42). The samples containing V600E had been stored for 354 and 97 days, while the samples containing V600K had been stored for 328 and 330 days before reprocessing. In the sequencing pools, BRAF V600E PPA was 100% (3/3) and NPA was 100% (23/23). V600K was not observed in the pools for a 100% NPA (26/26).

Real Time Reagent Stability

The real time stability study determined the shelf-life of PGDx ETC CDx over time by assessing variant concordance between the standard condition (0 months) and various timepoints (up to 19 months). A cell line blend with BRAF V600E was assessed in this study and was tested in triplicate across three unique lots at seven different timepoints: 0 months (T0), 3 months (T3), 4 months (T4), 7 months (T7), 13 months (T13), 18 months (T18), and 19 months (T19).

PGDx ETC CDx demonstrated stability concordance of BRAF V600E was 100% PPA (3/3) and 100% NPA (12/12) for each reagent kit lot and each time point in the diluted external control.

Based on the results from this real time stability study, concordance was not impacted when processed with kits being stored up to 19 months, giving a shelf-life claim of 18 months.

Transport Stability

The transport stability study assessed whether the shipping configurations developed for all components included in the PGDx ETC CDx reagent kit were adequate when shipped under the intended storage conditions or exposed to simulated transport conditions over a 72-hour timeframe. The kits were put under excessive heat and freezing temperature stresses. The time points from the study included baseline (T0) and immediately post-stress (T1). An additional long-term storage timepoint (T16, 16 months) was generated using kits that were stored under standard conditions for 16 months post manufacturing

and then subjected to the stress conditions. One reagent kit lot was tested for each condition, for a total of three kits. 1 cell line blend containing BRAF V600E and V600K was assessed in triplicate across all five conditions. Variant concordance was evaluated by comparing results from the T16 kits with those previously reported for the T0 baseline condition. The assay demonstrated concordance for all test conditions at all time points. BRAF V600E PPA was 100% (3/3) and NPA was 100% (12/12). CDx variants maintained robust performance under 72 hours simulated transport conditions, as BRAF V600E calls between samples processed with either a fresh kit or a kit that had undergone stress conditions were concordant.

Freeze/Thaw Stability
The freeze/thaw stability study demonstrated the in-use stability of PGDx ETC CDx reagent components by exposing the kit to a controlled series of thaw/re-freeze cycles of -20°C and 80°C reagents, at 2 timepoints: 0 months (T0) and 18 months (T18). A cell line blend containing BRAF V600E was run in triplicate, which was prepared using PGDx ETC reagents that had undergone 0, 4, or 5 thaw/re-freeze cycles, across both timepoints. Variant concordance was assessed by comparing each thaw/re-freeze condition at each timepoint to the standard condition. For all test conditions at time points, BRAF V600E PPA was 100% (3/3) and NPA was 100% (12/12). The assay demonstrated robust stability across two timepoints and up to five thaw/re-freeze cycles.

2. **Animal Studies**
No animal studies were conducted using PGDx ETC CDx.

3. **Additional Studies**
No additional studies were conducted using PGDx ETC CDx.

X. SUMMARY OF THE PRIMARY CLINICAL STUDY

Clinical validation of the companion diagnostic claim for PGDx ETC CDx was performed to demonstrate safety and effectiveness through a non-inferiority study via comparison to an FDA-approved comparator companion diagnostic (CCD) using intended use specimens that were not obtained from clinical trials. The study followed the methodology from Li (2016) for non-inferiority statistical testing. Samples were tested once on PGDx ETC CDx (FCD) and twice on the CCD (CCD1 and CCD2). Repeats were permitted as needed and followed the applicable test instructions for use. Non-inferiority was evaluated by comparing conditional agreements between FCD, CCD1, and CCD2 based on the methodology described by Li (2015).

Definitions and calculations used in non-inferiority analyses are provided below:
- PPA_{C1C2} is the proportion of CCD1 positive results in which CCD2 is positive
- PPA_{C1F} is the proportion of CCD1 positive results in which FCD is positive.
- PPA_{C2C1} is the proportion of CCD2 positive results in which CCD1 is positive.
- PPA_{C2F} is the proportion of CCD2 positive results in which FCD is positive.
- NPA_{C1C2} is the proportion of CCD1 negative results in which CCD2 is negative.

- NPA_{C1F} is the proportion of CCD1 negative results in which FCD is negative.
- NPA_{C2C1} is the proportion of CCD2 negative results in which CCD1 is negative.
- NPA_{C2F} is the proportion of CCD2 negative results in which FCD is negative.

The differences in agreement (zetas) were calculated as follows:
- ζPPA1 (PPA_{C1C2} - PPA_{C1F})
- ζPPA2 (PPA_{C2C1} - PPA_{C2F})
- ζNPA1 (NPA_{C1C2} - NPA_{C1F})
- ζNPA2 (NPA_{C2C1} - NPA_{C2F})

A. Study Design

The safety and effectiveness of PGDx elio tissue complete CDx as a follow-on companion diagnostic (FCD) was evaluated for the detection of BRAF V600E and V600K mutations in patients with melanoma who may be eligible for treatment with FDA-approved BRAF inhibitor therapies or BRAF/MEK inhibitor combination therapies. This was demonstrated through a non-inferiority study, in which the performance of PGDx elio tissue complete CDx (FCD) was compared to an FDA-approved comparator companion diagnostic (CCD) test using remnant deidentified samples representative of the intended use population specific to each CCD.

To select the study population, the following inclusion and exclusion criteria were used:

Inclusion criteria:
- Tumor purity ≥ 5%
- Available DNA ≥ 50 ng, but target 100 ng of DNA input
- Patients were diagnosed with advanced melanoma

Exclusion Criteria:
- Tumor purity < 5%
- Sample yields < 50 ng DNA
- Patients were not diagnosed with melanoma
- Patient samples not tested twice with comparator CDx (CCD) assay

B. Accountability of PMA Cohort

A total of 379 unique FFPE melanoma tissue samples were tested twice using the CCD and once using PGDx elio tissue complete CDx. Sample accountability for this study is shown in Figure 8 and described below.

A total of 563 samples were tested with the Biomeriux THxID BRAF Kit, the approved CDx device used as a comparator CDx (CCD) in the study, between February 2023 and October 2023. Of these 563 samples, 152 samples were excluded from enrollment in this clinical validation study for the following reasons:

- Insufficient Residual DNA (n=60)
- Low DNA concentration, < 10 ng/µl (n=85)

- Tumor purity < 5% (n=4)
- Samples were from NYS (New York State) or international source (n=3)

The remaining 411 samples were tested a second time with the CCD and 404 generated valid results. Seven samples did not yield a valid result for both CCD replicates. These 404 samples were shipped to the PGDx CLIA lab for testing, where 15 samples were then immediately excluded from the study due to insufficient material received (< 50 ng DNA), leaving 389 samples.

Of the 389 samples tested, 10 cases failed to produce a result on the PGDx ETC CDx assay or had insufficient DNA to repeat. These 10 failed cases were included in the assay success rate calculations but were excluded from variant concordance analysis. In total, 379 (389 minus 10) cases were included in the final variant concordance analysis, which included the following CCD results: 114 BRAF V600E-positive cases, 37 BRAF V600K-positive cases, 1 BRAF V600E&K-positive case, and 227 negative cases. Both CCD replicates for all 379 cases were concordant. The expected prevalence of BRAF V600E and V600K in melanoma is 29.6% (622/2098), and 5.2% (110/2098), respectively, based on TCGA data (https://www.cbioportal.org/). The observed prevalence in the validation cohort was similar, with 30.1% (114/379) positive for BRAF V600E, and 9.8% (37/379) positive for BRAF V600K.

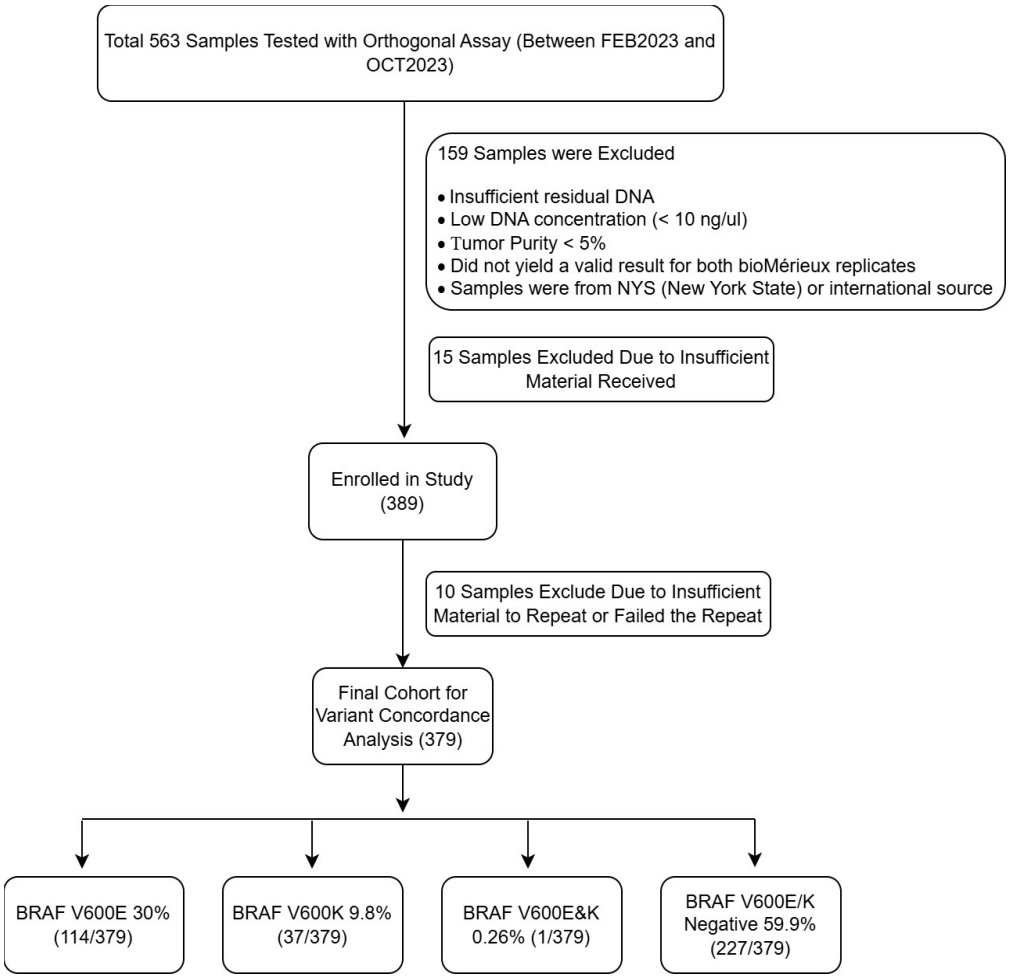

Figure 1: Sample Enrollment Summary
This figure describes the workflow of sample testing with the comparator CDx method, exclusion of samples failing to meet the inclusion criteria, and subsequent enrollment and testing with ETC CDx.

C. Safety and Effectiveness Results

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

2. Effectiveness Results
   To support the CDx indications listed in Table 1.1, the cohort was analyzed two ways. The first analysis was performed using only samples with V600E mutations and the second analysis was performed for samples including both V600E and V600K mutations as positive. Positive percent agreement (PPA), and Negative percent agreement (NPA) were calculated. Concordance between the CCD and the FCD (C1F or C2F) was

subtracted from the concordance between the 2 CCD replicates (C1C2 or C2C1) [refer to the Li et al (2015) methodology referenced above]. The primary endpoint was to test the hypothesis that individual agreement between FCD and CCD was non-inferior to the agreement of 2 CCD replicates by non-inferiority margins of $\delta_1 \leq 10\%$ for PPA and $\delta_0 \leq 10\%$ for the NPA, when comparing BRAF V600E or V600K mutations. Please see Table 19 below for analysis definitions to determine concordance between FCD and CCD.

Table 19: Table of Analysis Definitions

Analysis Scenario	**Samples Analyzed**	**Analysis Definition**	**Concordance Mapping FCD = CCD**
Amino Acid Matching Analysis	V600E Results (N=379)	Required exact amino acid match for reported V600E variant between follow-on assay and comparator assay.	V600E = V600E
	V600K Results (N=379)	Required exact amino acid match for reported V600K variant between follow-on assay and comparator assay.	V600K = V600K
	V600E or V600K Results, Tumor Purity ≥ 20 (N=372)	Inclusion of only samples with tumor purity greater than or equal to 20%. Sample level concordance of reporting V600E or V600K. Indicative of potential treatment if positive for either	V600E = V600E OR V600K = V600K
Amino Acid Matching Analysis (Tumor Purity ≥ 20)	V600E Results, Tumor Purity ≥ 20 (N=372)	Inclusion of only samples with tumor purity greater than or equal to 20%. Required exact amino acid match for reported V600E variant between follow-on assay and comparator assay.	V600E = V600E
Amino Acid Matching Analysis (Tumor Purity ≥ 20)	V600K Results, Tumor Purity ≥ 20 (N=372)	Inclusion of only samples with tumor purity greater than or equal to 20%. Required exact amino acid match for reported V600K variant between follow-on assay and comparator assay.	V600K = V600K

Concordance data is provided below in Table 20 (BRAF V600E results only), Table 21 (BRAF V600K results only), and Table 23 (BRAF V600E and V600K combined).

Concordance assessment of BRAF V600E variants yielded a PPA of 98.3% and an NPA of 98.9%. The upper bound confidence intervals (CIs) for discordance were 4.5% for ζPPA1 and ζPPA2, and 2.56% for ζNPA1 and ζNPA2.

Table 20: Amino Acid Match Analysis V600E Results

	CCD1+				**CCD1−**	
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
FCD+	113	0	113	0	3	3
FCD-	2	0	2	0	261	261
Total	115	0	115	0	264	264
PPA C1F	98.3% 113/115 (93.9%, 99.5%)		NPA C1F		98.9% 261/264 (96.7%, 99.6%)	
PPA C2F	98.3% 113/115 (93.9%, 99.5%)		NPA C2F		98.9% 261/264 (96.7%, 99.6%)	
PPA C1C2	100% 115/115 (96.76%, 100.0%)		NPA C1C2		100% 264/264 (98.56%, 100.0%)	
PPA C2C1	100% 115/115 (96.76%, 100.0%)		NPA C2C1		100% 264/264 (98.56%, 100.0%)	
ζppa1 (CI95)	0.0174 (0, 0.045)					
ζppa2 (CI95)	0.0174 (0, 0.045)					
ζnpa1 (CI95)	0.0114 (0, 0.0256)					
ζnpa2 (CI95)	0.0114 (0, 0.0256)					

CI95 refers to the 95% Confidence Intervals

Table 21: Amino Acid Match Analysis V600K Results

	CCD1+				**CCD1−**	
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
FCD+	35	0	35	0	0	0
FCD-	3	0	3	0	341	341
Total	38	0	38	0	341	341
PPA C1F	92.1% 35/38 (79.2%, 97.3%)		**NPA C1F**		100% 341/341 (98.88%, 100.0%)	

PPA C2F	92.1% 35/38 (79.2%, 97.3%)	**NPA C2F**	100% 341/341 (98.88%, 100.0%)
PPA C1C2	100% 38/38 (90.81%, 100.0%)	**NPA C1C2**	100% 341/341 (98.88%, 100.0%)
PPA C2C1	100% 38/38 (90.81%, 100.0%)	**NPA C2C1**	100% 341/341 (98.88%, 100.0%)
ζppa1 (CI95)	0.0789 (0, 0.175)		
ζppa2 (CI95)	0.0789 (0, 0.175)		
ζnpa1 (CI95)	0 (0, 0)		
ζnpa2 (CI95)	0 (0, 0)		

CI95 refers to the 95% Confidence Intervals

To determine the performance of PGDx ETC CDx detecting BRAF MNVs in general, PGDx also reviewed study data for dinucleotide BRAF V600 “E2” targets. PGDx identified 5/5 (100%) concordantly positive BRAF V600E dinucleotide variants present in the non-inferiority study (Table 22).

Table 22: BRAF V600E Dinucleotide Results

Sample Name	CCD Result	FCD Result	Genomic Location	Reference	Mutant	ETC VAF (%)
LVA000022T1	BRAF V600E	BRAF V600E	Chr7:140453135140453136	CA	TT	28.7
LVA000090T1	BRAF V600E	BRAF V600E	Chr7:140453135140453136	CA	TT	48.3
LVA000148T1	BRAF V600E	BRAF V600E	Chr7:140453135140453136	CA	TT	18.6

LVA000201T1	BRAF V600E	BRAF V600E	Chr7:140453135140453136	CA	TT	41.5
LVA000339T1	BRAF V600E	BRAF V600E	Chr7:140453135140453136	CA	TT	41.5

Since V600K is present in under 10% of population, BRAF V600E and V600K mutations was evaluated in aggregate, and demonstrated comparable performance of PGDx elio tissue complete CDx relative to the currently marketed CDx test, achieving 96.7% PPA and 99.5% NPA (Table 23). Upper bound 95% confidence intervals (CIs) were ≤3.27% for ζPPA1 and ζPPA2, and ≤0.5% for ζNPA1 and ζNPA2.

Table 23: Amino Acid Match Analysis V600E and V600K Results

	CCD1+			**CCD1−**		
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
FCD+	148	0	148	0	3	3
FCD-	5	0	5	0	602	602
Total	153	0	153	0	605	605
PPA C1F	96.7% 148/153 (92.6%, 98.6%)		**NPA C1F**		99.5% 602/605 (98.6%, 99.8%)	
PPA C2F	96.7% 148/153 (92.6%, 98.6%)		**NPA C2F**		99.5% 602/605 (98.6%, 99.8%)	
PPA C1C2	100% 153/153 (97.55%, 100.0%)		**NPA C1C2**		100% 605/605 (99.36%, 100.0%)	
PPA C2C1	100% 153/153 (97.55%, 100.0%)		**NPA C2C1**		100% 605/605 (99.36%, 100.0%)	
ζppa1 (CI95)	0.0327 (0.0068, 0.0633)					
ζppa2 (CI95)	0.0327 (0.0068, 0.0633)					
ζnpa1 (CI95)	0.005 (0, 0.0114)					
ζnpa2 (CI95)	0.005 (0, 0.0114)					

CI95 refers to the 95% Confidence Intervals

There were 5 false negative results. Three samples had VAF values (~0.25%) that were below the assay cutoff (0.4%). One sample did now have adequate number of reads that were above Q30, therefore it failed to provide a result, and one sample did not have adequate DNA to repeat.

Subgroup Analysis
No analyses were performed for sex-, age-, race-, ethnicity-, or any other relevant characteristic- specific subgroups.

Pediatric Extrapolation
PGDx elio tissue complete CDx is intended to be used as a companion diagnostic to identify melanoma patients who may benefit from treatment with BRAF-targeted therapies in accordance with the approved therapeutic product labeling. While rare in children, melanoma is the most common skin cancer in children, followed by basal cell carcinomas and squamous cell carcinomas. Melanoma accounts for about 3% of all cancers in children between 15 to 19 years of age, and annual incidence in the United States increases with age (https://www.cancer.gov/types/skin/hp/child-melanoma-treatment-pdq). There were 9 pediatric cases that were evaluated in the analytical accuracy study, with the youngest 6 years old.-In addition, the data from adult and pediatric patients with metastatic solid tumors who have BRAF V600E mutation demonstrated equivalent performance. Since there is no change in the assay workflow, including bioinformatics, based on the age of the patients, the validation data can be extrapolated to the pediatric population.

XII. FINANCIAL DISCLOSURE

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

- Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: [0]
- Significant payment of other sorts: [0]
- Proprietary interest in the product tested held by the investigator: [0]
- Significant equity interest held by investigator in sponsor of covered study: [1]

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

XIII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable

XIV. PANEL MEETING RECOMMENDATION AND FAD'S POST-PANEL ACTION

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

XV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

Effectiveness Conclusions

Analytical performance studies were conducted with PGDx elio tissue complete CDx using DNA extracted from FFPE tissue from melanoma. The performance for detecting the tested variants, when the test is used in accordance with the directions provided, has been characterized based on clinical and non-clinical studies conducted for the device as described above. The clinical benefit of PGDx elio tissue complete CDx as a follow-on companion diagnostic for the detection of BRAF V600E/K mutations in patients with melanoma was demonstrated through a clinical concordance study assessing non-inferiority (NI) using archived melanoma patient FFPE tissue with a previously approved CDx test.as the comparator. The non-inferiority (NI) statistical testing approach used passed the acceptance criteria specified in the protocol. The concordance observed between PGDx elio tissue complete CDx and the approved CDx test supports the effectiveness of PGDx elio tissue complete CDx to identify melanoma patients whose tumors are BRAF V600E/K positive for which PGDx elio tissue complete CDx results can be used to direct use of the associated therapeutics listed in the Intended Use above. These results provide \evidence that PGDx elio tissue complete CDx can accurately identify melanoma patients harboring BRAF V600E or V600K mutations, aiding in the selection of patients who may benefit from targeted BRAF inhibitor therapy.

Safety Conclusions

The risks of the device are associated with the potential mismanagement of patients resulting from false results of the test. PGDx elio tissue complete CDx is an IVD test, performed using DNA 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 result, and consequently inappropriate patient management decisions in melanoma treatment. Patients with false positive results may undergo treatment with the associated therapies listed in Table 1.1 of the intended use statement without expectation of 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, from which they might have received meaningful clinical benefit. There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy. Analytical performance of the assay demonstrates the test to be safe for use as indicated. The test presents no additional safety hazard to the patients being tested.

Benefit-Risk Determination

The PGDx elio tissue complete CDx test, provides probable benefit in detecting BRAF V600E or V600K mutations, to identify patients for treatment with BRAF inhibitors approved by FDA or BRAF/MEK inhibitor Combinations approved by FDA. The clinical evidence supporting the probable benefit of this test, was generated through a non-inferiority study design comparing one replicated of the PGDx elio tissue complete CDx test with two replicates of the FDA approved device. The top-line non-inferiority study results, for the detection of BRAF V600E, the device showed acceptable PPA and NPA (98.9%), when compared to an FDA approved comparator. In all, there were only two false negatives amongst 115 samples analyzed (that were positive by the comparator assay), for a PPA of this device conditional on the CCD1 or CCD2 result were 98.3% 113/115 (95% CI: 93.9- 99.5%). For the detection of BRAF V600E and V600K, the device showed acceptable PPA and NPA (99.5%), when compared to an FDA approved comparator. In all, there were only five false negatives amongst the 153 samples (148/153), for a PPA of this device conditional on the CCD1 or CCD2 results of 96.7% (95% CI: 92.6-98.6%), in a representative cohort. In addition, strong performance data was demonstrated for BRAF V600E2, which is also a dinucleotide mutation and the DNA level, mitigating the uncertainty around the risk of detecting BRAF V600K (which is also a dinucleotide variant). Taken together, there appears to be clinically acceptable performance and probable clinical benefit, for the PGDx elio tissue complete CDx test, to detect BRAF V600E or V600K mutations, in patients with melanoma for the treatment with BRAF inhibitors approved by FDA or BRAF/MEK inhibitor combinations approved by FDA.

There is probable risk associated with the use of this device, mainly due to 1) false positive, false negatives, or failure to provide a result, and 2) incorrect interpretation of test results by the user. The risks of the PGDx elio tissue complete CDx test are associated with the potential mismanagement of patients 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 which may lead to adverse events or patients may have delayed access to treatments that could be more beneficial. A false negative result may prevent a patient from accessing a therapeutic product or combination with meaningful clinical benefit. The risk of false results is partially mitigated by clinical and analytical studies presented above, for BRAF V600E and BRAF V600K in melanoma. However, the PGDx elio tissue complete CDx test, demonstrated non-inferiority to the FDA approved companion diagnostics comparator, and therefore does not introduce significant risks above this device.

Additional factors to be considered in determining probable risks and benefits for the PGDx elio tissue complete CDx test included: analytical performance of the device for this biomarker and substantial representation of these key variants in melanoma.

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, taking into account the totality of the analytical and clinical data, the probable benefits for the indicated use of this device are deemed to exceed the probable risks.

Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indication for use. Data from the analytical and clinical validation studies support the performance of PGDx elio tissue complete CDx as an aid in the identification of BRAF V600E/K mutation positive melanoma patients for whom the therapies listed in Table 1.1 of the Intended Use statement may be indicated.

XVI. CDRH DECISION

CDRH issued an approval order on July 30, 2026.

The applicant's manufacturing facility was inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820), which was in effect at the time of the inspection. As of February 2, 2026, the revised part 820, referred to as the Quality Management System Regulation (QMSR), is effective.

XVII. APPROVAL SPECIFICATIONS

Directions for use: See device labeling

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

Post-approval Requirement and Restrictions: None

XVIII. REFERENCES

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.