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:	Guardant360® CDx
Device Procode:	PQP
Applicant's Name and Address:	Guardant Health, Inc. 505 Penobscot Drive Redwood City, CA 94306 USA
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P200010/S021
Date of FDA Notice of Approval:	September 25, 2025

The original PMA (P200010) for Guardant360® CDx was approved on August 7, 2020 for the detection of genetic alterations in circulating cell-free DNA (cfDNA) from plasma of peripheral whole blood from patients who may benefit from one of the FDA-approved therapies for non-small cell lung cancer (NSCLC). Subsequently, additional PMA supplements were approved for expanding the indications for use of Guardant360® CDx for detecting *EGFR* exon 20 insertions, *KRAS* G12C mutations and *ERBB2/HER2* activating mutations (single nucleotide variants [SNVs] and exon 20 insertions) in patients with NSCLC and *ESR1* missense mutations between codons 310-547 in patients with breast cancer. The SSEDs to support the previously approved indications are available on the CDRH website.

The current panel-track supplement was submitted to expand the intended use and indications for use of Guardant360® CDx to include a companion diagnostic indication for the detection of specific *ESR1* mutation(s) (SNVs and V422 deletion) in patients with breast cancer who may benefit from treatment with INLURIYO (imlunestrant).

II. INDICATIONS FOR USE

Guardant360® CDx is a qualitative next generation sequencing-based *in vitro* diagnostic device that uses targeted high throughput hybridization-based capture technology for detection of single nucleotide variants (SNVs), insertions and deletions (indels) in 55 genes, copy number amplifications (CNAs) in two (2) genes, and fusions in four (4) genes. Guardant360 CDx utilizes circulating cell-free DNA (cfDNA) from plasma of peripheral whole blood collected in Streck Cell-Free DNA Blood Collection Tubes

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

Table 1. Companion Diagnostic Indications

Indication	Biomarker	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions, L858R, and T790M*	TAGRISSO® (osimertinib)
	EGFR exon 20 insertions	RYBREVANT® (amivantamab-vmjw)
	ERBB2/HER2 activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
	KRAS G12C	LUMAKRAS™ (sotorasib)
Breast cancer	*ESR1* missense mutations between codons 310 and 547	ORSERDU™ (elacestrant)
	ESR1 E380, V422del, S463, L469, L536, Y537, and D538 mutations	INLURIYO™ (imlunestrant)

A negative result from a plasma specimen does not assure that the patient's tumor is negative for genomic findings. Patients who are negative for the biomarkers listed in Table 1 should be reflexed to tissue biopsy testing for Table 1 biomarkers using an FDA-approved tumor tissue test, if feasible.

*The efficacy of TAGRISSO® (osimertinib) has not been established in the *EGFR* T790M plasma-positive, tissue-negative or unknown population and clinical data for T790M plasma-positive patients are limited; therefore, testing using plasma specimens is most appropriate for consideration in patients from whom a tumor biopsy cannot be obtained.

Additionally, the test is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with any solid malignant neoplasms. The test is for use with patients previously diagnosed with cancer and in conjunction with other laboratory and clinical findings.

Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

Guardant360 CDx is a single-site assay performed at Guardant Health, Inc.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

Warnings and precautions are listed below:

- Alterations reported may include somatic (not inherited) or germline (inherited) alterations. The assay filters germline variants from reporting except for pathogenic *BRCA1, BRCA2, ATM,* and *CDK12* alterations. However, if a reported alteration is suspected to be germline, confirmatory testing should be considered in the appropriate clinical context.
- The test is not intended to replace germline testing or to provide information about cancer predisposition.
- Somatic alterations in *ATM* and *CDK12* are not reported by the test as they are excluded from the test's reportable range.
- Genomic findings from cfDNA may originate from circulating tumor DNA (ctDNA) fragments, germline alterations, or non-tumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP).
- Allow the tube to fill completely until blood stops flowing into the tube. Underfilling of tubes with less than 5 mL of blood (bottom of the label indicates 5 mL fill when tube is held vertically) may lead to incorrect analytical results or poor product performance. This tube has been designed to fill with 10 mL of blood.

V. DEVICE DESCRIPTION

The Guardant360 CDx test includes reagents, software, and procedures for testing cfDNA from whole blood samples. The test uses 5-30 ng of cfDNA for library construction and next generation sequencing (NGS). Sequencing data is processed using a customized bioinformatics pipeline designed to detect several classes of genomic alterations, including nucleotide substitutions, indels, copy number amplifications, and genomic fusions / rearrangements. The device is designed to sequence 74 genes, but only report pre-defined and de novo alterations within the 55 genes outlined in **Table 2**. The test's reportable range for SNVs and indels covers approximately 46,000 bases.

Table 2. Genes Containing Alterations Detected by the Guardant360® CDx

Alteration Type	Genes
Single Nucleotide Variants (SNVs)	*AKT1, ALK, APC, AR, ARAF, ATM*, BRAF, BRCA1**, BRCA2**, CCND1, CDH1, CDK4, CDK6, CDK12*, CDKN2A, CTNNB1, EGFR, ERBB2, ESR1, FGFR1, FGFR2, FGFR3, GATA3, GNA11, GNAQ, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MET, MLH1, MTOR, MYC, NF1, NFE2L2, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, PTEN, RAF1, RET, RHEB, ROS1, SMAD4, SMO, STK11, TERT, TSC1, VHL*
Indels	*AKT1, ALK, APC, ATM*, BRAF, BRCA1**, BRCA2**, CDH1, CDK12*, CDKN2A, EGFR, ERBB2, ESR1, FGFR2, GATA3, HNF1A, HRAS, KIT, KRAS, MET, MLH1, NF1,*

Alteration Type	Genes
	PDGFRA, PIK3CA, PTEN, RET, ROS1, STK11, TSC1, VHL
Copy Number Amplifications (CNAs)	*ERBB2, MET*
Fusions / Rearrangements	*ALK, NTRK1, RET, ROS1*

*Reporting is enabled for pathogenic germline alterations only. Somatic alterations will not be reported.
**Reporting is enabled for both germline and somatic alterations.

Test Output

The test report includes variants reported in the following categories (**Table 3**):

Table 3. Category Definitions

Category	Guardant360 CDx			Comments
	Prescriptive use for a Therapeutic Product	**Clinical Performance**	**Analytical Performance**	
Category 1: Companion Diagnostic (CDx)	**Yes**	**Yes**	**Yes**	ctDNA biomarkers linked to the safe and effective use of the corresponding therapeutic product, for which Guardant360 CDx has demonstrated clinical performance shown to support therapeutic efficacy and strong analytical performance for the biomarker.
Category 2: ctDNA Biomarkers with Strong Evidence of Clinical Significance in ctDNA	No	No	**Yes**	ctDNA biomarkers with strong evidence of clinical significance presented by other FDA-approved liquid biopsy companion diagnostics for which Guardant360 CDx has demonstrated analytical reliability but not clinical performance.
Category 3A: Biomarkers with Evidence of Clinical Significance in tissue supported by: strong analytical validation using ctDNA	No	No	**Yes**	ctDNA biomarkers with evidence of clinical significance presented by tissue-based FDA-approved companion diagnostics or professional guidelines for which Guardant360 CDx has demonstrated analytical performance including analytical accuracy, and concordance of blood-based testing to tissue-based testing for the biomarker.

Category	Guardant360 CDx			Comments
	Prescriptive use for a Therapeutic Product	Clinical Performance	Analytical Performance	
Category 3B: Biomarkers with Evidence of Clinical Significance in tissue supported by: analytical validation using ctDNA	No	No	**Yes**	ctDNA biomarkers with evidence of clinical significance presented by tissue-based FDA-approved companion diagnostics or professional guidelines for which Guardant360 CDx has demonstrated minimum analytical performance including analytical accuracy.
Category 4: Other Biomarkers with Potential Clinical Significance	No	No	**Yes**	ctDNA biomarkers with emergent evidence based on peer-reviewed publications for genes/variants in tissue, variant information from well-curated public databases, or in-vitro pre-clinical models, for which Guardant360 CDx has demonstrated minimum analytical performance.

Biomarker Rules for *ERBB2* Activating Mutations Reported by Guardant360 CDx

- The following *ERBB2* activating mutations will be reported in Category 1 as a companion diagnostic (CDx) for patients with NSCLC for ENHERTU® (famtrastuzumab deruxtecan-nxki):

  A775_G776insYVMA, Y772_A775dup, P780_Y781insGSP, G778_P780dup, G776delinsVC, G776_V777delinsCVCG, G776delinsLC, V777_S779dup, G776_V777insL, V777_G778insG, G778_S779insLPS, V777_G778insCG, A775_G776insV, A775_G776insTVMA, G776_V777insVGC, G778dup, G778_S779insCPG, L755S, V777L, G776S, S310F, G776V, V777M, S310Y, R678Q, T733I, L755M, L755P, L755W, D769N, D769H, D769Y, L755A, I767M, V842I, T862I, L869R, R896C, R896H, G776C, G776_V777insVC, S779_P780insVGS, I767F, T798I

Biomarker Rules for *ESR1* Mutations Reported by Guardant360 CDx

- Missense mutations between codons 310 and 547 of *ESR1* will be reported in Category 1 as a companion diagnostic (CDx) for patients with breast cancer for ORSERDU (elacestrant).
- The following *ESR1* mutations will be reported in Category 1 as a companion diagnostic (CDx) for patients with breast cancer for INLURIYO (imlunestrant):

  E380A; E380D; E380K; E380Q; E380V; V422del; S463F; S463P; L469V; L536F; L536G; L536H; L536I; L536K; L536N; L536P; L536Q;

L536R; L536V; Y537C; Y537D; Y537G; Y537H; Y537N; Y537P; Y537Q; Y537S; D538E; D538G; D538H; D538N; D538V

Test Kit Contents

The test includes the Guardant360 CDx Blood Collection Kit (BCK), which is sent to ordering laboratories. Each BCK contains two blood collection tubes. The BCK also contains supporting packaging materials, instructions for use and a return shipping label. The BCK contains the following components:

- Streck blood collection tubes for specimen collection, stabilization, and transport of cfDNA; 2 per kit.
- Cushioning materials to prevent breakage of the blood collection tubes; 2 per kit
- Foam tray for protection of collection tubes during transport
- Absorbent sheet to be used during specimen shipping
- Biohazard specimen bag for protection during specimen transport
- Return shipping label for return of specimen to Guardant Health
- Barcodes for specimen identification and shipping instructions
- Instructions for Use for blood draw
- Patient welcome brochure which contains an overview of the test
- Test requisition form to complete to order Guardant360 CDx for a patient.

The test also includes the Guardant360 CDx Sample Preparation Kit (SPK), which is used in the Guardant Health Clinical Laboratory. The SPK contains reagents for library preparation, library enhancement, and cfDNA quantification/qualification. The kit is assembled into six (6) different boxes (referred to as box 1, 2, 3, 4a, 4b, and 4c) based on the usage of the reagents. The division of reagents amongst the boxes reflects different storage conditions and/or locations (e.g., different laboratory spaces).

Instruments

Guardant360 CDx is intended to be performed with serial number-controlled instruments as indicated in **Table 4**. All instruments are qualified by Guardant Health, Inc. under the Guardant Health Quality System.

Table 4. Serial Number Controlled Instruments for Use with the Guardant360 CDx Assay

Instrument
Agilent Technologies 4200 TapeStation Instrument
Hamilton Company Microlab STAR
Hamilton Company Microlab STARlet
Illumina NextSeq 550 Sequencer
Veriti 96-Well Thermal Cycler

Test Process

Whole Blood Collection and Shipping

The Guardant360 CDx Blood Collection Kit is used by ordering laboratories / physicians to collect whole blood specimens and ship them to the Guardant Health Clinical Laboratory. A minimum of 5 mL whole blood must be received in order to achieve optimal performance for the Guardant360 CDx assay. Underfilling of tubes with less than 5 mL of blood may lead to incorrect analytical results or poor product performance.

Plasma Isolation and cfDNA Extraction

Whole blood specimens are processed in the Guardant Health Clinical Laboratory within 7 days of blood collection. Plasma is isolated from both tubes of whole blood via centrifugation. One tube of plasma is stored, while the second tube is used for cfDNA extraction using the QIAGEN QIAsymphony SP Instrument and reagent system. The resulting cfDNA is quantified using the 4200 TapeStation. Input amounts ranging from 5 to 30 ng of cfDNA are further processed for each sample.

Library Preparation and Enrichment

Reagents from the Guardant360 CDx Sample Preparation Kit are used during library preparation, enrichment, enrichment wash, and quantitation steps using the Veriti 96-Well Thermal Cycler, Microlab STAR and STARlet, and 4200 TapeStation Instruments. During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation. The resulting DNA is amplified by PCR to create libraries suitable for enrichment.

Amplified libraries are enriched for genes of interest using hybrid target capture with custom biotinylated RNA probes. Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence. Amplified enriched libraries are pooled in equimolar amounts, denatured, and diluted to appropriate concentration for sequencing.

DNA Sequencing

Paired-end sequencing by synthesis is performed with the Illumina NextSeq 550 Sequencing system. The amplified cfDNA is analyzed by parallel sequencing of amplified target genes to an average depth of coverage of greater than 2,700 unique molecules.

Data Analysis and Reporting

The Guardant360 CDx Software uses a custom-developed analysis bioinformatics pipeline (BIP) software module. The BIP software module uses the raw data (output) from the targeted sequencing, partitions the data based on the sample index sequence (barcode) of each read to separate reads originating from individual samples, and executes a proprietary algorithmic reconstruction of the digitized sequencing signals based on molecular barcodes for high-fidelity molecule-based alteration calling downstream. The sequence data then undergoes an alignment process where it is mapped to the human genome (hg19) and an analysis of sequence alteration data is performed.

Alteration detection is conducted according to alteration calling metrics derived from clinical sample analysis. All alterations must pass alteration calling metrics as described in **Table 5**.

The SNV and indel cut-offs are defined in terms of mutant allele fraction (MAF) estimate, number and type of molecules supporting the alteration, pseudo-gene assessment, and likelihood ratio (LLR) score. The MAF estimate describes the calculated allelic fraction of an SNV or indel. The number of molecules describes the observed number of molecules meeting requirements for a particular alteration call. The LLR score is a calculated number that reflects how much observed support for the mutation exceeds expectations based on PCR and sequencing induced artifacts.

Table 5. Alteration Analytical Calling Threshold/Cut-Off Metrics

SNV Calling Property	**Metric**
DNA Molecule Support	≥ 2
MAF Estimate	≥ 0.001%
Log Likelihood Ratio	≥ 0
Indel Calling Property	**Metric**
DNA Molecule Support	≥ 2
MAF Estimate	≥ 0.01%
Log Likelihood Ratio	≥ 10
CNA Calling Property	**Metric**
ERBB2 copy number	≥ 2.18
ERBB2 Z-score	≥ 10
ERBB2 amplification is not associated with chromosome-arm aneuploidy	TRUE
MET copy number	≥ 2.16
MET Z-score	≥ 10
MET amplification is not associated with chromosome-arm aneuploidy	TRUE
Fusion Calling Property	**Metric**
MAPQ score of supporting molecule to fusion sequence	> 30
Number of unique fusion molecules	≥ 2

Number of unique fusion reads	> 2

The laboratory and physician receive a qualitative alteration-level result. A sample will receive an overall "Failed" result when any quality control (QC) metric is failed. Samples failing any QC metric are automatically held and not released. The laboratory may attempt to rerun a patient sample that has failed a QC metric by using stored plasma or intermediate products.

Results from samples passing all QC metrics are formatted onto an IVD results report with CDx relevant information (Category 1) and all other biomarkers (Categories 2-4).

Quality Control Measures

The Guardant360 CDx Sample Preparation Kit includes the Variant Control, which is engineered to contain known positive and negative alterations and is treated as a sample. Additionally, a no template negative control (NTC) is run in parallel with patient samples.

The Variant Control consists of a mixture of cfDNA from multiple human cancer cell lines containing all four alteration types, SNVs, indels, CNAs and fusions. The control is treated as a sample and processed starting from 15 ng cfDNA input through sequencing where it is analyzed for the presence and absence of the specific alterations.

Although the Variant Control does not contain all the alterations that the test is capable of detecting, concordant detection of alterations targeted in the Variant Control indicates that assay is performing as expected across the panel.

In addition to assessing Variant Control performance within a batch, the test is assessing multiple per-sample in-process and post-sequencing analytical metrics for each of the patient samples tested. These metrics provide in depth analytical QC information that complements Variant Control performance data and is specific and informative to that sample performance.

The NTC samples are absent of a DNA template, so cfDNA extraction, library preparation, and enrichment steps are expected to result in background level metrics.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA approved companion diagnostic (CDx) alternatives for the detection of some of the genetic alterations using cfDNA isolated from plasma samples to those that are listed in Table 1 of the Guardant360 CDx intended use statement. These approved alternative CDx tests are listed in **Table 6** below. Each alternative has its own advantages and disadvantages. A patient should fully discuss any alternative with their physician to select the most appropriate method that best meets expectations and lifestyle. For additional details see list of FDA Cleared or Approved Companion Diagnostic Devices at https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools

Table 6: List of FDA-Approved CDx Assays for Genes Targeted by the Guardant360 CDx

Gene and Variant	Indication	Therapy	Device (PMA #)	Company	Technology
EGFR T790M	NSCLC	TAGRISSO® (osimertinib)	cobas® EGFR Mutation Test v2	Roche Molecular Systems, Inc.	Polymerase Chain Reaction (PCR)
EGFR L858R and exon 19 deletions			FoundationOne® Liquid CDx	Foundation Medicine, Inc.	NGS

Note: There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of *KRAS* G12C mutations for the identification of patients with NSCLC eligible for treatment with LUMAKRAS™ (sotorasib). However, there is one FDA approved CDx alternative for the detection of *KRAS* G12C mutation in patients with NSCLC using tissue specimens for treatment with LUMAKRAS™ (sotorasib): QIAGEN *therascreen*® KRAS RGQ PCR Kit (See SSED for P110027/S012).

Similarly, there are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of *EGFR* exon 20 insertions for the identification of patients with NSCLC eligible for treatment with RYBREVANT® (amivantamab-vmjw). There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of *ERBB2* activating mutations (SNVs and exon 20 insertions) for the identification of patients with NSCLC eligible for treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki). There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of *ESR1* missense mutations in patients with breast cancer (BC) eligible for treatment with ORSERDU (elacestrant). Also, there are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of specific *ESR1* mutations (subject of this PMA Supplement) in patients with BC eligible for treatment with INLURIYO (imlunestrant).

However, there is an FDA approved CDx (Life Technologies Corporation's OncomineDx Target test) alternative for the detection of *EGFR* exon 20 insertions in NSCLC patients using tissue specimens for treatment with RYBREVANT® (amivantamab-vmjw) (See labeling for P160045/S027). There is also an FDA approved (Life Technologies Corporation's OncomineDx Target test) CDx alternative for the detection of *ERBB2* activating mutations (SNVs and exon 20 insertions) in patients with NSCLC using tissue specimens for treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki) (See SSED for P160045/S035).

VII. MARKETING HISTORY

The Guardant360 CDx Premarket Approval (PMA) application was FDA-approved on August 7, 2020, and subsequently commercialized in the USA. The approved PMA and its supplements that affected the Intended Use are listed in **Table 7**.

Table 7. Marketing History

Submission No.	Date of Approval	Biomarker	Patient Population	Drug
P200010	August 7, 2020	*EGFR* exon 19 deletions, L858R, and T790M	NSCLC	TAGRISSO® (osimertinib)
P200010/S001	May 21, 2021	*EGFR* exon 20 insertions	NSCLC	RYBREVANT® (amivantamab-vmjw)
P200010/S002	May 28, 2021	*KRAS* G12C	NSCLC	LUMAKRAS™ (sotorasib)
P200010/S008	August 11, 2022	*ERBB2/HER2* activating mutations (SNVs and exon 20 insertions)	NSCLC	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
P200010/S010	January 27, 2023	*ESR1* missense mutations between codons 310 and 547	BC	ORSERDU™ (elacestrant)

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 test results, and subsequently, may lead to inappropriate 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 indicated therapy.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

The primary evidence for supporting the performance of Guardant360 CDx in detecting *ESR1* E380 mutations, V422 deletion, Y537 mutations, S463P/F, L469V, D538 mutations, and L536 mutations (hereafter referred to as *ESR1* mutations) was from the data presented using intended use specimens across all validation studies. In addition to the existing platform-level and *ESR1*-specific validation data (P200010, P200010/S001, P200010/S002 and P200010/S010), analytical accuracy/concordance, limit of detection (LoD), and precision near the LoD studies, were conducted to support the INLURIYO (imlunestrant) indication for the specific *ESR1* mutation biomarker.

For Guardant360 CDx platform-level validation (P200010), performance characteristics were established using plasma-derived cfDNA samples from a wide range of cancer types. Each study included CDx variants, as well as a broad range of representative alteration types (SNVs, indels, CNAs, and fusions/rearrangements) in various genomic contexts across several genes. The platform validation studies included samples with *ESR1* mutations in plasma specimens from patients with BC and without BC. These results from the platform-level and *ESR1*-specific validation (P200010, P200010/S001,

P200010/S002 and P200010/S010) that have been leveraged to support Guardant360 CDx detection of *ESR1* mutations are referenced below. Additional *ESR1*-specific analytical validation studies are described below.

1. Analytical Accuracy/Concordance

An analytical accuracy study was conducted previously as part of P200010/S010 to demonstrate the concordance between Guardant360 CDx and an externally validated mass spectrometry-based comparator method for the detection of *ESR1* missense mutations. The study demonstrated a positive percent agreement (PPA) of 97.6% with the corresponding 95% two-sided exact confidence intervals (CIs) as 93.1% - 99.5% and a negative percent agreement (NPA) of 84.6% (95% CI: 77.2% - 90.3%). Further analysis demonstrated that the low NPA observed in the Accuracy Study was due to the low sensitivity of the comparator method. Please refer to the SSED for P200010/S010 (Section IX.A.1).

To validate the detection of V422 deletion by Guardant360 CDx, an additional Analytical Accuracy study was performed using an externally validated NGS-based method as the comparator (**Table 8**). Data was collected from two sets of samples. The first set included 5 samples with *ESR1* V422del with MAF values between 0.1-0.4%, representing typical MAF levels in the intended use population. The result demonstrated 80% PPA with 95% CI of 28.4%-99.5%. The second set included 5 samples with *ESR1* V422del with MAF values above the LoD (~1-3%), which were titrated to MAF values at 0.64%, 0.16%, and 0.04%. A PPA of 100% was observed at MAF levels 1-3% and 0.64%, while a PPA of 0% was observed at MAF levels of 0.16% and 0.04%, respectively. The reduced PPA observed at low MAF levels indicated that the detection of *ESR1* V422 deletion became more stochastic on both assays when MAF levels were well below the LoD.

Table 8. Summary of Concordance Between Guardant360 CDx and the Comparator for *ESR1* V422 Deletion

Sample Set (SS) #: MAF Range	Guardant360 CDx (+), Comparator (+)	Guardant360 CDx (+), Comparator (-)	Guardant360 CDx (-), Comparator (+)	Guardant360 CDx (-), Comparator (-)	Patients (n)	PPA (95% CI)*
SS1: 0.1-0.4%	4	0	1	0	5	80.0% (28.4%-99.5%)
SS2: 1-3%	5	0	0	0	5	100.0% (47.8%-100.0%)

Sample Set (SS) #: MAF Range	Guardant360 CDx (+), Comparator (+)	Guardant360 CDx (+), Comparator (-)	Guardant360 CDx (-), Comparator (+)	Guardant360 CDx (-), Comparator (-)	Patients (n)	PPA (95% CI)*
SS2: 0.64%	5	0	0	0	5	100.0% (47.8%-100.0%)
SS2: 0.16%	0	1	2	2	5	0.0% (0.0%-84.2%)
SS2: 0.04%	0	1	1	3	5	0.0% (0.0%-97.5%)

* The Clopper-Pearson Exact Method was used for the confidence interval (CI) analysis.

2. Analytical Sensitivity

a. Limit of Blank (LoB)

Please refer to the Summary of Safety and Effectiveness Data P200010 (Section IX.A.3.a) for Guardant360 CDx platform-level analytical sensitivity data for LoB. There were no false positives for *ESR1* missense mutations among 240 replicates tested across three unique reagent lots.

b. Limit of Detection (LoD)

Please refer to the Summary of Safety and Effectiveness Data P200010/S010 (Section IX.A.2.b) for the LoDs of Guardant360 CDx for *ESR1* missense mutations (E380Q, S463P, Y537S, and D538G).

An additional combined LoD Confirmation and Precision Study was performed to confirm the LoD for *ESR1* V422del using a sample pool from patients with BC at 5 ng cfDNA input harboring *ESR1* V422del at 1.0-1.5X the Guardant360 CDx panel-wide indel LoD (2.7%) across 24 replicates. V422del is an exon 8 in-frame deletion variant. Please refer to Section IX.A.4 for further details regarding the combined LoD Confirmation and Precision Study. The LoDs for *ESR1* E380Q, Y537S, D538G, S463P (See P200010/S010 for details) and V422del are summarized in **Table 9**.

Table 9. Summary of Established/Confirmed LoDs for *ESR1* Mutations in BC Clinical Samples

ESR1 Mutation	Exon #	LoD MAF (%)	
		5ng input	30ng input
E380Q^	7	1.0	0.3
Y537S^	10	1.0	0.3
D538G^	10	1.1	0.2
S463P*	9	2.8	NT
V422del*	8	3.4	NT

^LoD values were established for E380Q, Y537S and D538G
*LoD values were confirmed for S463P and V422del
MAF, Mutant Allele Fraction; **NT**, Not Tested.

3. **Analytical Specificity**
   Please refer to the Summary of Safety and Effectiveness Data P200010 and P200010/S002 (Section IX.A.4) for Guardant360 CDx platform validation of analytical specificity, including endogenous and microbial interfering substances and *in silico* specificity. The effect of potential exogenous interfering substances that may carry over from cfDNA extraction on assay performance was evaluated in the PMA supplement (P200010/S002).

4. **Precision**
   Please refer to the Summary of Safety and Effectiveness Data P200010/S010 (Section IX.A.4) where the precision (repeatability and within-site reproducibility) of Guardant360 CDx for detecting representative *ESR1* mutations E380Q, S463P, Y537S and D538G was demonstrated.

   An additional precision study was conducted for *ESR1* V422del with sample pools from patients with BC. Testing was performed using 5 ng cfDNA input at analyte levels both near (1.0-1.5X) and above (2.0-3.0X) LoD across six unique reagent lot-instrument-operator combinations with four replicates per combination (6 × 4 = 24 replicates). Together, these data provide an estimate of precision for alterations in all four exons and both alteration types (SNVs and V422del) specified in the imlunestrant biomarker definition (**Table 10**).

Table 10. Summary of Precision Results for Representative *ESR1* Mutations

ESR1 **Mutation**	**Exon #**	**Observed MAF%**	**Relative LoD Level (X)**	**Number Positive/ Number Expected**	**PPA (95% CI)*** **
E380Q	7	1.0	1.0*	24/24	100% (85.8%-100%)
V422del	8	3.4	1.3**	24/24	100% (85.8%-100%)
		7.5	2.8**	24/24	100% (85.8%-100%)
S463P	9	2.8	2.6*	24/24	100% (85.8%-100%)
Y537S	10	1.0	1.0*	23/24	95.8% (78.9% - 99.9%)
D538G	10	1.1	1.0*	23/24	95.8% (78.9% - 99.9%)

*Compared to the established LoD for the prevalent *ESR1* missense mutations E380Q, Y537S, and D538G.
**Compared to the Guardant360 CDx panel-wide indel LoD.
***The Clopper-Pearson Exact Method was used for the CI analysis.

The original PMA (P200010) comprised negative precision data from 72 self-declared cancer-free age-matched healthy donors. 240 replicates were tested at 30 ng inputs across three precision combinations of operator group, instrument combination, reagent lots and start dates. No *ESR1* false positive mutations were detected (NPA 100%, 240/240). These data are leveraged to support this PMA supplement. Please refer to the Summary of Safety and Effectiveness Data for P200010 (Section IX.A.5) for details on precision for mutation-negative samples, precision starting from plasma extraction, and precision studies in support of the Guardant360 CDx platform validation.

5. **Carryover/Cross-Contamination**
   Please refer to the Summary of Safety and Effectiveness Data of P200010 (Section IX.A.6) for platform-level carryover/cross-contamination data for Guardant360 CDx.

6. **Reagent Lot Interchangeability**
   Please refer to the Summary of Safety and Effectiveness Data P200010 (Section IX.A.7) for Guardant360 CDx platform validation of reagent lot interchangeability.

7. **Stability**
   Please see the Summary of Safety and Effectiveness Data P200010 (Section IX.A.8) for Guardant360 CDx platform level reagent and sample stability, including whole blood stability, plasma stability, cfDNA stability, and intermediate sample stability. Please also refer to the Summary of Safety and Effectiveness Data

P200010/S010 (Section IX.A.7) for additional stability studies that verified the platform level stability for whole blood, plasma, and cfDNA samples using samples from patients with BC harboring *ESR1* mutations.

8. **Guard Banding/Robustness**
   Please refer to the Summary of Safety and Effectiveness Data P200010/S002 (Section IX.A.6) for Guardant360 CDx platform level Guard Banding/Robustness study.

9. **General Lab Equipment and Reagent Evaluation**
   Please see the Summary of Safety and Effectiveness Data P200010 (Section IX.A.9) for Guardant360 CDx platform validation of general lab equipment and reagents, including cfDNA extraction as well as other instruments and reagents.

B. Animal Studies

No animal studies were conducted using Guardant360 CDx.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The safety and effectiveness of the Guardant360 CDx assay for identifying breast cancer patients with *ESR1* mutations who may benefit from treatment with INLURIYO (imlunestrant) was demonstrated through testing of cfDNA in pre-treatment plasma specimens from patients enrolled into the EMBER-3 (NCT04975308) clinical study. The EMBER-3 study was conducted to support the efficacy of INLURIYO (imlunestrant). A summary of the Guardant360 CDx clinical validation study is presented below.

A. EMBER-3 (NCT04975308) Clinical Study Design

The EMBER-3 clinical study is a Phase 3, randomized, open-label, active-controlled, multicenter trial of INLURIYO (imlunestrant) as continuous monotherapy (Arm A), investigator's choice endocrine therapy of either fulvestrant or exemestane (Arm B), and an additional investigational combination regimen in patients with ER+/HER2- locally advanced (not amenable to curative treatment by surgery) or metastatic breast cancer. The study enrolled patients who have been treated with an aromatase inhibitor (AI), alone or in combination with a CDK4/6 inhibitor. Eligible patients were randomized 1:1:1 between the Arm A, Arm B and an additional investigational combination regimen stratified by (1) previous treatment with any CDK4/6 inhibitor, (2) presence of visceral metastases and (3) region.

Subjects from the primary EMBER-3 registration population (Arm A and Arm B) with *ESR1* mutations detected by Guardant360 CDx were included in the diagnostic study primary clinical efficacy cohort to assess the clinical validity of Guardant360 CDx to aid in the selection of breast cancer patients with *ESR1* mutations for INLURIYO (imlunestrant) therapy.

1. **Clinical Study Inclusion and Exclusion Criteria**
   Subjects in the primary EMBER3 registration population were included in the diagnostic study efficacy cohort if the selection criteria were met. The key inclusion criteria are summarized below:

   - Must be at least 18 years of age.
   - Must have ER+ and HER2- tumor status confirmed per local laboratory testing as defined in the relevant American Society of Clinical Oncology (ASCO) / College of American Pathologists (CAP) Guidelines.
   - Patients were required to have progressed:
     - Within 12 months of completing neoadjuvant or adjuvant aromatase inhibitor therapy with no systemic treatment for recurrent disease or
     - Greater than 12 months after neoadjuvant or adjuvant endocrine therapy or de novo metastatic disease and had progressed on only one line of aromatase inhibitor therapy.
   - Measurable disease or non-measurable bone-only disease.

   Guardant360 CDx Diagnostic Study Efficacy Cohort Patient Inclusion Criteria
   - Patient randomized into either Arm A or Arm B of the EMBER-3 clinical study.
   - Patient is part of the EMBER-3 INLURIYO (imlunestrant) NDA registration population.
   - Adequate pre-treatment plasma sample available for testing by Guardant360 CDx.

2. **Follow-up Schedule**
   The Guardant360 CDx diagnostic study involved only testing and analysis of plasma samples; as such, no additional patient subject follow-up was conducted in regard to the diagnostic study.

3. **Clinical Endpoints**
   The clinical endpoint used to assess INLURIYO (imlunestrant) efficacy in the EMBER-3 (NCT04975308) clinical study primary objective was progression free survival (PFS) by RECIST version 1.1 as assessed by the investigator at the local site or death due to any cause in the absence of disease progression.

4. **Diagnostic Objective and Endpoints**
   The primary objective of the diagnostic clinical study was to demonstrate the safety and effectiveness of Guardant360 CDx as a companion diagnostic to aid in the selection of breast cancer patients with *ESR1* mutations for treatment with INLURIYO (imlunestrant) therapy.

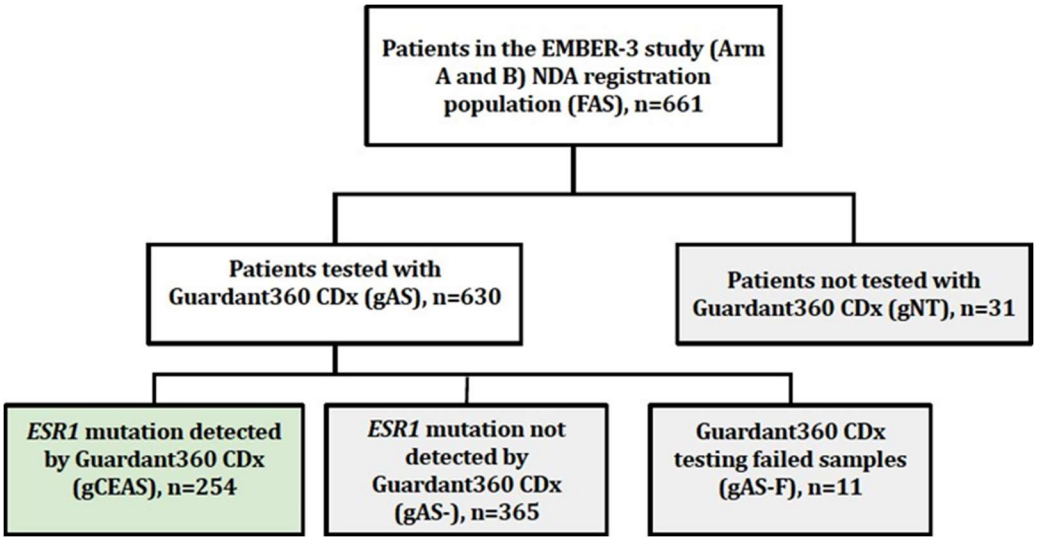
   This objective was assessed by comparing INLURIYO (imlunestrant) therapy efficacy relative to investigator's choice endocrine therapy of either fulvestrant or exemestane in the EMBER-3 registration population of patients with *ESR1* mutation(s) detected by Guardant360 CDx (*ESR1*-mut). The primary endpoint is

the same as that used for the clinical study, PFS per RECIST v1.1 as assessed by the investigator.

B. Accountability of PMA Cohort for the Guardant360 CDx Clinical Study for *ESR1* Mutations

Pre-treatment plasma samples from 630 of the primary EMBER-3 registration population subjects (Arms A and B, n=661) were tested with Guardant360 CDx, of which 254 subjects (40.3% of the Guardant360 CDx tested population) have one or more *ESR1* mutation(s) detected and 365 (57.9% of the Guardant360 CDx tested population) have none. Thirty-one subjects did not have samples available for testing, and 11 samples failed sample QC and did not generate valid Guardant360 CDx results (**Figure 1**).

Figure 1. Guardant360 CDx *ESR1* Mutations Efficacy Analysis Patient Accountability and Analysis Set Definitions



FAS: Full Analysis Set; **gAS**: All patients from the FAS tested with Guardant360 CDx; **gCEAS**: All patients in the gAS with an *ESR1* mutation detected by Guardant360 CDx, which is the primary clinical efficacy population (shaded in green); **gAS-**: All patients in the gAS with no *ESR1* mutations detected by Guardant360 CDx; **gAS-F**: All patients in the gAS that failed Guardant360 CDx testing; **gNT**: All patients in the FAS not tested by Guardant360 CDx

C. Study Population Demographics and Baseline Parameters

Demographics and baseline clinical characteristics of subjects enrolled in Arm A and Arm B of the EMBER-3 clinical study were categorized relative to the diagnostic study populations as defined by Guardant360 CDx results. As shown in **Tables 11** and **12**, the *ESR1*-mut and the *ESR1*-mut-nd populations are well balanced. The INLURIYO (imlunestrant) therapy and investigator's choice endocrine therapy (fulvestrant or exemestane) treatment arms are also well balanced.

Table 11. Demographic and Baseline Characteristics of Full Analysis Set Population

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**		**gAS-Unknown (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**			
Analysis set:	**136***	**172**	**118**	**193**	**254**	**365**	**42**	**661**	
Gender n (%)									
n	136	172	118	193	254	365	42	661	-
Female	136(100.0)	169(98.3)	118(100.0)	192(99.5)	254(100.0)	361(98.9)	41(97.6)	656(99.2)	
Male	0	3(1.7)	0	1(0.5)	0	4(1.1)	1(2.4)	5(0.8)	
Age									
n	136	172	118	193	254	365	42	661	0.432
Mean	60.7	59.9	59.6	62	60.2	61	59.9	60.6	
Std. Dev.	11.1	12.2	11.2	12.3	11.2	12.3	11.3	11.8	
Median	61	60.5	59.5	63	61	62	61	61	
Min	28	28	33	27	28	27	32	27	
Max	85	87	85	89	85	89	80	89	
Age Category (years) n (%)									
n	136	172	118	193	254	365	42	661	>.999
<65	90(66.2)	106(61.6)	78(66.1)	107(55.4)	168(66.1)	213(58.4)	27(64.3)	408(61.7)	
>=65	46(33.8)	66(38.4)	40(33.9)	86(44.6)	86(33.9)	152(41.6)	15(35.7)	253(38.3)	
Age Category 2 (years) n (%)									
n	136	172	118	193	254	365	42	661	0.910
<65	90(66.2)	106(61.6)	78(66.1)	107(55.4)	168(66.1)	213(58.4)	27(64.3)	408(61.7)	
>=65 - <75	30(22.1)	46(26.7)	24(20.3)	53(27.5)	54(21.3)	99(27.1)	12(28.6)	165(25.0)	
>=75 - <85	14(10.3)	16(9.3)	15(12.7)	28(14.5)	29(11.4)	44(12.1)	3(7.1)	76(11.5)	
>=85	2(1.5)	4(2.3)	1(0.8)	5(2.6)	3(1.2)	9(2.5)	0	12(1.8)	
Race n (%)									
n	136	172	118	193	254	365	42	661	0.646
American Indian or Alaska Native	7(5.1)	16(9.3)	3(2.5)	13(6.7)	10(3.9)	29(7.9)	0	39(5.9)	
Asian	33(24.3)	49(28.5)	31(26.3)	50(25.9)	64(25.2)	99(27.1)	25(59.5)	188(28.4)	
Black or African American	7(5.1)	4(2.3)	3(2.5)	4(2.1)	10(3.9)	8(2.2)	0	18(2.7)	
White	80(58.8)	94(54.7)	76(64.4)	111(57.5)	156(61.4)	205(56.2)	16(38.1)	377(57.0)	

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**		**gAS-Unknown (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**			
Multiple	1(0.7)	1(0.6)	1(0.8)	6(3.1)	2(0.8)	7(1.9)	0	9(1.4)	
Missing	8(5.9)	8(4.7)	4(3.4)	9(4.7)	12(4.7)	17(4.7)	1(2.4)	30(4.5)	
Ethnicity n (%)									
n	136	172	118	193	254	365	42	661	0.989
Hispanic or Latino	25(18.4)	51(29.7)	23(19.5)	55(28.5)	48(18.9)	106(29.0)	5(11.9)	159(24.1)	
Not Hispanic or Latino	97(71.3)	104(60.5)	83(70.3)	119(61.7)	180(70.9)	223(61.1)	31(73.8)	434(65.7)	
Not Reported	13(9.6)	17(9.9)	12(10.2)	19(9.8)	25(9.8)	36(9.9)	6(14.3)	67(10.1)	
Missing	1(0.7)	0	0	0	1(0.4)	0	0	1(0.2)	
Weight (kg)									
n	135	169	117	188	252	357	41	650	0.977
Mean	70.97	68.86	71.03	68.54	71	68.69	67.1	69.49	
Std. Dev.	15.2	17.44	16.23	16.11	15.65	16.73	12.7	16.12	
Median	69.5	66	69.8	66	69.6	66	64.5	67.1	
Min	42.5	37	38	39.9	38	37	47	37	
Max	111	142.7	127.3	129.1	127.3	142.7	105	142.7	
Missing	1	3	1	5	2	8	1	11	
Region									
n	136	172	118	193	254	365	42	661	0.972
East Asia	28(20.6)	45(26.2)	26(22.0)	43(22.3)	54(21.3)	88(24.1)	25(59.5)	167(25.3)	
North America/ Western Europe	63(46.3)	57(33.1)	54(45.8)	72(37.3)	117(46.1)	129(35.3)	8(19.0)	254(38.4)	
Other	45(33.1)	70(40.7)	38(32.2)	78(40.4)	83(32.7)	148(40.5)	9(21.4)	240(36.3)	

FAS = Full Analysis Set; **gAS** = All patients from the FAS tested with Guardant360 CDx; **gCEAS** = All patients in the gAS with an *ESR1* mutation detected by Guardant360 CDx, which is the primary clinical efficacy population (shaded in green); **gAS-** = All patients in the gAS with no *ESR1* mutations detected by Guardant360 CDx; **gAS-F** = All patients in the gAS that failed Guardant360 CDx testing; **gNT** = All patients in the FAS not tested by Guardant360 CDx; **Std. Dev.** = Standard Deviation; **Min** = Minimum; **Max** = Maximum; ***ESR1*-mut** = *ESR1* mutation detected; ***ESR1*-mut-nd**= *ESR1* mutation not detected.
*: Two subjects included in the drug efficacy analysis were not tested by Guardant360 CDx.

Table 12. Baseline Disease Characteristics of Full Analysis Set Population

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**		**gAS-Unknown (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
	***ESR1*-mut**	***ESR1*-mut nd**	***ESR1*-mut**	***ESR1*-mut nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**			
Analysis set:	**136***	**172**	**118**	**193**	**254**	**365**	**42**	**661**	
Duration of Disease (Months)									
n[a]	136	172	117	193	253	365	42	660	0.198
Mean	72.2	72.8	85.3	70.4	78.2	71.5	68.3	73.9	
Std.Dev.	76.1	70.9	85.6	71.4	80.8	71.1	66.5	74.6	
Median	39.6	49.6	45.7	45.2	41.6	46.3	48.6	45.5	
Min	8	1	5	2	5	1	3	1	
Max	408	355	377	324	408	355	283	408	
Missing	0	0	1	0	1	0	0	1	
Initial Pathological Diagnosis Type n(%)									
n[a]	136	168	118	189	254	357	41	652	0.567
Breast cancer	21(15.4)	28(16.7)	16(13.6)	38(20.1)	37(14.6)	66(18.5)	9(22.0)	112(17.2)	
Breast cancer metastatic	0	0	2(1.7)	0	2(0.8)	0	0	2(0.3)	
Invasive ductal breast carcinoma	101(74.3)	118(70.2)	87(73.7)	130(68.8)	188(74.0)	248(69.5)	28(68.3)	464(71.2)	
Invasive lobular breast carcinoma	11(8.1)	18(10.7)	12(10.2)	21(11.1)	23(9.1)	39(10.9)	4(9.8)	66(10.1)	
Mucinous breast carcinoma	3(2.2)	4(2.4)	1(0.8)	0	4(1.6)	4(1.1)	0	8(1.2)	
Initial Histopathological Diagnosis Grade n(%)									
n[a]	133	165	116	185	249	350	41	640	0.462
G1 - Low combined histologic grade (favorable)	11(8.3)	18(10.9)	5(4.3)	20(10.8)	16(6.4)	38(10.9)	2(4.9)	56(8.8)	

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**		**gAS-Unknown (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
	***ESR1*-mut**	***ESR1*-mut nd**	***ESR1*-mut**	***ESR1*-mut nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**			
G2 - Intermediate combined histologic grade (mod favorable)	68(51.1)	86(52.1)	69(59.5)	100(54.1)	137(55.0)	186(53.1)	16(39.0)	339(53.0)	
G3 - High combined histologic grade (unfavorable)	34(25.6)	37(22.4)	26(22.4)	40(21.6)	60(24.1)	77(22.0)	8(19.5)	145(22.7)	
GX - Grade cannot be assessed	20(15.0)	24(14.5)	16(13.8)	25(13.5)	36(14.5)	49(14.0)	15(36.6)	100(15.6)	
Study Entry Pathological Diagnosis Disease Stage n(%)									
n	136	172	118	193	254	365	42	661	0.626
Stage IIIB	1(0.7)	6(3.5)	0	4(2.1)	1(0.4)	10(2.7)	1(2.4)	12(1.8)	
Stage IIIC	3(2.2)	3(1.7)	1(0.8)	4(2.1)	4(1.6)	7(1.9)	0	11(1.7)	
Stage IV	132(97.1)	163(94.8)	117(99.2)	185(95.9)	249(98.0)	348(95.3)	41(97.6)	638(96.5)	
Baseline ECOG Performance Status n(%)									
n	136	172	118	193	254	365	42	661	0.603
0	84(61.8)	122(70.9)	77(65.3)	117(60.6)	161(63.4)	239(65.5)	27(64.3)	427(64.6)	
1	52(38.2)	50(29.1)	41(34.7)	76(39.4)	93(36.6)	126(34.5)	15(35.7)	234(35.4)	
Menopausal Status n(%)									
n[a]	136	169	118	192	254	361	41	656	>.999

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**		**gAS-Unknown (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
	***ESR1*-mut**	***ESR1*-mut nd**	***ESR1*-mut**	***ESR1*-mut nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**			
Post-Menopausal	120(88.2)	137(81.1)	105(89.0)	165(85.9)	225(88.6)	302(83.7)	35(85.4)	562(85.7)	
Pre-Menopausal	16(11.8)	32(18.9)	13(11.0)	27(14.1)	29(11.4)	59(16.3)	6(14.6)	94(14.3)	
Missing	0	3	0	1	0	4	1	5	
Prior CDK4/6i in any setting (CRF) n(%)									
n	136	172	118	193	254	365	42	661	0.495
No	44(32.4)	78(45.3)	33(28.0)	94(48.7)	77(30.3)	172(47.1)	28(66.7)	277(41.9)	
Yes	92(67.6)	94(54.7)	85(72.0)	99(51.3)	177(69.7)	193(52.9)	14(33.3)	384(58.1)	
Measurable Disease n(%)									
n	136	172	118	193	254	365	42	661	0.536
No	26(19.1)	35(20.3)	27(22.9)	48(24.9)	53(20.9)	83(22.7)	12(28.6)	148(22.4)	
Yes	110(80.9)	137(79.7)	91(77.1)	145(75.1)	201(79.1)	282(77.3)	30(71.4)	513(77.6)	
Number of Organs Involved n(%)									
n	136	172	118	193	254	365	42	661	0.7
1	35(25.7)	64(37.2)	35(29.7)	74(38.3)	70(27.6)	138(37.8)	16(38.1)	224(33.9)	
2	44(32.4)	52(30.2)	39(33.1)	54(28.0)	83(32.7)	106(29.0)	14(33.3)	203(30.7)	
>=3	57(41.9)	56(32.6)	44(37.3)	65(33.7)	101(39.8)	121(33.2)	12(28.6)	234(35.4)	
Visceral Metastasis (CRF) n(%)									

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**		**gAS-Unknown (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
	***ESR1*-mut**	***ESR1*-mut nd**	***ESR1*-mut**	***ESR1*-mut nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**			
n	136	172	118	193	254	365	42	661	0.610
No	54(39.7)	76(44.2)	51(43.2)	93(48.2)	105(41.3)	169(46.3)	21(50.0)	295(44.6)	
Yes	82(60.3)	96(55.8)	67(56.8)	100(51.8)	149(58.7)	196(53.7)	21(50.0)	366(55.4)	

FAS = Full Analysis Set; **gAS** = All patients from the FAS tested with Guardant360 CDx; **gCEAS** = All patients in the gAS with an *ESR1* mutation detected by Guardant360 CDx, which is the primary clinical efficacy population (shaded in green); **gAS-** = All patients in the gAS with no *ESR1* mutations detected by Guardant360 CDx; **gAS-F** = All patients in the gAS that failed Guardant360 CDx testing; **gNT** = All patients in the FAS not tested by Guardant360 CDx; **Std. Dev.** = Standard Deviation; **Min** = Minimum; **Max** = Maximum; ***ESR1*-mut** = *ESR1* mutation detected; ***ESR1*-mut-nd**= *ESR1* mutation not detected; **n**= number of subjects, **n**[a]= number of subjects with non-missing data, used as denominator for percentage calculation; **CRF**=case report form.
*: Two subjects included in the drug efficacy analysis were not tested by Guardant360 CDx.

D. Safety and Effectiveness Results

1. Safety Results

   Data regarding the safety of INLURIYO (imlunestrant) therapy are presented in the original drug approval. Refer to the INLURIYO (imlunestrant) label for more information. No adverse events were reported in the conduct of the diagnostic studies used to support this PMA supplement.

   For the specific adverse events that occurred in the clinical studies, please see the INLURIYO (imlunestrant) FDA approved package insert which is available at Drugs@FDA.

2. Effectiveness Results

   PFS in Patients with *ESR1* Mutations Detected by Guardant360 CDx

   To demonstrate the clinical validity of Guardant360 CDx for the selection of ER+/HER2- locally advanced/metastatic breast cancer patients with *ESR1* mutations for treatment with INLURIYO (imlunestrant), the primary diagnostic study objective was assessed by comparing the efficacy of INLURIYO (imlunestrant) therapy relative to investigator's choice endocrine therapy in *ESR1*-mut patients.

   The PFS hazard ratio (HR) observed in the *ESR1*-mut population (gCEAS) treated with INLURIYO (imlunestrant) therapy vs. investigator's choice endocrine

therapy (fulvestrant or exemestane) was 0.635 (95% CI: [0.478, 0.844], p=0.0017, **Table 13**). The clinical efficacy of INLURIYO (imlunestrant) therapy in the *ESR1*-mut population is further supported by the clear separation of the treatment arms in the Kaplan-Meier plot of PFS (**Figure 2**).

The median PFS in the *ESR1*-mut population treated with INLURIYO (imlunestrant) therapy was 5.49 months (95% CI: 3.78, 7.16) vs. investigator's choice endocrine therapy (fulvestrant or exemestane) 3.84 months (95% CI: 3.68, 5.52) (**Table 13**).

Figure 2. Kaplan-Meier Plot of Investigator-Assessed PFS for INLURIYO (imlunestrant) Therapy vs. Investigator's Choice Endocrine Therapy (fulvestrant or exemestane) in *ESR1*-Mut Subjects (Date Cutoff Date = 24-JUN-2024; Note: Two subjects included in the drug efficacy analysis were not tested by Guardant360 CDx.**)**

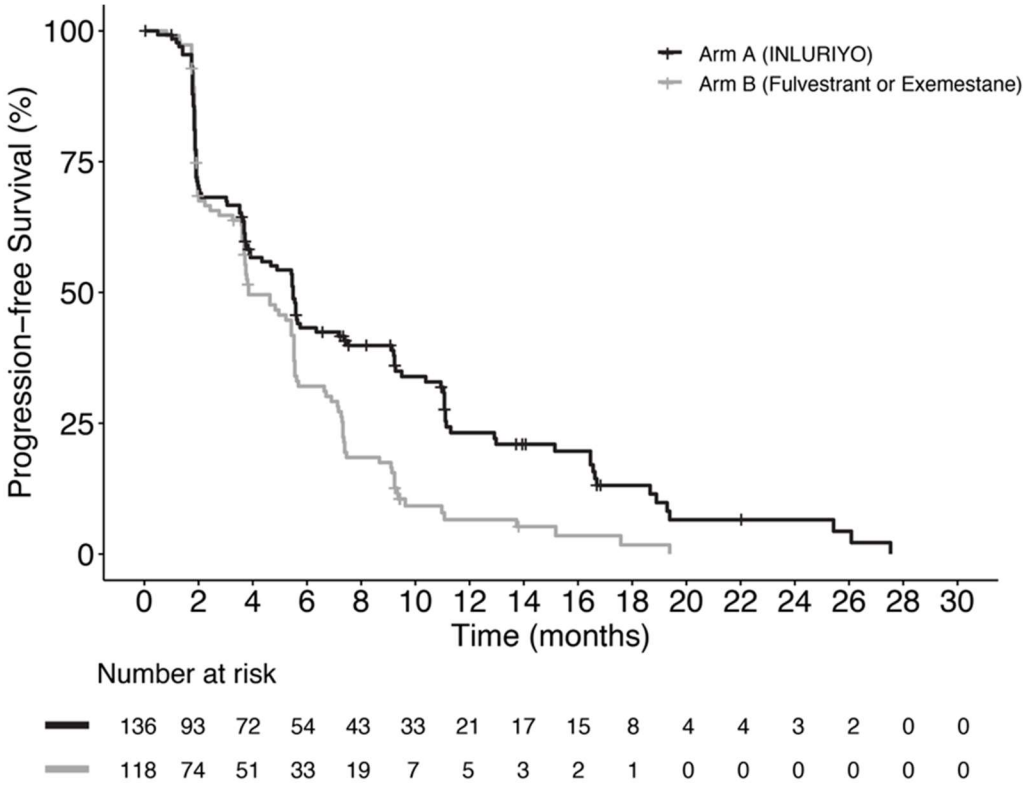


Table 13. Summary of Investigator-Assessed PFS for INLURIYO (imlunestrant) Therapy vs. Investigator's Choice Endocrine Therapy (fulvestrant or exemestane) in *ESR1*-Mut Subjects

	INLURIYO Therapy (N=136*)	**Fulvestrant or Exemestane (N=118)**
Number of Events, n (%)	109 (80.1)	102 (86.4)

	INLURIYO Therapy (N=136*)	Fulvestrant or Exemestane (N=118)
Median PFS Months (95% CI)	5.49 (3.78, 7.16)	3.84 (3.68, 5.52)
Hazard Ratio (95% CI)	0.635 (0.478, 0.844)	
p-value (stratified log-rank)	0.0017	

*Two subjects included in the drug efficacy analysis were not tested by Guardant360 CDx.

To assess the potential impacts of missing test result data, sensitivity analysis was conducted using multiple imputation (MI) approach based on building a classifier-based logistic regression model. Sensitivity analysis demonstrated similar results for PFS (HR=0.624, 95% CI: 0.469, 0.830).

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

E. Financial Disclosure

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

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

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

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

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

For the intended use of identifying breast cancer patients with *ESR1* mutations for treatment with INLURIYO (imlunestrant), the effectiveness of Guardant360 CDx was demonstrated through analytical studies using patient samples from the intended use population and a diagnostic clinical validation study utilizing Guardant360 CDx results and outcome data from the EMBER-3 (NCT04975308) clinical study. The data from the analytical validation and clinical studies support the reasonable assurance of safety and effectiveness of Guardant360 CDx when used in accordance with the indications for use. Data from the EMBER-3 clinical study show that patients who had qualifying *ESR1* mutations received benefit from treatment with INLURIYO (imlunestrant) and support the addition of the CDx indication to Guardant360 CDx.

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. Guardant360 CDx is an *in vitro* diagnostic test, which involves testing of cfDNA extracted from the plasma of whole blood routinely collected as part of the diagnosis and patient care.

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions in cancer treatment. Patients with false positive results may undergo treatment with one of the therapies listed in Table 1 of the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with the indicated therapy.

C. Benefit-Risk Determination

The clinical benefit of Guardant360 CDx for the identification of breast cancer patients with *ESR1* mutation(s) for treatment with INLURIYO (imlunestrant) was demonstrated in a prospective analysis of efficacy and safety data obtained from the EMBER-3 (NCT04975308) clinical study. The supporting clinical validation analysis demonstrates PFS HR (0.635, 95% CI: 0.478, 0.844) for subjects from the EMBER-3 clinical study positive by Guardant360 CDx for *ESR1* mutation(s) (*ESR1*-mut) treated with INLURIYO (imlunestrant) relative to investigator's choice endocrine therapy (fulvestrant or exemestane) which provides for a meaningful clinical benefit, given the patient population. These results support the use of Guardant360 CDx as an aid in identification of breast cancer patients with *ESR1* mutation(s) for INLURIYO (imlunestrant) treatment.

There is a potential risk associated with the use of Guardant360 CDx, for identification of breast cancer patients with *ESR1* mutation(s) for treatment with INLURIYO (imlunestrant), 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 Guardant360 CDx 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 may have delayed access to treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial drug.

The likelihood of false results was assessed by analytical and clinical validation studies, which partially mitigate the probable risk of the Guardant360 CDx, discussed above.

The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with acceptable accuracy, mitigating the potential for false results.

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

In conclusion, given the above information, the data support that for the indications of the Guardant360 CDx device discussed above, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the analytical and clinical studies support the use of Guardant360 CDx as an aid for the identification of patients with breast cancer with qualifying *ESR1* mutation(s) (E380 mutations, V422 deletion, Y537 mutations, S463P/F, L469V, D538 mutations, and L536 mutations) that may benefit from INLURIYO (imlunestrant) therapy.

XIII. CDRH DECISION

CDRH issued an approval order on September 25, 2025.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XV. REFERENCES

None.