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/S010
Date of FDA Notice of Approval:	January 27, 2023

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 (SNVs and exon 20 insertions) in NSCLC patients. 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 *ESR1* missense mutations between codons 310 and 547 in breast cancer patients who may benefit from treatment with ORSERDU™ (elacestrant).

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)

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

Guardant360 CDx is a single-site test performed at Guardant Health, Inc. The 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, PDGFRA, PIK3CA, PTEN, RET, ROS1, STK11, TSC1, VHL*

Alteration Type	Genes
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* Missense 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).

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) within the LIMS system. The IVD results report will be populated with patient-specific information and may be merged with additional information provided by Guardant Health as a professional service prior to approval and release by the laboratory director or designee.

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). Also, there are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of *ESR1* missense mutations (subject of this PMA Supplement) in patients with breast cancer (BC) eligible for treatment with ORSERDU (elacestrant).

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

Guardant Health, Inc. initially designed and developed the Guardant360 laboratory developed test (Guardant360 LDT), and the first commercial sample was tested in 2012 to detect the presence of genomic alterations in plasma isolated from whole blood. The Guardant360 LDT is not FDA-cleared or -approved.

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)

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* missense mutations between codons 310-547 (hereafter referred to as *ESR1* missense mutations) was from the data presented using intended use specimens across all validation studies. In addition to the existing platform-level validation data (P200010, P200010/S001 and P200010/S002), analytical accuracy/concordance, limit of detection (LoD), precision at the LoD and sample stability (whole blood, plasma, cfDNA) studies, were conducted to support the indication for the *ESR1* missense 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* missense mutations in plasma specimens from patients with BC and without BC. These results from the platform-level validation (P200010, P200010/S001, and P200010/S002) that have been leveraged to support Guardant360 CDx detection of *ESR1* missense mutations are referenced below. Additional *ESR1*-specific analytical validation studies are described below.

1. Analytical Accuracy/Concordance

Accuracy for the detection of *ESR1* missense mutations was established relative to an externally validated mass spectrometry-based comparator method by comparing the results from 259 samples from the EMERALD (NCT03778931) clinical study. The analysis included 258 samples that passed QC on both assays. Among the 258 samples, two pairs of samples were identified to be duplicate as each sample pair was drawn from the same subject on different days. Therefore, these two pairs of samples (n=4) were excluded from the analysis and 254 samples were included in the Accuracy Study.

A summary of positive percent agreement (PPA), negative percent agreement (NPA), and the corresponding 95% two-sided exact confidence intervals (CIs) is provided in Table 8. Out of the 254 remaining samples, 121 samples (47.6%) were positive for an eligible *ESR1* variant by both Guardant360 CDx and the comparator method [Guardant360 CDx (+) / Comparator (+)], while 110 samples (43.3%) were negative by both platforms [Guardant360 CDx (-) / Comparator (-)]. Three samples (1.2%) were negative by Guardant360 CDx but positive by the comparator method [Guardant360 CDx (-) / Comparator (+)], and 20 samples (7.9%) were positive by Guardant360 CDx but negative by the comparator method [Guardant360 CDx (+) / Comparator (-)].

Table 8. Summary of Concordance Between Guardant360 CDx and the Comparator for *ESR1* Missense Mutations

Alteration Type	Guardant360 CDx (+), Comparator (+)	Guardant360 CDx (+), Comparator (-)	Guardant360 CDx (-), Comparator (+)	Guardant360 CDx (-), Comparator (-)	Patients (n)	PPA (95% CI)*	NPA (95% CI)*
ESR1 missense mutations	121	20	3	110	254	97.6% (93.1% - 99.5%)	84.6% (77.2% - 90.3%)

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

Among the 254 samples, 191 samples were enrolled in the EMERALD (NCT03778931) clinical study and have valid drug efficacy data, i.e., progression free survival (PFS) data. To understand if the low NPA of the Accuracy Study was due to the low sensitivity of the comparator method or the erroneous detection of the Guardant360 CDx, drug response data for the patients with discordant samples [Guardant360 CDx (+) / Comparator (-)], who were enrolled in the clinical study based on the CDx result, were analyzed.

Patients with discordant [Guardant360 CDx (+) / Comparator (-)] results in the elacestrant arm had a median PFS of 4.58 months, which is similar to the median PFS (3.78 months) observed in the patients assigned to the elacestrant arm in the clinical trial. Moreover, the discordant samples [Guardant360 CDx (+) / Comparator (-)] in the standard of care (SOC) arm had a median PFS of 1.87 months, which is the same median PFS observed in the total subjects assigned to the SOC arm. Refer to Section X for further details regarding the EMERALD (NCT03778931) clinical study.

The results show that Guardant360 CDx effectively detects *ESR1* missense mutations in patients with BC. The discordant samples [Guardant360 CDx (+) / Comparator (-)] had MAF values ranging from 0.04% to 0.56%, with the majority of samples (17 of 20 samples) harboring MAF values below the LoD (Refer to Section IX.A.2.b for further details regarding the LoD study). The discordance between the comparator method and Guardant360 CDx may be due to the stochastic detection of mutations below LoD of both assays, along with sensitivity differences between Guardant360 CDx and the comparator method.

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)

The LoDs for *ESR1* E380Q, Y537S and D538G were established using patient sample pools derived from patients with BC at 5 ng and 30 ng cfDNA input. E380Q is located in exon 7, and Y537S and D538G are located in exon 10 of the *ESR1* gene. All of these mutations are representative of prevalent *ESR1* missense mutations. Trial pools targeting 2.16% MAF at 5 ng input (pool 1) and 0.4% MAF at 30 ng input (pool 2) were characterized with Guardant360 CDx. For cfDNA at 5 ng input, the LoD was estimated using 5 MAF levels ranging from 0.10% to 2.16% (0.10%, 0.65%, 0.97%, 1.45% and 2.16%) with 24 replicates per level. For cfDNA at 30 ng input, the LoD was estimated using 5 MAF levels ranging from 0.017% to 0.40% (0.017%, 0.12%, 0.18%, 0.27% and 0.40%) with 14 replicates per level. Since fewer than three observed MAF levels had detection rates between 10% and 90%, an empirical estimate of the LoD was used, which was defined as the lowest MAF observed for which all MAF levels at or above this MAF had a hit rate ≥ 95%. The LoDs for *ESR1* E380Q, Y537S and D538G are summarized in Table 9.

The established LoDs were confirmed for these prevalent *ESR1* mutations by testing clinical sample pools from patients with BC near the established LoDs (refer to Table 9) across 24 replicates at 5 ng input using a combined LoD Confirmation and Precision Study. Refer to Section IX.A.4 for further details regarding the combined LoD Confirmation and Precision Study.

Table 9. Summary of Established LoDs for Prevalent *ESR1* Missense Mutations in BC Clinical Samples

ESR1 Missense Mutation	LoD, 5ng input MAF (%)	LoD, 30 ng input MAF (%)
E380Q	1.0	0.3
Y537S	1.0	0.3
D538G	1.1	0.2

In addition to confirming the LoDs for the prevalent mutations, a combined LoD Confirmation and Precision Study was also performed using sample pools from patients with BC at 5 ng cfDNA input with a 2-3% targeted MAF (2-3x LoD for the prevalent mutations) across 24 replicates for three rare *ESR1* mutations (H356D, G442A and S463P). The *ESR1* mutations H356D, G442A and S463P are located in exons 6, 8 and 9, respectively. The LoDs for *ESR1* G442A and S463P were confirmed; however, the study result of the LoD confirmation and precision study for *ESR1* H356D has a PPA of 83.3% or 91.7%, which did not achieve agreement of ≥ 95% across all conditions for H356D at 5 ng of cfDNA input (Table 10).

Table 10. Summary of Confirmed LoDs for *ESR1* Missense Mutations in Clinical Samples from Patients with BC

ESR1 Missense Mutation	LoD, 5ng input (MAF%)	Number Positive/ Number Expected	PPA (95% CI)**
H356D	2.1^	20/24	83.3% (62.6% - 95.3%)
H356D	3.1^	22/24	91.7% (73.0% - 99.0%)
G442A	2.3*	24/24	100% (85.8%-100%)
S463P	2.8*	24/24	100% (85.8%-100%)

^LoD values were not confirmed for H356D as the PPA did not achieve agreement of ≥95%.
*LoD values were confirmed at 2.1x (G422A) and 2.6x (S463P) of the prevalent *ESR1* mutation LoD values.
**The Clopper-Pearson Exact Method was used for the CI analysis.

Coverage analysis showed that the mean coverage at the H356D locus (independent of variant detection) was lower than the coverage for the other two *ESR1* missense mutations (G442A and S463P). The mean Non-Singleton Coverage (NSC) of H356D is 522.52, while that of G442A and S463P are 583.00 and 567.83, respectively. The reason for the study did not demonstrate

agreement of ≥ 95% H356D may be due to stochastic, near-threshold effects at a reduced coverage. Given these reasons and the rarity of H356D mutations (0.88% of the patients enrolled in the EMERALD (NCT03778931) clinical study were observed to harbor a H356D mutation), the study result was deemed acceptable. The LoD confirmation study results (Table 10) suggest that the LoDs for theses variants are higher (~2x-3x higher) than the established LoDs for the variants in Table 9 at 5 ng cfDNA input.

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**

The purpose of the precision study was to demonstrate the repeatability and within-site reproducibility of Guardant360 CDx for detecting representative *ESR1* missense mutations (H356D, E380Q, G442A, S463P, Y537S and D538G) through closeness of agreement between measured qualitative detection in replicates using different combinations of reagent lots, instruments, operators, and days. The study was conducted with sample pools from patients with BC harboring three prevalent missense mutations (E380Q, Y537S and D538G) or three rare mutations (H356D, G442A and S463P).

For prevalent mutations (E380Q, Y537S and D538G), precision was evaluated using 5 ng input with MAF levels targeted at 1x LoD. Each variant was tested across six unique reagent lot-instrument-operator combinations with four replicates per combination (6x4=24 samples). This study successfully verified the precision of Guardant360 CDx for detecting the three representative *ESR1* prevalent mutations (E380Q, Y537S and D538G) within and between different reagent lots, instrument sets, and operator groups with samples near LoD processed on different runs and days in the Guardant Health Clinical Laboratory (Table 11).

For rare mutations (H356D, G442A and S463P), the precision study was performed using 5 ng input at analyte levels targeted to 2.0-3.0% MAF (1.8x-2.9x of the established LoD (i.e., 1.1% MAF) for the prevalent *ESR1* missense mutations). Each variant was tested across six unique reagent lot-instrument-operator combinations, with four replicates per combination (6x4=24 samples). The PPA of *ESR1* SNVs G442A and S463P were observed to be 100%; however, the *ESR1* H356D mutation showed a PPA of 83.3% or 91.7% at 2.1% MAF and 3.1% MAF, respectively, which did not demonstrate agreement of ≥ 95% (Table 11). By evaluating 25 additional replicates from the same sample pool for H356D, PPA of 95.9% was achieved.

Table 11. Summary of Precision Results for Representative *ESR1* Missense Mutations

ESR1 Missense Mutation	Observed MAF%	Relative LoD Level*	Number Positive/ Number Expected	PPA (95% CI)***
E380Q	1.0	1.0x	24/24	100% (85.8%-100%)
Y537S	1.0	1.0x	23/24	95.8% (78.9% - 99.9%)
D538G	1.1	1.0x	23/24	95.8% (78.9% - 99.9%)
H356D	2.1**	2.0x	20/24	83.3% (62.6% - 95.3%)
H356D	3.1**	2.9x	22/24	91.7% (73.0% - 99.0%)
G442A	2.3	2.1x	24/24	100% (85.8%-100%)
S463P	2.8	2.6x	24/24	100% (85.8%-100%)

*Compared to the established LoD for the prevalent *ESR1* missense mutations.
**Note that the observed MAF is the average variant MAF from all samples with a reported variant (i.e., excluding dropouts).
***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. Additional stability studies were performed to verify the platform level stability for whole blood, plasma and cfDNA samples, which evaluated samples from patients with BC harboring *ESR1* missense mutations.

a. Whole Blood Stability

The purpose of this study was to demonstrate the stability of whole blood specimens used for Guardant360 CDx collected in the Guardant360 BCK, across the expected range of sample transport and storage conditions for up to 7 days after blood collection prior to plasma isolation. While whole blood stability from patients with multiple different cancer types has been examined previously (P200010), this study focuses specifically on specimens derived from patients with metastatic breast cancer (mBC).

A total of four BCTs were drawn from each of 11 patients with mBC and subjected to the conditions described in Table 12 below. For each patient, a single tube of blood was processed to plasma one day after the blood draw (stored at room temperature). Plasma was then shipped on dry ice to Guardant Health. This constituted the reference condition. In addition to the reference tube, three more blood tubes per donor were shipped as whole blood to Guardant Health and subjected to Condition 1 (Summer profile), Condition 2 (Winter profile) or Condition 3 (Room temperature), as described in Table 12. After conditioning, plasma was isolated on the 8th day after blood collection and run using the Guardant360 CDx assay.

Table 12. Description of Whole Blood Storage Conditions

Condition	BCT per Patient	Storage Condition / Processing
Reference (Ref)	1	Reference condition: Plasma processing (day 1 after blood collection).
Condition 1 (C1)	1	Summer Profile Storage: 4h at 22°C, 6h at 37°C and 56h at 22°C, 6h at 37°C) plus remaining time at room temperature.
Condition 2 (C2)	1	Winter Profile Storage: 4h at 18°C, 6h at 0°C, 56h at 10°C, and 6h at 0°C plus remaining time at room temperature.
Condition 3 (C3)	1	Room Temperature Storage: Storage at room temperature 18-25°C.

Among the 44 samples, one sample failed the "On Target Rate" post-sequencing QC metric, and four samples from one patient were excluded from the analysis because the reference condition sample had less than 5ng input. The remaining 39 samples resulted in 10 evaluable patient groups for the

Winter Profile (C2) and Room Temperature (C3) conditions and 9 evaluable patient groups for the Summer Profile condition (C1). All storage conditions demonstrated acceptable performance (Table 13). All samples in each group demonstrated acceptable sample-level molecule recovery, as assessed by depth of NSC coverage across the panel. The fold change of median NSC in the test condition over the reference condition or time zero ranged from 0.87 to 1.00.

Table 13. Whole Blood Stability Summary Results

				Room Temp
Sample-Level Molecule Recovery	Median of NSC fold (condition / reference)	0.87	0.94	1.00
Exon-Level Molecule Recovery	90% LCL of Fraction of Exons with Expected Coverage	98.26%	98.71%	98.55%
Variant Call Concordance*	PPA (n/N)	83.33% (10/12)	75.00% (9/12)	75.00% (9/12)
	PPA (≥ 1X LoD) (n/N)	100.00% (6/6)	100.00% (6/6)	100.00% (6/6)
	NPA (n/N)	>99.99% (25,315/ 25,316)	100.00% (27,849/ 27,849)	>99.99% (27,848/ 27,849)
Variant Call Concordance (*ESR1*)**	PPA (n/N)	85.71% (6/7)	85.71% (6/7)	71.43% (5/7)
	PPA (≥ 1X LoD) (n/N)	100.00% (3/3)	100.00% (3/3)	100.00% (3/3)
	NPA (n/N)	99.51% (204/205)	100.00% (226/226)	99.56% (225/226)

LCL, Lower Confidence Limit.
*The variant call concordance analysis is based on the results from all detectable variants.
**The variant call concordance analysis is limited to the *ESR1* variants.

Exon-level coverage was also acceptable for all conditions evaluated. The fraction of exons with relative exon level coverage difference between each condition and the reference (Time zero) within 2σ (2 × 0.204) was 98.56%-98.95%, which demonstrate that there was no preferential drop-out of relative exon-level coverage exceeding expected levels due to random variation. The

lower bound of the 90% exact binomial CI for the fraction of genomic targeted exonic regions with relative exon-level NSC is shown in Table 13.

PPAs were also calculated for the SNVs, insertion-deletion mutations (indels) and copy number alterations (CNAs) in the reportable range: 11 SNVs and 1 indel. All conditions showed variant call concordance PPA of 75.00% - 83.33%. The low PPA for variant call concordance is probably due to the sub-LoD sample level (0.14x - 0.90x LoD) for some samples. PPA above LoD was 100.00% for all conditions (Table 13). For the NPA analysis, a total of 2,533 genomic positions were considered for each sample pair. The ratio of genomic positions in agreement and the total number of assessed positions are shown in parentheses.

To demonstrate biomarker-specific variant call concordance, the PPA and NPA calculations were repeated for *ESR1* missense mutations within the biomarker definition (i.e., codons 310-547). The PPA was observed to be 85.71% for both the Summer and Winter Profiles and 71.43% for the Room Temperature Profile. One variant - Y537N (c.1609T>A), which had a sample level of 0.25x LoD, was not detected in all storage conditions; the other variant - L536Q (c.1607_1608delTCinsAG), which had a sample level of 0.90x LoD, was not detected in C3 (Room Temperature). Detection below or close to the LoD threshold is expected to be stochastic. For variants ≥ 1x LoD in the reference condition, the PPA was observed to be 100% for all storage conditions. The NPA calculations were limited to only consider the 21 genomic locations that overlapped the biomarker definition that were found in either this study or in P200010. The NPA was observed to be 99.50% in the summer profile, 100.00% in the Winter profile, and 99.56% in the Room Temperature profile (Table 13).

The results confirm the observations from P200010 for BC samples that whole blood storage in Guardant360 BCKs preserves cfDNA quantity and quality for up to seven days and in extreme weather shipping conditions.

b. Plasma Stability

The purpose of this study was to demonstrate the stability of frozen plasma samples at -80°C ± 10°C, which is representative of the expected range of sample storage conditions for the specimen type. While plasma stability has been examined previously (P200010) for all variant types across multiple cancer indications, this study focuses specifically on specimens derived from patients with advanced breast cancer.

The study samples were selected in reverse chronological order from remnant BC plasma specimens in the Guardant Health biobank that were sequenced using Guardant360 CDx at least 46 days before the study start date. Two BCTs from 22 cancer patients, 44 samples in total, were collected and run on Guardant360 CDx, with plasma stored at the specified storage conditions (Table 14).

Table 14. Description of Plasma Storage Conditions

Condition	BCT per Patient	Storage Condition / Processing
Reference	1	Reference condition: cfDNA extraction after plasma isolation within 48 hours of delivery.
Condition	1	Storage condition: Storage of plasma at -80 ± 10°C for ≥ 45 days before cfDNA extraction.

All 44 samples passed their respective in-process and post-sequencing QC metrics. In the tested storage condition, samples demonstrated acceptable sample-level molecule recovery, relative exon-level coverage, variant call concordance for all variants, including variant call concordance for *ESR1* (Table 15).

Sample level molecule recovery showed a NSC fold change of 0.94. The lower bound of the 90% CI for the relative exon coverage metric for the storage condition was 98.05%. The PPA was also calculated for a total of 43 variants (37 SNVs and 6 Indels). The storage condition showed variant call concordant PPA of 88.37%. The PPA above LoD was 96.97%. The NPA across the reportable range was 99.99% (Table 15).

Table 15. Plasma Stability Summary Results

Study Endpoint	Metric	Study Result
Sample-Level Molecule Recovery	Median of NSC fold (condition/reference)	0.94
Exon-Level Molecule Recovery	90% LCL of Fraction of Exons with Expected Coverage	98.05%
Variant Call Concordance*	PPA (n/N)	88.37% (38/43)
	PPA (≥ 1X LoD) (n/N)	96.97% (32/33)
	NPA (n/N)	99.99% (55,958/55,961)
Variant Call Concordance (*ESR1*)**	PPA (n/N)	73.68% (14/19)
	PPA (≥ 1X LoD) (n/N)	88.89% (8/9)
	NPA (n/N)	100.00% (510/510)

* The variant call concordance analysis is based on the results from all detectable variants.
**The variant call concordance analysis is limited to the *ESR1* variants.

The PPA and NPA calculations were also performed for *ESR1* missense mutations within the biomarker definition. The PPA was observed to be 73.68%. Among the five discordant variants, four variants were detected below or near the LoD threshold (0.10x – 0.87x LoD). Detection below or close to the LoD threshold is expected to be stochastic. For variants ≥ 1x LoD in the reference condition, the PPA was observed to be 88.89% for the storage condition, and one variant – D351N (c.1051G>A), which had a sample level of 1.77x LoD was not detected in the storage condition. The NPA was observed to be 100% in the storage condition (Table 15).

Based on these study results, plasma may be stored at -80 ± 10°C for ≤ 45 days before cfDNA extraction.

c. cfDNA Stability

The purpose of this study was to evaluate the stability of cfDNA samples stored at -20°C ± 5°C for 45 days (Table 16). While cfDNA stability has been examined previously (P200010) for all variant types across multiple cancer indications, this study focused specifically on specimens derived from patients with advanced breast cancer. In total, 56 samples were collected from 28 patients. One sample failed the post-enrichment quantification step due to high molarity and was excluded from the analysis. The remaining 55 samples passed all QC metrics with a 98.2% pass rate, resulting in 27 evaluable sample pairs.

Table 16. Description of cfDNA Storage Conditions

Condition	BCT per Patient	Storage Condition / Processing
Reference	1	Reference condition: Quantitation, dilution and library preparation on the same day as cfDNA extraction.
Condition	1	Quantitation and dilution on the same day as cfDNA extraction, followed by storage of cfDNA at -20°C ± 5°C for 46 days plus 1 freeze/thaw cycle before library preparation.

In the tested storage condition, samples demonstrated acceptable sample-level molecule recovery, relative exon-level coverage, and variant call concordance (Table 17).

Sample level molecule recovery showed a NSC fold change of 1.05. The lower bound of the 95% CI for the relative exon coverage metric for the storage condition was 90.30%. The PPA was also calculated for a total of 106 variants (96 SNVs and 10 Indels). The storage condition showed a variant call concordance PPA of 89.62%, and the PPA above LoD was 96.05%. The NPA across the reportable range was 99.97% (Table 17).

In total, 74 *ESR1* SNVs were detected in the reference condition. The PPA was observed to be 89.19%. The variant call discordance is probably due to the sub-LoD detection in the reference condition (variants at 0.13x - 0.90x LoD sample level). For variants ≥ 1x LoD in the reference condition, the PPA was observed to be 96.00% for the storage condition. The NPA was observed to be 99.36% in the storage condition (Table 17).

Table 17. cfDNA Stability Summary Results

Study Endpoint	Metric	Study Result
Sample-Level Molecule Recovery	Median of NSC fold (condition/reference)	1.05
Exon-Level Molecule Recovery	95% LCL of Fraction of Exons with Expected Coverage	90.30%
Variant Call Concordance*	PPA (n/N)	89.62% (95/106)
	PPA (≥ 1X LoD) (n/N)	96.05% (73/76)
	NPA (n/N)	99.97% (69,018/69,040)
Variant Call Concordance (*ESR1*)**	PPA (n/N)	89.19% (66/74)
	PPA (≥ 1X LoD) (n/N)	96.00% (48/50)
	NPA (n/N)	99.36% (1,243/1,251)

*The variant call concordance analysis is based on the results from all detectable variants.
**The variant call concordance analysis is limited to the *ESR1* variants.

Based on these study results, extracted cfDNA may be stored at -20°C for ≤ 45 days for Guardant360 CDx analysis.

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 patients with mBC with *ESR1* missense mutations between codons 310 and 547 (hereafter referred to as *ESR1* missense mutations) who may benefit from treatment with ORSERDU (elacestrant) was demonstrated through testing of cfDNA in pre-treatment plasma specimens from patients enrolled into the EMERALD (NCT03778931) clinical study, which was conducted to support the efficacy of ORSERDU (elacestrant). In the EMERALD clinical study, patients were prospectively stratified based on Guardant360 CDx detection of *ESR1* missense mutations. A summary of the Guardant360 CDx clinical validation study is presented below.

A. EMERALD (NCT03778931) Clinical Study Design

The EMERALD clinical study was an international, multicenter, randomized, open-label, active-controlled, event-driven, Phase 3 clinical study comparing the safety and efficacy of ORSERDU (elacestrant) to the standard of care (SOC) treatment options of fulvestrant or an aromatase inhibitor (AI) in post-menopausal women and men with ER+/HER2- mBC. Eligible patients were randomized in a 1:1 ratio to either ORSERDU (elacestrant) or SOC and stratified by *ESR1* mutation status using Guardant360 CDx and other criteria described in the clinical study protocol.

Patients from the primary EMERALD registration population positive for *ESR1* mutations by Guardant360 CDx were included in the diagnostic study primary clinical efficacy cohort to assess the clinical validity of Guardant360 CDx to identify BC patients with *ESR1* missense mutations to aid in the selection of patients for treatment with ORSERDU (elacestrant) therapy.

1. Clinical Study Inclusion and Exclusion Criteria

Patients in the primary EMERALD registration population were included in the diagnostic study efficacy cohort if the selection criteria were met. The key inclusion criteria are summarized below:

- Male or postmenopausal female
- Histologically- or cytologically-proven adenocarcinoma of the breast with evidence of either locally advanced disease not amenable to resection or radiation therapy with curative intent or metastatic disease not amenable to curative therapy
- Must be appropriate candidates for endocrine monotherapy
- Must have ER+ and HER2- tumor status confirmed
- Must have previously received at least one and no more than two lines of endocrine therapy, either a monotherapy or as a combination therapy with another agent; prior treatment with a CDK4/6 inhibitor in combination with either fulvestrant or an AI; and no more than one line of cytotoxic chemotherapy in the mBC setting
- Measurable disease or non-measurable bone-only disease.

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 ORSERDU (elacestrant) efficacy in the EMERALD (NCT03778931) clinical study primary objective was progression free survival (PFS) by RECIST version 1.1 as assessed by independent central review (ICR) or death from any cause.

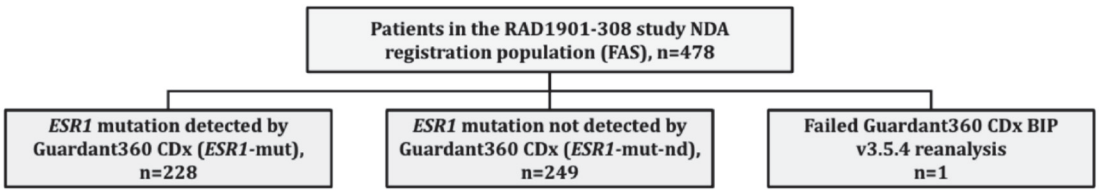
4. **Diagnostic Objective and Endpoints**
   The primary objective of the diagnostic clinical study was to demonstrate the safety and effectiveness of Guardant360 CDx as a companion diagnostic to aid in the selection of breast cancer patients with *ESR1* missense mutations for ORSERDU (elacestrant) therapy. This was a co-development study utilizing plasma samples and the clinical outcome data from the EMERALD (NCT03778931) clinical study. Guardant360 CDx was used to test all available samples.

   The primary objective was assessed by comparing the efficacy of ORSERDU (elacestrant) to that of subjects on SOC therapy in patients harboring *ESR1* mutations detected by Guardant360 CDx. The primary endpoint is the same as that used for the clinical study, PFS by RECIST 1.1 as assessed by ICR.

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

The EMERALD (NCT03778931) clinical study registration population (Full Analysis Set, FAS) included 478 subjects, of which 228 subjects had *ESR1* missense mutations detected by Guardant360 CDx, while 249 subjects did not have an *ESR1* missense mutation detected by Guardant360 CDx (Figure 1). Of note, one patient was excluded from the diagnostic study efficacy analysis due to QC failure with the final Guardant360 CDx bioinformatics software.

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



C. Study Population Demographics and Baseline Parameters for the Guardant360 CDx Clinical Study for *ESR1* Missense Mutations

Demographics and baseline clinical characteristics of patients enrolled in the EMERALD (NCT03778931) clinical study was categorized relative to the diagnostic study populations, as defined by Guardant360 CDx results. As shown in Tables 18 and 19, the diagnostic study primary efficacy population *ESR1*-mutated (*ESR1*-mut) and *ESR1*-mutation-not-detected (*ESR1*-mut-nd) were well balanced. The ORSERDU (elacestrant) and SOC treatment arms were also well balanced.

Table 18. Clinical Effectiveness Analysis Subgroup Demographics

	Elacestrant		**SOC**		**Total**	
	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**
Analysis set:	**115**	**124**	**113**	**125**	**228**	**249**
Age, years						
N (missing)	115 (0)	124 (0)	113 (0)	125 (0)	228 (0)	249 (0)
Mean	62.7	62.4	62.0	64.4	62.4	63.4
SD	12.25	11.91	11.74	10.03	11.98	11.03
Median	64.0	63.0	63.0	64.0	63.0	64.0
Min	28	24	32	41	28	24
Max	89	84	83	82	89	84
Age Group (years) n (%) n (missing)	115 (0)	124 (0)	113 (0)	125 (0)	228 (0)	249 (0)
>=18 - <50	15 (13.0)	18 (14.5)	19 (16.8)	10 (8.0)	34 (14.9)	28 (11.2)
>=50 - <65	47 (40.9)	55 (44.4)	43 (38.1)	55 (44.0)	90 (39.5)	110 (44.2)
>=65 - <75	36 (31.3)	28 (22.6)	34 (30.1)	31 (24.8)	70 (30.7)	59 (23.7)
>=75	17 (14.8)	23 (18.5)	17 (15.0)	29 (23.2)	34 (14.9)	52 (20.9)
<65	62 (53.9)	73 (58.9)	62 (54.9)	65 (52.0)	124 (54.4)	138 (55.4)
>=65	53 (46.1)	51 (41.1)	51 (45.1)	60 (48.0)	104 (45.6)	111 (44.6)
Race n (%) [1]						
N (missing)	94 (21)	96 (28)	92 (21)	102 (23)	186 (42)	198 (51)
American Indian or Alaska Native	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Asian	5 (5.3)	11 (11.5)	8 (8.7)	8 (7.8)	13 (7.0)	19 (9.6)
Black or African American	4 (4.3)	1 (1.0)	4 (4.3)	3 (2.9)	8 (4.3)	4 (2.0)
Native Hawaiian or Other Pacific Islander	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
White/Caucasian	84 (89.4)	84 (87.5)	80 (87.0)	90 (88.2)	164 (88.2)	174 (87.9)
Other	1 (1.1)	0 (0.0)	0 (0.0)	1 (1.0)	1 (0.5)	1 (0.5)
Gender n (%) n (missing)	115(0)	124 (0)	113 (0)	125 (0)	228 (0)	249 (0)
Male	0 (0.0)	6 (4.8)	0 (0)	1 (0.8)	0 (0.0)	7 (2.8)
Female	115 (100.0)	118 (95.2)	113 (100.0)	124 (99.2)	228 (100.0)	242 (97.2)
Ethnicity n (%) n (missing)	115 (0)	124 (0)	113 (0)	125 (0)	228 (0)	249 (0)

	Elacestrant		SOC		Total	
	ESR1-mut	*ESR1*-mut-nd	*ESR1*-mut	*ESR1*-mut-nd	*ESR1*-mut	*ESR1*-mut-nd
Hispanic or Latino	10 (8.7)	9 (7.3)	10 (8.8)	8 (6.4)	20 (8.8)	17 (6.8)
Non-Hispanic or Latino	92 (80.0)	102 (82.3)	88 (77.9)	102 (81.6)	180 (78.9)	204 (81.9)
Unknown	13 (11.3)	13 (10.5)	15 (13.3)	15 (12.0)	28 (12.3)	28 (11.2)
Height (cm) n (missing)	113 (2)	123 (1)	112 (1)	124 (1)	225 (3)	247 (2)
Mean	161.98	162.62	160.65	161.24	161.27	161.93
SD	7.454	8.230	6.482	7.743	6.998	8.003
Median	160.00	161.00	160.40	162.00	160.30	162.00
Min	143.0	144.8	145.0	142.0	143.0	142.0
Max	183.0	190.0	173.0	183.0	183.0	190.0
Weight (kg) n (missing)	115 (0)	124 (0)	113 (0)	125 (0)	228 (0)	249 (0)
Mean	73.41	72.04	71.87	72.83	72.65	72.43
SD	17.145	15.092	16.455	16.443	16.787	15.758
Median	69.00	70.00	69.10	72.00	69.05	70.45
Min	42.0	44.0	44.0	42.0	42.0	42.0
Max	135.0	125.7	124.0	132.3	135.0	132.3
BMI (kg/m2) n (missing)	113 (2)	123 (1)	112 (1)	124 (1)	225 (3)	247 (2)
Mean	28.07	27.13	27.88	27.95	27.97	27.55
SD	6.058	4.901	6.012	5.752	6.023	5.350
Median	26.30	27.03	27.41	26.75	26.48	26.85
Min	17.5	18.2	16.9	16.5	16.9	16.5
Max	52.7	40.9	45.1	47.8	52.7	47.8
ECOG Performance Status n (%) n (missing)	115 (0)	124 (0)	113 (0)	125 (0)	228 (0)	249 (0)
0	67 (58.3)	76 (61.3)	62 (54.9)	73 (58.4)	129 (56.6)	149 (59.8)
1	48 (41.7)	48 (38.7)	51 (45.1)	51 (40.8)	99 (43.4)	99 (39.8)
>1	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.4)

ESR1-mut = *ESR1*-mutated, *ESR1*-mut-nd = *ESR1*-mutation-not-detected, SD = Standard Deviation, Min = Minimum, Max = Maximum, BMI = Body Mass Index, ECOG = Eastern Cooperative Oncology Group,
[1] Subjects may select more than 1 race.

Table 19. Clinical Effectiveness Analysis Subgroup Baseline Clinical Characteristics

			SOC		Total	
						ESR1-mut-nd
Years Since Initial Diagnosis						
N (missing)	115 (0)	124 (0)	113 (0)	125 (0)	228 (0)	249 (0)
Mean	7.49	8.63	8.41	8.90	7.95	8.77
SD	6.527	6.372	6.985	7.742	6.759	7.080
Median	4.92	6.76	5.75	6.42	5.42	6.63
Min	0.2	0.7	0.9	0.5	0.2	0.5
Max	28.4	32.2	31.0	40.1	31.0	40.1
Stage at Initial Diagnosis						

			SOC		Total			
							ESR1-mut-nd	
I	15	(13.0)	20	(16.1)	11	(9.7)	18	(14.4)	26	(11.4)	38	(15.3)
II	27	(23.5)	53	(42.7)	39	(34.5)	42	(33.6)	66	(28.9)	95	(38.2)
III	1	(0.9)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.4)	0	(0.0)
IIIA	5	(4.3)	14	(11.3)	6	(5.3)	14	(11.2)	11	(4.8)	28	(11.2)
IIIB	4	(3.5)	3	(2.4)	0	(0.0)	3	(2.4)	4	(1.8)	6	(2.4)
IIIC	4	(3.5)	7	(5.6)	6	(5.3)	1	(0.8)	10	(4.4)	8	(3.2)
IV	42	(36.5)	20	(16.1)	38	(33.6)	38	(30.4)	80	(35.1)	58	(23.3)
Unknown	17	(14.8)	7	(5.6)	12	(10.6)	9	(7.2)	29	(12.7)	16	(6.4)
T Stage at Initial Diagnosis												
T1	18	(15.7)	29	(23.4)	20	(17.7)	23	(18.4)	38	(16.7)	52	(20.9)
T2	29	(25.2)	48	(38.7)	40	(35.4)	49	(39.2)	69	(30.3)	97	(39.0)
T3	13	(11.3)	18	(14.5)	6	(5.3)	11	(8.8)	19	(8.3)	29	(11.6)
T4	11	(9.6)	7	(5.6)	10	(8.8)	13	(10.4)	21	(9.2)	20	(8.0)
Unknown	3	(2.6)	2	(1.6)	5	(4.4)	2	(1.6)	8	(3.5)	4	(1.6)
N Stage at Initial Diagnosis												
N0	16	(13.9)	35	(28.2)	15	(13.3)	38	(30.4)	31	(13.6)	73	(29.3)
N1	34	(29.6)	45	(36.3)	37	(32.7)	31	(24.8)	71	(31.1)	76	(30.5)
N2	14	(12.2)	14	(11.3)	10	(8.8)	14	(11.2)	24	(10.5)	28	(11.2)
N3	9	(7.8)	7	(5.6)	11	(9.7)	11	(8.8)	20	(8.8)	18	(7.2)
Unknown	1	(0.9)	3	(2.4)	8	(7.1)	4	(3.2)	9	(3.9)	7	(2.8)
M Stage at Initial Diagnosis												
M0	41	(35.7)	82	(66.1)	51	(45.1)	67	(53.6)	92	(40.4)	149	(59.8)
M1	27	(23.5)	15	(12.1)	26	(23.0)	27	(21.6)	53	(23.2)	42	(16.9)
Unknown	6	(5.2)	7	(5.6)	4	(3.5)	4	(3.2)	10	(4.4)	11	(4.4)
Stage at Baseline												
IIA	1	(0.9)	0	(0.0)	0	(0.0)	1	(0.8)	1	(0.4)	1	(0.4)
IIIA	0	(0.0)	2	(1.6)	0	(0.0)	0	(0.0)	0	(0.0)	2	(0.8)
IIIC	0	(0.0)	1	(0.8)	1	(0.9)	0	(0.0)	1	(0.4)	1	(0.4)
IV	8	(7.0)	4	(3.2)	7	(6.2)	11	(8.8)	15	(6.6)	15	(6.0)
IVA	1	(0.9)	2	(1.6)	2	(1.8)	1	(0.8)	3	(1.3)	3	(1.2)
IVB	1	(0.9)	2	(1.6)	1	(0.9)	2	(1.6)	2	(0.9)	4	(1.6)
IVC	0	(0.0)	1	(0.8)	1	(0.9)	0	(0.0)	1	(0.4)	1	(0.4)
Unknown	91	(79.1)	103	(83.1)	88	(77.9)	103	(82.4)	179	(78.5)	206	(82.7)
T Stage at Baseline												
T1	2	(1.7)	6	(4.8)	2	(1.8)	3	(2.4)	4	(1.8)	9	(3.6)
T2	6	(5.2)	7	(5.6)	8	(7.1)	7	(5.6)	14	(6.1)	14	(5.6)
T3	3	(2.6)	3	(2.4)	0	(0.0)	4	(3.2)	3	(1.3)	7	(2.8)
T4	8	(7.0)	4	(3.2)	4	(3.5)	7	(5.6)	12	(5.3)	11	(4.4)
Unknown	24	(20.9)	30	(24.2)	25	(22.1)	29	(23.2)	49	(21.5)	59	(23.7)
N Stage at Baseline												
N0	8	(7.0)	6	(4.8)	3	(2.7)	9	(7.2)	11	(4.8)	15	(6.0)
N1	4	(3.5)	10	(8.1)	6	(5.3)	7	(5.6)	10	(4.4)	17	(6.8)
N2	4	(3.5)	3	(2.4)	3	(2.7)	4	(3.2)	7	(3.1)	7	(2.8)
N3	3	(2.6)	3	(2.4)	1	(0.9)	4	(3.2)	4	(1.8)	7	(2.8)
Unknown	24	(20.9)	28	(22.6)	27	(23.9)	27	(21.6)	51	(22.4)	55	(22.1)
M Stage at Baseline												
M0	3	(2.6)	5	(4.0)	0	(0.0)	6	(4.8)	3	(1.3)	11	(4.4)
M1	27	(23.5)	33	(26.6)	25	(22.1)	37	(29.6)	52	(22.8)	70	(28.1)
Unknown	13	(11.3)	13	(10.5)	14	(12.4)	10	(8.0)	27	(11.8)	23	(9.2)
Sites of Disease												
Breast	24	(20.9)	15	(12.1)	21	(18.6)	28	(22.4)	45	(19.7)	43	(17.3)
Bone	101	(87.8)	91	(73.4)	93	(82.3)	91	(72.8)	194	(85.1)	182	(73.1)

	Elacestrant		SOC		Total	
	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**
Bone only	14 (12.2)	24 (19.4)	14 (12.4)	15 (12.0)	28 (12.3)	39 (15.7)
Lymph Nodes	34 (29.6)	34 (27.4)	27 (23.9)	41 (32.8)	61 (26.8)	75 (30.1)
Visceral [1]	81 (70.4)	82 (66.1)	83 (73.5)	85 (68.0)	164 (71.9)	167 (67.1)
Brain	3 (2.6)	1 (0.8)	2 (1.8)	1 (0.8)	5 (2.2)	2 (0.8)
Liver	60 (52.2)	62 (50.0)	64 (56.6)	49 (39.2)	124 (54.4)	111 (44.6)
Lung	27 (23.5)	29 (23.4)	31 (27.4)	37 (29.6)	58 (25.4)	66 (26.5)
Other Sites	26 (22.6)	21 (16.9)	18 (15.9)	30 (24.0)	44 (19.3)	51 (20.5)
Abdominal Cavity	2 (1.7)	3 (2.4)	1 (0.9)	4 (3.2)	3 (1.3)	7 (2.8)
Adrenal Gland	5 (4.3)	3 (2.4)	5 (4.4)	4 (3.2)	10 (4.4)	7 (2.8)
Cervix Uteri	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)	1 (0.4)	0 (0.0)
Chest Wall	0 (0.0)	3 (2.4)	3 (2.7)	8 (6.4)	3 (1.3)	11 (4.4)
Esophagus	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.8)	0 (0.0)	2 (0.8)
Head And Neck	1 (0.9)	1 (0.8)	0 (0.0)	0 (0.0)	1 (0.4)	1 (0.4)
Intestine	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.4)
Kidney	1 (0.9)	0 (0.0)	0 (0.0)	2 (1.6)	1 (0.4)	2 (0.8)
Mediastinum	6 (5.2)	3 (2.4)	1 (0.9)	2 (1.6)	7 (3.1)	5 (2.0)
Other	1 (0.9)	4 (3.2)	1 (0.9)	4 (3.2)	2 (0.9)	8 (3.2)
Pancreas	1 (0.9)	2 (1.6)	0 (0.0)	1 (0.8)	1 (0.4)	3 (1.2)
Pericardium	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.8)	1 (0.4)	1 (0.4)
Skin	6 (5.2)	3 (2.4)	5 (4.4)	4 (3.2)	11 (4.8)	7 (2.8)
Soft Tissue	5 (4.3)	1 (0.8)	3 (2.7)	2 (1.6)	8 (3.5)	3 (1.2)
Spleen	1 (0.9)	1 (0.8)	0 (0.0)	0 (0.0)	1 (0.4)	1 (0.4)
Stomach	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)
Thyroid Gland	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.4)
Number of Metastatic Sites						
0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1	16 (13.9)	35 (28.2)	19 (16.8)	27 (21.6)	35 (15.4)	62 (24.9)
2	43 (37.4)	31 (25.0)	34 (30.1)	38 (30.4)	77 (33.8)	69 (27.7)
>=3	44 (38.3)	34 (27.4)	41 (36.3)	41 (32.8)	85 (37.3)	75 (30.1)

[1] Includes lung, liver, brain, pleural, and peritoneal involvement

D. Safety and Effectiveness Results

1. Safety Results

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

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

2. Effectiveness Results

   PFS in Patients Positive by Guardant360 CDx for *ESR1* Missense Mutations

   To demonstrate the clinical validity of Guardant360 CDx for the identification of patients with ER+/HER2- mBC with *ESR1* missense mutations for treatment with

ORSERDU (elacestrant), the primary diagnostic study objective was assessed by comparing the efficacy of ORSERDU (elacestrant) relative to SOC in patients positive for *ESR1* missense mutations by Guardant360 CDx.

The PFS hazard ratio (HR) observed in the *ESR1*-mut population treated with ORSERDU (elacestrant) vs. SOC was 0.55 (95% CI 0.39 – 0.77, p=0.0005, Figure 2). Similar results were seen in the sensitivity analysis using an unstratified Cox Proportional Hazard model with an observed HR of 0.53 (95% CI 0.38 - 0.74, p=0.0002). Demonstration of clinical efficacy in the *ESR1*-mut population is further supported by clear separation of the treatment arms in the Kaplan-Meir plot of PFS (Figure 2). The median PFS in the *ESR1*-mut population treated with ORSERDU (elacestrant) was 3.78 months (95% CI 2.17 – 7.26) vs. 1.87 months for SOC (95% CI 1.87 – 2.14) (Table 20).

Figure 2. Progression-Free Survival for Elacestrant versus SOC in *ESR1*-mut Subjects

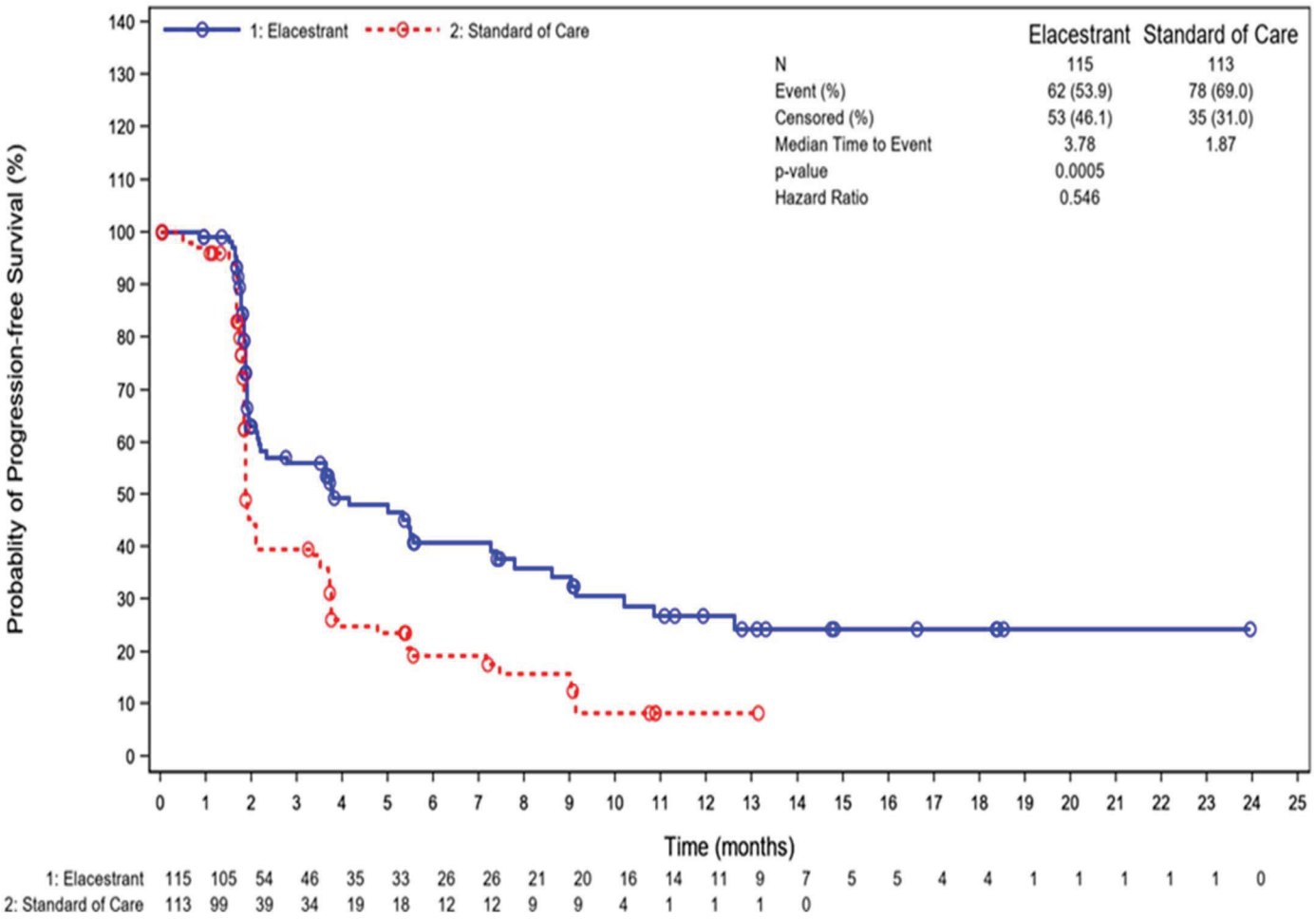


Table 20. Efficacy Results for EMERALD (Patients with *ESR1* Missense Mutations)

	ORSERDU (N = 115)	SOC (Fulvestrant or an Aromatase Inhibitor) (N=113)
Progression-free Survival (PFS)[a]		
Number of PFS Events, n (%)	62 (53.9)	78 (69.0)
Median PFS months[b] (95% CI)	3.78 (2.17, 7.26)	1.87 (1.87, 2.14)
Hazard ratio[c] (95% CI)	0.55 (0.39, 0.77)	
p-value[d] (stratified log-rank)	0.0005	
Overall Survival (OS)		
Number of OS Events, n (%)	61 (53.0)	60 (53.1)
Hazard ratio[c] (95% CI)	0.90 (0.63, 1.30)	
p-value[d] (stratified log-rank)	NS[e]	

[a] PFS results based on blinded imaging review committee.
[b] Kaplan-Meier estimate; 95% CI based on the Brookmeyer-Crowley method using a linear transformation.
[c] Cox proportional hazards model stratified by prior treatment with fulvestrant (yes vs no) and visceral metastasis (yes vs no).
[d] Stratified log-rank test two-sided p-value.
[e] NS – Not statistically significant.

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 patients with BC with *ESR1* missense mutations for treatment with ORSERDU (elacestrant), the effectiveness of Guardant360 CDx was demonstrated through analytical studies using patient samples from the intended use population and a diagnostic study utilizing Guardant360 CDx results and outcome data from the EMERALD (NCT03778931) 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 EMERALD (NCT03778931) clinical study show that patients with BC who had qualifying *ESR1* missense mutations received benefit from treatment with ORSERDU (elacestrant) 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* missense mutation(s) for treatment with ORSERDU (elacestrant) was demonstrated in a prospective analysis of efficacy and safety data obtained from the EMERALD (NCT03778931) clinical study. The supporting clinical validation analysis demonstrates PFS HR (0.55, 95% CI 0.39 – 0.77) for subjects from the EMERALD clinical study positive by Guardant360 CDx for *ESR1* mutation(s) (*ESR1*-mut) treated with ORSERDU (elacestrant) relative to SOC 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* missense mutation(s) for ORSERDU (elacestrant) treatment.

There is a potential risk associated with the use of Guardant360 CDx, for identification of breast cancer patients with *ESR1* missense mutation(s) for treatment with ORSERDU (elacestrant), 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 *ESR1* missense mutation(s) between codons 310 and 547 that may benefit from ORSERDU (elacestrant) therapy.

XIII. CDRH DECISION

CDRH issued an approval order on January 27, 2023.

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.