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/S008
Date of FDA Notice of Approval:	August 11, 2022

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 derived 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 and *KRAS* G12C mutations 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 *ERBB2* activating mutations (SNVs and exon 20 insertions) in NSCLC patients who may benefit from treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki).

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)

A negative result from a plasma specimen does not assure that the patient's tumor is negative for genomic findings. NSCLC 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; see 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	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	
using ctDNA				
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.

ERBB2 activating mutations reported by Guardant360® CDx

NSCLC patients with the following *ERBB2* activating mutations will be reported in Category 1 as a companion diagnostic (CDx) 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

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 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 L858R and exon 19 deletions	NSCLC	TAGRISSO® (osimertinib)	cobas® EGFR Mutation Test v2	Roche Molecular Systems, Inc.	Polymerase Chain Reaction (PCR)
			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 NSCLC patients eligible for treatment with LUMAKRAS™ (sotorasib). However, there is one FDA approved CDx alternative for the detection of *KRAS* G12C mutation in NSCLC patients 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 NSCLC patients eligible for treatment with RYBREVANT™ (amivantamab-vmjw). Also, 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 NSCLC patients (subject of this SSED) eligible for treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki). 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 NSCLC patients 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 CDx was FDA-approved on August 7, 2020 and subsequently commercialized in the USA.

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.

For the specific adverse events that occurred in the clinical studies, please see the ENHERTU® (fam-trastuzumab deruxtecan-nxki) FDA approved package insert which is available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

The primary evidence for supporting the performance of Guardant360® CDx in detecting *ERBB2* activating mutations (SNVs and exon 20 insertions) 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), and precision at LoD studies were conducted to support the indication for *ERBB2* activating mutations (SNVs and exon 20 insertion mutations).

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 *ERBB2* SNVs and exon 20 insertion mutations in NSCLC and non-NSCLC plasma specimens. These results from the platform-level validation (P200010, P200010/S001, and P200010/S002) have been leveraged to support Guardant360 CDx detection of *ERBB2* activating mutations (SNVs and exon 20 insertion mutations). In lung cancer, the most frequent *ERBB2* mutations occurred in exon 20 (48%), with Y772dupYVMA mutation comprising 34% of all *ERBB2* mutations. Other most common *ERBB2* mutations include S310F/Y, L755P/S, and V777L/M. Additional validation studies using the above mentioned most prevalent mutations to support expansion of the intended use to include *ERBB2* activating mutations (SNVs and exon 20 insertion mutations) are described below.

1. Analytical Accuracy/Concordance

Accuracy for the detection of *ERBB2* activating mutations (SNVs and exon 20 insertion mutations) was established relative to an externally validated, Next Generation Sequencing-based comparator method by comparing the results from 205 samples from the intended use population. The 205 samples were composed of 116 *ERBB2* mutated [28 available samples from the DS8201-A-U204 clinical study and 88 samples from Guardant Health's biobank selected based on historical testing to enrich for *ERBB2* positive samples (Biomarker enriched)] and 89 *ERBB2* mutation unselected patients (Biomarker Unselected) from Guardant Health's biobank. Two samples out of 205 failed testing on Guardant360 CDx and were excluded from the subsequent analysis. A total of 203 out of 205 samples tested for the study passed all QC metrics for Guardant360 CDx.

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 7. Since the samples were selected from different sources based on different assays, the unadjusted agreements in Table 7 may be subject to potential bias. Positive agreement was 98.8% while negative agreement was 91.5% for the detection of *ERBB2* activating mutations (SNVs and exon 20 insertion mutations). It was noted that the discordance was primarily driven by the stochastic detection of mutations below the LoD

of both assays, along with sensitivity differences between Guardant360 CDx and the comparator method.

Table 7. Summary of Concordance Between Guardant360 CDx and the Comparator for *ERBB2* activating mutations (SNVs and Exon 20 insertion mutations)

Alteration Type	Guardant360 CDx(+), Comparator (+)	Guardant360 CDx(+), Comparator (-)	Guardant360 CDx(-), Comparator (+)	Guardant360 CDx(-), Comparator (-)	Patients (n)	PPA (95% CI)	NPA (95% CI)
ERBB2 activating mutations (SNVs and exon 20 insertions)	85	10	1	107	203	98.8% (93.7% - 100.0%)	91.5% (84.8% - 95.8%)

To further investigate the origin of the 10 Guardant360 CDx (+) Comparator (-) samples, agreement between Guardant360 CDx and the comparator assay was calculated for each sample source independently (Table 8). As shown in Table 8, all 10 discordant samples were from cohorts enriched for *ERBB2* SNVs and exon 20 insertion mutations, including nine samples from the Guardant Health biobank and one sample from the clinical study.

Table 8. Summary of Concordance Between Guardant360 CDx and the Comparator for *ERBB2* activating mutations (SNVs and Exon 20 insertion mutations) by Sample Source

Sample Source	Guardant360 CDx(+), Comparator (+)	Guardant360 CDx(+), Comparator (-)	Guardant360 CDx(-), Comparator (+)	Guardant360 CDx(-), Comparator (-)	Patients (n)	PPA (95% CI)	NPA (95% CI)	PPV (95% CI)	NPV (95% CI)
Clinical Study	25	1	0	2	28	100.0% (86.3% - 100.0%)	66.7% (9.4% - 99.2%)	96.2% (80.4%- 99.9%)	100.0% (15.8%- 100.0%)
Biomarker Enriched	59	9	1	17	86	98.3% (91.1% - 100.0%)	65.4% (44.3% - 82.8%)	86.8% (76.4%- 93.8%)	94.4% (72.7%- 99.9%)

Biomarker Unselected	1	0	0	88	89	100.0% (2.5% - 100.0%)	100.0% (95.9% - 100.0%)	100.0% (1.5%- 100.0%)	100.0% (94.9%- 100.0%)

Note: PPA/NPA and PPV/NPV were not adjusted for the distribution of samples in the accuracy study.

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 *ERBB2* activating mutations (SNVs and exon 20 insertion mutations) among 240 replicates tested across three unique reagent lots.

b. Limit of Detection (LoD)

The LoDs for *ERBB2* variants was established using pools of cfDNA harboring two *ERBB2* exon 20 insertions (*ERBB2* A775_G776insYVMA and *ERBB2* G778_P780dup) and three *ERBB2* SNVs in exons 8, 19, and 20 (*ERBB2* S310F, *ERBB2* L755P, and *ERBB2* V777L) from non-small cell lung cancer (NSCLC) patient samples. The LoD was established with these clinical sample pools at 5 ng and 30 ng input, using a combination of probit and empirical approaches. Samples were titrated with wild-type cfDNA pooled from multiple NSCLC clinical samples to target five different MAF levels and tested across 24 replicates for 5 ng input and 14 replicates for 30 ng input. The established LoDs of *ERBB2* exon 20 insertions and SNVs are summarized on Table 9.

Table 9. Summary of Established LoDs for *ERBB2* activating mutations (SNVs and Exon 20 insertions) in NSCLC Clinical Samples

***ERBB2* Mutation**	**Alteration Type**	**LoD, 5ng input (MAF%)**	**LoD, 30 ng input (MAF%)**
A775_G776ins YVMA	Insertion	1.0	0.4
G778_P780dup	Insertion	1.3	ND
V777L	SNV	1.0	0.2
S310F	SNV	1.2	0.3
L755P	SNV	1.8	0.5

ND: Not determined

The established LoD was confirmed for these variants by testing NSCLC clinical patient pools targeting 1-1.4x of the established LoD (refer to Table 9) across at least 22 replicates at 5 ng input using a combined LoD Confirmation and Precision Study. The variants used for LoD establishment were also used for LoD confirmation [two *ERBB2* exon 20 insertions (*ERBB2* A775_G776insYVMA and *ERBB2* G778_P780dup) and three *ERBB2* SNVs in

exons 8, 19, and 20 (*ERBB2* S310F, *ERBB2* L755P, and *ERBB2* V777L)]. In this combined LoD and Precision study (see also Section IX.A.4. below) samples were tested across six reagent lot-instrument-operator combinations. The confirmed LoDs of *ERBB2* exon 20 insertions and SNVs are summarized on Table 10.

Table 10. Combined LoD Confirmation and Precision Study Summary Results for *ERBB2* activating mutations (SNVs and Exon 20 insertions)

***ERBB2* Mutation**	**Alteration Type**	**Observed MAF% (x LoD)**	**Number Positive/ Number Expected**	**PPA (95% CI)**
A775_G776ins YVMA	Insertion	1.0 (1x)	23/23	100% (85.2%-100%)
G778_P780dup	Insertion	1.3 (1x)	24/24	100% (85.8%-100%)
V777L	SNV	1.2 (1.2x)	24/24	100% (85.8%-100%)
S310F	SNV	1.68 (1.4x)	24/24	100% (85.8%-100%)
L755P	SNV	1.8 (1x)	22/23	95.7% (78.1%-99.9%)

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 see the Summary of Safety and Effectiveness Data for P200010 (Section IX.A.5) for Guardant360 CDx platform-level validation of precision, including precision from cfDNA pools, precision from plasma extraction, and precision from alteration negative samples.

a. Precision for *ERBB2* activating mutations (SNVs and Exon 20 insertions) from NSCLC cfDNA Clinical Sample Pools

The purpose of the precision study was to demonstrate the repeatability and within-site reproducibility of Guardant360 CDx for detecting *ERBB2* activating mutations (SNVs and exon 20 insertion mutations) through closeness of agreement between qualitative detection in replicates using different combinations of reagent lots, instruments, operators, and days. The study was conducted with pooled NSCLC patient samples harboring two *ERBB2* exon 20 insertions (*ERBB2* A775_G776insYVMA and *ERBB2* G778_P780dup) and three *ERBB2* SNVs in exons 8, 19, and 20 (*ERBB2* S310F, *ERBB2* L755P, and *ERBB2* V777L).

Precision was established at the most challenging 5 ng input level at targeted MAF levels of 1-1.4x LoD. Each variant was tested across six unique reagent lot-instrument-operator combinations with four replicates per combination (6x4=24 samples). At least 22 samples were assessed per variant (see Table 11). This study successfully verified the precision of Guardant360 CDx for detecting *ERBB2* activating mutations (SNVs and exon 20 insertions) 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). All *ERBB2* mutation variants demonstrated acceptable precision (PPA 95.7%-100.0%), Table 11.

Table 11. Summary of Precision Results for *ERBB2* SNVs and Exon 20 insertion mutations

***ERBB2* Mutation**	**Alteration Type**	**Fold LoD (x)**	**Number Positive/ Number Expected**	**PPA (95% CI)**
A775_G776ins YVMA	Insertion	1x	23/23	100% (85.2%-100%)
G778_P780dup	Insertion	1x	24/24	100% (85.8%-100%)
V777L	SNV	1.2x	24/24	100% (85.8%-100%)
S310F	SNV	1.4x	24/24	100% (85.8%-100%)
L755P	SNV	1x	22/23	95.7% (78.1%-99.9%)

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 *ERBB2* false positive mutations were detected (NPA 100%, 240/240). These data are leveraged to support this PMA supplement.

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 see 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.

8. Guardbanding/Robustness

Please see the Summary of Safety and Effectiveness Data P200010/S002 (Section IX.A.6) for Guardant360 CDx platform level Guardbanding/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(IES)

The safety and effectiveness of the Guardant360 CDx for selecting NSCLC subjects who may benefit from treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki) was demonstrated through testing of DNA in pre-treatment plasma specimens from patients enrolled into one of two Daiichi Sankyo Studies [DS8201-A-U204 (DESTINY Lung 01)] used to support the efficacy of fam-trastuzumab deruxtecan-nxki (ENHERTU®). A clinical bridging study was conducted to assess clinical agreement between samples with *ERBB2* activating mutations (SNVs and exon 20 insertions) status tested with the clinical trial assay (CTA) and the Guardant360® CDx in the intent-to-test population. A summary of the Guardant360® CDx clinical validation study is presented below.

A. DESTINY Lung 01 (DS8201-A-U204) Study Design

The DS8201-A-U204 clinical study is a Phase 2, multicenter, open-label, 3-cohort, clinical study of intravenously administered ENHERTU® in subjects with unresectable and/or metastatic NSCLC. The DS8201-A-U204 clinical study population comprises *ERBB2* activating mutation-positive subjects from Cohort 2 of the DS8201-A-U204 study whose disease progressed on or after standard therapy and who were treated with at least one dose of ENHERTU®. Ninety-one subjects were enrolled based on the presence of *ERBB2* activating mutations (SNVs and exon 20 insertions) by local tissue testing or clinical trial assays (CTA).

Guardant360® CDx Bridging Study Design for *ERBB2* activating mutations (SNVs and Exon 20 insertions)

Pre-treatment plasma samples from 89 out of 91 (two subjects did not have pre-treatment plasma samples available for testing) subjects from Cohort 2 of DS8201-A-U204 clinical study (89/91, 97.8%) were tested with Guardant360 CDx. The DS8201-A-U204 clinical study did not include patients negative for *ERBB2* activating mutations (*ERBB2* SNVs and exon 20 insertions) and therefore did not represent the

Guardant360 CDx (+), tissue-based CTA (-) [Guardant360 CDx+ CTA-] subgroup of the Guardant360 CDx intended use population. As such, supplemental matched tissue and plasma samples were obtained from subjects available through commercial vendors using subject selection criteria similar to those of the DS8201-A-U204 clinical study, and a sensitivity analysis was performed to evaluate the potential impact of the Guardant360 CDx (+)/CTA (-) population on the efficacy in the Guardant360 CDx intended use population.

1. Clinical Inclusion and Exclusion Criteria

The criteria for inclusion into the clinical bridging study are summarized below:

- Inclusion criteria for plasma samples from the DS8201-A-U204 clinical study efficacy cohort
    - Pathologically documented unresectable and/or metastatic NSCLC.
    - Has relapsed from or is refractory to standard treatment or for whom no standard treatment is available.
    - Documented CLIA or equivalent laboratory tissue test result demonstrating the presence of an eligible *ERBB2* mutation.
    - Presence of at least one measurable lesion assessed by the investigator based on RECIST version 1.1.
- Guardant360 CDx Diagnostic Study Efficacy Cohort Inclusion Criteria
    - Subject enrolled in Cohort 2 of the DS8201-A-U204 clinical study with informed consent for blood samples used for diagnostic development.
    - Subjects had adequate pre-treatment plasma sample available for Guardant360 CDx testing.
- Guardant360 CDx Diagnostic Study Sensitivity Analysis Prevalence Sub-Study Cohort Inclusion Criteria
    - Pathologically documented, locally advanced or metastatic NSCLC.
    - Subject must either be previously untreated or have active disease progression and were not receiving active cancer therapy at the time of blood collection.
    - Subjects must provide archived tumor tissue samples (unstained slides and/or an FFPE tissue block collected within 5 years of the matched plasma sample) with sufficient tumor content and quantity for testing as defined by the central testing laboratory requirements.
    - Subject must provide plasma sample available for Guardant360 CDx testing.

2. Follow-up Schedule

The Guardant360 CDx clinical bridging study involved retrospective testing of plasma samples; as such, no additional patient follow-up was conducted in regard to the clinical bridging study.

3. Clinical Endpoints

The clinical endpoint used to assess ENHERTU® efficacy in the DS8201-A-U204 clinical study was objective response rate (ORR) by RECIST version 1.1 as assessed by independent central review (ICR).

4. Diagnostic Objective and Endpoints

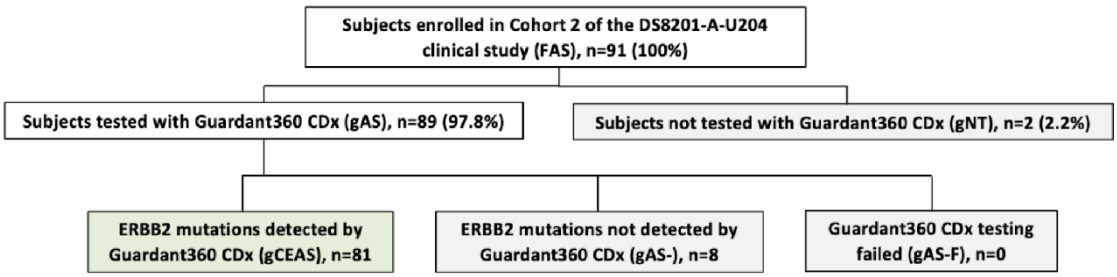
The primary objective of the clinical bridging study is to demonstrate the safety and effectiveness of Guardant360 CDx for the selection of NSCLC patients with *ERBB2* SNVs and exon 20 insertion mutations for treatment with ENHERTU®. The primary endpoint is ORR by RECIST version 1.1 as assessed by ICR and compared to the benchmark ORR of the DS8201-A-U204 clinical study.

A sensitivity analysis was conducted to model the impact of the Guardant360 CDx(+) CTA(-) population on the efficacy in the intended use population.

B. Accountability of PMA Cohort for the Guardant360 CDx Clinical Bridging Study for *ERBB2* activating mutations (SNVs and Exon 20 insertions)

The Guardant360 CDx clinical bridging study included 89 (97.8%) of the 91 subjects enrolled in Cohort 2 of the DS8201-A-U204 (Figure 1). Of these, 81 subjects (89%) tested *ERBB2* mutation positive by Guardant360 CDx and were included in the primary objective analysis set, while 8 (8.8%) tested negative, and two (2.2.%) subjects enrolled in the DS8201-A-U204, 2 [Guardant360 CDx not tested (gNT), 2.2%] were not tested because plasma was unavailable. No samples failed testing by Guardant360 CDx. Thus, a total of 91 patients were in the final full analysis set (FAS) for the diagnostic clinical validation (bridging) study.

Figure 1. Guardant360® CDx *ERBB2* Activating Mutation Bridging Study Efficacy Analysis Subject Accountability and Analysis Set Definitions

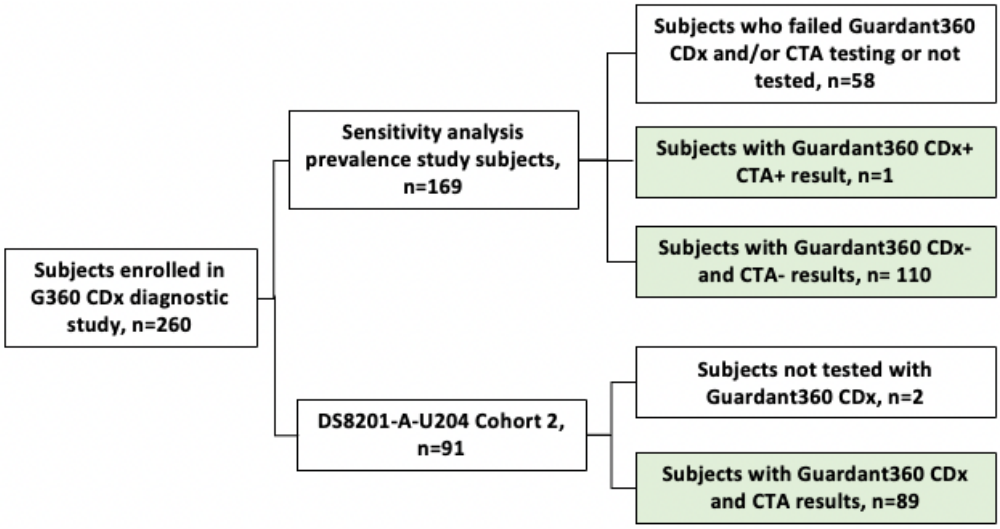


Note: Clinical efficacy subgroup (gCEAS) shaded in green. Clinical efficacy comparator subgroups shaded in gray.

Plasma samples from biomarker-negative (negative for *ERBB2* SNVs and exon 20 insertion mutations) patients in Study DS8201-A-U204 was not available to represent Guardant360 CDx(+) CTA(–) subgroup of the Guardant360® CDx-positive intended use population. Therefore, for sensitivity analysis, matched tissue and plasma samples were procured from commercial vendors and/or other clinical study sources to estimate the prevalence of *ERBB2* activating mutation (SNVs and exon 20 insertions) Guardant360 CDx(+) CTA(–) population and to assess the potential impact of this population on

clinical efficacy. The samples procured from commercial vendors were tested using NGS based tests, which were representative of CTAs used to enroll subjects into the DS8201-A-U204 clinical study. The sensitivity analysis prevalence sub study set included 169 subjects with matched plasma and tissue samples (Figure 2). Of those 169 subjects, 58 (34.3%) failed or were not tested by either Guardant360 CDx and/or tissue-based CTA testing. This includes 36 samples that failed CTA testing but have Guardant360 CDx results; 17 samples that failed Guardant360 CDx testing but have CTA results; 2 samples that failed both CTA and Guardant360 CDx testing; and 3 samples that were unable to be tested by Guardant360 CDx and/or CTA. This resulted in 111 subjects with valid Guardant360 CDx and tissue CTA results. Of these, one subject had Guardant360 CDx+ CTA+ status, no subjects had Guardant360 CDx+ CTA- status or Guardant360 CDx- CTA+ status, and 110 subjects had Guardant360 CDx- CTA- status.

Figure 2. Guardant360 CDx *ERBB2* Sensitivity Analysis Prevalence Sub-Study Subject Accountability



Note: Assay agreement subgroup (AAAS) shaded in green.

Concordance Between Guardant360® CDx and Tissue Testing

Concordance between Guardant360® CDx and tissue-based CTA testing using matched plasma and tissue samples from Cohort 2 of the DS8201-A-U204 clinical study, along with the sensitivity analysis prevalence sub-study group, is shown in Table 12 below. While all samples from the DS8201-A-U204 clinical study population were positive for *ERBB2* activating mutations (SNVs and exon 20 insertions) by tissue testing as a requirement for enrollment in the clinical study, the sensitivity analysis prevalence sub-study subject samples were identified and commercially procured in an effort to represent the *ERBB2* mutation-negative population.

Table 12. Concordance Between Guardant360 and Tissue-based CTA

	Tissue-based CTA Testing		
	ERBB2 Mutation +	*ERBB2* Mutation -	Total
Guardant360 CDx			
ERBB2 Mutation +	82*	0	82
ERBB2 Mutation -	8	110	118
Total	90	110	200
PPA (95% CI)	91.1% (83.2% - 96.1%)		
NPA (95% CI)	100.0% (96.7% - 100.0%)		
OPA (95% CI)	92.5% (88.8% - 95.1%)		

*Includes one *ERBB2* mutation positive sample identified from biomarker negative population used for sensitivity analysis.

C. Study Population Demographics and Baseline Parameters

Demographics and baseline clinical characteristics of subjects enrolled in Cohort 2 of the DS8201-A-U204 clinical study were categorized relative to the diagnostic study populations as defined by Guardant360® CDx.

As shown in Table 13 and Table 14, the diagnostic study efficacy population (gCEAS) demographics and baseline clinical characteristics closely resemble those of the overall DS8201-A-U204 (DESTINY Lung 01) diagnostic clinical study population (FAS).

Table 13. Demographics of the Clinical Effectiveness Analysis Subgroups

	DESTINY Lung 01 -6.4 mg/kg				
	gCEAS N=81	**gAS- N=8**	**gAS N=89**	**gNT N=2**	**Total (FAS) N=91**
Age (years)					
n	81	8	89	2	91
Mean	59.8	65.9	60.4	55.5	60.3
SD	11.26	14.74	11.64	28.99	11.94
Median	60	62.5	60	55.5	60
Min, Max	29, 79	48, 88	29, 88	35, 76	29, 88
Sex (n (%))					
Female	52 (64.2)	6 (75.0)	58 (65.2)	2 (100.0)	60 (65.9)
Male	29 (35.8)	2 (25.0)	31 (34.8)	0	31 (34.1)
Race (n (%))					
White	34 (42.0)	5 (62.5)	39 (43.8)	1 (50.0)	40 (44.0)
Black or African American	1 (1.2)	0	1 (1.1)	0	1 (1.1)
Asian	28 (34.6)	3 (37.5)	31 (34.8)	0	31 (34.1)

	DESTINY Lung 01 -6.4 mg/kg				
	gCEAS N=81	**gAS- N=8**	**gAS N=89**	**gNT N=2**	**Total (FAS) N=91**
American Indian or Alaska Native	0	0	0	0	0
Native Hawaiian or other Pacific Islander	0	0	0	0	0
Hispanic	0	0	0	0	0
Other	18 (22.2)	0	18 (20.2)	1 (50.0)	19 (20.9)
Missing/Unknown	0	0	0	0	0
Ethnicity (n (%))					
Hispanic or Latino	2 (2.5)	0	2 (2.2)	0	2 (2.2)
Not Hispanic or Latino	60 (74.1)	7 (87.5)	67 (75.3)	1 (50.0)	68 (74.7)
Not Applicable	19 (23.5)	1 (12.5)	20 (22.5)	1 (50.0)	21 (23.1)

FAS = all subjects in Cohort 2 of the DS8201-A-U204 clinical study; gAS = all subjects from the FAS tested with Guardant360 CDx; gAS- = All subjects in the gAS who tested negative by Guardant360 CDx for *ERBB2* SNVs and exon 20 insertion mutations; gCEAS = all subjects in the gAS who tested positive by Guardant360 CDx for *ERBB2* SNVs and exon 20 insertion mutations; gNT = all subjects from the FAS not tested by Guardant360 CDx.

Table 14. Baseline Clinical Characteristics of the Clinical Effectiveness Analysis Subgroups

	DESTINY Lung 01 -6.4 mg/kg				
	gCEAS N=81	**gAS- N=8**	**gAS N=89**	**gNT N=2**	**Total (FAS) N=91**
Histology (n (%))					
Adenocarcinoma	81 (100.0)	8 (100.0)	89 (100.0)	2 (100.0)	91 (100.0)
Large Cell	0	0	0	0	0
Squamous	0	0	0	0	0
Other	0	0	0	0	0
Tumor Stage at Study Entry (n (%))					
I-II	0	0	0	0	0
IIIA	1 (1.2)	1 (12.5)	2 (2.2)	0	2 (2.2)
IIIB	2 (2.5)	0	2 (2.2)	0	2 (2.2)
IIIC	1 (1.2)	0	1 (1.1)	0	1 (1.1)

	DESTINY Lung 01 -6.4 mg/kg				
	gCEAS N=81	**gAS- N=8**	**gAS N=89**	**gNT N=2**	**Total (FAS) N=91**
IV	18 (22.2)	1 (12.5)	19 (21.3)	1 (50.0)	20 (22.0)
IVA	19 (23.5)	3 (37.5)	22 (24.7)	1 (50.0)	23 (25.3)
IVB	40 (49.4)	3 (37.5)	43 (48.3)	0	43 (47.3)
ECOG Score [n (%)]					
0	20 (24.7)	2 (25.0)	22 (24.7)	1 (50.0)	23 (25.3)
1	61 (75.3)	6 (75.0)	67 (75.3)	1 (50.0)	68 (74.7)

FAS = all subjects in Cohort 2 of the DS8201-A-U204 diagnostic clinical study; gAS = all subjects from the FAS tested with Guardant360 CDx; gAS- = All subjects in the gAS who tested negative by Guardant360 CDx for *ERBB2* SNVs and exon 20 insertion mutations; gCEAS = all subjects in the gAS who tested positive by Guardant360 CDx for *ERBB2* SNVs and exon 20 insertion mutations; gNT = all subjects from the FAS not tested by Guardant360 CDx.

D. Safety and Effectiveness Results

1. Safety Results

The safety of ENHERTU® was evaluated at two dose levels: 6.4 mg/kg DESTINY-Lung 01 DS8201-A-U204 and 5.4 mg/kg DESTINY-Lung 02 DS8201-A-U206. ENHERTU is being approved at the lower dose (5.4 mg/kg) due to increased rates of Interstitial Lung Disease/pneumonitis at the higher dose. Adverse events observed with the higher dose are unrelated to the Guardant360 CDx.

Data regarding the safety of ENHERTU® (fam-trastuzumab deruxtecan-nxki) therapy are presented in the original drug approval. Refer to the ENHERTU® (fam-trastuzumab deruxtecan-nxki) label for more information. No adverse events were reported in the conduct of the diagnostic studies used to support this sPMA as these involved retrospective testing of banked plasma specimens only.

2. Effectiveness Results

a. ORR in Patients Positive by Guardant360 CDx for *ERBB2* activating mutations (SNVs and exon 20 insertions)

The efficacy of fam-trastuzumab deruxtecan-nxki (ENHERTU®) was evaluated in Daiichi Sankyo DS8201-A-U204 (DESTINY Lung 01, n=91) and DS8201-A-U206 (DESTINY Lung 02, n=52) studies. Demographic and baseline disease characteristics were similar for patients in both the DESTINY-Lung 01 and DESTINY-Lung 02 studies. Also, the response rates were consistent across the evaluated dose levels (5.4 mg/kg and 6.4 mg/kg). The efficacy of ENHERTU (fam-

trastuzumab deruxtecan-nxki) in both study populations (DESTINY Lung 01 and DESTINY Lung 02) and in those subjects positive for *ERBB2* SNVs and exon 20 insertion mutations by Guardant360 CDx is shown in Table 15. The observed diagnostic clinical bridging study ORR (58.0%, 95% CI 46.5% - 68.9%) based on the DESTINY Lung 01 study population is similar to the ORR (53.8%, 95% CI: 39.5% - 67.8%) from the ENHERTU efficacy population (DESTINY Lung 02). The lower limit of the 95% CI exceeds the benchmark ORR of 30% from the DS8201-A-U204 and DS8201-A-U206 clinical studies. The duration of response (DOR) for Guardant360 clinical efficacy population (gCEAS) was 9.25 months (95% CI: 5.67, 18.23).

Table 15. ORR in the gCEAS and ENHERTU Study Populations Assessed by Independent Central Review

	gCEAS (n=81)	**DESTINY Lung 01 (n=91) - 6.4 mg/kg**	***DESTINY Lung 02 (n=52)- 5.4 mg/kg**
Objective Response Rate, n (%) (95% CI)	47 (58.0) (46.5, 68.9)	50 (54.9) (44.2, 65.4)	30 (57.7) (43.2, 71.3)
Complete response (CR)	1 (1.2)	1 (1.1)	1 (1.9)
Partial response (PR)	46 (56.8)	49 (53.8)	29 (55.8)
Duration of Response (DOR)			
Median[a], months (95% CI)	9.25 (5.7, 18.2)	9.3 (5.7, 14.7)	8.7 (7.1, NE)

*This is the primary efficacy population for the approval of fam-trastuzumab deruxtecan-nxki (ENHERTU®). [a]Estimated by the Kaplan-Meier Method. NE- Not Estimable, CI= confidence interval

The 95% CI is calculated using the Exact (Clopper-Pearson) method.

b. Sensitivity Analysis

The primary objective analysis described above demonstrated ENHERTU® (fam-trastuzumab deruxtecan-nxki) efficacy in the Guardant360 CDx(+) CTA(+) subset of the Guardant360 CDx intended use population. As subjects in the DS8201-A-U204 clinical study were enrolled based on positive tissue testing for *ERBB2* activating mutations (SNVs and exon 20 insertions), a sensitivity analysis was assessed using matched tissue and plasma samples (procured from vendors according to the selection criteria similar to the DS8201-A-U204 clinical study). The sensitivity analysis, modeling efficacy in the entire Guardant360 CDx intended use population, demonstrates robustness to the contribution of the unrepresented Guardant360 CDx (+) CTA (-) subjects, with estimated ORRs highly similar to the observed (Table 15 vs. Table 16) due to the high NPA (100%) of Guardant360 CDx relative to tissue testing. The lower limit of the 95% CI for the estimated ORRs across the modeled conditions (Table 16) is greater than the benchmark ORR of 30% in the clinical study, which demonstrates ENHERTU® (fam-trastuzumab deruxtecan-nxki) efficacy across the entire

Guardant360 CDx intended use population, irrespective of efficacy in the modeled Guardant360 CDx(+) CTA(-) population.

Table 16. Sensitivity Analysis for the Guardant360 CDx(+) CTA(-) Population

Assumed Effect in CDx^+/CTA^-	1% *ERBB2* Prevalence, Simulated ORR in CDx+ (95% CI)	2% *ERBB2* Prevalence, Simulated ORR in CDx+ (95% CI)
100% × Observed ORR in CDx^+/CTA^+	0.58 (0.47,0.68)	0.58 (0.47,0.68)
75% × Observed ORR in CDx^+/CTA^+	0.58 (0.47,0.68)	0.58 (0.47,0.68)
50% × Observed ORR in CDx^+/CTA^+	0.58 (0.47,0.68)	0.58 (0.47,0.68)
25% × Observed ORR in CDx^+/CTA^+	0.58 (0.47,0.68)	0.58 (0.47,0.68)
0% × Observed ORR in CDx^+/CTA^+	0.58 (0.47,0.68)	0.58 (0.47,0.68)

E. Pediatric Extrapolation

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

F. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included one investigator 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 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 NSCLC patients with *ERBB2* activating mutations (SNVs and exon 20 insertions) for treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki), the effectiveness of Guardant360 CDx was demonstrated through analytical studies using patient samples from the intended use population and a clinical bridging study using plasma from Cohort 2 of the DS8201-A-U204 clinical study. The data from the analytical validation and clinical bridging study support the reasonable assurance of safety and effectiveness of Guardant360 CDx when used in accordance with the indications for use. Data show that patients who had qualifying *ERBB2* activating mutations (SNVs and exon 20 insertions) received benefit from treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki) 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 sPMA 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

Treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki) provides a meaningful clinical benefit to NSCLC patients with *ERBB2* activating mutations (SNVs and *ERBB2* exon 20 insertions), as demonstrated in the DS8201-A-U204 (DESTINY Lung 01) and DS8201-A-U206 (DESTINY Lung 02) clinical studies.

To expand the intended use/indications for use of the Guardant360 CDx test to identify NSCLC patients harboring *ERBB2* activating mutations (SNVs and *ERBB2* exon 20 insertions), who can benefit from treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki), the probable clinical benefit of Guardant360 CDx was demonstrated by a retrospective bridging analysis of plasma samples obtained from the Phase 2,

multicenter, open-label, 2-cohort DS8201-A-U204 clinical study. The clinical concordance and efficacy agreement between Guardant360 CDx and CTA were calculated using the CTA results as the reference. The point estimates of PPA, NPA, and OPA were 91.0%, 100%, and 92.5%, respectively. In addition, key clinical efficacy endpoints of DS8201-A-U204 study were reported in the population positive for *ERBB2* activating mutations (SNVs and *ERBB2* exon 20 insertions) by the Guardant 360 CDx Test (clinical efficacy). The efficacy in the Guardant 360 CDx cohort (ORR 58% 95% CI 46.5% – 68.9%,), was clinically meaningful, given the patient population, and supported the efficacy observed as reported in the drug label (ORR 53.8%, 95% CI 39.5% - 67.8%) trial (DS8201-A-U206). Given the available information and the analytical and clinical data provided in the submission, the data supports the conclusion that the Guardant360 Test has probable benefit in selecting NSCLC patients with *ERBB2* activating mutations (SNVs and *ERBB2* exon 20 insertions), for treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki).

There is a potential risk associated with the use of Guardant360 CDx, for identification of NSCLC patients with ERBB2 activating mutations (SNVs and ERBB2 exon 20 insertions), 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 patient's treatment resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial 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 therapeutic regimen. The risks of erroneous results are partially mitigated by the analytical performance of the device.

The likelihood of false results was assessed by analytical and clinical validation studies, which partially mitigate the probable risk of the Guardant360® CDx %]). The performance of the Guardant360 CDx in the analytical accuracy study (PPA=98.8% [93.7% - 100.0%], NPA=91.5% [84.8% - 95.8]) and in the bridging study, partially mitigate the risks of this device. However, due to potential false negativity, identified in the clinical bridging concordance study, patients negative for this biomarker should be reflexed to a tissue test, if feasible. Also, supportive of the performance of the test was a limit of blank study, which demonstrated a false-positive rate of 0%.

Together, the totality of the clinical and analytical data support the use of Guardant360 CDx as an aid in selecting NSCLC patients with *ERBB2* activating mutations (SNVs and exon 20 insertions) for ENHERTU® (fam-trastuzumab deruxtecan-nxki) treatment, and that the probable benefit of the use of this device for this indication exceeds the probable risk.

The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with high 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 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 *ERBB2* activating mutations (SNVs and exon 20 insertions) in NSCLC patients for whom ENHERTU® (fam-trastuzumab deruxtecan-nxki) may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order on August 11, 2022.

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.