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 94063 USA

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P200010

Date of FDA Notice of Approval:

Breakthrough Device: Granted breakthrough device status (formerly known as the Expedited Access Pathway, or EAP) on January 29, 2018 because the device (1) is intended to provide more effective diagnosis of a life threatening or irreversibly debilitating disease or condition (2) represents a breakthrough technology that provides a clinically meaningful advantage over existing legally marketed technology, and (3) the availability of the device is in the best interest of patients.

A De Novo (DEN200001) for Streck Cell-Free DNA Blood Collection Tubes (BCTs) was also submitted for the use of the Streck Cell-Free BCTs with the Guardant360 CDx. DEN200001 was authorized on August 7, 2020 in conjunction with the approval of P200010.

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 non-small cell lung cancer (NSCLC) patients who may benefit from treatment with the targeted therapy 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)

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 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. 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*
Copy Number Amplifications (CNAs)	*ERBB2, MET*
Fusions	*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 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 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.

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 (refered 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

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
Log likelihood ratio	≥ 10
MAF Estimate	≥ 0.01%
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 is an FDA approved companion diagnostic (CDx) alternative for the detection of genetic alterations using cfDNA, as listed in Table 1 of the Guardant360 CDx intended use statement. The approved CDx test is detailed below: for additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm?source=govdelivery

- cobas® EGFR Mutation Test v2 (Roche Molecular Systems, Inc.)
  - Technology: polymerase chain reaction (PCR)
  - Therapy: Tagrisso (osimertinib)
  - Indication: Non-small cell lung cancer (NSCLC)

Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

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. Guardant360 LDT has been used to detect the presence of genomic alterations in plasma isolated from whole blood. The Guardant360 LDT is not FDA-cleared or -approved.

Guardant360 CDx has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect 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 TAGRISSO® (osimertinib) FDA approved package insert which is available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Guardant360 CDx performance characteristics were established using clinical samples from patients with a wide range of cancer types, including those with NSCLC. The clinical samples consisted of pools of cfDNA from clinical samples from multiple cancer types, pools of cfDNA from clinical samples derived from one cancer type (e.g., samples from patients with NSCLC) or un-pooled clinical samples. Studies included CDx variants, as well as a broad range of representative alteration types (SNVs, indels, CNAs, and fusions) in various genomic contexts across several genes in the assay's reportable range. Due to limitations in clinical sample availability and due to the rarity of the fusions reported by the Guardant360 CDx, contrived samples were utilized for some analytical validation studies. A contrived sample functional characterization

(CSFC) study was conducted to demonstrate comparable performance of contrived samples made of cell line cfDNA and clinical sample cfDNA so that fusion cell line cfDNA material could be used in some analytical validation studies. Fusion positive clinical samples were used to confirm the estimated limit of detection, analytical accuracy and precision.

1. Analytical Accuracy/Concordance with an Orthogonal Method

The detection of alterations by Guardant360 CDx was compared to results of an externally validated NGS assay. Samples from 386 donors with different cancer types were collected for the study. Sixteen (16) samples failed testing with the comparator assay due to instrument failures, while eleven (11) samples failed testing with the Guardant360 CDx assay due to an instrument failure due to a power outage. 359 samples remained comprising three collection sets as follows.

Collection set one consisted of 100 donor samples selected with the comparator assay consecutively without selection for any specific variants. Since the first sample collection was expected to lack many rare variants, in the second collection set, a set of 100 positive samples were selected with the comparator assay. Collection set three consisted of 159 samples selected from the Guardant Health biobank based on Guardant360 LDT results to include additional rare variants including fusions which were not available from collection sets 1 and 2.

Of 359 patients, no samples failed QC on Guardant 360 CDx, and three samples failed QC with the comparator NGS assay. In total, 356 donor samples across 18 cancer types, which all passed every QC metric were used for the concordance analysis. The cancer types represented in this study included lung (178), gastrointestinal (82), colon (25), breast (17), head and neck (13), prostate (12), genitourinary (7), bladder (3), stomach (3), pancreas (3), endocrine (2), liver (2), ovarian (2), kidney (2), gynecologic (1), esophagus (1), skin (1), and other (5). A summary of Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) with 95% confidence intervals (CI) is provided in Table 6 for CDx alterations in samples from the intended use population, i.e., patients with NSCLC. Agreement rates for each of the CDx variants ranged from 95% to 100% for PPA, and from 98.1% to 99.9% for NPA. The reported PPA and NPA were not adjusted for the distribution of samples from collection set 3 selected using Guardant LDT results.

Table 6. Summary of Concordance Between Guardant360 CDx and NGS Comparator for CDx variants

Alteration Type	Guardant360 CDx(+), Comparator(+)	Guardant360 CDx(+), Comparator (-)	Guardant360 CDx(-), Comparator(+)	Guardant360 CDx(-), Comparator(-)	Possible Variants (n)	Patients (n)	PPA (95% CI)	NPA (95% CI)
EGFR T790M	19	3	1	153	1	176	95.0% (75.1%, 99.9%)	98.1% (94.5%, 99.6%)
EGFR L858R	18	1	0	157	1	176	100.0% (81.5%, 100.0%)	99.4% (96.5%, 100.0%)
EGFR exon 19 deletions	30	1	1	1024	6	176	96.8% (83.3%, 99.9%)	99.9% (99.5%, 99.9%)

A summary of PPA and NPA for other clinically significant variant categories and for panel wide for SNVs and indels over all sample collections is provided in Table 7 and Table 8, respectively. The reported PPA and NPA were not adjusted for the distribution of samples from collection set 3 that were selected using Guardant LDT results.

Positive agreement rates were evaluable for nine (9) patients with clinical Category 2 variants, which consisted of clinically relevant *PIK3CA* mutations in breast cancer patients that included E545A, E542K, E545K, H1047R, and H1047L variants. Concordance analysis resulted in 100% PPA and 100% NPA for the Category 2 variants.

Positive agreement rates for clinical Categories 3 and 4 variants resulted in 93.5% PPA and 86.1% PPA, respectively. Variants in clinical category 3 and 4 showed 99.8% and 100.0% NPA.

MET amplifications had a PPA of 56%, which is attributed to differences in reporting of copy number amplifications by the Guardant360 CDx and the comparator assay. The Guardant360 CDx reports on only focal amplifications and not chromosome-arm amplifications, while the NGS comparator assay reports all amplifications.

Table 7. Summary of Concordance Between Guardant360 CDx and NGS Comparator for other clinically significant variant categories.

Alteration Type	Guardant360 CDx(+), Comparator(+)	Guardant360 CDx(+), Comparator (-)	Guardant360 CDx(-), Comparator(+)	Guardant360 CDx(-), Comparator(-)	Possible Variants (n)	Patients (n)	PPA (95% CI)	NPA (95% CI)
Category 2 variants	9	0	0	76	5	17	100.0 % (66.4%, 100.0%)	100% (95.3%, 100.0%)
Category 3 variants	115	11	8	6191	50	N/A*	93.5% (87.6%, 97.2%)	99.8% (99.7%, 99.9%)
Category 4 variants	420	58	68	137582	388	356	86.1% (82.7%, 89.0%)	100.0% (99.9%, 100.0%)
MET CNAs	13	3	10	330	1	356	56.5% (34.5%, 76.8%)	99.1% (97.4%, 99.8%)
ERBB2 CNAs	15	0	2	339	1	356	88.2% (63.6%, 98.5%)	100.0% (98.9%, 100.0%)
NTRK1 Fusions	5	0	0	351	1	356	100.0% (47.8%, 100.0%)	100.0% (98.9%, 100.0%)
RET Fusions	11	2	1	342	1	356	91.7% (61.5%, 99.8%)	99.4% (97.9%, 99.9%)
ALK Fusions	10	2	0	344	1	356	100.0% (69.2%, 100.0%)	99.4% (97.9%, 99.9%)
ROS1 Fusions	11	0	0	345	1	356	100.0% (71.5%, 100.0%)	100.0% (98.9%, 100.0%)

* For Category 3, no number is given for the number of patients. This is because Category 3 is a merge of many different variants, each with a specific set of cancer types that qualify the variant to belong in Category 3. This means that a different number of patients was associated with each variant within Category 3. For this level, the concordantly negative population was computed as the sum of the concordantly negative populations if each variant in this category was treated independently.

A summary of PPA and NPA for panel wide SNVs and indels over all sample collections is provided in Table 8. The study demonstrated a PPA of 82.5% for indels, 91.4% for SNVs and >99% NPA for the entire reportable range, demonstrating the analytical accuracy of the device.

Table 8. Summary of Concordance Between Guardant360 CDx and NGS Comparator panel wide for SNVs and indels.

Alteration Type	Guardant360 CDx(+), Comparator(+)	Guardant360 CDx(+), Comparator (-)	Guardant360 CDx(-), Comparator(+)	Guardant360 CDx(-), Comparator(-)	Possible Variants (n)	Patients (n)	PPA (95% CI)	NPA (95% CI)
Panel-wide SNVs	428	48	40	13,726,844	38,560	356	91.5% (88.5%, 93.8%)	99.9% (99.9%, 99.9%)
Panel-wide Indels	118	19	25	15,717,238	44,150	356	82.5% (75.3%, 88.4%)	99.9% (99.9%, 99.9%)

2. Contrived Sample Functional Characterization (CSFC) Study

A CSFC study was performed to demonstrate comparable performance between contrived samples that consisted of fusion cell line cfDNA material and fusion positive clinical sample cfDNA material. The CSFC study was performed using 5 ng DNA input (the lowest cfDNA input for the assay) to compare the performance of the Guardant360 CDx with cfDNA derived from cell lines and cfDNA derived from multiple clinical samples from multiple cancer types with *ALK*, *NTRK1*, *RET*, and *ROS1* fusions. The cell line and clinical cfDNA sample pools contained known fusion events that were diluted with pools of wild-type (WT) cfDNA from multiple clinical specimens from multiple cancer types to pre-determined MAF levels (targeted levels were above and below LoD; see Table 9). Cell line cfDNA sample pools were tested across 13-20 replicates, 13 replicates for level 6, 14 replicates for level 2, and 20 replicates for the other levels at 5 ng cfDNA input. Clinical cfDNA sample pools from multiple cancer types were tested with 14 replicates at 5 ng cfDNA input. Both cell line and clinical cfDNA sample pools were tested with the orthogonal method to confirm MAF levels. Detection rates of the 4 fusions, for each titration level, and for each of the two types of pools, are presented in Table 9.

Based on these analyses, the results (Table 9) demonstrate that the performance of the Guardant360 CDx is similar for both fusion positive contrived cfDNA samples and for fusion positive clinical cfDNA samples.

Table 9. Fusion Detection Rate in the CSFC study

Fusion	Sample Type	Detection Rate (95% confidence interval)					
		Level 1 Target MAF 0.07%	**Level 2 Target MAF 0.175%**	**Level 3 Target MAF 0.35%**	**Level 4 Target MAF 0.7%**	**Level 5 Target MAF 1.4%**	**Level 6 Target MAF 1.8%**
EML4-ALK	Cell line	5.0% (0.1%, 24.9%)	28.6% (8.4%, 58.1%)	50.0% (27.2%, 72.8%)	90.0% (68.3%, 98.8%)	100.0% (83.2%, 100.0%)	100.0% (75.3%, 100.0%)
EML4-ALK	Clinical	7.1% (0.2%, 33.9%)	28.6% (8.4%, 58.1%)	50.0% (23.0%, 77.0%)	85.7% (57.2%, 98.2%)	100.0% (76.8%, 100.0%)	100.0% (76.8%, 100,0%)
CCDC6-RET	Cell line	15.0% (3.2%, 37.9%)	35.7% (12.8%, 64.9%)	80.0% (56.3%, 94.3%)	95.0% (75.1%, 99.9%)	100.0% (83.2%, 100%)	100.0% (75.3%, 100%)
TRIM33-RET	Clinical	7.1% (0.2%, 33.9%)	14.3% (1.8%, 42.8%)	64.3% (35.1%, 87.2%)	85.7% (57.2%, 98.2%)	100.0% (76.8%, 100%)	100.0% (76.8%, 100%)
ROS1-SLC34A2	Cell line	0.0% (0.0%, 16.8%)	21.4% (4.7%, 50.8%)	50.0% (27.2%, 72.8%)	75.0% (50.9%, 91.3%)	100.0% (83.2%, 100.0%)	100.0% (75.3%, 100,0%)
ROS1-CD74	Clinical	7.1% (0.2%, 33.9%)	42.9% (17.7%, 71.1%)	85.7% (57.2%, 98.2%)	100.0% (76.8%, 100%)	100.0% (83.9%, 100.0%)	ND
TPM3-NTRK1	Cell line	15.0% (3.2%, 37.9%)	50.0% (23.0%, 77.0%)	40.0% (19.1%, 63.9%)	90.0% (68.3%, 98.8%)	100.0% (83.2%, 100.0%)	100.0% (75.3%, 100.0%)
PLEKHA6-NTRK1	Clinical	21.4% (4.7%, 50.8%)	35.7% (12.8%, 64.9%)	85.7% (57.2%, 98.2%)	100.0% (76.8%, 100.0%)	ND	100.0% (76.8%, 100.0%)

ND: Not Determined

3. Analytical Sensitivity

a. Limit of Blank (LoB)

The LoB was established by evaluating whole blood samples from age-matched healthy donors. Sixty-two (62) donor samples confirmed to be mutation negative based on sequencing with an externally validated orthogonal method were processed using 30 ng of cfDNA input with the Guardant360 CDx (highest DNA input for the assay) across three lots of reagents, operator groups, and instruments. Of the 62 donor samples, 58 donor samples were tested with 4 replicates, while 4 donors were tested with 2 replicates for a total of 240 replicates analyzed to assess the false positive rate of Guardant360 CDx.

This study demonstrated a near zero false positive rate across the entire assay reportable range, as shown in Table 10. The false positive rate was zero for Category 1 (CDx) and Category 2 variants.

Table 10. LoB Study Summary Results

Category	Per Position False Positive Rate	Per Sample False Positive Rate
Category 1: *EGFR* L858R	0%	0 (0/240)
Category 1: *EGFR* T790M	0%	0 (0/240)
Category 1: *EGFR* Exon 19 deletions	0%	0 (0/240)
Category 2	0%	0 (0/240)
Panel-wide SNVs (38,560 bp)	<0.00005% (4/(38,560*240)	1.67% (4/240)
Panel-wide Indels (44,150 bp)	<0.00002% (2/(44,150*240)	0.83% (2/240)
Panel-wide CNAs (2 genes)	0.2% (1/(2*240))	0.42% (1/240)
Panel-wide Fusions (4 genes)	0%	0 (0/240)

b. Limit of Detection (LoD)

The LoD for the Guardant360 CDx variants with CDx claims, representative SNVs and indels, and all reportable CNAs and fusions was established at the lowest and highest claimed cfDNA input amounts (5 and 30ng). LoD established for fusions using cfDNA derived from cell lines was confirmed at 5ng cfDNA input using cfDNA derived from clinical patient samples. LoDs were further confirmed in the clinical pools of relevant cancer types for CDx variants and additional representative variants, including long indels and homopolymers in a combined LoD confirmation and precision study.

For SNVs, indels, including CDx variants and for CNAs, the Guardant360 CDx LoD was established by combining cfDNA from clinical plasma samples from multiple cancers to create pools of material comprising multiple known alterations. The LoD was established with these clinical cfDNA sample pools at 5 ng and 30 ng input, using a combination of probit and empirical approaches. Samples were titrated at 5 different MAF values that included levels above and below the LoD for SNVs, and indels or copy numbers values for CNAs and tested across 20 replicates for 5 ng input and 14 replicates for 30 ng input across two reagent lots.

The LoDs of three (3) CDx alterations representing *EGFR* T790M, *EGFR* L858R, and *EGFR* exon 19 deletions established using pools of cfDNA from clinical plasma samples from multiple cancer types are summarized in Table 11. The LoD was

confirmed for CDx variants using cfDNA sample pools from patients with NSCLC only; refer to Table 13 below.

Table 11. Summary of Established LoD for Alterations Associated with CDx Claims using pools of cfDNA from Clinical Plasma Samples from Multiple Cancer Types

Alteration	Alteration Type	LoD, 5ng input (MAF)	LoD, 30 ng input (MAF)
EGFR T790M	SNV	1.1 %	0.2%
EGFR L858R	SNV	1.0 %	0.2%
EGFR exon 19 deletion, p.Glu746_Ala750del	Indel (15 bp)	1.5%	0.2%

The LoD estimates for SNV, indels, and CNA alterations established using pools of cfDNA from clinical plasma samples from multiple cancer types are summarized in Table 12.

For fusions, the Guardant360 CDx LoD was established using cfDNA from cell lines with known fusions titrated into wild-type (WT) cfDNA from clinical plasma samples. Samples were titrated at 5 different MAF values for fusions across 20 replicates for 5 ng cfDNA input and 14 replicates for 30 ng cfDNA input across two reagent lots. The established LoD was then confirmed using fusion positive cfDNA from clinical plasma samples at 5 ng cfDNA input only. Fusion positive cfDNA from clinical samples were titrated across 5 concentrations with 14 replicates across 2 reagent lots. The higher of the LoD values established using cell lines and confirmed using clinical samples were used to claim the LoD performance levels of the test for fusions at 5 ng (Table 12).

Table 12. LoD Establishment Study Summary Results for Representative Variants using Pools of cfDNA Clinical Plasma Samples from Multiple Cancer Types

Alteration	Alteration Type	LoD, 5 ng (MAF/CN)	LoD, 30 ng (MAF/CN)
BRAF V600E	SNV	1.8%	0.2%
KRAS G12V	SNV	1.5%	0.5%
NRAS Q61R	SNV	3.0%	0.8%
BRCA1 p.E23fs	Indel (2 bp)	2.6%	0.8%
BRCA2 p.S1982fs	Indel (1 bp)	1.3%	0.4%
EGFR exon 20 insertion, p.Ala767_Val769dup	Indel (9 bp)	0.8%	0.2%

Alteration	Alteration Type	LoD, 5 ng (MAF/CN)	LoD, 30 ng (MAF/CN)
ERBB2 exon 20 insertion, p.A775_G776insYVMA	Indel (12 bp)	1.1%	0.2%
MET	CNA	2.4	2.4
ERBB2	CNA	2.3	2.3
NTRK1	Fusion	0.9% (0.9%)	(0.2%)
RET	Fusion	1.1% (0.7%)	(0.1%)
ROS1	Fusion	1.9% (1.2%)	(0.2%)
ALK	Fusion	1.4% (1.5%)	(0.2%)

The numbers in parentheses represent LoD established using cell line derived cfDNA. MAF: Mutant Allele Fraction. CN: copy number.

The established LoD was confirmed for CDx variants by testing clinical patient pools exclusively from NSCLC patients targeting 1-1.5xLoD of the established LoD (refer to Table 11) across at least 20 replicates at 5 ng input using a combined LoD Confirmation and Precision Study. Similarly, the established LoD was confirmed for SNVs and indels in clinical pools made exclusively from the relevant cancer type source material prepared with 5 ng cfDNA input targeting 1-1.5x LoD and run in at least 20 replicates targeting 5 distinct variants. Established LoD targets were used for 5 variants (*EGFR* L858R, *EGFR* T790M, *EGFR* exon 19 deletion, p.E746_A750del, *KRAS* G12C, and *ROS1* fusions), while *in silico* LoD targets were used for 10 additional variants to target variants to 1-1.5x LoD.

In this combined LoD and Precision study (see Section IX.A.5. below for additional studies demonstrating assay precision starting from cfDNA extraction, and with additional mutation positive and negative samples) samples were tested across three precision combinations that evaluated three operator groups, three instrument combinations, and three SPK reagent lots over at least three different start dates. The higher of the LoD values established using clinical sample pools from cancer patients and confirmed using clinical samples exclusively from the relevant cancer type source material were used to claim LoD performance of the test at 5 ng input as summarized in Table 13.

Table 13. Combined LoD Confirmation and Precision Study Summary Results for CDx Variants and Representative Variants

Alteration	MAF	Alteration Type	Cancer Type	Number Positive / Number Expected	PPA
EGFR L858R	1.5%*	SNV	NSCLC	20/20	100.0%
EGFR T790M	1.4%*	SNV	NSCLC	19/20	95.0%
EGFR exon 19 deletion, p.E746_A750del	1.5%*	Indel (15bp)	NSCLC	20/20	100.0%
EGFR exon 19 deletion, A750_I759delinsPT	2.3%^	Indel (29 bp)	NSCLC	20/20	100.0%
KIT V654A	2.5%^	SNV	Prostate	20/20	100.0%
KRAS G12C	1.8%*	SNV	NSCLC	19/20	95.0%
PIK3CA E545K	2.4%^	SNV	Breast	21/21	100.0%
PIK3CA H1047L	1.7%^	SNV	Breast	21/21	100.0%
EGFR exon 20 insertion, H773_V774insHPH	3.5%^	Indel (9 bp)	NSCLC	22/22	100.0%
MET exon 14 skipping 7.116412041.AAGGTATATTTCAGTT>A	2.7%^	Indel (15 bp)	NSCLC	20/20	100.0%
BRCA2 p.T3033fs	4.4%^	Indel (1 bp), homopolymer	NSCLC	21/21	100.0%
BRCA2 p.I605fs	5.0%^	Indel (1 bp), homopolymer	Prostate	20/20	100.0%
BRCA2 p.V1532fs	4.2%^	Indel (1 bp), homopolymer	Prostate	20/20	100.0%
STK11 p.L282fs	4.7%^	Indel (1 bp), homopolymer	NSCLC	21/21	100.0%
ROS1	1.8%*	Fusion	NSCLC	21/21	100.0%

* Observed MAF level in LoD Confirmation Study. LoD confirmed with single cancer type clinical pool and ≥95% detection rate is within 1-1.5x LoD MAF level from the original establishment study range.
^ Observed MAF at the level tested with ≥95% detection rate for variants without direct prior LoD establishment data.

Panel-wide SNV and indels detected by Guardant360 CDx is summarized in Table 14 as median values.

Table 14. Summary of LoD for Alterations Associated with Panel-Wide Claims

Alteration	Median LoD, 5ng (MAF)	Median LoD, 30ng (MAF)
Panel-wide SNVs	1.8%	0.2%
Panel-wide Indels	2.7%	0.2%

4. Analytical Specificity

a. Endogenous and Microbial Interfering Substances

To evaluate the potential impact of endogenous and microbial interfering substances on the performance of Guardant360 CDx, this study evaluated whole blood samples representing 13 cancer types. Whole blood was collected from at least 10 advanced cancer patients per condition. A total of 4 BCTs per patient were collected. Whole blood from a single BCT from each donor was spiked with an interfering substance at a pre-defined concentration.

A total of 146 samples (73 reference-condition pairs, 12 sample pairs in albumin condition, 11 sample pairs in bilirubin (conjugated) condition, 11 sample pairs in bilirubin (unconjugated) condition, 11 sample pairs in hemoglobin condition, 11 sample pairs in *Staphylococcus epidermidis* condition and 17 sample pairs in triglyceride condition) were tested to analyze the effect of 6 interfering substances on assay performance. After QC checks, a total of 130 samples (65 reference-condition pairs, 10 sample pairs in albumin condition, 11 sample pairs in bilirubin (conjugated) condition, 11 sample pairs in bilirubin (unconjugated) condition, 10 sample pairs in hemoglobin condition, 11 sample pairs in *Staphylococcus epidermidis* condition and 12 sample pairs in triglyceride condition) were eligible for analysis.

Substances were considered as non-interfering if, when compared to no interferent controls, the sample level molecule recovery, exon-level molecule recovery, and variant call concordance met pre-defined acceptance thresholds.

Sample level molecule recovery was determined by the depth of non-singleton molecule (NSC) coverage across the panel. Median non-singleton molecule coverage across targeted regions was evaluated to demonstrate that microbial or interfering substances do not impact assay performance to sequence unique molecules. Recovery of unique molecules across interfering substance conditions did not show a negative impact of interfering substances (fold change of median NSC in spike condition over reference condition ranged from 0.88 to 1.08).

Relative exon coverage calculated as the ratio of median exon coverage to sample level coverage for each of the 508 exon regions was compared for each condition-reference sample pair. Aggregating across all samples contributing to the analysis, the

total fraction of all exonic regions within expected level of differences defined as 2* σ, where σ is the pooled standard deviation of the differences observed in historical (σ =0.108) were calculated. Under normal distribution assumption, the fraction of such regions is expected to be 95%. The fraction of exons with relative exon level coverage difference between condition and reference within 2σ (2 * 0.108) was 94.3-99.7%, which demonstrate that there was no preferential drop-out of relative exon-level coverage exceeding expected levels due to random variation, and the entire panel was covered consistently between reference and interfering substance conditions. The lower bound of the 95% exact binomial CI for the fraction of genomic targeted exonic regions with relative exon-level non-singleton coverage is shown in Table 15.

The results were aggregated across all variants across all ten whole blood samples, and concordance was assessed within each treatment category across variants. PPAs were calculated for 62 SNVs, 24 indels, and 3 CNAs. The 6 conditions tested showed variant call concordant PPAs ranging from 83.3%-100.0%. PPA ≥ 1x LoD ranged from 90.0%-100.0% for all 6 interferents, Table 15.

The panel-wide NPAs were also calculated for SNVs and indels within the reportable range. The discordant negative variants were defined as those negative variants that were positive in the non-reference condition. The panel-wide NPA was 99.9%-100.0% for all conditions (Table 15).

In conclusion no interference was found in albumin (60 g/L), bilirubin (conjugated) (342 μmol/L), bilirubin (unconjugated) (342 μmol/L), hemoglobin (2 g/L), *Staphylococcus epidermidis* (106 cfu) and triglycerides (15 g/L).

Table 15. Summary of Interference Results

Condition	PPA	≥ 1x LoD PPA	NPA	Sample Level Molecule Recovery Median of NSC fold (condition / reference)	Relative Exon Level Coverage Lower bound of 95% CI
Albumin (60 g/L)	100.0% (13/13)	100.0% (9/9)	100.0% (462217/462217)	0.96	99.5%
Bilirubin (conjugated) (342 μmol/L)	90.9% (10/11)	100.0% (9/9)	99.9% (508440/508442)	0.98	93.8%
Bilirubin (unconjugated) (342 μmol/L)	84.6% (11/13)	100.0% (8/8)	99.9% (508439/5084440)	0.88	96.0%
Hemoglobin (2 g/L)	83.3% (10/12)	100.0% (7/7)	100.0% (462218/462218)	1.08	96.1%

Condition	PPA	≥ 1x LoD PPA	NPA	Sample Level Molecule Recovery Median of NSC fold (condition / reference)	Relative Exon Level Coverage Lower bound of 95% CI
Staphylococcus epidermidis (106 cfu)	90.9% (10/11)	90.0% (9/10)	100.0% (508442/508442)	1.03	98.9%
Triglyceride (15 g/L)	89.7% (26/29)	100.0% (25/25)	99.9% (554644/554647)	0.96	93.7%

The effect of potential exogenous interfering substances that may carry over from cfDNA extraction on assay performance was not evaluated. A post-market study will be conducted to evaluate the effect of exogenous interfering substances on assay performance. See Section XIII.

b. *In silico* Specificity Analysis

An *in silico* specificity study was conducted to evaluate the potential for Guardant360 CDx test probes to amplify non-specific products from human DNA and to assess the potential for incorrect results due to commensal microorganism contamination. For this *in silico* study, the probe sequences were mapped against a reference genome that included unplaced contigs and decoy sequences designed to capture known problematic reads in human genome sequence.

In addition, a modified version of the human genome was generated by adding genomic contigs from common bacterial, fungal, and viral sources that can be found in the blood or on the skin. Finally, sequencing primer sequences were checked for specificity using NCBI's primer BLAST tool.

When mapped to the human genome with decoy sequences, unplaced contigs, and representative microbial contaminant genomes, 97.6% of the probes uniquely mapped to the intended targets (MAPQ ≥ 60). None of the probes mapped to the representative microbial contaminant genomes. No off-target amplicons <1000 bp were predicted for the primers using the web-based NCBI Primer-BLAST tool against a database of Ref-Seq representative genomes.

Based on the results, all of the panel coverage results satisfy the desired performance. There is minimal risk of Guardant360 CDx producing a false positive patient result due to detection of microbial sequences or mis-alignment of off-target sequences in the human genome.

5. Precision

The purpose of the precision studies was to demonstrate the repeatability and within-site reproducibility of Guardant360 CDx through closeness of agreement between

measured qualitative output obtained in replicate testing using different combinations of reagent lots, instruments, operators, and days. Additional runs were conducted (1) on mutation negative samples to demonstrate precision of analytically blank samples, and (2) on plasma samples to understand the influence of extraction on test. All studies were conducted exclusively with patient material and no cell line material was used.

a. Precision from three distinct cfDNA clinical sample pools

Precision was evaluated for alterations associated with CDx claims, as well as representative and specific alterations to support platform-level performance. Repeatability including intra-run performance (run on the same plate under the same conditions) and reproducibility including inter-run performance (run on different plates under different conditions) were assessed and compared across three different precision combinations (PC) of instrument sets, reagent lots, and operators over multiple days. Three distinct precision clinical cfDNA sample pools from multiple cancer types, containing a total of 16 targeted alterations across all pools were prepared targeting 1-1.5 x LoD, and tested at 5ng cfDNA input. Ten (10) replicates per three (3) pools were tested for each of three (3) precision combinations (90 replicate samples total) and comprised of three (3) different reagent lots (G360 SPK, Ampure XP beads, and NextSeq 550 sequencing, reagent lots), three (3) different instrument sets and three (3) different operator groups. Each combination was tested on two (2) batches, sequenced on four (4) flow cells. The QIAsymphony instrument was not paired within each of the three (3) precision combination sets, since the sample pools were generated from previously extracted and stored cfDNA. Precision starting from cfDNA extraction was evaluated in a separate study described in Section IX.A.5.b. In total, 480 alterations were assessed across 90 samples tested. Qualitative results were used to calculate PPA and NPA.

The final levels for the targeted variants tested ranged from 0.7x to 2.6x LoD. Three variants were below 1x LoD (*ROS1* fusion at 0.9x LoD, *MET* amplification at 0.8x LoD , and *NRAS* Q61R at 0.7x LoD), 8 were within 1-1.5x range, including the CDx variants, and 5 variants were in the 1.7x – 2.6x LoD range.

Across 960 expected negative targeted sites (32 targeted negative variants across 3 sample pools * 30 replicates), the observed NPA was 100.0%.

All CDx alterations demonstrated acceptable precision (PPA 96.7%-100.0%), Table 16.

Table 16. Precision CDx Variant PPA Summary

Alteration	Number Positive / Number Expected	PPA (95% CI)
EGFR T790M	30/30	100.0% (88.4%, 100%)
EGFR L858R	30/30	100.0% (88.4%, 100%)

EGFR Exon 19 Del, E746_A750del	29/30	96.7% (82.8%, 99.9%)

The variant level PPA for all targeted variants were above 90.0% across all instrument, reagent, and operator combinations, except for *MET* amplification in pool 1, which may be attributed to the 0.8x LoD range achieved in the titration pool (Table 17). *ROS1* fusion detection demonstrated 93.3% PPA, consistent with the achieved 0.9x LoD titration level. *BRCA1* E23fs also resulted in a lower variant level PPA (90.0%) than expected. However, the 90.0% detection rate is consistent with the variant being located in a more challenging area of the panel with respect to coverage. Specifically the variant is considered to be in a more challenging area because it is in a region with relatively low GC content and has below average DNA molecule recovery.

In summary, across 480 alterations (150 SNVs, 150 indels, 60 CNAs, and 120 fusions) in a set of 90 cfDNA sample replicates containing 16 unique alterations across 3 cfDNA sample pools made from cfDNA from multiple cancer types, all alterations demonstrated PPA of 86.7%-100.0%. The PPA across all targeted alterations for each condition was evaluated. The PPA across all targeted alterations per precision combination (PC) ranged from 96.3%- 99.4%.

Table 17. Variant Level PPA for Four Variant Classes

Alteration Class	Alteration	Number Positive / Number Expected	PPA (95% CI)
SNV	*KRAS* G12V	30/30	100.0% (88.4%, 100%)
SNV	*NRAS* Q61R	30/30	100.0% (88.4%, 100%)
SNV	*BRAF* V600E	30/30	100.0% (88.4%, 100%)
SNV	Panel-wide	150/150	100.0% (97.6%, 100%)
Indel	*ERBB2* A775_G776insYVMA	30/30	100.0% (88.4%, 100%)
Indel	EGFR A767_V769dup	30/30	100.0% (88.4%, 100%)
Indel	*BRCA1* E23fs	27/30	90.0% (73.5%, 97.9%)
Indel	*BRCA2* S1982fs	30/30	100.0% (88.4%, 100%)
Indel	Panel-wide	146/150	97.3% (93.3%, 99.3%)
CNA	*ERBB2*	30/30	100.0% (88.4%, 100%)
CNA	*MET*	26/30	86.7% (69.3%, 96.2%)
Fusion	*EML4-ALK*	30/30	100.0% (88.4%, 100%)
Fusion	*TPM3-NTRK1*	30/30	100.0% (88.4%, 100%)
Fusion	*TRIM33-RET*	30/30	100.0% (88.4%, 100%)
Fusion	*ROS1-CCDC6*	28/30	93.3% (77.9%, 99.2%)

Precision from clinical pools with samples from a single clinically relevant cancer type was confirmed in the combined LoD confirmation and precision study described in Section IX.A.3.b above.

b. Precision from plasma evaluation of extraction precision and precision of downstream steps

The purpose of this study was to show the precision of variant calling for the entire sample workflow (from cfDNA extraction through sequencing) with un-pooled clinical samples.

This study utilized clinical plasma samples from 53 unique patients. Each plasma sample with positive variants (as detected by Guardant360 LDT) and high cfDNA yields was split into six aliquots or six replicates per patient.

The LoD was established for inputs of 5 ng and 30 ng, which are the lower and upper limit of cfDNA mass input for library preparation. Since the purpose of this precision study was to test the full spectrum of sample yields that would be observed in normal use, sample inputs ranged from 5 ng to 30 ng of cfDNA input. The corresponding LoD range was between 1x for the 30 ng LoD MAFs, and 1.5x for the 5 ng LoD MAFs. Variants that were previously observed in this MAF range in the Guardant360 LDT run were selected for this study and evaluated for call agreement.

Eighteen (18) different tumor types were evaluated in this study to support a pan-cancer tumor profiling indication for Guardant360 CDx (see Section IX.A.10. for more details on pan-cancer claim). Each donor specimen was processed in duplicate across three lots for a total of 6 replicates. “Lot” refers to different reagent lots, as well as different combinations of operators, days, and instruments to evaluate precision. The targeted variants evaluated in the study are shown in Table 18.

Table 18. Targeted Variants Amongst the 53 Donor Samples Selected for Study

Category	Variant	Number of Eligible Based on MAF/CN
ERBB2	CNA	3
MET	CNA	3
ALK	fusion	2
RET	fusion	2
EGFR exon 19 deletion	indel	6
EGFR exon 20 insertion	indel	2
Long indel (>30 bp)	indel	1
MET exon 14 skipping	indel	1
BRAF V600E	SNV	3
EGFR L858R	SNV	6
EGFR T790M	SNV	4
KRAS G12C	SNV	3
PIK3CA E542K	SNV	3

PIK3CA E545K	SNV	4
PIK3CA H1047L/R	SNV	2
PIK3CA C420R	SNV	3

A total of 315 replicates passed QC and were analyzed for within-condition and between-condition precision.

For each eligible variant, pairwise comparisons of variant detection were made between the technical replicates in each lot. From the study design with three lots and two replicates within each lot, there were 3 pairs for each variant in calculating within-lot average positive agreement (APA) and 12 pairs for each variant in calculating between-lot APA.

The APA results for eligible SNVs, indels, fusions, CNAs and all three together are shown in Table 19. Workflow or sample QC failures mean there were fewer than 3 lots per variant tested in some cases. The within lot APA for all variant types together was 97.3% as shown in Table 19.

Table 19. Within-Reagent Lot APA Summary

Variant Type	Variant Lot Comparisons	Concordant (C)	Discordant (D)	APA
SNV	150	141	9	96.9%
Indel	35	35	0	100.0%
CNA	15	13	2	92.9%
Fusion	12	12	0	100.0%
ALL	212	201	11	97.3%

The within-lot average negative agreement (ANA) was 99.9%. This statistic includes all called variant sites panel-wide, not just the eligible variant sites based on LoD in the source samples, so this statisitic includes positions with expected stochastic detection due to low mutant molecule count. The number of positions evaluated was 46,217 unique SNV and indel reportable positions, 2 CNAs, and 4 fusions.

The between lot APA for eligible SNVs, indels, fusions, CNAs, and all reportable variants together are shown in Table 20. For each of these variants, there were 12 pairwise comparisons.

Table 20. Between-Lot APA Summary

Variant Type	Variant Lot Comparisons	Concordant	Discordant	APA
SNV	47	531	26	97.6%
indel	11	132	0	100.0%
CNA	8	53	6	94.6%

fusion	4	48	0	100.0%
ALL	70	764	32	98.0%

The between-lot APA for all variant types together was 98.0%. The between lot ANA was 99.9% across all reportable positions and variants. This statistic includes all called variant sites, not just the eligible variants sites based on LoD in the source samples, so includes positions with expected stochastic detection due to low mutant molecule count. The number of positions evaluated was 46,217 unique SNV and indel reportable positions, 2 CNAs, and 4 fusions.

Notably, for *ERBB2* amplifications, within and between-lot APA were observed to be 80.0% and 85.0%, respectively, due to variation in focality determination. Specifically some of the replicates were determined to be focally amplified, and thus reported by the assay, and some were determined to be aneuploid and thus reported negative as the Guardant360 CDx reports CNAs only for focal amplifications and not chromosome-arm amplifications.

In addition to the main study, supplementary samples, starting from plasma, were processed to evaluate precision from extraction. Fusion samples were created by diluting cfDNA extracted from cell lines harboring *ROS1* and *NTRK1* fusions into plasma of clinical lung cancer samples negative for fusions. These contrived plasma samples were evaluated in lieu of clinical samples for this study due to the rarity of these alterations. Plasma was processed from extraction to sequencing on the same batches as the rest of the study samples. The fusion cfDNA was diluted to < 0.2% MAF for *ROS1* and *NTRK1* at ~30 ng input. There was 100% detection (6/6) across reagent lots for both fusions when tested at 0.15% MAF at approximately 30 ng of cfDNA.

c. Precision from mutation negative samples

Samples from healthy donors were pre-screened by an externally validated orthogonal method. Mutation negative samples by the orthogonal method were tested by Guardant360 CDx in three reproducibility conditions (i.e., different reagent lots, operators, instruments, and days). Four replicates from each donor were tested with Guardant360 CDx across the different reproducibility conditions. The study demonstrated a sample-level, within-condition ANA of 97.4% and sample-level between-condition ANA of 97.3%. The within-condition ANA was 99.6% and between-condition ANA was 99.6% for 7 variants that had a positive call in at least one condition. Within-condition and between-condition ANA values were 100.0% for all CDx variants (*EGFR* L858R, *EGFR* T790M, and *EGFR* exon 19 deletions) and category 2 variants.

6. Carryover/Cross-Contamination

The carryover/cross-contamination study evaluated the prevalence of cross-contamination when material is transferred between samples in the same batch and

carry-over when material is transferred between samples across batches processed sequentially on the same instrument using Guardant360 CDx.

A total of 352 plasma samples across 8 batches (44 samples/batch x 8 batches) were run in a consecutive order across instruments within the analytical accuracy study and sequenced on 16 flowcells.

There was no evidence of high positive variants from near-by wells detected in negative samples. In conclusion, no carryover or cross-contamination was observed in 352 samples processed across 8 consecutive batches.

7. Reagent Lot Interchangeability

The Guardant360 CDx Sample Preparation Kit (G360 SPK), a single use kit, provides the reagents necessary to process a batch of patient cfDNA samples and prepare libraries for sequencing (Guardant360 workflow steps: library preparation & enrichment). This study evaluated the interchangeability of each G360 SPK box (Box 1, 2, 3, and 4). Box 4 has three components 4a, 4b, 4c, which have to be used together from the same lot.

Reagent lot interchangeability was assessed by testing cfDNA sample pools in five replicates using two different lots of Guardant360 CDx Sample Preparation Kit in seven different lot combinations. Two sample pools contained in total 16 known variants, 9 variants in pool 1 and 7 variants in pool 2. Variant positive cfDNA diluted in WT clinical cfDNA to levels near the LoD were prepared from clinical cfDNA (pool 1) and a mixture of clinical cfDNA and cell line-derived cfDNA (pool 2). Cell-line cfDNA was used to evaluate fusions. For the sample replicates that proceeded to sequencing, all met the performance metrics.

To test the interchangeability of the Guardant360 SPK boxes, 5 replicates of two sample pools harboring 16 targeted variants near LoD were tested in 7 interchangeability run conditions. Kit Lot Interchangeability of G360 SPK boxes was evaluated based on the rate of positive agreement for detection of targeted variants.

Out of 70 samples, 68 passed QC metrics (97% pass rate). The rate of qualitative agreement rate (QDR), i.e., the agreement with the majority call for baseline reagent was calculated. QDR was defined as the number of positively detected targeted variants across eligible samples (D) divided by the total number of targeted variants tested across eligible samples (N), expressed as a percentage (100 * D/N). QDR ranged from 91.6% to 98.7%. There was 100.0% negative agreement among expected negative sites within respective pool replicates.

The panel-wide assessment of NPA was 99.9% calculated from negative variant sites across the Guardant360 CDx reportable range that are not detected in the reference condition represents SPK Lot A for all combinations tested.

8. Stability

a. Reagent Stability

The objective of this study was to evaluate the closed-container stability and establish the shelf-life of the unused Guardant360 CDx Sample Preparation Kit. Three lots of identical reagents with the same specifications were stored under the specified storage conditions for each box and then tested with two sample pools at defined time points following the same protocol.

Two sample pools contained in total 16 known variants, 9 variants in pool 1 and 7 variants in pool 2. Variant positive cfDNA diluted in WT clinical cfDNA to levels near the LoD were prepared from clinical cfDNA (pool 1) and a mixture of clinical cfDNA and cell line-derived cfDNA (pool 2). Cell-line cfDNA was used to evaluate fusions. These variants are both clinically relevant and are representative of the entire Guardant360 CDx panel based on variation in GC content, sequence context, and coverage.

Three (3) lots of G360 SPK boxes 1-4 were used for this study and were tested as soon as feasible after manufacturing and QC. Time zero (T0) was defined as the first testing timepoint, and reagents were aged and used to test samples at or after 3, 4, 7, 10, 13, and 19 months to support a shelf-life stability claim of 2, 3, 6, 9, 12, or 18 months, respectively. When possible, testing at all time points was conducted using frozen aliquots of the same large sample pool.

The G360 SPK boxes were tested at each timepoint with five (5) replicates per each of the two unique sample pools. At least 4 replicates had to pass sample level sequencing QC metrics for a valid timepoint, and only results from samples passing QC were evaluated against the acceptance criteria. If less than 4 replicates passed sequencing level QC, and after failed samples were re-run, the testing timepoint would be considered failed due to unstable SPK reagents for that lot. Replicates were tested at the minimum (5 ng) cfDNA input, as this represents the most challenging condition.

208 of these sample replicates passed all expected QC metrics (96.2% pass rate) and were included in this study (up to 19 months of testing), representing 2 different pools, processed in 5 replicates each, for each of the 3 SPK lots used, and the 7 time points (0, 3, 4, 7, 10, 13, and 19 months).

Qualitative detection rates (QDR), which is based on the agreement with the majority call at T0 for the number of targeted variants detected, were assessed per lot/per timepoint (Table 21). QDR was defined as the number of positively detected targeted variants that were positively detected in the baseline condition across eligible samples (D) divided by the total number of positively detected targeted variants tested across eligible samples (N), expressed as a percentage (100 * D/N). The study shows no significant difference between time points compared to T0 for all three lots (alpha = 0.05), demonstrating that there was no significant decline in detection rates over the

course of the study (Table 21). All of the expected negative variants were observed as negative calls across all replicates, indicating 100% negative agreement among all targeted variants expected to be negative across study conditions. The panel-wide assessment of NPA was 99.9% calculated from negative variant sites across the Guardant360 CDx reportable range that are not detected in the reference condition representing time 0 for all time points tested.

Table 21. SPK Concordance Per Timepoint and Lot Summary Results

Lot	Metric	T0	T3	T4	T7	T10	T13	T19
Lot 1	Detected / Expected = QDR	80/80 = 100.0%	80/80 = 100.0%	69/71 = 97.2%	78/80 = 97.5%	77/80 = 96.3%	80/80 = 100.0%	117/121= 96.7%
	QDR LLCI	95.5%	95.5%	90.2%	91.3%	89.4%	95.5%	91.8%
	chi squared statistic	N/A*	0.637	0.506	1.359	N/A*	N/A*	1.313
	p-value	N/A*	0.212	0.238	0.122	N/A*	N/A*	0.126
Lot 2	Detected / Expected = QDR	71/73 = 97.3%	69/71 = 97.2%	80/80 = 100.0%	77/80 = 96.3%	80/80 = 100.0%	71/71 = 100.0%	70/71= 98.6%
	QDR LLCI	90.5%	90.2%	95.5%	89.4%	95.5%	94.9%	92.4%
	chi squared statistic	0	0.605	0	0.605	0.479	0	0
	p-value	0.5	0.782	0.5	0.782	0.756	0.5	0.5
Lot 3	Detected /Expected = QDR	76/80 = 95.0%	80/80 = 100.0%	62/64 = 96.9%	79/80 = 98.8%	80/80 = 100.0%	80/80 = 100.0%	79/80= 98.8%
	QDR LLCI	87.7%	95.5%	89.2%	93.2%	95.5%	95.5%	93.2%
	chi squared statistic	2.3	0.02	0.83	2.3	2.3	2.3	0.2
	p-value	0.9	0.6	0.8	0.9	0.9	0.9	0.7

*chi-square statistic of proportion test is not defined for the case when the two sample proportions of detection are 100%

Based on the time points tested (up to the 19-month time point) the results support a maximum of 18 months of shelf life for the Guardant360 Sample Preparation Kit at the recommended stored conditions.

b. <u>Whole Blood Stability</u>

The objective of this study was to demonstrate the stability of whole blood specimens used for Guardant360 CDx collected in the Guardant360 BCK, that is in Streck Cell-

Free DNA BCTs, across the expected range of sample transport and storage conditions for up to 7 days after blood collection prior to plasma isolation.

A total of four BCTs were drawn from each of 16 cancer patients and subjected to the conditions described in Table 22 below. From each patient, one tube was processed to plasma 1 day after blood draw (storage 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). After conditioning, plasma was isolated on the 8th day after blood collection and run on the Guardant360 CDx.

Table 22. Description of Whole Blood Storage Conditions

Condition	BCT # from Each Patient	Storage Condition / Processing
Reference	1	Reference condition: Plasma processing (day 1 after blood collection).
1	2	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.
2	3	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.
3	4	Room Temperature Storage: Storage at room temperature 18-25°C.

All 64 samples passed all QC and were included in analysis. All storage conditions demonstrated acceptable performance (Table 23). All samples in each group demonstrated acceptable sample-level molecule recovery as assessed by depth of NSC coverage across the panel. Fold change of median NSC in test condition over the reference condition or time zero ranged from 0.90 to 0.97.

Exon-level coverage was also acceptable for all conditions evaluated. The fraction of exons with relative exon level coverage difference between condition and reference (Time zero) within 2σ (2 * 0.108) was 95.3-96.3%, which demonstrate that there was no preferential drop-out of relative exon-level coverage exceeding expected levels due to random variation, and the entire panel was covered consistently between reference and interfering substance conditions. The lower bound of the 95% exact binomial CI for the fraction of genomic targeted exonic regions with relative exon-level non-singleton coverage is shown in Table 23.

PPAs were also calculated for the SNVs and indels in the reportable range: 10 SNVs and 6 indels. All conditions showed variant call concordant PPA of 87.5% - 93.8%.

PPA above LoD was 100% for all conditions (Table 23). The data indicate acceptable sensitivity and specificity when using samples across the storage conditions.

The panel-wide NPAs were also calculated for SNVs and indels within the reportable range within 55 genes, CNAs and fusions. The total set of negative variants was set to the reportable range excluding variants found to be positive in the reference condition. The discordant negative variants were defined as those negative variants that were positive in the non-reference condition. The panel wide NPA was 99.9% for condition 1 (739,550 out of 739,552 variants), 99.9% (739,550 out of 739,552 variants) for condition 2, and 99.9% (739,548 out of 739,552 variants) for condition 3 (Table 23).

Table 23. Whole Blood Stability Summary Results

Study Endpoint	Metric	Summer profile	Winter profile	Room temperature
		Study Result	Study Result	Study Result
Sample-level Molecule Recovery Difference (fold change)	Median of NSC fold (condition / reference)	0.90	0.97	0.97
Relative Exon-Level Coverage	Lower bound of 95% CI for fraction of exons outside expected coverage	95.4%	94.8%	95.8%
Variant Call Concordance	PPA	87.5% (14/16)	93.8% (15/16)	93.8% (15/16)
	PPA (≥ 1xLoD)	100.0% (13/13)	100.0% (13/13)	100.0% (13/13)
	NPA	99.9% (739,550/ 739,552)	99.9% (739,550/ 739,552)	99.9% (739,548/ 739,552)

The whole blood stability study described above was supplemented by an additional study with two objectives (1) to demonstrate the concordance between samples processed into plasma on the same day as blood collection and the samples processed into plasma the day after collection; (2) robustness to changes in relative humidity (RH) that tubes may be exposed to during shipping.

A total of four BCTs were drawn from 19 healthy donors. For each donor, one BCT was processed to plasma within 4 hours after blood collection and shipped to Guardant Health on dry ice on the same day. This served as the reference condition. The other 3 BCTs will be subjected to conditions described below:

- Test condition 1. Intact whole blood in BCTs packed in BCKs was shipped overnight to Guardant Health and plasma isolation was done on the day of receipt (Day 1 after blood collection).
- Test condition 2. Exposure of whole blood in BCT starting on the day of blood collection and for 1 day to low humidity (25% RH, at 23℃) storage profile, followed by storage at Room temperature for 1 day. Plasma isolation occurred on Day 2 after blood collection.
- Test condition 3. Storage of whole blood in BCT starting on the day of blood collection and for 1 day at Room temperature, followed by exposure to high-humidity (90% RH, at 23℃) storage profile for 1 day. Plasma isolation occurred on Day 2 after blood collection.

Out of 76 samples processed, 24 study samples (6 distinct donor samples for all 4 conditions) had cfDNA underloading in some samples and overloading in some other samples due to a Guardant operator error. After QC check, 52 samples from 13 donors passed all sample QC metrics and were included in the analysis. Recovery of unique molecules across the 3 conditions did not show a negative impact of Day 1 processing and exposure of tubes to high (90% RH) and low (25% RH) relative humidity conditions. Fold change of median NSC in storage condition over reference condition ranged from 0.95 to 0.99. For the reportable range of the device, the fraction of exons with relative coverage within 2σ (2 * 0.108) ranged 98.1 – 99.0%.

Based on the evidence from preservation of overall coverage and relative exon coverage the quantity and quality of cfDNA are not impacted by: (1) whole blood collection at vendor site and overnight shipping to Guardant Health at room temperature, followed by standard plasma isolation on day 1 after collection, (2) exposure of whole blood in BCT starting on the day of blood collection and for 1 day to low relative humidity (25% RH, at 23℃) storage profile, followed by storage at Room temperature for 1 day and plasma isolation on Day 2 after blood collection, and (3) Storage of whole blood in BCT starting on the day of blood collection and for 1 day at Room temperature, followed by exposure to high relative humidity (90% RH, at 23℃) storage profile for 1 day and plasma isolation on Day 2 after blood collection.

Based on these study results, whole blood may be stored in Cell-Free DNA BCTs for up to 7 days after blood collection and prior to plasma isolation, and can withstand winter and summer shipping conditions.

c. Plasma Stability

To define the storage conditions and evaluate the stability of plasma isolated from whole blood, stability at defined temperatures and durations was assessed. Four

BCTs from 12 cancer patients, 48 samples in total, were collected and run on Guardant360 CDx, with plasma stored at the specified storage conditions (Table 24). Plasma from one BCT was processed through cfDNA extraction on the same day as a reference condition (Ref), plasma from a second BCT was stored at 2-8°C for 25 hours before cfDNA extraction (for a 24-hour stability claim at 2-8°C) as condition 1 (C1), plasma from a third BCT was stored at -80°C ± 10°C with two freeze/thaw cycles for 46 days before cfDNA extraction (for a 45-day stability claim at -80°C ± 10°C) as condition 2 (C2), and plasma from a fourth BCT was stored at -80°C ± 10°C for one year before cfDNA extraction to support usage of stored plasma for analytical validation (AV) studies (C3). Extracted cfDNA from each condition was stored at -20°C ± 5°C until further processing.

Table 24. Description of Plasma Storage Conditions

Condition	BCT # from Each Patient	Storage Condition / Processing
Reference (Ref)	1	Reference condition: cfDNA extracted directly after plasma isolation on the same day.
Condition 1 (C1)	2	Storage of plasma at 2-8°C for 25 hours before cfDNA extraction (for a 24-hour stability claim at 2-8°C).
Condition 2 (C2)	3	Storage of plasma at -80°C ± 10°C plus 2 freeze/thaw cycles for 46 days before cfDNA extraction (for a 45-day stability claim at -80°C ± 10°C).
Condition 3 (C3)	4	Storage of plasma at -80°C ± 10°C plus 2 freeze/thaw cycles for one year to support usage of stored plasma for analytical validation (AV) studies.

Out of 48 samples processed, 40 study samples (11 samples in reference condition, 8 samples in Condition 1, 10 samples in Condition 2 and 11 samples in Condition 3) passed their respective in-process and post-sequencing QC metrics and had at least one reference-condition sample pair, thus were included in the final analysis. In the three tested storage conditions, samples demonstrated acceptable performance (Table 23). In the three tested storage conditions, samples demonstrated acceptable sample-level molecule recovery, relative exon-level coverage, and variant call concordance.

Sample level molecule recovery showed fold change of 0.93, 1.10 and 0.99, less than 25% change with 95% confidence interval in all three conditions.. Exon-level relative coverage demonstrated 92.8%-97.1% fraction of exons within 2σ of expected relative coverage. The lower bound of the 95% exact binomial CI for the fraction of genomic targeted exonic regions with relative exon-level non-singleton coverage is shown in Table 25.

PPAs were also calculated for the SNVs and indels in the reportable range within 55 genes that are reportable by test, as well as the reportable CNA and fusion genes: 14 SNVs, 1 indel and 1 CNA. Three conditions showed variant call concordant PPA of 76.9% - 78.6%. PPA above LoD was 90.9% - 91.7% for all conditions (a single variant was discordant, see Table 25). NPA across the reportable range was 99.9% - 99.9%.

Based on these study results, plasma may be stored at 2-8°C for 24 hours or at -80°C ± 10°C plus 2 freeze/thaw cycles for 1 year before cfDNA extraction.

Table 25. Plasma Stability Summary Results

Study Endpoint	Metric	Condition 1	Condition 2	Condition 3
		Result	Result	Result
Sample-level Molecule Recovery	Median of NSC fold (condition / reference)	0.93	1.10	0.99
Relative Exon-level Coverage	Lower Bound of 95% CI	92.0%	96.6%	96.6%
Variant Call Concordance	PPA	76.9% (10/13)	78.6% (11/14)	78.6% (11/14)
	PPA (≥ 1xLoD)	90.9% (10/11)	91.7% (11/12)	91.7% (11/12)
	NPA	99.9% (369,770/ 369,771)	99.9% (462,215/ 462,216)	99.9% (508,437/508,439)

d. cfDNA Stability

To define the storage conditions and evaluate the stability of cfDNA extracted from the plasma of whole blood, stability at defined temperatures and durations was assessed. Eighty-eight (88) samples were collected from 22 patients and run on Guardant360 CDx, with cfDNA stored in the specified storage conditions (Table 26). Samples were split into two extraction arms (with quantification either before or after freezing) to establish stability of cfDNA under both measurement workflows.

Eighty eight (88) samples were processed for the reference and 3 conditions below.

Table 26. Description of cfDNA Storage Conditions

Condition	BCT # from Each Patient	Storage Condition / Processing	
		A. Post-Extraction Quantitation	**B. Post-Storage Quantitation**
Reference A&B	1	Reference condition: Quantitation, dilution, and library preparation post-extraction on the same day.	Reference condition: Quantitation, dilution, and library preparation post-extraction on the same day.
Condition 1 A&B	2	Quantitation and dilution post-extraction on the same day, followed by storage of cfDNA at 2-8°C for 25 hours (in FluidX tubes) before library preparation (for a 24-hour stability claim at 2-8°C).	Storage of cfDNA at 2-8°C for 25 hours (in Biorad elution plate), followed by quantitation and library dilution, before library preparation (for a 24-hour stability claim at 2- 8°C).
Condition 2 A&B	3	Quantitation and dilution post-extraction on the same day, followed by storage of cfDNA at -20°C ± 5°C plus 2 freeze/thaw cycles for 46 days (in FluidX tubes) before library preparation (for a 45-day stability claim at -20°C ± 5°C).	Storage of cfDNA at -20°C ± 5°C plus 2 freeze/thaw cycles for 46 days (in Biorad elution plate), followed by quantitation and library dilution, before library preparation (for a 45-day stability claim at -20°C ± 5°C).
Condition 3 A&B	4	Quantitation and dilution post-extraction on the same day, followed by storage of cfDNA at -20°C ± 5°C plus 5 freeze/thaw cycles for one year to support usage of stored cfDNA for AV studies in FluidX tubes before library preparation.	Storage of cfDNA at -20°C ± 5°C plus 5 freeze/thaw cycles for one year to support usage of stored cfDNA for AV studies (in Biorad elution plate), followed by quantitation and library dilution, before library preparation.

Out of 88 samples processed, 87 study samples passed QC metrics and were included in the final analysis. In the 3 tested storage conditions in both arms, samples demonstrated acceptable performance (Table 27).

The recovery of unique molecules across storage conditions did not show a negative impact of storage: fold change of median NSC in storage condition over reference condition ranged from 0.93 to 1.06 in arm A (quantitation post-extraction); and from 0.90 to 0.96 in arm B (quantitation post-storage).

Relative exon coverage was also compared for each of the 508 exon regions in 55 genes reported by the test. The fraction of exons with relative exon level coverage difference between condition and reference within 2σ was 92.3-97.3% in Arm A, and 87.4-93.9% in Arm B. The lower bound of the 95% exact binomial CI for the fraction of genomic targeted exonic regions with relative exon-level non-singleton coverage is shown in Table 27. The data show that there was no preferential drop out of relative exon-level coverage in excess of what is expected due to random variation, and the panel was covered consistently between reference and storage conditions.

PPAs were also calculated for the SNVs and indels, i.e., 12 SNVs and 3 indels in Arm A, and 11 SNVs and 2 indels in Arm B. Three conditions showed variant call concordant PPA of 93.3%-100% in Arm A and 92.3% - 100% in Arm B. PPA above LoD were all 100% for all conditions in Arm A and Arm B (Table 27).

Together, these results demonstrated that cfDNA was stable at -20°C ± 5°C for one year and 5 freeze/thaw cycles and 2-8°C for 24 hours. The stability of the stopping point in the workflow for storage of cfDNA at 2-8°C for 24 hours post-extraction pre-quantification was also established.

Table 27. cfDNA Stability Summary Results

Arm A		**Condition 1 2-8°C, 24 hours**	**Condition 2 -20 ± 5°C, 45 days**	**Condition 3 -20 ± 5°C, 1 year**
Study Endpoint	**Metric**	**Study Result**	**Study Result**	**Study Result**
Sample-level Molecule Recovery	Median of NSC fold (condition / reference)	1.06	1.00	0.93
Relative Exon-Level Coverage	Lower bound of 95% CI	96.8%	91.6%	91.5%
Variant Call Concordance	PPA	93.3% (14/15)	100.0% (14/14)	100.0% (15/15)
	PPA (≥ 1 x LoD)	100.0% (13/13)	100.0% (13/13)	100.0% (13/13)
	NPA	99.9% (508,437/508,438)	100.0% (462,216/462,216)	100.0% (508,438/508,438)
Arm B		**Condition 1 2-8°C, 24 hours**	**Condition 2 -20 ± 5°C, 45 days**	**Condition 3 -20 ± 5°C, 1 year**
Study Endpoint	**Metric**	**Study Result**	**Study Result**	**Study Result**
Sample-level Molecule Recovery	Median of NSC fold (condition / reference)	0.96	0.91	0.90
Relative Exon-Level Coverage	lower bound of 95% CI	93.2%	86.5%	91.5%
Variant Call Concordance	PPA	100.0% (13/13)	100.0% (13/13)	92.3% (12/13)
	PPA (≥ 1 x LoD)	100.0% (12/12)	100.0% (12/12)	100.0% (12/12)
	NPA	100.0% (508,440/508,440)	99.9% (508,439/508,440)	100.0% (508,440/508.440)

e. <u>Intermediate Sample Stability</u>

To define the storage conditions and evaluate the stability of intermediate products, i.e., library plate, enriched library plate, and sequencing pool, used for repeat testing

in the Guardant360 CDx workflow, stability at defined temperatures and durations was assessed. Samples were stored across all conditions (-20°C ± 5°C for 13, 15, or 22 days; or 2-8°C for 31 hours) with an additional thirty (30) samples of fresh intermediate product for reference. Calls from the stored intermediate product were compared to the fresh intermediate product (i.e. the reference condition).

A total of 90 samples containing the sample pools from the precision study from three distinct cfDNA clinical sample pools were used for the study. Sixty samples were processed to test 4 intermediate stability conditions (library plate, enriched library plate, 20 pM sequencing pool, 2.2 pM sequencing pool) and stored as described in Table 28. The intermediate products tested for library plate and enriched library plate were subjected to 2 freeze/thaw cycles. The 20 pM sequencing pool was subjected to 3 freeze/thaw cycles.

Each condition was tested on 3 pools in 5 replicates (3x5) for a total of 15 samples. All 4 sample intermediate product conditions resulted in a total of 60 samples (15x4) passing QC. Additionally, 30 samples from the 2 analytical precision batches (15x2) were used as reference for the analysis of this study.

Table 28. Description of Intermediate Product Storage Conditions

Intermediate Product	Storage	Target Storage Claim	Stability Testing
Enriched Library Plate	-20°C ± 5°C	14 days (including 2 freeze/thaw cycles)	At least 15 days (including 2 freeze/thaw cycles)
Library Plate	-20°C ± 5°C	21 days (including 2 freeze/thaw cycles)	At least 22 days (including 2 freeze/thaw cycles)
20 pM Pool	-20°C ± 5°C	12 days (including 2 freeze/thaw cycles)	At least 13 days (including 2 freeze/thaw cycles)
2.2 pM Pool	2-8°C	30 hours	At least 31 hours

The Qualitative Detection Rate (QDR) for a storage condition was calculated which is equivalent to PPA relative to the reference condition. QDR was defined as the number of positively detected targeted variants that were positively detected in the reference condition across eligible samples (D) divided by the total number of positively detected targeted variants tested across eligible samples (N), expressed as a percentage (100 * D/N). QDR relative to reference conditions ranged from 97.7% to 100% across all stored intermediate product conditions compared to reference conditions (Table 29). NPA was calculated from all negative variant sites across the Guardant360 CDx reportable range that are not detected in the reference condition. The total number of distinct variants in the final reportable range is 46,223, representing 46,217 SNVs and indels, 2 CNAs and 4 fusions. From this list, all called variants in study samples for each of the 3 pools were removed as expected positive sites for replicates of the same pool in the remaining study conditions. NPA was greater than 99.9%.

Table 29. Intermediate Product Stability Summary Results

Stored Intermediate Product	D	N	QDR	LLCI	ULCI
Enriched Library Plate	85	87	97.7	91.9	99.7
Library Plate	88	88	100.0	95.9	100.0
20 pM Sequencing Pool	86	86	100.0	95.8	100.0
2.2 pM Sequencing Pool	83	84	98.8	93.5	100.0

D: number of positive calls, N: Number of variants in eligible samples

Based on these study results, intermediate products may be stored at -20°C ± 5°C for 14 days (enriched library plate), 21 days (library plate), or 12 days (20 pM Pool). Additionally, the 2.2 pM pool intermediate product may be stored at 2-8°C for 30 hours.

9. General Lab Equipment and Reagent Evaluation

a. cfDNA Extraction

The performance of the cfDNA extraction from plasma samples was evaluated on multiple QIAsymphony SP instruments and reagent kit lots to characterize performance of the Guardant360 CDx extraction process. A retrospective analysis of clinical whole blood samples processed on the Guardant360 LDT implementation of the Guardant360 CDx device system were evaluated to characterize the variability between instruments as well as the variability between runs on the same instrument. Clinical blood samples processed on the Guardant360 CDx system (N=11,267 processed samples across 79 cancer types), including second tubes re-processed for a quality failure of the first tube or clinical need, were evaluated to characterize the variability between instruments as well as the variability between runs on the same instrument. Second tube extraction is used to estimate the reproducibility of the extraction process for the same blood sample.

All combinations of QIAsymphony kit/instruments used had a successful extraction rate greater than 94% with a range of 94.9%-100% (Table 30).

Table 30. QIAsymphony Extraction Performance Summary Results

QIAsymphony Extraction Performance Summary Results	QIAsymphony DSP Circulating DNA Kit Lot ID				
QIAsymphony SP Instrument ID	1	2	**3**	4	Success Rate by Instrument
1	97.2% (254)	97.3% (1022)	94.7% (912)	NA	96.2% (2188)
2	98.7% (79)	97.8% (1043)	95.8% (1088)	NA	96.8% (2210)
3	NA	100.0% (42)	98.6% (283)	NA	98.8% (325)
4	100.0% (230)	96.9% (912)	97.6% (489)	NA	97.6% (1631)
6	NA	98.6% (69)	98.7% (312)	NA	98.7% (381)
7	100.0% (36)	97.6% (82)	97.7% (885)	NA	97.8% (1003)
7	NA	98.6% (1452)	97.2% (2036)	100.0% (41)	97.8% (3529)
Success Rate by Reagent Lot	98.7% (599)	97.8% (4622)	96.8% (6005)	100.0% (41)	Total success rate: 97.3% (11267)

Note: NA values indicate no samples were processed with that combination of instrument/kit.
The number of samples processed by each combination in parentheses.

Post-sequencing quality metrics were retrieved for all samples that passed laboratory quality control thresholds. Variance component analysis revealed that the percent of variation explained by the QIAsymphony kit and instrument was no more than 2.1% in cfDNA extraction yield.

b. <u>Other Instruments and Reagents</u>

The other general lab instrument/reagent systems (4200 TapeStation, Microlab STAR, Microlab STARlet, NextSeq 550 Sequencing, and Veriti 96-Well Thermal Cycler) were assessed in combination in the precision study. Instruments and reagents varied in 3 precision combinations. Three sample pools were created at 5ng cfDNA inputs. Ten replicates per pool were tested for each of three precision combinations for a total of 6 batches sequenced on 12 flowcells. All 90 study samples passed respective QC metrics and were included in the final analysis.

Acceptable alteration PPA and NPA results were demonstrated across instruments (Table 31). Acceptable sequencing QC parameters were demonstrated across precision combinations (Table 32).

Table 31. Sequencer PPA and NPA Across Precision Combinations

Instrument #	PPA	95% CI	NPA	95% CI
1	98.1% (210/214)	[95.3%, 99.5%]	100.0% (40/40)	[91.2%, 100.0%]
2	98.1% (52/53)	[89.9%, 100.0%]	100.0% (10/10)	[69.2%, 100.0%]
3	98.1% (156/159)	[94.6%, 99.6%]	100.0% (30/30)	[88.4%, 100.0%]
4	96.3% (52/54)	[87.3%, 99.5%]	100.0% (10/10)	[69.2%, 100.0%]

Table 32. Sequencing Flowcell Level QC Parameters Across Precision Combinations

QC Parameters (threshold)	Mean	SD	CV%
Cluster Density (≥170000, ≤ 280000)	223,333	9610	4.3
Percentage of Clusters Passing Filter (≥70.0)	89.1	1.2	1.3
Quality Score (Q30) in read 1 (≥70.0)	89.1	0.7	0.8
Quality Score (Q30) in read 2 (≥70.0)	87.0	0.8	0.9
Quality Score (Q30) in index (≥70.0)	95.3	0.4	0.5
Prephasing index (≤0.01)	0	0	N/A
Prephasing 1 (≤0.01)	0.0012	0.00008	6.9
Prephasing 2 (≤0.01)	0.0014	0.00005	3.8
Phasing index (≤0.01)	0	0	N/A
Phasing 1 (≤0.01)	0.0014	0.00022	14.9
Phasing 2 (≤0.01)	0.0017	0.00018	10.5

In conclusion, the critical general lab instruments and reagents demonstrated acceptable performance for use with the Guardant360 CDx test.

10. <u>Pan Cancer Analysis</u>

Guardant360 CDx performance characteristics were established using cfDNA derived from a wide range of cancer types. In total, 929 patient samples representing 20 cancer categories were included across the analytical validation studies performed for Guardant360 CDx.

cfDNA fragment size distributions were compared across samples from multiple cancer types. For this analysis, clinical samples were selected from analytical validation studies representing 8 different cancer types: NSCLC, breast, colorectal cancer (CRC), prostate, and uterine. The electropherograms of cfDNA post-extraction from plasma on the TapeStation show a mono-nucleosomal peak that is consistent across cancer types and with published literature. Based on these observations, cfDNA fragment size distributions are similar across cancer types and would generate qualitatively similar inputs into the assay workflow.

To further understand the performance of the Guardant360 CDx across cancer types, pre-sequencing quality metrics (cfDNA extraction and library enrichment), post-sequencing quality metrics (non-singleton coverage, in-process contamination, coverage exceptions, GC bias, and on target rate), as well as the clinically relevant metrics of overall QC success rate and detectable levels of tumor shedding (as measured by the maximum allelic fraction of detected somatic variants) across samples tested with Guardant360 CDx candidate assay implemented in Guardant's CLIA laboratory as an LDT test were analyzed. The Guardant360 LDT assay in this analysis refered to as an LDT implementation of the CDx utilizing the exact configuration. This test has been operated in the Guardant Health Clinical Laboratory to process over 10,000 clinical samples. The quality thresholds are equivalent between both versions with the exception of an additional 5 ng minimum input amount requirement for Guardant360 CDx and an upper limit to the cluster density per flowcell. These additional requirements were applied retrospectively to the Guardant360 LDT results to infer success rates for Guardant360 CDx (note that a single flowcell, out of 640, fails the upper limit of cluster density for the Guardant360 CDx).

The pan-cancer analysis evaluated 11,097 samples processed across 23 cancer categories. For each cancer category, quality pass rates were measured, and the overall patient success rate was >98% for all cancer categories. The frequency of failures for each of the individual metrics was similar across cancer types, Table 33.

Table 33. Sample success rate across 23 cancers

Category Data			**Sample Preparation QC Data, % Pass**			**Patient Sample Sequencing QC Data, % Pass (median value)**			**Patient Outcome Metrics**		
Cancer Category	Total Patients	First Tube Success	cfDNA Ex. Sample QC Pass %	Library Enrich. Sample QC Pass %	In process Contam-ination %	Coverage Exception	GC Bias	Non-singleton Coverage	On Target Rate	Overall Sample Pass Rate	Maximum MAF: median (standard deviation)
Breast	1516	95.2	96.6	99.1	100 (0.01)	99.2 (0.0)	99.7 (1.36)	99.8 (2766)	99.3 (88.04)	99.9	2.9 (17.5)
CUP	258	95.0	98.8	99.2	100 (0.01)	96.9 (0.0)	99.2 (1.38)	99.2 (2981)	98.4 (88.63)	100	4.9 (19.7)
Cholangio-carcinoma	302	96.0	98.6	99.3	99.7 (0.01)	99.0 (0.0)	99.3 (1.45)	100 (2911)	99.3 (88.95)	100	1.2 (13.5)
Colorectal	1041	96.5	98.8	99.5	100 (0.01)	97.8 (0.0)	98.7 (1.36)	99.8 (2832)	99.3 (88.33)	100	5.3 (21.1)
Gastroeso-phageal	443	96.2	99.0	100	100 (0.01)	98.2 (0.0)	98.4 (1.37)	100 (2790)	99.7 (88.34)	100	3.1 (17.7)
Gyneco-logical	322	95.4	98.0	99.7	100 (0.01)	97.5 (0.0)	98.7 (1.30)	100 (2771)	99.7 (88.15)	99.1	3.1 (18.5)

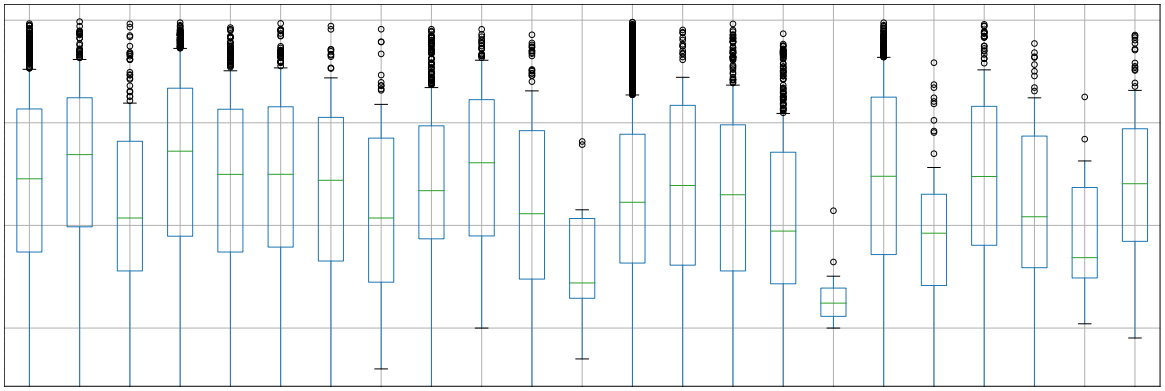
Category Data			Sample Preparation QC Data, % Pass			Patient Sample Sequencing QC Data, % Pass (median value)			Patient Outcome Metrics		
Cancer Category	Total Patients	First Tube Success	cfDNA Ex. Sample QC Pass %	Library Enrich. Sample QC Pass %	In process Contam-ination %	Coverage Exception	GC Bias	Non-singleton Coverage	On Target Rate	Overall Sample Pass Rate	Maximum MAF: median (standard deviation)
Head and Neck	98	94.9	96.7	100	99.0 (0.01)	99.0 (0.0)	100 (1.23)	99.0 (2399)	100 (87.85)	100	2.8 (17.0)
Liver	67	91.0	100	100	100 (0.01)	97.0 (0.0)	100 (1.50)	98.5 (2880)	97.0 (88.68)	100	1.2 (16.5)
Lung Squamous Cell	584	97.6	98.2	99.6	100 (0.01)	99.8 (0.0)	100 (1.27)	100 (2812)	99.7 (88.31)	100	2.2 (14.7)
Lung cancer, NOS	152	93.4	95.6	100	100 (0.01)	98.7 (0.0)	98.7 (1.39)	100 (2837)	99.3 (88.01)	99.3	4.1 (19.1)
Melanoma	174	90.8	90.4	99.4	100 (0.01)	99.4 (0.0)	100 (1.25)	100 (2439)	100 (87.90)	98.8	1.3 (15.3)
Mesoth-elioma	12	100	100	100	100 (0.01)	100 (0.0)	100 (1.20)	100 (2968)	100 (87.72)	100	0.3 (2.5)
NSCLC	4111	96.1	97.6	99.4	100 (0.01)	99.0 (0.0)	99.5 (1.29)	99.9 (2671)	99.4 (88.04)	99.9	1.7 (14.3)
Neuro-endocrine	100	90	93.6	98.9	100 (0.01)	98 (0.0)	100 (1.41)	100 (2758)	98 (87.91)	98	2.5 (21.7)
Other	419	95.7	97.95	99.5	100 (0.01)	97.8 (0.0)	99.3 (1.30)	99.3 (2730)	98.8 (88.11)	99.0	2.0 (17.3)
Pancreatic	581	95.9	97.6	98.5	100 (0.01)	99.0 (0.0)	100 (1.35)	100 (2843)	99.3 (88.12)	100	0.9 (13.9)
Primary CNS	47	93.6	93.3	100	100 (0.01)	100 (0.0)	100 (1.35)	100 (2431)	100 (88.28)	100	0.2 (0.3)
Prostate	770	94.9	98.0	99.3	100 (0.01)	97.53 (0.0)	99.09 (1.34)	99.9 (2706)	98.6 (88.14)	99.5	3.0 (19.6)
Renal	89	95.5	97.6	98.8	100 (0.01)	100 (0.0)	100 (1.28)	100 (2739)	98.9 (87.63)	100	0.8 (6.8)
SCLC	136	95.6	98.5	99.3	100 (0.01)	99.26 (0.0)	100 (1.34)	100 (2701)	98.5 (88.34)	100	3.0 (24.5)
Soft Tissue	91	98.9	98.9	100	100 (0.01)	100 (0.0)	100 (1.36)	100 (2844)	100 (88.26)	100	1.2 (12.8)

Category Data			Sample Preparation QC Data, % Pass			Patient Sample Sequencing QC Data, % Pass (median value)			Patient Outcome Metrics		
Cancer Category	Total Patients	First Tube Success	cfDNA Ex. Sample QC Pass %	Library Enrich. Sample QC Pass %	In process Contam-ination %	Coverage Exception	GC Bias	Non-singleton Coverage	On Target Rate	Overall Sample Pass Rate	Maximum MAF: median (standard deviation)
Thyroid	47	97.9	97.6	100	100 (0.01)	100 (0.0)	100 (1.33)	100 (2809)	100 (87.76)	100	0.5 (3.2)
Urothelial	147	99.3	99.3	100	100 (0.01)	98.64 (0.0)	98.64 (1.26)	100 (2660)	100 (87.82)	100	2.6 (15.2)

To assess the impact of cancer type on the variation of continuous QC metrics and ctDNA shedding level, the percent of variation explained by cancer type with variance component analysis was estimated. Variant component analysis was performed for cfDNA yield, enrichment molarity, GC bias, non-singleton coverage, on target rate, and maximum MAF. Cancer types explained no more than 2.9% of the variance across all metrics tested, including factors linked to assay sensitivity such as cfDNA yields, depth of coverage after library preparation and sequencing, and the levels of ctDNA shedding.

ctDNA shedding levels are shown below (Figure 1) by cancer type. Maximum MAF served as a proxy for ctDNA shedding, and maximum MAF ranges were similar for all cancer types, except primary CNS tumors. The difference in ctDNA shedding rated may be explained by CNS tumors being located behind the blood-brain barrier, which impairs the transfer of ctDNA from the CNS to the periphery, with a concomitant decrease in typical ctDNA level and detection rate. ctDNA detection is high in NSCLC and CRC, in which the most common genomic alterations are represented on the Guardant360 CDx panel; however, ctDNA detection rates are lower in mesothelioma and renal cell carcinoma, as mutations in the Guardant360 CDx reportable range are less common in these tumor types, resulting in lower ctDNA detection rate.

Figure 1. Maximum MAF distribution by cancer type



In addition to these QC metrics, cfDNA fragment distributions in a large cohort of clinical patient samples was examined to demonstrate similarity of profiles across cancer types. Similar to other QC metrics, cancer type explained less than 1% of the variance in the locations of the cfDNA fragment size profile peak.

11. Comparison to Guardant360 LDT

A study was performed to establish the concordance between Guardant360 CDx and Guardant360 LDT. The purpose of this study was to compare the Guardant360 CDx against a Guardant360 LDT configuration used to generate historical data and is intended to support the use of those results as representative of Guardant360 CDx results.

The design and composition of these two devices is similar, as they share the same principles of operation. The primary differences in design are the panel with which the device is operated. The Guardant360 LDT version used for data generation in support of concordance to the for Guardant360 CDx test in this study was operated with version 2.10 of the panel, which covers 73 genes. The Guardant CDx is operated with version 2.11 of the panel, which covers 74 genes. While the Guardant360 CDx can detect alterations in 74 genes, it only reports select SNVs and indels in 55 genes, CNAs in two (2) genes, and fusions in four (4) genes. The concordance analysis between the Guardant360 CDx and the Guardant360 LDT is limited to 55 gene restricted reportable range. This concordance analysis utilized the bioinformatics pipeline software corresponding to each assay version.

This study evaluated a set of 258 samples with alterations in genes interrogated by both assays, after removing 2 samples that failed QC metrics. The study included cfDNA derived from 22 cancer types, comprising two distinct sample sets. The first set was selected consecutively from among samples from patients with NSCLC positive for Guardant360 CDx variants according to Guardant360 LDT variant calling rules, targeting to obtain a minimum of 50 valid sample results for *EGFR* L858R, 50 for *EGFR* exon 19 deletions, and 75 for *EGFR* T790M mutation. The second set was selected consecutively without consideration for tumor type or previous testing results. Per the study protocol samples with specific set of rare variants were excluded from the study. "Rare" here was defined by Guardant Health as <1% prevalence or to rare fusion events (e.g. *NTRK1, ROS1*), and *MET* exon 14 skipping variants. In addition, when known to Guardant Health based on prior LDT testing or pathology reports, samples from patients for whom tumors are considered tumor mutational burden (TMB) high, microsatellite instability high (MSI-H), or PD-L1 positive were also excluded. In total, only 1 sample was excluded, as it contained an *ALK* fusion.

The cancer types represented in this concordance study were obtained from patients with NSCLC (195), gastrointestinal tumors (22), genitourinary tumors (20), breast cancer (14), gynecological tumors (4), and other solid tumors (4).
PPA and NPA between Guardant360 CDx and Guardant360 LDT, using the Guardant360 LDT assay as the reference method, was calculated for all alterations. A total of 279 SNVs, 117 indels, and 23 CNAs met the alteration inclusion criteria. A summary of PPA and NPA is provided in Table 34. PPA for the CDx variants as well as panel-wide SNVs, indels, and clinically significant variants showed was above 94% in all cases, whereas positive agreement levels were low for *ERBB2* and *MET* amplifications. Agreement levels were low for *ERBB2* and *MET* amplifications as amplification levels for 70% of samples tested were near the decision boundary (< 1.5x LoD). High NPA was observed in all classes.

Concordance between the Guardant360 CDx and the Guardant360 LDT for the four fusions reported by the Guardant360 CDx (*ROS1, ALK, NTRK1*, and *RET*) is unknown as it was not evaluated.

Table 34. Summary of Concordance Between Guardant360 CDx and Guardant360 LDT

Alteration Type	CDx+ LDT+	CDx− LDT+	CDx+ LDT−	CDx− LDT−	PPA (95% CI)	NPA (95% CI)
EGFR T790M	87	4	5	99	95.6% (89.1%, 98.8%)	95.2% (89.1%, 98.4%)
EGFR L858R	52	1	4	138	98.1% (89.9%, 100%)	97.2% (92.9%, 99.2%)
EGFR exon 19 deletions	89	3	2	101	96.7% (90.8%, 99.3%)	98.1% (93.2%, 99.8%)
Clinically Significant	282	16	14	97498	94.6% (91.4%,96.9%)	99.98% (99.97%,99.99%)
Panel-Wide SNV	242	15	21	105647	94.2% (90.6%,96.7%)	99.98% (99.97%,99.99%)
Panel-Wide Indel	102	5	7	50768	95.3% (89.4%,98.5%)	99.99% (99.97%,99.99%)
MET CNA	12	4	0	242	75.0% (47.6%,92.7%)	100% (98.5%,100%)
ERRB2 CNA	5	2	0	251	71.4% (29.0%,96.3%)	100% (98.5%,100%)

The concordance study also compared the Guardant360 CDx to the Guardant360 LDT which was also used in the FLAURA and AURA3 clinical studies to support the *EGFR* CDx indication.

The concordance analysis presented below in Table 35 is for the *EGFR* CDx variants in NSCLC patient samples only (195 out of 258). Concordance analyses between the Guardant360 CDx and Guardant360 LDT utilized the bioinformatics pipeline software corresponding to the Guardant360 CDx applied to the Guardant360 LDT results.

Table 35. Summary of Concordance Between Guardant360 CDx and Guardant360 LDT for CDx Variants

Alteration Type	CDx+ LDT+	CDx− LDT+	CDx+ LDT−	CDx− LDT−	PPA (95% CI)	NPA (95% CI)
EGFR T790M	87	4	5	99	95.6% (89.1%, 98.8%)	95.2% (89.1%, 98.4%)
EGFR L858R	52	1	4	138	98.1% (89.9%, 100%)	97.2% (92.9%, 99.2%)
EGFR exon 19 deletions	89	3	2	101	96.7% (90.8%, 99.3%)	98.1% (93.2%, 99.8%)

In addition to the concordance study described above, the analytical performance with regards to LoD and precision was found to be comparable between the Guardant360 CDx and the Guardant360 LDT with regards to the CDx variants.

B. Animal Studies

No animal studies were conducted using Guardant360 CDx.

C. Additional Studies

1. Blood Collection Tube Concordance

The purpose of this study was to establish concordance between the Streck Cell-Free DNA BCTs and BCT used in the clinical trials (hereafter referred to as BCT-CTA) to enable use of Guardant360 CDx data generated from the FLAURA and AURA3 clinical trials (refer to Section X below).

Blood from NSCLC Stage III or IV patients, prescreened externally for CDx positive and negative markers *EGFR* L858R, *EGFR* T790M, *EGFR* exon 19 deletions, were collected by utilizing two BCT-CTAs and two Streck Cell-Free DNA BCTs. The second BCT-CTA was not processed for this study. A total of 59 patients were enrolled, some with and others without CDx variants, and whole blood samples were tested from three tubes, two Streck Cell-Free DNA BCTs and one BCT-CTA.

The performance of BCT-CTAs relative to Streck Cell-Free DNA BCTs was evaluated through a call agreement analysis which tests the difference of the PPA of Streck Plasma Aliquot 2 (S2) to Streck Plasma Aliquot 1 (S1) and the PPA of BCT-CTA Plasma Aliquot 1 (C1) to S1 (difference denoted as ΔPPA1). ΔPPA2 is calculated similarly except that S2 is considered the reference instead of S1. For negative agreement, ΔNPA1 and ΔNPA2 are also calculated in a similar fashion.

Of the one-hundred and seventy-seven (177) aliquots (59 samples across 3 tube designations), 176 (99.4%) passed in-process and post-sequencing QC metrics. Of the 176 passing post-sequencing metrics, 2 failed sample QC, leaving 174 of 177 (98.3%) samples passing QC metrics. Three of the 59 patients with S1, S2, and C1 runs were excluded from call concordance analyses because of QC failures of at least one of 3 replicates. In total 56 patients met study criteria for inclusion, including 26 distinct CDx variants observed in at least one tube.

The PPA and NPA values across the entire set of CDx variants (aggregated), and for each CDx variants were calculated. BCT-CTAs and Streck Cell-Free DNA BCTs demonstrated expected levels of positive agreement, PPA 92 % – 95.5 % for CDx variants. Discordant detection was observed below LoD, with agreement above LoD being 100%. BCT-CTAs and Streck tubes demonstrated expected levels of negative agreement, NPA 97.3% – 100 % for CDx variants. The delta PPA and delta NPA values were within acceptable limits.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

Two clinical bridging studies were conducted to demonstrate the safety and effectiveness of the Guardant360 CDx to aid in the identification of NSCLC patients who may be eligible for treatment with TAGRISSO® (osimertinib, AstraZeneca) therapy based on the detection of *EGFR* exon 19 deletions or mutations in L858R and/or T790M. In the first study, pre-treatment plasma samples and clinical outcome data from patients randomized in the AstraZeneca FLAURA clinical study (NCT02296125) were used to support the safety and effectiveness of Guardant360 CDx to aid in the selection of previously untreated metastatic NSCLC patients with *EGFR* exon 19 deletions and/or L858R mutations for TAGRISSO therapy. In the second study, pretreatment plasma samples and clinical outcome data from the AstraZeneca AURA3 clinical study (NCT02151981) were used to assess the safety and effectiveness of the Guardant360 CDx to aid in identifying NSCLC patients whose disease has progressed on or after EGFR tyrosine kinase inhibitor (TKI) therapy and who may be eligible for TAGRISSO therapy based on a *EGFR* T790M mutation-detected result.

A. Guardant360 CDx Clinical Bridging Study Design for *EGFR* Exon 19 Deletions and L858R Mutations

FLAURA Clinical Study Design

The FLAURA clinical study was a Phase III, double-blind, randomized study assessing the efficacy and safety of TAGRISSO versus standard of care (SoC) EGFR TKI therapy (gefitinib or erlotinib) in the first-line treatment of patients with locally advanced and metastatic NSCLC whose tumors have *EGFR* exon 19 deletions or exon 21 L858R mutations. Patients were enrolled based on the presence of *EGFR* exon 19 deletions or exon 21 L858R mutation in their tumor as determined by the cobas® *EGFR* Mutation Test at a central laboratory orat a CLIA-certified or accredited laboratory. This clinical study was used to support the approval of TAGRISSO under NDA 208065 Supplement 8.

Guardant360 CDx *EGFR* Exon 19 Deletions or L858R Mutations Bridging Study Design

Pre-treatment blood samples were collected and clinical outcome data from patients positive for *EGFR* mutations by tissue testing randomized in the FLAURA clinical study were used to assess the safety and effectiveness of Guardant360 CDx for the selection of previously untreated metastatic NSCLC patients with *EGFR* exon 19 deletions or L858R mutations for TAGRISSO therapy.

Pretreatment plasma samples from 189 FLAURA patients (34% of the randomized population) were tested with Guardant360 LDT as part of an exploratory analysis. This Guardant360 LDT testing took place before the diagnostic clinical bridging study was initiated.

All patient samples would ideally have been tested using Guardant360 CDx for this diagnostic study's efficacy analysis. However, pre-treatment plasma samples were

only available for the 252 patients (45% of the randomized population) not previously tested with Guardant360 LDT.

The use of this population alone in the diagnostic study was not feasible due to the bias introduced by selection of patients for exploratory testing. Specifically, patients selected for exploratory testing using Guardant360 LDT were those who had progressed and/or discontinued treatment at the time of sample selection for testing, which created a selection bias that is expected to result in longer PFS in patients tested with Guardant360 CDx relative to those tested with Guardant360 LDT and, therefore, relative to the FLAURA randomized population as a whole.

In order to minimize this selection bias, the diagnostic study primary objective analysis includes all FLAURA patients with pretreatment plasma available for testing using Guardant360 CDx, supplemented by patients for whom data was previously generated on Guardant360 LDT. This combined patient group is expected to represent the full randomized patient population in a more robust manner. The analytical concordance study described above, supplemented by demonstration of the comparability of key performance characteristics, i.e., LoD and precision between the Guardant360 CDx and LDT, was performed to support the validity of combining data generated on Guardant360 CDx and LDT test versions for the detection of *EGFR* Exon 19 deletions and L858R mutations (Refer to Section III.A.9. Guardant360 CDx-LDT Concordance Study results). The potential impact of the discordance observed from these studies on the effectiveness of the device was further evaluated through sensitivity analyses (Refer to Section X.A.8.b.ii below). Further a blood collection concordance study establishing the concordance between samples collected in Streck Cell-Free DNA BCTs and the BCTs used in the clinical trial was conducted to support the validity of the data generated by testing samples collected in BCT-CTA (Refer to Section IX.C.2).

No plasma from FLAURA patients negative for *EGFR* mutations by tissue testing was available to represent the Guardant360 CDx-positive, tissue-negative portion of the Guardant360 CDx-positive intended use population. As such, supplemental matched tissue and plasma samples from the Noninvasive vs. Invasive Lung Evaluation clinical study (the NILE study, NCT03615443) were used to estimate the prevalence of patients positive for *EGFR* exon 19 deletions or L858R mutations by Guardant360 CDx but negative by tissue testing to evaluate the potential impact of this population on clinical efficacy.

1. <u>Clinical Bridging Study Inclusion and Exclusion Criteria</u>

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

- Inclusion Criteria for plasma samples from the FLAURA clinical study
  - Patient screened for the FLAURA clinical study with documented informed consent for blood sample use for diagnostic development
  - Pre-treatment time point plasma sample available for testing using Guardant360

- Exclusion Criteria for plasma samples from the FLAURA clinical study
  - Absence of plasma for testing on Guardant360
  - Informed consent withdrawn
  - China mainland patients

- Inclusion Criteria for samples from the NILE clinical study
  - Patient enrolled in the NILE clinical study with documented informed consent
  - Pre-treatment plasma sample available for testing with Guardant360 CDx
  - Availability of unstained slides and/or a tissue block of formalin-fixed paraffin-embedded tissue with sufficient tumor content and quantity for testing as defined by the central testing laboratory requirements for cobas® EGFR Mutation Test testing. Tumor tissue must be from the same disease process as the NILE study plasma sample

- Exclusion Criteria for samples from the NILE clinical study
  - Absence of available plasma or tissue for Guardant360 CDx and cobas® EGFR Mutation Test testing, respectively
  - Informed consent withdrawn

2. Follow-up Schedule

The Guardant360 CDx *EGFR* exon 19 deletions and L858R mutations bridging study involved only retrospective testing of plasma samples; as such, no additional patient follow-up was conducted.

3. Clinical Endpoints

The clinical endpoint used to assess TAGRISSO efficacy in the FLAURA clinical study primary objective was investigator-assessed progression-free survival (PFS), which was defined as the time interval between randomization and the first RECIST progression or mortality event. The Guardant360 CDx *EGFR* exon 19 deletions or L858R mutations bridging study uses the same clinical endpoint for its primary objective.

c. Diagnostic Objective and Endpoint

The primary objective of the diagnostic study was to demonstrate the safety and effectiveness of the Guardant360 CDx for the selection of metastatic NSCLC patients with *EGFR* exon 19 deletions or L858R mutations for treatment with TAGRISSO. This objective was assessed by comparing the efficacy, PFS to RECIST v1.1 by investigator assessment, of single-agent TAGRISSO compared with SoC EGFR TKI therapy in the tissue-positive, Guardant360 CDx-positive patients enrolled in FLAURA.

The possible influence of tissue-negative Guardant360 CDx-positive patients in the effectiveness of the Guardant360 CDx was assessed through a sensitivity analysis. As no plasma samples from FLAURA patients negative for *EGFR* mutations by tissue testing were available to represent the Guardant360 CDx-positive, tissue-negative portion of the Guardant360 CDx-positive intended use population, samples from the NILE clinical

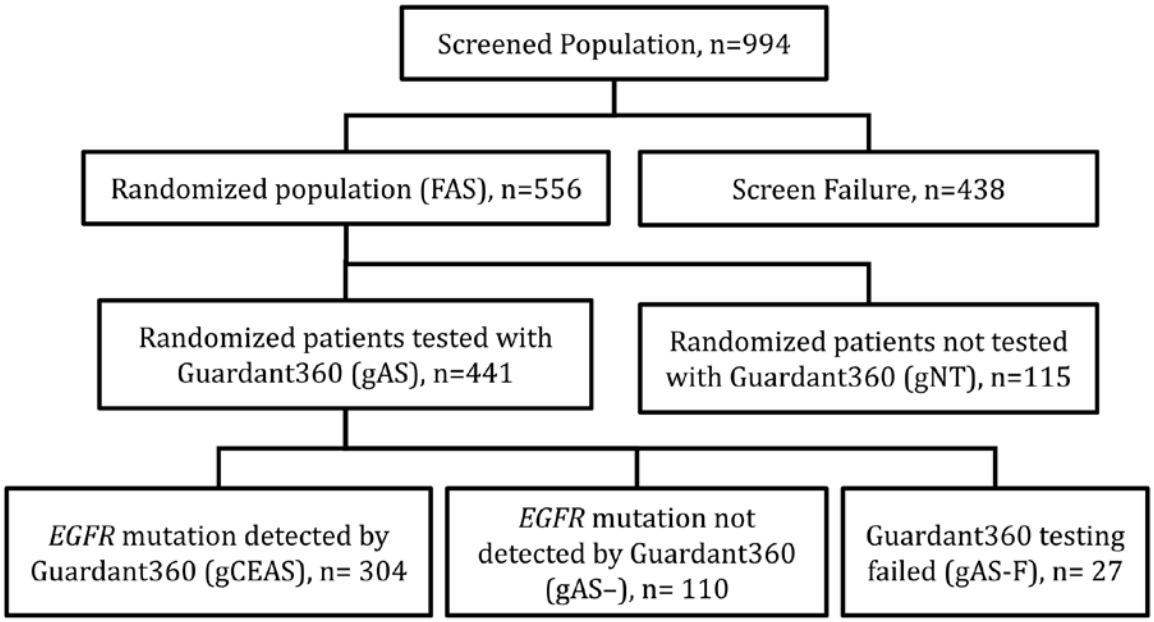
study were tested with Guardant360 CDx and the cobas® EGFR Mutation Test using tissue to calculate the NPA for the sensitivity analysis to evaluate the potential impact of this hypothetical population on clinical efficacy. The sensitivity analysis was performed using data generated by analyzing supplemental tissue samples from the NILE clinical study using the cobas® EGFR Mutation Test and by analyzing residual plasma samples from those same patients using Guardant360 CDx.

B. Accountability of PMA Cohort for Guardant360 CDx Clinical Bridging Study for *EGFR* Exon 19 Deletions and L858R Mutations

The FLAURA diagnostic study included 441 of the total 556 (79.3%) patients randomized in the FLAURA clinical study (Figure 2). The analysis sets comprise diagnostic data generated using Guardant360 CDx (252/441, 57.1%) supplemented by data previously generated on Guardant360 LDT (189/441, 42.9%) as described above. Hereafter, Guardant360 CDx and LDT test versions results combined are referred to as Guardant360 results.

Of these, 304 patients (54.7% of the total population) tested positive by the Guardant360 were included in the primary objective analysis set, while 110 (24.9%) tested negative, and 27 (6.1%) failed testing.

Figure 2. Guardant360 CDx *EGFR* exon 19 Deletions and or L858R Mutations Bridging Study Patient Accountability and Analysis Set Definitions



Concordance Between Guardant360 and the cobas® EGFR Mutation Test Using Plasma and Tissue

Concordance between Guardant360, i.e., Guardant360 CDx and LDT test versions results combined, and the cobas® EGFR Mutation Test using tissue for all matched plasma-tissue from the FLAURA study is shown in Table 36 below.

Table 36. Concordance Between Guardant360 and the cobas® EGFR Mutation Test Using Tissue in Samples from the FLAURA Clinical Study

***EGFR* Exon 19 Deletions**	**cobas® EGFR Mutation Test Using Tissue**			
	Positive	**Negative**	**Failed**	**Total**
Guardant360				
Positive	185	1	2	188
Negative	53	141	3	197
Failed	14	12	1	27
Total	252	154	6	412
PPA (95% CI) [a]	77.7% [71.9%, 82.9%]			
NPA (95% CI) [a]	99.3% [96.1%, 100.0%]			
***EGFR* L858R**	**cobas® EGFR Mutation Test Using Tissue**			
	Positive	**Negative**	**Failed**	**Total**
Guardant360				
Positive	96	2	2	100
Negative	40	242	3	285
Failed	12	14	1	27
Total	148	258	6	412
PPA (95% CI) [a]	70.6% [62.2%, 78.1%]			
NPA (95% CI) [a]	99.2% [97.1%, 99.9%]			
***EGFR* Exon 19 Deletions or L858R**	**cobas® EGFR Mutation Test Using Tissue**			
	Positive	**Negative**	**Failed**	**Total**
Guardant360				
Positive	281	2	4	287
Negative	93	4	1	98
Failed	26	0	1	27
Total	400	6	6	412
PPA (95% CI) [a]	75.1% [70.4%, 79.4%]			
NPA (95% CI) [a]	NC			

[a] PPA and NPA with 95% CIs are calculated based on valid test results (positive or negative). The 95% exact (Clopper-Pearson) CI is calculated. NC = not calculated

Concordance relative to Guardant360 CDx alone is similar to the concordance obtained with the Guardant360 combined data, i.e., Guardant360 CDx and LDT test versions results combined. The point estimates of PPA and NPA and corresponding 95% CIs for *EGFR* exon 19 deletions are 73.8% (65.7%, 80.8%) and 100% (95%, 100%) respectively. The point estimates of PPA and NPA and corresponding 95% CIs for *EGFR* L858R mutations are 68.6% (56.4%,79.1%) and 98.6% (95.0%, 99.8%) respectively. The PPA for *EGFR* exon 19 deletions or L858R was 72.0% with a corresponding 95% CI of 65.5%, 78.0%.

As no plasma samples from FLAURA patients negative for *EGFR* mutations (exon 19 deletions or L858R) by tissue testing were available, NPA could not be calculated using samples from FLAURA. The NPA for *EGFR* exon 19 deletions or L858R relative to the cobas® EGFR Mutation Test using tissue was calculated using samples from the NILE clinical study shown in Table 37 below. Of note, the single sample that tested positive for by Guardant360 CDx but negative by the cobas® EGFR Mutation Test using tissue comprised an uncommon *EGFR* exon 19 deletion, p.T751_I759delinsN, which is not targeted by the cobas® EGFR Mutation Test.

Table 37. Concordance of Central cobas® EGFR Mutation Test Tissue Testing with Guardant360 CDx in Supplemental NILE Study Samples

***EGFR* Exon 19 Deletions or L858R**	**cobas® EGFR Mutation Test Using Tissue**			
	Positive	**Negative**	**Failed**	**Total**
Guardant360				
Positive	14	1	0	15
Negative	0	73	2	75
Failed	0	2	0	2
Total	14	76	2	92
PPA (95% CI) [a]	100% [76.8%, 100.0%]			
NPA (95% CI) [a]	98.7% [92.7%, 100.0%]			

[a] PPA and NPA with 95% CIs are calculated based on valid test results (positive or negative). The 95% exact (Clopper-Pearson) CI is calculated.

C. Study Population Demographics and Baseline Parameters for Guardant360 CDx Clinical Bridging Study for *EGFR* Exon 19 Deletions and L858R Mutations

Demographic and baseline clinical characteristics of patients randomized in the FLAURA clinical study, full analysis set (FAS), were categorized relative to patients randomized in FLAURA positive for *EGFR* exon 19 deletions and/or L858R mutations by tissue and by Guardant360 (Guardant360 primary clinical efficacy analysis set; gCEAS) and assessed for treatment arm balance.

As shown in Table 38, demographics and baseline clinical characteristics in the clinical efficacy analysis subgroups were well-balanced between treatment arms, maintaining approximately a 1:1 randomization within each group.

Table 38. Clinical Effectiveness Analysis Subgroup Demographics and Baseline Clinical Characteristics

Characteristic		gCEAS		FAS	
		TAGRISSO (n=146)	EGFR TKI (gefitinib or erlotinib) (n=158)	TAGRISSO (n=279)	EGFR TKI (gefitinib or erlotinib) (n=277)
Age (years)	Median (range)	63 (32-83)	63 (35-87)	64 (26-85)	64 (35-93)
Age group (years), n (%)	<65	81 (55.5)	92 (58.2)	153 (54.8)	142 (52.3)
	≥65	65 (44.5)	66 (41.8)	126 (45.2)	132 (47.7)
Sex, n (%)	Female	95 (65.1)	103 (65.2)	178 (63.8)	172 (62.1)
Race, n (%)	Asian	83 (56.8)	94 (59.5)	174 (62.4)	173 (62.5)
Smoking status, n (%)	Never	99 (67.8)	100 (63.3)	182 (65.2)	175 (63.2)
	Current	1 (0.7)	4 (2.5)	8 (2.9)	9 (3.2)
	Former	46 (31.5)	54 (34.2)	89 (31.9)	93 (33.6)
AJCC staging at diagnosis	I-III	15 (10.3)	15 (9.5)	52 (18.6)	47 (17.0)
	IV	131 (89.7)	143 (90.5)	226 (81.0)	230 (83.0)
	Unknown	0 (0)	0 (0)	1 (0.4)	0 (0)
Overall disease classification	Metastatic	141 (96.6)	155 (98.1)	264 (94.6)	262 (94.6)
	Locally advanced	4 (2.7)	3 (1.9)	14 (5.0)	15 (5.4)
	Missing	1 (0.7)	0 (0)	1 (0.4)	0 (0)
Histology type	Adenocarcinoma	137 (93.8)	145 (91.8)	246 (88.2)	251 (90.6)
	Other	9 (6.2)	13 (8.2)	33 (11.8)	26 (9.4)

Demographic and baseline clinical characteristics of patients enrolled in the FLAURA clinical study, full analysis set (FAS), were also categorized relative FLAURA patients with plasma available for testing in this diagnostic study (gAS) and those without (gNT) to evaluate comparability (Table 39).

Baseline clinical characteristics were well-balanced within each population by treatment arm for all demographics and baseline clinical characteristics. Demographics and baseline clinical characteristics between gAS and gNT were well-balanced with the exception of age ≥ 65 (48.3% gAS vs. 39.1% gNT, p = 0.0791), never smoking status (62.8% gAS vs. 69.6% gNT, p = 0.1785), AJCC stage at diagnosis I-III (16.1% gAS vs. 24.3% gNT, p = 0.0354), and metastatic overall disease classification (95.5% gAS vs. 91.3% gNT, p = 0.0603).

Table 39. Comparison of Demographics and Baseline Clinical Characteristics Between FLAURA Patients with Plasma Available for Testing (gAS) and Those Without (gNT)

Characteristic		gAS			gNT			
		TAGRISSO (n=219)	EGFR TKI (n=222)	Total (n=441)	TAGRISSO (n=60)	EGFR TKI (n=55)	Total (n=115)	2-sided p value [a]
Age group (years), n (%)	<65	112 (51.1)	116 (52.3)	228 (51.7)	41 (68.3)	29 (52.7)	70 (60.9)	0.0791
	≥65	107 (48.9)	106 (47.7)	213 (48.3)	19 (31.7)	26 (47.3)	45 (39.1)	
Sex, n (%)	Female	137 (62.6)	142 (63.5)	279 (63.3)	41 (68.3)	30 (54.5)	71 (61.7)	0.7628
Race, n (%)	Asian	137 (62.6)	141 (63.5)	278 (63.0)	37 (61.7)	32 (58.2)	69 (60.0)	0.5117
Smoking status	Never	137 (62.6)	140 (63.1)	277 (62.8)	45 (75.0)	35 (63.6)	80 (69.6)	0.1785
	Current/ Former	82 (37.4)	82 (36.9)	164 (37.2)	15 (25.0)	20 (36.4)	35 (30.4)	
AJCC stage at diagnosis	I-III	38 (17.4)	33 (14.9)	71 (16.1)	14 (23.3)	14 (25.5)	28 (24.3)	0.0354
	IV	181 (82.6)	189 (85.1)	370 (83.9)	45 (75.0)	41 (74.5)	86 (74.8)	
	Missing	0	0	0	1 (1.7)	0	1 (0.9)	
Overall disease classification	Metastatic	208 (95.0)	213 (95.9)	421 (95.5)	56 (93.3)	49 (89.1)	105 (91.3)	0.0603
	Locally advanced	10 (4.6)	9 (4.1)	19 (4.3)	4 (6.7)	6 (10.9)	10 (8.7)	
	Missing	1 (0.5)	0	1 (0.2)	0	0	0	
Histology type	Adenocarci-noma	209 (95.4)	204 (91.9)	413 (93.7)	56 (93.3)	54 (98.2)	110 (95.7)	0.4185
Other	Other	10 (4.6)	18 (8.1)	28 (6.3)	4 (6.7)	1 (1.8)	5 (4.3)	

[a] 2-sided p-value is based on Chi-square test for the comparisons. Statistical comparison is based on non-missing values.

The demographic and baseline clinical characteristics of patients screened for the FLAURA and enrolled in the NILE clinical studies were in general well-balanced between the subgroups used in the supplementary Guardant360-positive, tissue-negative prevalence analysis with the exception of race and smoking status, Table 40.

Table 40. Supplementary Guardant360-Positive, Tissue-Negative Prevalence Analysis Subgroup Demographics and Baseline Clinical Characteristics

Characteristic		FLAURA Patients			NILE Patients
		FAS	Screen Failure	Total	
		(n=556)	(n=438)	(n=994)	(n=92)
Age Group (years), n (%)	<65	298 (53.6)	249 (56.8)	547 (55.0)	40 (43.5)
	≥65	258 (46.4)	189 (43.2)	447 (45.0)	52 (56.5)
Sex, n (%)	Female	350 (62.9)	228 (52.1)	578 (58.1)	57 (62.0)
Race, n (%)	Asian	347 (62.4)	221 (50.5)	568 (57.1)	5 (5.4)
Smoking Status	Never	357 (64.2)	251 (57.3)	608 (61.2)	21 (22.8)
	Current	17 (3.1)	57 (13.0)	74 (7.4)	22 (23.9)
	Former	182 (32.7)	130 (29.7)	312 (31.4)	46 (50.0)
	Missing	0	0	0	3 (3.3)
AJCC staging at diagnosis	I-III	99 (17.8)	0	99 (10.0)	17 (18.5)
	IV	456 (82.0)	0	456 (45.9)	75 (81.5)
	Missing	1 (0.2)	438 (100)	439 (44.2)	0
Overall disease classification	Metastatic	526 (94.6)	0	526 (52.9)	89 (96.7)
	Locally advanced	29 (5.2)	0	29 (2.9)	3 (3.3)
	Missing	1 (0.2)	438 (100)	439 (44.2)	0
Histology type	Adenocarcinoma	523 (94.1)	0	523 (52.6)	88 (95.7)
	Other	33 (5.9)	0	33 (3.3)	4 (4.3)
	Missing	0	438 (100)	438 (44.1)	0

SF = patients that failed FLAURA clinical study screening due to a negative EGFR tissue test result
NILE = patients from the NILE clinical study meeting criteria for inclusion into the Guardant360 CDx FLAURA bridging study

D. Safety and Effectiveness Results for Guardant360 CDx Clinical Bridging for *EGFR* Exon 19 Deletions and L858R Mutations

a. Safety Results

Data regarding the safety of TAGRISSO therapy were presented in the original drug approval and are summarized in the drug label. Refer to the TAGRISSO label for more information. No adverse events were reported in the conduct of the diagnostic studies used to support this PMA as these involved retrospective testing of banked specimens only.

b. Effectiveness Results

i. PFS in Patients Positive by Guardant360 for *EGFR* Exon 19 Deletions and/or L858R Mutations

The efficacy of single-agent TAGRISSO relative to EGFR TKI therapy in patients randomized in FLAURA positive for *EGFR* exon 19 deletions or L858R mutations by tissue and by Guardant360 (gCEAS) is shown in Table 41. The observed PFS hazard ratio (HR) of 0.41 (95% CIl 0.31, 0.54) is similar to that for the full FLAURA

randomized population (FAS, PFS HR 0.46, 95% CI 0.37, 0.57). The clinical efficacy observed in the tissue and plasma positive portion of the Guardant360 intended use population, gCEAS, is consistent with that in the FAS.

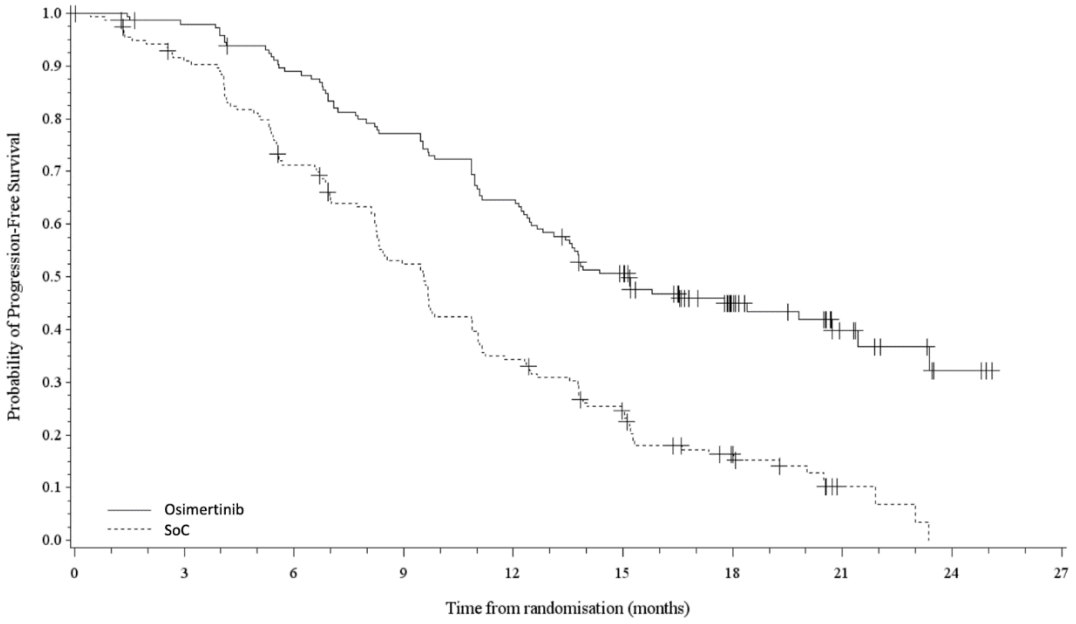
Kaplan-Meier analysis of PFS in the gCEAS is presented in Figure 3.

Table 41. Investigator-Assessed PFS in the Guardant360 Positive/Tissue Positive and FAS

				Comparison between treatments	
Population	**Treatment**	**N**	**Number (%) of patients with events [a]**	**Hazard Ratio (95% CI)**	**2-sided p-value**
gCEAS [b]	TAGRISSO	146	83 (56.8)	0.41 (0.31, 0.54)	<0.0001
	EGFR TKI	158	132 (83.5)		
FAS [b]	TAGRISSO	279	136 (48.7)	0.46 (0.37, 0.57)	<0.0001
	EGFR TKI	277	206 (74.4)		

[a] Progression events that do not occur within 2 scheduled visits (plus visit window) of the last evaluable assessment (or randomization) are censored and therefore excluded in the number of events. Progression includes deaths in the absence of RECIST (v1.1) progression.
[b] The analysis was performed using a log rank test stratified by mutation status and race. A hazard ratio < 1 favors TAGRISSO

Figure 3. Kaplan-Meier Plot of Investigator-Assessed PFS for the gCEAS



Number of patients at risk

Months	0	3	6	9	12	15	18	21	24	27
Osimertinib	146	142	128	111	93	70	35	15	3	0
SoC	158	139	108	78	51	34	17	3	0	0

ii. Sensitivity Analysis

Imputation of Missing Guardant360 Test Results Primary Analysis for the investigator-assessed PFS

The robustness of the study conclusions was assessed by evaluating the impact of missing Guardant360 results on the effectiveness of the device. The missing Guardant360 results were imputed in the randomized (tissue positive) population using an imputation model under missing at random assumption.

There were 115 out of 556 (21%) randomized patients in FLAURA without Guardant360 test results. One of the 115 patients had missing baseline covariates and is therefore removed from the analysis as this patient's probability Guardant360 positive (G360+) could not be predicted from the selected model. Baseline covariates included in the Logit model were:

- PFS (in months, post-baseline data)
- Age group (<65 years, ≥65 years)
- Smoking status (never, current/former)
- AJCC stage at diagnosis (I-III, IV)
- Overall disease classification (Metastatic, locally advanced)
- Cobas® EGFR Mutation Test using plasma test result (positive, negative, failure, missing)

Results based on 1,000 imputations are presented in Table 42 which shows robust and consistent TAGRISSO benefit in both the gCEAS defined by existing Guardant360 test results and the gCEAS (observed and imputed), in which missing Guardant360 test results were imputed via the specified Logit model. These results demonstrate that the missing data has no meaningful impact on the robustness of the efficacy result observed in the FLAURA study.

Table 42. Primary analysis for the investigator-assessed PFS for the gCEAS (observed) and gCEAS (observed and imputed)

				Comparison between treatments	
Population	**Treatment**	**N**	**Number (%) of patients with events [a]**	**Hazard Ratio**	**95% Confidence Interval**
gCEAS (observed)	TAGRISSO	146	83 (56.8)	0.41	0.31, 0.54
	EGFR TKI	158	132 (83.5)		
gCEAS (observed and imputed) [b]	TAGRISSO	173	93 (53.8)	0.42	0.37, 0.57
	EGFR TKI	192	154 (80.2)		

[a]Log rank method with adjustment of the study stratification factors is used for the comparison between treatments.[b] For each imputation, the analysis was performed using a log rank test stratified by mutation status and race. The average HR with 95% CI from 1,000 imputations is presented.

PFS Imputation Analysis to Evaluate the Effect of Observed Guardant360 CDx-LDT Discordance

An imputation analysis modeling the potential effect of Guardant360 CDx-Guardant360 LDT discordance on the PFS HR observed in the primary objective analysis was conducted. The sensitivity analysis by imputation analysis modelling was performed based on the NPA and PPA accounting for MAF between the Guardant360 CDx and Guardant360 LDT. The potential effect of Guardant360 CDx-Guardant360 LDT discordance on the PFS HR was calculated by the Log rank model. The identity between the observed investigator- assessed PFS HR of 0.41 (95% CI 0.31, 0.54) and the imputation results (0.40, 95% confidence 0.31, 0.54) demonstrates that the level of observed Guardant360 CDx-LDT discordance does not impact the observed results. These results support the combination of data derived from Guardant360 LDT and Guardant360 CDx for the primary objective analysis.

Sensitivity analysis for the investigator-assessed PFS in the Guardant360 positive population

A sensitivity analysis was performed by assuming a range of clinical efficacies in the Guardant360-positive, tissue-negative population (i.e. assumed HR (tissue-, G360+)), and the analysis results are presented in Table 43. The sensitivity analysis results support the primary analysis results, with consistent clinical benefit, due to the high PPV of Guardant360 relative to tissue tests. The PPV calculation shown in Table 43 for patients screened in FLAURA used a prevalence of 67%.

Table 43. Sensitivity analysis for investigator-assessed PFS (Guardant360 positive irrespective of tissue result

	Estimated P(Tissue+|Guardant360+) with 95% CI		Estimated HR (Guardant360+) with 95% CI		
	PPV Point Estimate	95% CI	Assumed HR (Tissue- and Guardant360+)	Estimated HR	95% CI
gCEAS (observed)	0.99	0.97, 1.00	0.41 0.50 0.75 1.00	0.41 0.41 0.41 0.41	0.31, 0.54 0.31, 0.54 0.31, 0.54 0.31, 0.54
gCEAS (observed and imputed)	0.99	0.97, 1.00	0.42 0.50 0.75 1.00	0.42 0.42 0.42 0.42	0.32, 0.54 0.32, 0.54 0.32, 0.54 0.32, 0.55

Log rank method with adjustment of the study stratification factors is used to estimate HR with 95%CI for the patients in the gCEAS (observed) and gCEAS (observed and imputed).

Further, because the demographic and baseline clinical characteristics of patients screened for the FLAURA and enrolled in the NILE clinical studies were not well-balanced for race and smoking status, an additional analysis was conducted to determine the minimum PPV that will lead to a unity (1.0) hazard ratio at the two-sided 95% upper confidence bound for Guardant360 positive population. Assuming fixed prevalence of the EGFR marker and PPA observed from the FLAURA samples, the NPA corresponding to this tipping point PPV was determined to help to address the robustness of the study results. This analysis demonstrated that NPA value corresponding to the PPV tipping point associated with an HR upper limit of the 95% CI = 1.0 was significantly less than the observed NPA of 98.7% (in Table 37) supporting the robustness of the study results.

E. Guardant360 CDx Clinical Bridging Study Design for *EGFR* T790M

AURA3 Clinical Study Design

AURA3 was a Phase III, multicenter international , open-label, randomized study to assess the efficacy and safety of TAGRISSO versus platinum-based doublet chemotherapy as second-line therapy in patients with locally advanced or metastatic *EGFR* T790M mutation-positive NSCLC, who had progressed following treatment with 1 line treatment with an approved EGFR-TKI agent. Patients were randomized in a 2:1 ratio to TAGRISSO or pemetrexed plus cisplatin /carboplatin.

Patients were enrolled based on the presence of *EGFR* T790M in their tumor as determined by the cobas® EGFR Mutation Test in a central laboratory. This clinical

study was used to support the approval of TAGRISSO under NDA 208065 Supplement 6.

Guardant360 CDx EGFR T790M Bridging Study Design

Pretreatment blood samples were collected and clinical outcome data from the AURA3 clinical study were used to assess the safety and effectiveness of the Guardant360 CDx for the selection of patients for TAGRISSO therapy with *EGFR* T790M mutation-positive metastatic NSCLC whose disease has progressed on or after EGFR TKI therapy.

Pretreatment samples from 287 AURA3 patients (68% of the randomized population) were tested with Guardant360 LDT in the research setting as part of an exploratory analysis. This Guardant360 LDT testing took place before this diagnostic study was initiated.

All patient samples would ideally have been tested using Guardant360 CDx for this diagnostic study's efficacy analysis. However, pre-treatment plasma samples were available for only 265 patients (63% of the randomized population). As such, this sample set was supplemented by 35 patients for whom data was previously generated on Guardant360 LDT but for whom no plasma remains available for testing with Guardant360 CDx. The analytical concordance study described above, supplemented by demonstration of the comparability of key performance characteristics, i.e., LoD and precision between the Guardant360 CDx and LDT, was performed to support the validity of combining data generated on Guardant360 CDx and LDT test versions for the detection of *EGFR* T790M mutation (Refer to above to the Guardant360CDx-LDT Concordance Study results). Further a blood collection concordance study establishing the concordance between samples collected in Streck Cell-Free DNA BCTs and the BCT used in the trial was conducted to support the valitidy of the data generated by testing samples collected in BCT-CTAs (Refer to Section IX.C.).

1. Clinical Bridging Study Inclusion and Exclusion Criteria

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

- Inclusion Criteria for plasma samples from the AURA3 clinical study
  - Patient screened for the AURA3 clinical study with documented informed consent for blood sample use for diagnostic development
  - Pre-treatment time point plasma sample available for testing using Guardant360

- Exclusion Criteria for plasma samples from the AURA3 clinical study
  - Absence of plasma for testing on Guardant360
  - Informed consent withdrawn
  - China mainland patients

2. Follow-up Schedule

The Guardant360 CDx *EGFR* T790M bridging study involved only retrospective testing of plasma samples; as such, no additional patient follow-up was conducted.

3. Clinical Endpoints

The clinical endpoint used to assess TAGRISSO efficacy in the AURA3 clinical study primary objective was investigator-assessed PFS, which was defined as the time interval between randomization and the first RECIST progression or mortality event. The Guardant360 CDx *EGFR* T790M bridging study uses the same clinical endpoint for its primary objective.

a. Diagnostic Objective and Endpoint

The primary objective of the study was to demonstrate the safety and effectiveness of Guardant360 CDx for the selection of NSCLC patients who have progressed on or after EGFR TKI therapy with *EGFR* T790M mutations for treatment with TAGRISSO. This objective was assessed by comparing the efficacy as determined by PFS to RECIST v1.1 by investigator assessment of single-agent TAGRISSO compared with chemotherapy in the tissue-positive, Guardant360 CDx-positive patients enrolled in AURA3.

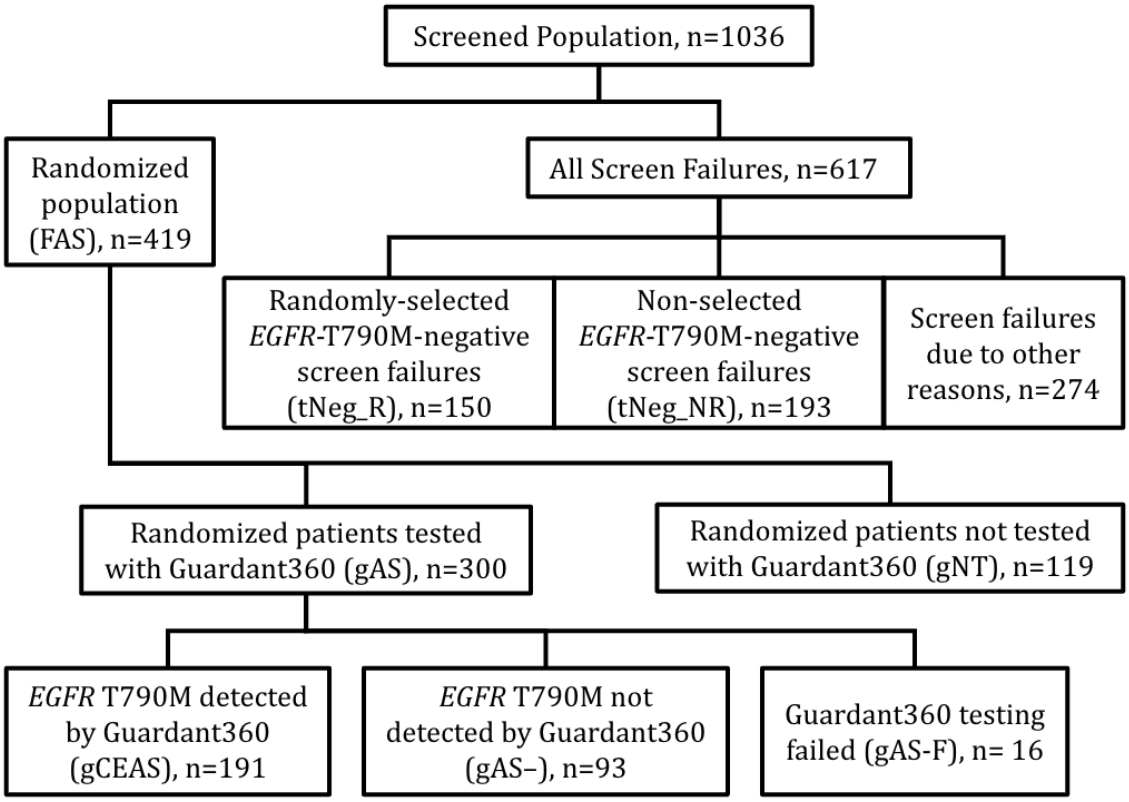
The possible influence of tissue-negative Guardant360 CDx-positive patients in the effectiveness of the Guardant360 CDx was assessed through sensitivity analysis based on randomly selected tissue-negative AURA3 screen-failure samples.

F. Accountability of PMA Cohort for Guardant360 CDx Clinical Bridging for *EGFR* T790M Mutations

The AURA3 diagnostic study included 300 of the total 419 (71.6%) patients randomized in the AURA3 clinical study (Figure 4). Of these, 191 patients (45.6% of the total population) tested positive by Guardant360 and were included in the primary objective analysis set, 93 (31.0%) tested negative, and 16 (5.3%) failed testing. The analysis sets comprise diagnostic data generated using Guardant360 CDx (265/300, 88.3%) supplemented by data previously generated on Guardant360 LDT (35/300, 11.7%) as described above. Hereafter, Guardant360 CDx and LDT test versions results combined are referred to as Guardant360 results.

As AURA3 randomized patients comprised only those positive by tissue testing for *EGFR* T790M mutations, a sensitivity analysis to assess the possible influence of tissue-negative, Guardant360 plasma-positive patients was also performed using 150 randomly selected samples derived from the screened population of AURA3 that failed screening due to a negative *EGFR* T790M tissue test result (150/343, 43.7%).

Figure 4. Guardant360 CDx EGFR T790M Bridging Study Patient Accountability and Analysis Set Definitions



Concordance Between Guardant360 and the cobas® EGFR Mutation Test Using Tissue

Concordance between Guardant360, i.e., Guardant360 CDx and LDT test versions results combined,and the cobas® EGFR Mutation Test using tissue for all matched plasma-tissue samples from the AURA3 study is shown in Table 44 below.

Table 44. Concordance Between Guardant360 and the cobas® EGFR Mutation Test using Tissue

***EGFR* T790M**	**cobas® EGFR Mutation Test Using Tissue**			
	Positive	**Negative**	**Failed**	**Total**
Guardant360				
Positive	190	48	0	238
Negative	92	98	0	190
Failed	15	4	0	19
Total	297	150 [b]	0	447
PPA (95% CI) [a]	67.4% [61.6%, 72.8%]			
NPA (95% CI) [a]	67.1% [58.9%, 74.7%]			

[a] PPA and NPA with 95% CIs are calculated based on valid test results (positive or negative). The 95% exact (Clopper-Pearson) CI is calculated.
[b] Includes 2 patients negative for *EGFR* T790M randomized into the FAS in error.

Concordance relative to Guardant360 CDx alone is similar. The point estimates of PPA and NPA and corresponding 95% CIs for *EGFR* T790M are 66.9% (60.7%, 72.8%) and 67.1% (58.9%, 74.7%) respectively.

G. Study Population Demographics and Baseline Parameters for Guardant360 CDx Clinical Bridging for *EGFR* T790M Mutations

Demographic and baseline clinical characteristics of patients randomized in the AURA3 clinical study were categorized relative to patients randomized in AURA3 positive for *EGFR* T790M mutations by tissue and by Guardant360 (Guardant360 primary clinical efficacy analysis set; gCEAS) and assessed for treatment arm balance.

As shown in Table 45, demographics and baseline clinical characteristics in the clinical efficacy analysis subgroups were well-balanced between treatment arms, maintaining approximately a 2:1 randomization within each group.

Table 45. Baseline Clinical Characteristics of the gCEAS, and FAS

Characteristic		gCEAS		FAS	
		TAGRISSO (n=138)	Chemo-therapy (n=53)	TAGRISSO (n=279)	Chemo-therapy (n=140)
Age (years)	Median (range)	61.0 (34-82)	63.0 (20-80)	62.0 (25- 85)	63.0 (20-90)
Age group (years), n (%)	<65	86 (62.3)	28 (52.8)	165 (59.1)	77 (55.0)
	≥65	52 (37.7)	25 (47.2)	114 (40.9)	63 (45.0)
Sex, n (%)	Male	50 (36.2)	13 (24.5)	107 (38.4)	43 (30.7)
	Female	88 (63.8)	40 (75.5)	172 (61.6)	97 (69.3)
Race, n (%)	Asian	74 (53.6)	35 (66.0)	182 (65.2)	92 (65.7)
Smoking status, n (%)	Never	95 (68.8)	39 (73.6)	189 (67.7)	94 (67.1)
	Current	5 (3.6)	1 (1.9)	14 (5.0)	8 (5.7)
	Former	38 (27.5)	13 (24.5)	76 (27.22)	38 (27.1)
AJCC staging at diagnosis	I-III	20 (14.5)	10 (18.9)	52 (18.6)	31 (22.1)
	IV	117 (84.8)	43 (81.1)	225 (80.6)	109 (77.9)
	Missing	1 (0.7)	0	2 (0.7)	0
Overall disease classification	Metastatic	134 (97.1)	53 (100.0)	266 (95.3)	138 (98.6)
	Locally advanced	4 (2.9)	0	13 (4.7)	2 (1.4)
Histology type	Adenocarcinoma	137 (99.3)	53 (100.0)	277 (99.3)	140 (100)
	Other	1 (0.7)	0	2 (0.7)	0

Also of interest in this analysis is the comparison between AURA3 patients with plasma available for testing in this diagnostic study (gAS) and those without (gNT) to evaluate comparability (Table 46).

Demographics and baseline clinical characteristics were well-balanced between treatment arms for both the gAS and gNT with the exception of Asian race (89.1% osimertinib vs. 65.5% chemotherapy) and sex (56.3% osimertinib vs. 70.9% chemotherapy) in the gNT. Demographics and baseline clinical characteristics between gAS and gNT were comparable, with the exception of age ≥ 65 (45.0% gAS vs. 35.3% gNT, p = 0.0697), Asian race (60.3% gAS vs. 78.2% gNT, p = 0.0005), and never smoking status (65.7% gAS vs. 72.3% gNT, p = 0.1931).

Table 46. Comparison Between AURA3 Patients with Plasma Available for Testing in this Diagnostic Study (gAS) and Those Without (gNT)

Characteristic		gAS			gNT			
		TAGRISSO (n=215)	Chemo-therapy (n=85)	Total (n=300)	TAGRISSO (n=64)	Chemo-therapy (n=55)	Total (n=119)	2-sided p value [a]
Age group (years), n (%)	<65	121 (56.3)	44 (51.8)	165 (55.0)	44 (68.8)	33 (60)	77 (64.7)	0.0697
	≥65	94 (43.7)	41 (48.2)	135 (45.0)	20 (31.2)	22 (40)	42 (35.3)	
Sex, n (%)	Female	136 (63.3)	58 (68.2)	194 (64.7)	36 (56.3)	39 (70.9)	75 (63.0)	0.7520
Race, n (%)	Asian	125 (58.1)	56 (65.9)	181 (60.3)	57 (89.1)	36 (65.5)	93 (78.2)	0.0005
Smoking status	Never	141 (65.6)	56 (65.9)	197 (65.7)	48 (75.0)	38 (69.1)	86 (72.3)	0.1931
	Current/ Former	74 (34.4)	29 (34.1)	103 (34.3)	16 (25.0)	17 (30.9)	33 (27.7)	
AJCC stage at diagnosis	I-III	39 (18.1)	23 (27.1)	62 (20.7)	13 (20.3)	8 (14.5)	21 (17.6)	
	IV	174 (80.9)	62 (72.9)	236 (78.7)	51 (79.7)	47 (85.5)	98 (82.4)	0.4657
	Missing	2 (0.9)	0 (0)	2 (0.7)	0 (0)	0 (0)	0 (0)	
Overall disease classification	Metasta-tic	204 (94.9)	84 (98.8)	288 (96.0)	62 (96.9)	54 (98.2)	116 (97.5)	
	Locally advan-ced	11 (5.1)	1 (1.2)	12 (4.0)	2 (3.1)	1 (1.8)	3 (2.5)	0.5712
Histology type	Adeno-carcino-ma	214 (99.5)	85 (100)	299 (9.7)	64 (100)	55 (100)	119 (100)	1.000
	Other	1 (0.5)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)	

[a] 2-sided p-value is based on Chi-square test for the comparisons. Statistical comparison is based on non-missing values.

H. Safety and Effectiveness Results for Guardant360 CDx Clinical Bridging for *EGFR* T790M Mutations

a. Safety Results

Data regarding the safety of TAGRISSO therapy were presented in the original drug approval and are summarized in the drug label. Refer to the TAGRISSO label for more information. No adverse events were reported in the conduct of the diagnostic studies used to support this PMA as these involved retrospective testing of banked specimens only.

b. Effectiveness Results

i. PFS in Patients Positive by Guardant360 for *EGFR* T790M

The efficacy of single-agent TAGRISSO relative to chemotherapy in patients positive for *EGFR* T790M mutations by Guardant360 (gCEAS) is shown in Table 47. The observed PFS HR of 0.34 (95% CI 0.22, 0.53) was similar to the full AURA3 randomized population (FAS, PFS HR 0.30, 95% CI 0.23, 0.41). This demonstrates clinically relevant osimertinib efficacy in the Guardant360 intended use population.

Kaplan-Meier analysis of PFS in the gCEAS is presented in Figure 5.

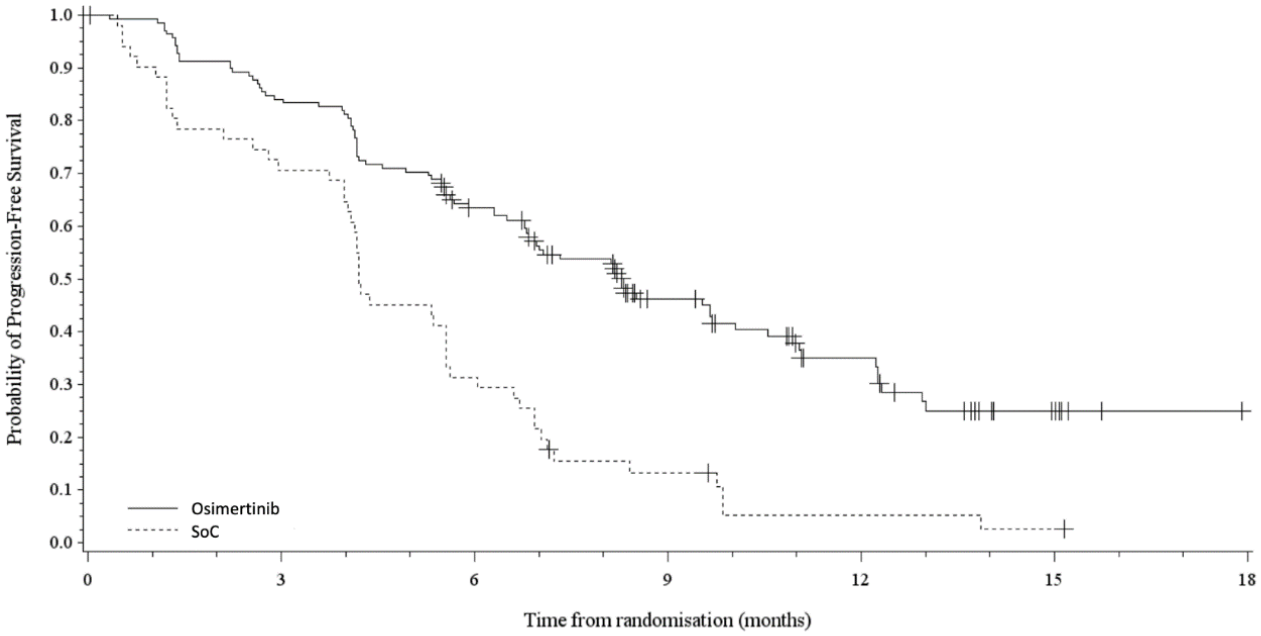
Table 47. Investigator-Assessed PFS in the gCEAS and FAS

				Comparison between treatments	
Population	**Treatment**	**N**	**Number (%) of patients with events [a]**	**Hazard Ratio (95% CI)**	**2-sided p-value**
gCEAS [b]	TAGRISSO	138	85 (61.6)	0.34 (0.22, 0.53)	<0.0001
	Chemotherapy	53	48 (90.6)		
FAS [b]	TAGRISSO	279	140 (50.2)	0.30 (0.23, 0.41)	<0.0001
	Chemotherapy	140	110 (78.6)		

[a] Progression events that do not occur within 2 scheduled visits (plus visit window) of the last evaluable assessment (or randomization) are censored and therefore excluded in the number of events. Progression includes deaths in the absence of RECIST (v1.1) progression.

[b] The analysis was performed using a log rank test stratified by race. A hazard ratio < 1 favors TAGRISSO

Figure 5. Kaplan-Meier Plot of Investigator-Assessed PFS for gCEAS



Number of patients at risk

Months	0	3	6	9	12	15	18
Osimertinib	138	116	80	41	22	7	0
Chemotherapy	53	36	16	6	2	1	0

ii. Sensitivity Analysis

Imputation of missing Guardant360 test results Primary analysis for the investigator-assessed PFS

The robustness of the study conclusions were assessed by evaluating the impact of missing Guardant360 results on the effectiveness of the device. The missing Guardant360 results were imputed in the randomized (tissue positive) population using an imputation model under missing at random assumption. There are 119 (300/419, 28%) randomised patients in AURA3 with missing Guardant360 test results, each of the 119 patients with missing Guardant360 test results is to be imputed via a specified Logit model. Baseline covariates included in the Logit model are:

- PFS (in months, post-baseline data)
- Age group (<65 years, ≥65 years)
- Race (Asian, Non-Asian)
- Smoking status (never, current/former)
- cobas® EGFR Mutation Test using plasma test result (positive, negative, failed, not tested, missing)

Results based on 1,000 imputations are presented in Table 48 and show robust and consistent TAGRISSO benefit in the gCEAS defined by the observed Guardant360 test results and the gCEAS (observed and imputed), in which missing Guardant360 test results were imputed via the specified Logit model. The consistency of these results demonstrates that the missing G360 data have no meaningful impact on the robustness of the efficacy result observed in the AURA3 study.

Table 48. Primary Analysis for the Investigator-Assessed PFS for the gCEAS (Observed) and gCEAS (Observed and Imputed)

				Comparison between treatments	
Population	**Treatment**	**N**	**Number (%) of patients with events [a]**	**Hazard Ratio**	**95% Confidence Interval**
gCEAS (observed)	TAGRISSO	138	85 (61.6)	0.34	0.22, 0.53
	Chemotherapy	53	48 (90.6)		
gCEAS (observed and imputed) [b]	TAGRISSO	182	102 (56.0)	0.35	0.24, 0.51
	Chemotherapy	92	74 (80.4)		

[a]Log rank method with adjustment of the study stratification factors is used for the comparison between treatments. [b] For each imputation, the analysis was performed using a log rank test stratified by mutation status and race. The average HR with 95% CI from 1,000 imputations is presented.

PFS Imputation Analysis to Evaluate the Effect of Observed Guardant360 CDx-LDT Discordance

An imputation analysis modeling the potential effect of Guardant360 CDx-Guardant360 LDT discordance on the PFS HR observed in the primary objective analysis was conducted. The sensitivity analysis by imputation analysis modelling was performed accounting for MAF. The potential effect of Guardant360 CDx-Guardant360 LDT discordance on the PFS HR was calculated by the Log rank model. The identity between the observed investigator- assessed PFS HR of 0.34 (95% CI 0.22, 0.53) and the imputation results (0.34, 95% confidence 0.22, 0.53) demonstrates that the level of observed Guardant360 CDx-LDT discordance does not impact the observed results. These results support the combination of data derived from Guardant360 LDT and Guardant360 CDx for the primary objective analysis.

Sensitivity analysis for the investigator-assessed PFS in the Guardant360 positive population

The analysis above demonstrated TAGRISSO efficacy in the Guardant360-positive, tissue-positive subset of the Guardant360 CDx intended use population. As shown in Table 49, sensitivity analysis modeling efficacy in the entire Guardant360 CDx intended use population demonstrates robustness to the contribution of the

Guardant360-positive, tissue-negative patients not represented in the AURA3 clinical study, with statistically-significant efficacy maintained across the entire Guardant360 CDx intended use population, including the modeled Guardant360-positive, tissue-negative subgroup. The PPV calculation shown in Table 49 for patients screened in AURA3 used a prevalence of 55%.

Table 49. Sensitivity Analysis for Investigator-Assessed PFS (Guardant360 positive irrespective of tissue result)

	Estimated P(Tissue+|Guardant360+) with 95% CI		**Estimated HR (Guardant360+) with 95% CI**		
	PPV Point Estimate	**95% CI**	**Assumed HR (Tissue- and Guardant360+)**	**Estimated HR**	**95% CI**
gCEAS (observed)	0.72	0.66, 0.77	0.34 0.50 0.75 1.00	0.34 0.38 0.43 0.46	0.22, 0.53 0.27, 0.53 0.30, 0.60 0.33, 0.65
gCEAS (observed and imputed)	0.72	0.66, 0.77	0.35 0.50 0.75 1.00	0.36 0.39 0.43 0.47	0.24, 0.51 0.29, 0.52 0.32, 0.59 0.35, 0.64

Log rank method with adjustment of the study stratification factors is used to estimate HR with 95%CI for the patients in the gCEAS (observed) and gCEAS (observed and imputed).

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

I. 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 because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The analytical performance of Guardant360 CDx for the detection of SNVs, indels, select amplifications, and select fusions in plasma-derived cfDNA across solid tumors was established in the analytical studies reported above. Analytical accuracy, sensitivity, specificity, and precision are reported in Section IX.

The clinical benefit of Guardant360 CDx to select patients with NSCLC whose tumors have *EGFR* exon 19 deletions, L858R mutations, and/or T790M mutations for osimertinib therapy was demonstrated in two bridging studies using pretreatment samples from the FLAURA and AURA3 clinical studies and is summarized in Section X above. In both bridging studies, osimertinib efficacy relative to standard of care therapy was similar in patients positive for *EGFR* mutations by Guardant360 versus the total randomized population of each study. Additional sensitivity analyses to impute missing data (Guardant360-positive, tissue-negative patients not represented in the FLAURA and AURA3 clinical studies) consistently support clinical benefit in patients with these specific *EGFR* mutations.

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B. Safety Conclusions

The risks of the device are based on nonclinical laboratory studies as well as data collected in clinical studies conducted to support PMA approval as described above. As an *in vitro* diagnostic test for the detection of *EGFR* mutations that can inform treatment selection in a NSCLC patient's blood sample, failure of Guardant360 CDx to perform as expected or incorrect interpretations results may lead to inappropriate patient management decisions in NSCLC treatment. Since a patient with a negative result (including a false negative result) from the Guardant360 CDx will be reflexed to having their *EGFR* status determined from an formalin-fixed paraffin-embedded (FFPE) tissue specimen, the risks of the Guardant360CDx are largely associated with a false positive result in a patient, who may then undergo treatment with TAGRISSO

(osimertinib) with inappropriate expectation of therapeutic benefit and experience side effects.

The patient population included in the AURA3 study used to support the safety and effectiveness of the Guardant360 CDx for selecting patients for treatment with TAGRISSO (osimertinib) was originally selected using FFPE tumor specimens. Clinical data for the *EGFR* T790M plasma-positive, tissue-negative (or unknown) population is limited, and therefore additional clinical data is needed to establish the clinical performance of the test in this population (see Section XIII below). Given the lack of data, testing with the Guardant360 CDx is considered most appropriate for patients from whom a tumor biopsy cannot be obtained for the purpose of identifying patients with EGFR T790M status.

C. Benefit-Risk Determination

Treatment with TAGRISSO provides meaningful clinical benefit to patients with NSCLC harboring *EGFR* exon 19 deletions, *EGFR* L858R mutations or T790M mutations. The benefit of the Guardant360 CDx was demonstrated using archived pre-treatment plasma samples from the FLAURA and AURA3 studies. In the FLAURA study, pre-treatment plasma samples and clinical outcome data from patients randomized in the AstraZeneca FLAURA clinical study (NCT02296125), supporting the probable benefit of Guardant360 CDx in identifying patients with previously untreated metastatic NSCLC with *EGFR* exon 19 deletions and/or L858R mutations for TAGRISSO, based on the estimated hazard ratio of 0.41-0.42, providing evidence of probable benefit. In the AURA3, pretreatment plasma samples and clinical outcome data from the AstraZeneca AURA3 clinical study (NCT02151981) were used to support the probable benefit of the Guardant360 CDx in identifying NSCLC patients whose disease has progressed on or after EGFR TKI therapy who were eligible for TAGRISSO therapy based on a *EGFR* T790M mutation-detected result, with a hazard ratio range of 0.34-0.47 on sensitivity analysis, providing evidence of probable benefit. In addition, the Guardant360 CDx device has probable benefit in tumor mutation profiling of circulating cell-free DNA (cfDNA) from plasma for cancer patients with a solid malignant neoplasm, to be used by qualified health care professionals in accordance with professional guidelines.

There is potential risk associated with the use of this device, mainly due to 1) false positive, false negatives, or failure to provide a result and 2) incorrect interpretation of test results by the user.

The risks of the Guardant360 CDx for the selection of NSCLC patients with *EGFR* exon 19 deletions, *EGFR* L858R mutations or T790M mutations, for treatment with TAGRISSO, 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 combination that is not beneficial and may lead to adverse events or may have delayed access to other treatments that could be more beneficial. A false negative result may prevent a patient from accessing a

potentially beneficial therapeutic regimen. However, this risk is partially mitigated by reflex testing recommendation for negative results on these biomarkers.

The risks of false results are partially mitigated by the analytical validation results summarized above. In addition, the risks of false negative results are partially mitigated by a recommendation that those patients whose plasma generates a negative result for those alterations included in Table 1 should have their tumor mutation status for Table 1 alterations verified by using a FDA approved tumor test, if feasible. Though the Guardant360 CDx assay has been analytically validated as summarized above, multiple post-market studies are also planned. The overall clinical and analytical data support that for the Guardant360 CDx assay, and the indications noted in the intended use statement, the probable benefits outweigh the probable risks.

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

In conclusion, given the available information above, the data support that for the selection of NSCLC patients with *EGFR* exon 19 deletions, *EGFR* L858R, or T790M mutations for treatment with TAGRISSO 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 clinical studies support the use of Guardant360 CDx in the identification of patients for whom treatment with the therapies listed in the Intended Use Statement may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order on August 7, 2020. The final conditions of approval cited in the approval order are described below.

1. Guardant Health, Inc. must provide data from additional testing of clinical samples to supplement the analytical accuracy of Guardant360 CDx for biomarkers to be reported in the tumor profiling indication. Clinical samples should adequately represent the range of biomarkers that are detected by Guardant360 CDx and include the corresponding cancer type under which the biomarker will be reported.
2. Guardant Health, Inc. must provide data from a guardbanding study to test the tolerance of Guardant360 CDx to variation in critical parameters, i.e., cell-free DNA input, adapter volume, hybridization time, and hybridization Wash Buffer according to the agreed upon study protocol. The data from this study must be adequate to support that variation in critical parameters do not adversely impact Guardant360 CDx results.

3. Guardant Health, Inc. must provide data evaluating the effects of potential exogenous interfering substances that may carry over from cfDNA extraction on performance of Guardant360 CDx according to the agreed upon study protocol. The data from this study must be adequate to support that potential exogenous interfering substances do not adversely impact Guardant360 CDx results.

The final study data, study conclusions, and labeling revisions should be submitted within one (1) year of the PMA approval date.

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.