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/S002

Date of FDA Approval: May 28, 2021

The original PMA (P200010) for Guardant360® CDx was approved on August 7, 2020 for the detection of genetic alterations in circulating cell-free DNA (cfDNA) from plasma of peripheral whole blood derived from patients who may benefit from one of the FDA-approved therapies for non-small cell lung cancer (NSCLC). Subsequently, another PMA supplement was approved for expanding the indications for use of Guardant360 CDx since its original approval. The SSED to support the previously approved indication is available on the CDRH website.

The current panel-track supplement was submitted to expand the intended use and indications for use of Guardant360 CDx to include a companion diagnostic indication for the detection of *KRAS* G12C in NSCLC patients who may benefit from treatment with LUMAKRAS™ (sotorasib).

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 therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1. Companion Diagnostic Indications

Indication	Biomarker	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions, L858R, and T790M*	TAGRISSO® (osimertinib)
	EGFR exon 20 insertions	RYBREVANT™ (amivantamab-vmjw)
	KRAS G12C	LUMAKRAS™ (sotorasib)

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

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

Additionally, the test is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with any solid malignant neoplasms. The test is for use with patients previously diagnosed with cancer and in conjunction with other laboratory and clinical findings. Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product. Guardant360 CDx is a single-site assay performed at Guardant Health, Inc.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

Warnings and precautions are listed below:

- Alterations reported may include somatic (not inherited) or germline (inherited) alterations. The assay filters germline variants from reporting except for pathogenic *BRCA1, BRCA2, ATM,* and *CDK12* alterations. However, if a reported alteration is suspected to be germline, confirmatory testing should be considered in the appropriate clinical context.
- The test is not intended to replace germline testing or to provide information about cancer predisposition.
- Somatic alterations in *ATM* and *CDK12* are not reported by the test as they are excluded from the test's reportable range.
- Genomic findings from cfDNA may originate from circulating tumor DNA (ctDNA) fragments, germline alterations, or non-tumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP).

- Allow the tube to fill completely until blood stops flowing into the tube. Underfilling of tubes with less than 5 mL of blood (bottom of the label indicates 5 mL fill when tube is held vertically) may lead to incorrect analytical results or poor product performance. This tube has been designed to fill with 10 mL of blood.

V. DEVICE DESCRIPTION

Guardant360 CDx is a single-site test performed at Guardant Health, Inc. The test includes reagents, software, and procedures for testing cfDNA from whole blood samples. The test uses 5-30 ng of cfDNA for library construction and next generation sequencing. 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 / Rearrangements	*ALK, NTRK1, RET, ROS1*

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

Test Output

The test report includes variants reported in the following categories: see Table 3:

Table 3. Category Definitions

Category	Guardant360 CDx			Comments
	Prescriptive use for a Therapeutic Product	Clinical Performance	Analytical Performance	
Category 1: Companion Diagnostic (CDx)	**Yes**	**Yes**	**Yes**	ctDNA biomarkers linked to the safe and effective use of the corresponding therapeutic product, for which Guardant360 CDx has demonstrated clinical performance shown to support therapeutic efficacy and strong analytical performance for the biomarker.
Category 2: ctDNA Biomarkers with Strong Evidence of Clinical Significance in ctDNA	No	No	**Yes**	ctDNA biomarkers with strong evidence of clinical significance presented by other FDA-approved liquid biopsy companion diagnostics for which Guardant360 CDx has demonstrated analytical reliability but not clinical performance.
Category 3A: Biomarkers with Evidence of Clinical Significance in tissue supported by: strong analytical validation 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 (referred to as box 1, 2, 3, 4a, 4b, and 4c) based on the usage of the reagents. The division of reagents amongst the boxes reflects different storage conditions and/or locations (e.g. different laboratory spaces).

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

Table 4. Serial Number Controlled Instruments

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

Test Process

Whole Blood Collection and Shipping
The Guardant360 CDx Blood Collection Kit is used by ordering laboratories / physicians to collect whole blood specimens and ship them to the Guardant Health Clinical Laboratory. A minimum of 5 mL whole blood must be received in order to achieve optimal performance for the Guardant360 CDx assay. Underfilling of tubes

with less than 5 mL of blood may lead to incorrect analytical results or poor product performance.

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

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

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

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

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

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

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

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

SNV Calling Property	**Metric**
DNA Molecule Support	≥ 2
MAF Estimate	≥ 0.001%
Log Likelihood Ratio	≥ 0
Indel Calling Property	**Metric**
DNA Molecule Support	≥ 2
MAF Estimate	≥ 0.01%
Log Likelihood Ratio	≥ 10
CNA Calling Property	**Metric**
ERBB2 copy number	≥ 2.18
ERBB2 Z-score	≥ 10
ERBB2 amplification is not associated with chromosome-arm aneuploidy	TRUE
MET copy number	≥ 2.16
MET Z-score	≥ 10
MET amplification is not associated with chromosome-arm aneuploidy	TRUE
Fusion Calling Property	**Metric**
MAPQ score of supporting molecules to fusion sequence	> 30
Number of unique fusion molecules	≥ 2
Number of unique fusions 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 *EGFR* exon 19 deletions, *EGFR* L858R, and *EGFR* T790M genetic alterations using cfDNA, for the TAGRISSO® (osimertinib) therapeutic. 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)

- FoundationOne® Liquid CDx (Foundation Medicine, Inc.)
  - Technology: Next Generation Sequencing (NGS)
  - 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.

In addition, there are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of *EGFR* Exon 20 insertions for the identification of NSCLC patients eligible for treatment with RYBREVANT™ (amivantamab-vmjw).

There is one FDA approved CDx alternative for the detection of *KRAS* G12C mutation in NSCLC patients using tissue specimens for treatment with LUMAKRAS™ (sotorasib): *therascreen*® KRAS RGQ PCR Kit (P110027/S012)

There is no FDA approved CDx alternative for the detection of *KRAS* G12C mutations in NSCLC patients for LUMAKRAS™ (sotorasib).

VII. MARKETING HISTORY

Guardant Health, Inc. initially designed and developed the Guardant360 laboratory developed test (Guardant360 LDT), and the first commercial sample was tested in 2012 to detect the presence of genomic alterations in plasma isolated from whole blood. The Guardant360 CDx was FDA-approved on August 7, 2020 and subsequently commercialized in the USA.

Guardant360 CDx has not been marketed in 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 LUMAKRAS™ (sotorasib) FDA approved package insert which is available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

The primary evidence for supporting the performance of Guardant360 CDx in detecting *KRAS* G12C was from the data presented using intended use specimens across all validation studies. In addition to the existing platform-level validation data (P200010), analytical accuracy/concordance and precision at the limit of detection (LoD) studies were conducted to support the indication for *KRAS* G12C CDx biomarker. Further, results from exogenous interference and guardbanding studies

that were completed to fulfil the conditions of approval for the original PMA P200010 and that utilized samples carrying *KRAS* G12C mutations, were also included to support this PMA supplement.

For Guardant360 CDx platform-level validation (P200010), performance characteristics were established using cfDNA isolated from plasma derived from a wide range of cancer types. Each study included CDx variants as well as a broad range of representative alteration types (SNVs, indels, CNAs, and fusions/rearrangements) in various genomic contexts across several genes. The platform validation studies included samples with *KRAS* G12C mutation in NSCLC and non-NSCLC plasma specimens. These results from the platform-level validation (P200010) have been leveraged to support Guardant360 CDx detection of *KRAS* G12C mutations. Additional *KRAS* G12C-specific additional analytical validation studies are described below.

1. Analytical Accuracy/Concordance

a. Analytical Accuracy/Concordance with an Orthogonal Method for *KRAS* G12C

An analytical accuracy study was performed using clinical plasma specimens from 106 *KRAS* G12C mutation-positive NSCLC patients and 107 *KRAS* G12C mutation-negative NSCLC patients to demonstrate the concordance between Guardant360 CDx and an externally validated mass spectrometry-based comparator assay for the detection of *KRAS* G12C. This study evaluated a set of 214 NSCLC plasma specimens from three (3) cohorts, including 53 NSCLC samples positive for *KRAS* G12C mutation by tissue testing from the clinical study (cohort 1), 53 NSCLC samples obtained without consideration for biomarker status from the clinical sensitivity study (cohort 2), 69 NSCLC samples positive for *KRAS* G12C mutation by Guardant360 LDT from the Guardant Health biobank of previously collected samples (cohort 3), and 39 NSCLC samples selected without consideration for biomarker status from the Guardant Health biobank (cohort 3). One sample failed QC metrics on Guardant360 CDx, resulting in 213 evaluable samples. A summary of positive percent agreement (PPA) and negative percent agreement (NPA) and corresponding two-sided Clopper-Pearson 95% confidence intervals (CIs) is provided in Table 6.

The concordance for *KRAS* G12C mutations across all evaluable samples was 96% PPA and 94% NPA. The discordance (10 samples) listed in Table 6 occurred in samples with circulating free DNA amounts near or below the LoD, which resulted in stochastic detection due to random sampling effects. The reported PPA and NPA (Table 6) were not adjusted for the distribution of samples from the Guardant Health biobank collected using the Guardant360 LDT.

Table 6. Summary of Concordance Between Guardant360 CDx and a Comparator for CDx Variants

Alteration Type	Guardant360 CDx (+), Comparator (+)	Guardant360 CDx (+), Comparator (-)	Guardant360 CDx (-), Comparator (+)	Guardant360 CDx (-), Comparator (-)	Patients (n)	PPA (95% CI)	NPA (95% CI)	PPV (95% CI)	NPV (95% CI)
KRAS G12C	102	6	4	101	213	96% (91%, 99%)	94% (88%, 98%)	94% (88%, 98%)	96% (91%, 99%)

To further investigate the origin of the six Guardant360 CDx^{+} Comparator^{-} samples, agreement between Guardant360 CDx and the comparator assay was calculated for each sample source independently (Table 7). As shown in Table 7, all six discordant samples were from cohorts enriched for *KRAS* G12C, including four positive samples from the Guardant Health biobank and two samples from the clinical study.

Table 7. Summary of Concordance Between Guardant360 CDx and Comparator for *KRAS* G12C by Cohort

Sample Cohort	Guardant360 CDx (+), Comparator (+)	Guardant360 CDx (+), Comparator (-)	Guardant360 CDx (-), Comparator (+)	Guardant360 CDx (-), Comparator (-)	PPA (95% CI)	NPA (95% CI)	PPV (95% CI)	NPV (95% CI)
CV_ITT (N=53)	39	2	1	11	98% (87%, 100%)	85% (55%, 98%)	95% (84%, 99%)	92% (62%, 100%)
CV_ Prevalence (N=53)	3	0	0	50	100% (29%, 100%)	100% (93%, 100%)	100% (29%, 100%)	100% (74.87%, 99.05%)
GH_ Biobank Unselected (N=39)	3	0	0	36	100% (29%, 100%)	100% (90%, 100%)	100% (29%, 100%)	100% (93%, 100%)

GH_Biobank Positive (N=68)	57	4	3	4	95% (86%, 99%)	50% (16%, 84%)	93% (84%, 98%)	57% (18%, 90%)

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

2. Analytical Sensitivity

Please see the Summary of Safety and Effectiveness Data P200010 (Section IX.A.3) for Guardant360 CDx platform validation of analytical sensitivity, including Limit of Blank (LoB) and Limit of Detection (LoD).

Per P200010, the LoB was established by evaluating 62 whole blood samples from healthy age-matched donor samples. 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.

No false positive *KRAS* G12C alterations were detected among 240 replicates tested across three unique reagent lots.

In PMA P200010 the LoD for *KRAS* G12V was established to be 1.5% MAF at 5 ng cfDNA input and 0.5% MAF at 30 ng cfDNA input using patient samples from multiple cancers. The established LoD was further confirmed in P200010 using clinical samples to be 1.8% MAF at 5 ng cfDNA input and 0.5% MAF at 30 ng cfDNA input by testing 20 and 14 replicates, respectively, with 3 sets of reagents lots. These confirmed LoD values were utilized in other performance studies (e.g., precision, guardbanding and interference) provided in this supplemental PMA. Further, the LoD values at high and low DNA input levels for *KRAS* G12C were confirmed in a precision study using NSCLC patient samples near these confirmed LoD values (see section 4 below).

3. Analytical Specificity

Please see the Summary of Safety and Effectiveness Data P200010 (Section IX.A.4) for Guardant360 CDx platform validation of analytical specificity, including endogenous and microbial interfering substances and *in silico* specificity. However, during original PMA (P200010) approval, the effect of potential exogenous interfering substances that may carry over from cfDNA extraction on assay performance was not evaluated. Therefore, the following study was performed to fulfill the obligation of a condition of approval for P200010.

a. Exogenous Interference

To evaluate the effect of extraction wash buffer carry-over on the performance of Guardant360 CDx, this study evaluated whole blood samples containing 10 variants representative of SNVs (*KRAS* G12C, *EGFR* T790M, *EGFR* L858R, *PIK3CA* E545A/D, *PIK3CA* H1047L/R), indels (*EGFR* E746_A750del, *EGFR* A767_V769dup), CNAs (*MET*), and fusions (*EML4-ALK*, *TPM3-NTRK1),* including CDx alterations. Two variant pools were prepared by diluting either clinical or cell line-derived cfDNA fusion samples (targeting 1-2 x LoD) positive for a given biomarker with mutation-negative cfDNA derived from either NSCLC or breast cancer. A total number of 30 samples were included in this study with 2 different pools, processed in 6 replicates each at 5 ng input using extraction wash buffer volume of 10% (v/v). Twenty-five (25) of the 30 sequenced samples passed QC metrics and were used for analysis.

The reference condition showed a quantitative detection rate (QDR) of 98.3%, with one variant (*EGFR* A767_V769dup) missing in one sample, while the QDR of the test condition was 100% (Table 8). For each condition, the lower limits of the 95% confidence interval were higher than 80% meeting the acceptance criteria. The chi-square test also showed a non-significant difference of QDRs (p-value = 0.49) between the test condition and reference condition.

Pool 1 and 2 were designed to have no shared targeted variants; thus, NPA was analyzed by assessing for the targeted variants designed out of each pool. None of the expected negative calls were observed across samples, showing a 100% per-sample NPA at the targeted variants across all conditions.

Table 8. Exogenous Interference Summary Results

QDR	Reference	Test
Positive Detected Variants Across Samples	59	64
QDR	59/60 = 98.33%	64/64 = 100%
95% CI	[91.06%, 99.96%]	[94.40%, 100%]

The QDR, defined as the number of positively detected targeted variants tested across eligible samples (D) divided by the total number of targeted variants tested across eligible samples (N) expressed as a percentage (100 * D / N), and the 95% confidence intervals.

4. Precision

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

a. Precision for *KRAS* G12C from NSCLC cfDNA Clinical Sample pools

The purpose of the precision study was to demonstrate the repeatability and within-site reproducibility of Guardant360 CDx for detecting *KRAS* G12C mutation through closeness of agreement between qualitative detection in replicates using different combinations of reagent lots, instruments, operators, and days. The study was conducted with pooled NSCLC patient samples harboring *KRAS* G12C mutations.

Two cfDNA sample pools harboring *KRAS* G12C were prepared at targeted MAF levels of 1-1.5 x LoD and tested at the 5 ng (2.4% MAF, 1.33 x LoD) and 30 ng (0.7% MAF, 1.4 x LoD) cfDNA input amounts. For the 5ng and 30ng input amounts, seven (7) and three (3) replicates were tested, respectively, for each of the six (6) precision combinations composed of three different reagent lots, two different instrument sets, and two different operator groups. In total, 42 samples tested at the 5ng input level and 18 samples were assessed at the 30ng input level.

This study successfully verified the precision of Guardant360 CDx for detecting *KRAS* G12C mutations within and between different reagent lots, instrument sets, and operator groups with samples near LoD processed on different runs and days in the Guardant Health Clinical Laboratory (Table 9). The acceptance criteria were met with a positive precision of 100% at both 5 and 30 ng cfDNA input.

Table 9. Summary of Precision Results for *KRAS* G12C

Input Amount	Concordant / Expected Positives	PPA [95% CI]
5 ng	42/42	100% [91.6% - 100%]
30 ng	18/18	100% [81.5% - 100%]

Samples from healthy donors pre-screened by an externally validated orthogonal method were reanalyzed specifically for *KRAS* G12C mutations to determine if false positives were detected across replicates or conditions. The study demonstrated a sample-level, within-condition average negative agreement (ANA) of 100% and a sample-level between-condition ANA of 100% for *KRAS* G12C.

5. Carryover/Cross-Contamination

Refer to Summary of Safety and Effectiveness Data P200010 (Section IX.A.6).

6. Guardbanding

During original PMA (P200010) approval, the guardbanding study was not performed. Therefore, this study was performed as part of fulfillment of the conditions of approval of P200010 as well as to support this indication.

The purpose of the guardbanding study was to evaluate cfDNA input at the minimum (5 ng) and maximum (30 ng) input amounts, adapter volume tolerances for ligation steps, hybridization time tolerances in the enrichment process and wash buffer 2 temperature tolerances in the enrichment process (Table 10).

Table 10. Guardbanding Study Overview

Guardbanding Condition	Reference Condition	Condition 1	Condition 2
cfDNA input amount	5 ng	2.5 ng	4 ng
cfDNA input amount	30 ng	36 ng	45 ng
Adapter volume	18.0 µl	16.2 µl	19.8 µl
Hybridization Time	12 hours	24 hours	N/A
Wash Buffer Temperature	71^0C	70^0C	72^0C

Ten targeted variants representative of SNVs, indels, CNAs, and fusions were tested in 2 variant pools. Each variant pool was prepared by diluting either clinical or cell line-derived cfDNA samples positive for a given biomarker with mutation-negative cfDNA derived from either NSCLC or breast cancer patients targeting each variant to 1-2X LoD. One hundred four (104) of the 126 samples passed post-sequencing QC metrics, with only the 2.5 ng cfDNA input condition failing to reach the minimum sample number.

All QDRs (Qualitative Detection Rates) were 100%, except for the 4 ng input condition, which showed a QDR of 97.2%, with one variant (*EGFR* A767_V769dup) missing in one of 4 ng input samples (Table 11). The QDR was 100% with a QDR lower limit of the 95% confidence interval (LLCI) of 85.47%. For each tested guardbanding condition, all LLCI were higher than 80%, meeting the acceptance criteria.

NPA was analyzed by assessing for the variants targeted in each sample pool. None of the targeted variants were observed across samples, resulting in a 100% per-sample NPA across all conditions.

Table 11. Guardbanding Summary Results

Guardbanding Condition	Reference Condition	Condition 1	Condition 2
cfDNA Input Amount (5 ng) QDR [95% CI]	56/56 = 100% [93.62%, 100%]	N/A (by design, the QC metric failed at this level)	35/36 = 97.22% [85.47%, 99.93%]
cfDNA Input Amount (30 ng) QDR [95% CI]	50/50 = 100% [92.89%, 100%]	46/46 = 100% [92.29%, 100%]	50/50 = 100% [92.89%, 100%]
Adapter Volume QDR [95% CI]	56/56 = 100% [93.62%, 100%]	60/60 = 100% [94.04%, 100%]	50/50 = 100% [92.89%, 100%]

Hybridization Time QDR [95% CI]	56/56 = 100% [93.62%, 100%]	60/60 = 100% [94.04%, 100%]	N/A
Wash Buffer Temperature QDR [95% CI]	56/56 = 100% [93.62%, 100%]	60/60 = 100% [94.04%, 100%]	60/60 = 100% [94.04%, 100%]

N/A: Not Applicable (see Table 10); QDR: qualitative detection rate.

These results demonstrate the robustness of Guardant 360 CDx to variation in cfDNA input (4 ng to 45 ng), enrichment wash buffer temperature, enrichment hybridization time, and library adapter volume.

7. Reagent Lot Interchangeability

Please see the Summary of Safety and Effectiveness Data P200010 (Section IX.A.7) for Guardant360 CDx platform validation of reagent lot interchangeability.

8. Stability

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

9. General Lab Equipment and Reagent Evaluation

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

B. Animal Studies

No animal studies were conducted using Guardant360 CDx.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

In the Amgen 20170543 clinical trial, patients were enrolled based on the presence of the *KRAS* G12C mutation in tissue specimens confirmed using the QIAGEN *therascreen* KRAS RGQ PCR test. Pre-treatment plasma samples from all patients enrolled in the clinical study registration population were collected. A clinical bridging study using pre-treatment plasma samples and clinical outcome data from patients enrolled in the Amgen 20170543 clinical study was conducted to demonstrate the safety and effectiveness of Guardant360 CDx to aid in the identification of NSCLC patients who may be eligible for treatment with LUMAKRAS™ (sotorasib, Amgen) therapy based on the detection of *KRAS* G12C mutation.

A. Guardant360 CDx Clinical Bridging Study for *KRAS* G12C

Amgen 20170543 Clinical Study Design

The Amgen 20170543 clinical study was a phase 1/2 multicenter, non-randomized, open-label study of orally administered LUMAKRAS™ (sotorasib) in subjects with NSCLC. The primary sotorasib registration population comprises *KRAS* G12C mutation-positive subjects from the Amgen 20170543 study whose disease progressed after prior therapy (immunotherapy / chemotherapy) and who were treated with at least one dose of the recommended phase 2 dose (RP2D) of sotorasib. Patients were enrolled based on the presence of *KRAS* G12C mutations in their tumors as confirmed by central tissue testing. This clinical study was used to support the approval of LUMAKRAS™ (sotorasib) under NDA 214665.

Guardant360 CDx Bridging Study Design for *KRAS* G12C Mutations

Pre-treatment plasma samples from 112 Amgen 20170543 clinical study patients (88.9% of 126 the primary registration population) were tested with Guardant360 CDx. The Amgen 20170543 clinical study did not include patients negative for *KRAS* G12C mutation and therefore did not represent the Guardant360 CDx-positive, tissue-negative portion of the Guardant360 CDx-positive intended use population. As such, supplemental matched tissue and plasma samples were obtained from subjects in other Amgen clinical studies and commercial vendors using subject selection criteria similar to those of the Amgen 20170543 clinical study and used to estimate the prevalence of patients positive for *KRAS* G12C mutations by Guardant360 CDx but negative by tissue testing to evaluate the potential impact of this population on clinical efficacy.

1. Clinical Bridging Study Inclusion and Exclusion Criteria

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

- Inclusion criteria for plasma samples from the Amgen 20170543 clinical study efficacy cohort
    - Subject included in the primary sotorasib registration population with informed consent for blood sample use for diagnostic development.
    - Adequate pretreatment sample available for Guardant360 CDx testing as defined in the device Instructions for Use (IFU).
- Inclusion criteria for samples for the diagnostic study sensitivity analysis prevalence sub-study
    - Subject provided informed consent for blood and tissue sample use for development purposes.

- Pathologically documented locally advanced or metastatic NSCLC.
- Subjects must have active disease progression and must not be receiving therapy at the time of blood collection.
- Subjects must provide an archived tumor tissue sample (unstained slides and/or an FFPE tissue block collected within 5 years of the matched plasma sample) with sufficient tumor content and quantity for testing as defined by the central testing laboratory requirements.
- Subject must provide a whole blood or plasma specimen that meets the requirements for Guardant360 CDx testing.

2. Follow-up Schedule

The Guardant360 CDx *KRAS* G12C mutation 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 LUMAKRAS™ (sotorasib) efficacy in the Amgen 20170543 clinical study primary objective was objective response rate (ORR) by response evaluation criteria in solid tumors (RECIST) 1.1 as assessed by independent radiologic review (IRR). The Guardant360 CDx bridging study for NSCLC patients with a *KRAS* G12C mutation uses the same clinical endpoint for its primary objective.

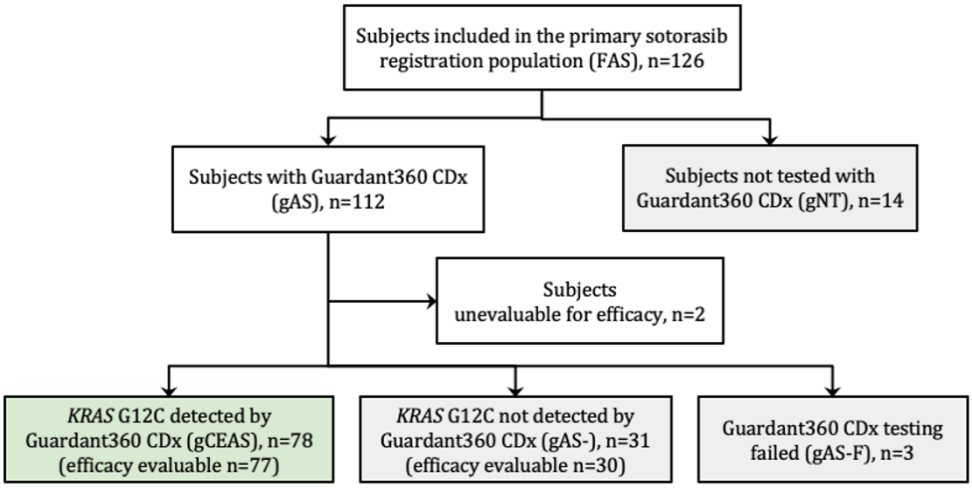
4. Diagnostic Objective and Endpoints

The primary objective of the clinical bridging study was to demonstrate the safety and effectiveness of Guardant360 CDx for the selection of metastatic NSCLC patients with *KRAS* G12C mutations for treatment with LUMAKRAS™ (sotorasib). The primary endpoint is ORR by RECIST 1.1 as assessed by IRR.

B. Accountability of the PMA Cohort for the Guardant360 CDx Clinical Bridging Study for *KRAS* G12C Mutations

The Guardant360 CDx clinical bridging study included 112 (89%) of the total 126 patients in the Amgen 20170543 registration population (Figure 1). Of these, 78 (70%) tested positive by Guardant360 CDx and were included in the primary objective analysis set, while 31 (28%) tested negative, and 3 (3%) failed testing. Two (2) of the 126 subjects in the initial primary sotorasib registration population were later found to be unevaluable for response due to the absence of radiographically measurable lesions at baseline. Thus, a total of 124 patients were the final full analysis set (FAS).

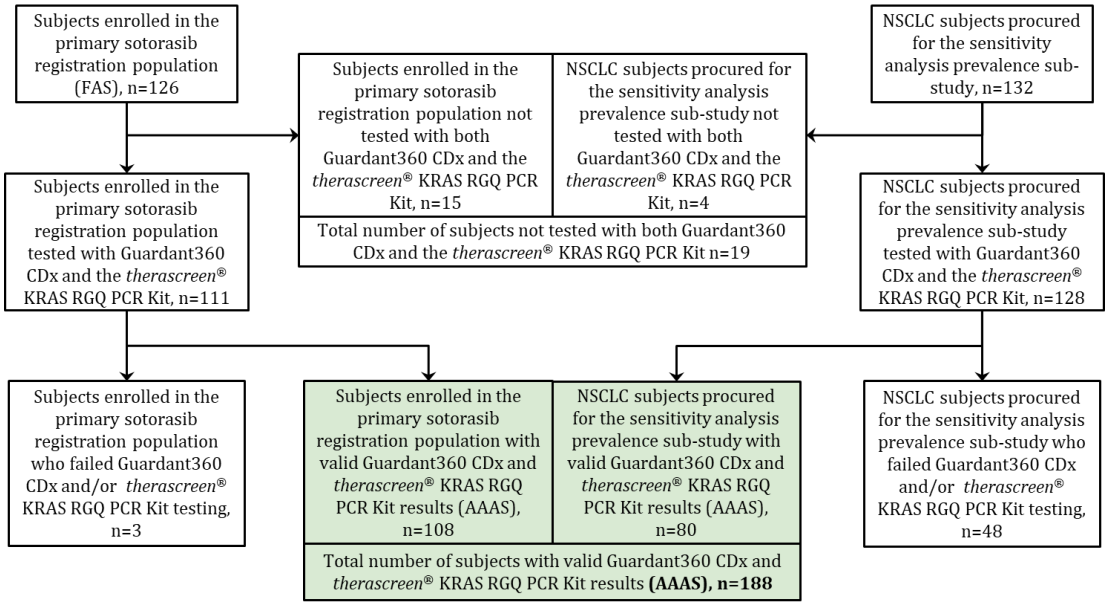
Figure 1. Guardant360 CDx *KRAS* G12C Mutation Bridging Study Efficacy Analysis Patient Accountability and Analysis Set Definitions



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

The Guardant360 CDx assay agreement analysis included 188 patients with Guardant360 CDx and *therascreen* KRAS RGQ PCR Kit using tissue test results from both the Amgen 20170543 clinical study (FAS) and the sensitivity analysis prevalence sub-study group (Figure 2).

Figure 2. Guardant360 CDx *KRAS* G12C Assay Agreement Analysis Patient Accountability and Analysis Set Definitions



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

Concordance Between Guardant360 CDx and the *therascreen* KRAS RGQ PCR Kit using Tissue

Concordance between Guardant360 CDx and the *therascreen* KRAS RGQ PCR Kit using tissue for all matched plasma and tissue samples from the Amgen 20170543 clinical study and the sensitivity analysis prevalence sub-study group is shown in Table 12 below. While all samples sourced from the primary sotorasib registration population were positive by the *therascreen* KRAS RGQ PCR Kit as a condition of their enrollment in the clinical study, the prevalence study subjects were recruited without regard for biomarker status and thus comprised both *KRAS* G12C-positive and -negative subjects at a natural prevalence (Figure 2).

For the concordance analysis (Table 12), when assessing the positive percent agreement (PPA), 108 tissue-positive samples were evaluated from the primary sotorasib registration population. In addition, one sample that was not evaluable for efficacy (Figure 1) was still considered as part of the concordance analysis which results in a total of 109 samples for PPA calculation. Of the 109 tissue-positive patients in the primary sotorasib registration population, 78 samples were positive and 31 were negative by Guardant360 CDx (Figure 1 and Table 12).

Of the 80 samples from the sensitivity analysis prevalence sub-study, i.e., samples without regard for biomarker status and comprising both *KRAS* G12C-positive and -negative subjects at a natural prevalence, 72 were negative by both Guardant360 CDx and the *therascreen KRAS* RGQ PCR test using tissue. The remaining 8 were positive by the *therascreen KRAS* RGQ PCR test, of which 4 were positive by the Guardant360 CDx, and 4 were negative by the Guardant360 CDx. Samples with negative results from *therascreen KRAS* RGQ PCR test were used for negative percent agreement (NPA) calculation (Table 12).

Table 12. Concordance Between Guardant360 CDX and *therascreen* KRAS RGQ PCR Kit using Tissue

	***therascreen* KRAS RGQ PCR Kit Positive (CTA)**	***therascreen* KRAS RGQ PCR Kit Negative**	**Total**
Guardant360 CDx Positive (n) (%)	78 (71.6)	0 (0.0)	78 (43.1)
Guardant360 CDx Negative (n) (%)	31 (28.4)	72 (100.0)	103 (56.9)
Total	109	72	181
Positive Percent Agreement (95% CI)	71.6% (62.1% – 79.8%)		
Negative Percent Agreement (95% CI)	100% (95.0% – 100%)		

C. Study Population Demographics and Baseline Parameters for the Guardant360 CDx Clinical Bridging Study for *KRAS* G12C Mutations

Demographics and baseline clinical characteristics of patients enrolled in the Amgen 20170543 clinical study were categorized relative to the diagnostic study populations as defined by Guardant360 CDx results and assessed for treatment arm balance.

As shown in Table 13 and Table 14, the clinical bridging study efficacy population (gCEAS) demographics and baseline clinical characteristics closely resemble those of the overall registration population (FAS). Demographic and baseline clinical characteristics of patients with plasma available for testing in this diagnostic study (gAS) and those without (gAS-Unk which is a combination of samples not tested and those for whom Guardant360 CDx testing failed) were also comparable to FAS and gCEAS.

Table 13. Baseline Demographics of the FAS and Sub-groups

	FAS	gCEAS	gAS	gAS-UNK
Sex n (%)				
Male	63 (50.0)	36 (46.2)	58 (51.8)	7 (41.2)
Female	63 (50.0)	42 (53.8)	54 (48.2)	10 (58.8)
Ethnicity - n (%)				
Hispanic or Latino	2 (1.6)	1 (1.3)	1 (0.9)	1 (5.9)
Not Hispanic or Latino	116 (92.1)	73 (93.6)	104 (92.9)	14 (82.4)
Missing	8 (6.3)	4 (5.1)	7 (6.3)	2 (11.8)
Race - n (%)				
American Indian or Alaska Native	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Asian	19 (15.1)	11 (14.1)	19 (17.0)	0 (0.0)
Black or African American	2 (1.6)	1 (1.3)	1 (0.9)	1 (5.9)
Native Hawaiian or Other Pacific Islander	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
White	103 (81.7)	65 (83.3)	90 (80.4)	16 (94.1)
Multiple	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other	2 (1.6)	1 (1.3)	2 (1.8)	0 (0.0)
Age (years)				
n	126	78	112	17
Mean	62.9	62.7	62.6	65.3
SD	9.3	9.7	9.4	7.9
Median	63.5	63.0	63.0	65.0
Q1, Q3	56.0, 70.0	56.0, 72.0	56.0, 70.0	61.0, 70.0
Min, Max	37, 80	37, 78	37, 80	46, 79
Age Group (years)				
18 - 64 years	67 (53.2)	43 (55.1)	61 (54.5)	7 (41.2)

	FAS	gCEAS	gAS	gAS-UNK
65 - 74 years	49 (38.9)	29 (37.2)	44 (39.3)	7 (41.2)
75 - 84 years	10 (7.9)	6 (7.7)	7 (6.3)	3 (17.6)
≥ 85 years	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 14. Baseline Clinical Characteristics of the FAS and Sub-groups

	FAS	gCEAS	gAS	gAS-UNK
ECOG status at baseline - n (%)				
0	38 (30.2)	20 (25.6)	35 (31.3)	5 (29.4)
1	88 (69.8)	58 (74.4)	77 (68.8)	12 (70.6)
2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Weight (kg)				
n	126	78	112	17
Mean	71.08	71.18	71.35	67.92
SD	17.14	17.38	17.06	18.30
Median	70.65	70.15	71.00	70.00
Q1, Q3	58, 83	58, 83	58, 83	57, 82
Min, Max	37, 123	37, 123	37, 123	40, 108
Height (cm)				
n	123	77	110	16
Mean	168	168	168	168
SD	9.2	8.9	8.9	11.6
Median	169	168	169	168
Q1, Q3	161, 175	161, 175	161, 175	156, 175
Min, Max	146, 188	151, 188	151, 188	146, 183
Prior line of anti-cancer therapy - n (%)				
0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1	54 (42.9)	33 (42.3)	48 (42.9)	8 (47.1)
2	44 (34.9)	28 (35.9)	38 (33.9)	7 (41.2)
3	28 (22.2)	17 (21.8)	26 (23.2)	2 (11.8)
≥ 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Median (number of prior lines)	2	2	2	2
Type of prior anti-cancer therapy - n (%)				
Chemotherapy	115 (91.3)	73 (93.6)	104 (92.9)	14 (82.4)
Platinum-base chemotherapy	113 (89.7)	72 (92.3)	102 (91.1)	14 (82.4)
Immunotherapy	116 (92.1)	72 (92.3)	102 (91.1)	16 (94.1)
Checkpoint inhibitor	116 (92.1)	72 (92.3)	102 (91.1)	16 (94.1)
Anti PD-1 or anti PD-L1	115 (91.3)	72 (92.3)	101 (90.2)	16 (94.1)
Platinum-base chemotherapy and anti PD-1 or anti PD-L1[c]	102 (81.0)	66 (84.6)	91 (81.3)	13 (76.5)
Hormonal therapy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

	FAS	gCEAS	gAS	gAS-UNK
Targeted biologics	30 (23.8)	17 (21.8)	28 (25.0)	2 (11.8)
Anti-VEGF biological therapy	25 (19.8)	15 (19.2)	24 (21.4)	1 (5.9)
Targeted small molecules	9 (7.1)	3 (3.8)	6 (5.4)	3 (17.6)
Other	1 (0.8)	1 (1.3)	1 (0.9)	0 (0.0)
Disease stage at initial diagnosis - n (%)				
Stage I	11 (8.7)	6 (7.7)	10 (8.9)	1 (5.9)
Stage II	14 (11.1)	6 (7.7)	12 (10.7)	2 (11.8)
Stage III	22 (17.5)	19 (24.4)	21 (18.8)	1 (5.9)
Stage IV	78 (61.9)	46 (59.0)	68 (60.7)	13 (76.5)
Missing	1 (0.8)	1 (1.3)	1 (0.9)	0 (0.0)
Disease stage at screening - n (%)				
Stage I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Stage II	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Stage III	5 (4.0)	4 (5.1)	5 (4.5)	0 (0.0)
Stage IV	121 (96.0)	74 (94.9)	107 (95.5)	17 (100.0)
Differentiation - n (%)				
Well differentiated	6 (4.8)	4 (5.1)	4 (3.6)	2 (11.8)
Moderately differentiated	15 (11.9)	6 (7.7)	12 (10.7)	4 (23.5)
Poorly differentiated	24 (19.0)	16 (20.5)	19 (17.0)	5 (29.4)
Undifferentiated	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	81 (64.3)	52 (66.7)	77 (68.8)	6 (35.3)
PD-L1 protein expression - n (%)				
< 1%	33 (26.2)	18 (23.1)	30 (26.8)	3 (17.6)
≥ 1% and < 50%	24 (19.0)	16 (20.5)	22 (19.6)	3 (17.6)
≥ 50%	35 (27.8)	24 (30.8)	31 (27.7)	5 (29.4)
Unknown	34 (27.0)	20 (25.6)	29 (25.9)	6 (35.3)
Histopathology type - n (%)				
Squamous	1 (0.8)	1 (1.3)	1 (0.9)	0 (0.0)
Adenosquamous carcinoma	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Squamous cell carcinoma	1 (0.8)	1 (1.3)	1 (0.9)	0 (0.0)
Non-squamous	125 (99.2)	77 (98.7)	111 (99.1)	17 (100.0)
Adenocarcinoma	120 (95.2)	75 (96.2)	106 (94.6)	16 (94.1)
Mucinous	8 (6.3)	5 (6.4)	8 (7.1)	0 (0.0)
Large cell carcinoma	3 (2.4)	2 (2.6)	3 (2.7)	1 (5.9)
Bronchoalveolar carcinoma	2 (1.6)	0 (0.0)	2 (1.8)	0 (0.0)
Sarcomatoid	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Undifferentiated	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

	FAS	gCEAS	gAS	gAS-UNK
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Metastatic - n (%)				
Yes	122 (96.8)	74 (94.9)	108 (96.4)	17 (100.0)
No	4 (3.2)	4 (5.1)	4 (3.6)	0 (0.0)
Number of body sites of metastatic disease - n (%)				
0	4 (3.2)	4 (5.1)	4 (3.6)	0 (0.0)
1	51 (40.5)	26 (33.3)	46 (41.1)	7 (41.2)
2	30 (23.8)	20 (25.6)	28 (25.0)	2 (11.8)
3	24 (19.0)	17 (21.8)	21 (18.8)	3 (17.6)
> 3	17 (13.5)	11 (14.1)	13 (11.6)	5 (29.4)
Liver metastasis (n%)				
Yes	26 (20.6)	17 (21.8)	21 (18.8)	7 (41.2)
No	100 (79.4)	61 (78.2)	91 (81.3)	10 (58.8)
Brain metastasis (n%)				
Yes	26 (20.6)	17 (21.8)	22 (19.6)	5 (29.4)
No	100 (79.4)	61 (78.2)	90 (80.4)	12 (70.6)
Bone metastasis (n%)				
Yes	61 (48.4)	41 (52.6)	52 (46.4)	10 (58.8)
No	65 (51.6)	37 (47.4)	60 (53.6)	7 (41.2)
Smoking history - n (%)				
Never	6 (4.8)	4 (5.1)	6 (5.4)	0 (0.0)
Current	15 (11.9)	7 (9.0)	14 (12.5)	3 (17.6)
Former	102 (81.0)	66 (84.6)	89 (79.5)	14 (82.4)
Missing	3 (2.4)	1 (1.3)	3 (2.7)	0 (0.0)
Region n (%)				
North America	79 (62.7)	50 (64.1)	68 (60.7)	12 (70.6)
Europe	30 (23.8)	18 (23.1)	27 (24.1)	5 (29.4)
Asia	12 (9.5)	7 (9.0)	12 (10.7)	0 (0.0)
Rest of the world	5 (4.0)	3 (3.8)	5 (4.5)	0 (0.0)
Best response to last prior line of therapy - n (%)				
Complete response	1 (0.8)	1 (1.3)	1 (0.9)	0 (0.0)
Partial response	12 (9.5)	9 (11.5)	12 (10.7)	1 (5.9)
Stable disease	33 (26.2)	19 (24.4)	28 (25.0)	5 (29.4)
Progressive disease	48 (38.1)	33 (42.3)	44 (39.3)	5 (29.4)
Unevaluable	1 (0.8)	0 (0.0)	0 (0.0)	1 (5.9)
Unknown / not applicable / not done	27 (21.4)	15 (19.2)	23 (20.5)	5 (29.4)
Missing	4 (3.2)	1 (1.3)	4 (3.6)	0 (0.0)

To assess potential bias arising from plasma sample availability, baseline demographic information and baseline clinical disease characteristics of subjects with a valid Guardant360 CDx result (gAS-E) and those without (gAS-Unk) were compared and the associated p values reported in Table 15 and Table 16. No meaningful differences were observed.

Table 15. Comparison of Baseline Demographics Between gAS-E and gAS-Unk

	gAS-E	gAS-Unk	p-value
Sex - n (%)			
Male	56 (51.4)	7 (41.2)	0.4340
Female	53 (48.6)	10 (58.8)	
Ethnicity - n (%)			
Hispanic or Latino	1 (0.9)	1 (5.9)	0.2390
Not Hispanic or Latino	102 (93.6)	14 (82.4)	
Race - n (%)			
American Indian or Alaska Native	0 (0.0)	0 (0.0)	0.0769
Asian	19 (17.4)	0 (0.0)	
Black or African American	1 (0.9)	1 (5.9)	
Native Hawaiian or Other Pacific Islander	0 (0.0)	0 (0.0)	
White	87 (79.8)	16 (94.1)	
Multiple	0 (0.0)	0 (0.0)	
Other	2 (1.8)	0 (0.0)	
Age group - n (%)			
18 - 64 years	60 (55.0)	7 (41.2)	0.2354
65 - 74 years	42 (38.5)	7 (41.2)	
75 - 84 years	7 (6.4)	3 (17.6)	
>= 85 years	0 (0.0)	0 (0.0)	

Table 16. Comparison of Baseline Clinical Characteristics Between gAS-E and gAS-Unk

	gAS-E	gAS-Unk	p-value
ECOG status at baseline - n (%)			
0	33 (30.3)	5 (29.4)	0.9425
1	76 (69.7)	12 (70.6)	
2	0 (0.0)	0 (0.0)	
Weight (kg)			
Mean	71.57	67.92	0.4158

	gAS-E	gAS-Unk	p-value
Height (cm)			
Mean	168.00	166.73	0.6089
Prior line of anti-cancer therapy - n (%)			
0	0 (0.0)	0 (0.0)	0.5304
1	46 (42.2)	8 (47.1)	
2	37 (33.9)	7 (41.2)	
3	26 (23.9)	2 (11.8)	
>= 4	0 (0.0)	0 (0.0)	
Type of prior anti-cancer therapy - n (%)			
Chemotherapy	101 (92.7)	14 (82.4)	0.1690
Immunotherapy	100 (91.7)	16 (94.1)	1.0000
Platinum-base chemotherapy and anti PD-1 or anti PD-L1	89 (81.7)	13 (76.5)	0.7395
Hormonal therapy	0 (0.0)	0 (0.0)	NA
Targeted biologics	28 (25.7)	2 (11.8)	0.3575
Targeted small molecules	6 (5.5)	3 (17.6)	0.1028
Other	1 (0.9)	0 (0.0)	1.0000
Disease stage at initial diagnosis - n (%)			
Stage I	10 (9.2)	1 (5.9)	0.6104
Stage II	12 (11.0)	2 (11.8)	
Stage III	21 (19.3)	1 (5.9)	
Stage IV	65 (59.6)	13 (76.5)	
Disease stage at screening - n (%)			
Stage I	0 (0.0)	0 (0.0)	1.0000
Stage II	0 (0.0)	0 (0.0)	
Stage III	5 (4.6)	0 (0.0)	
Stage IV	104 (95.4)	17 (100.0)	
Differentiation - n (%)			
Well differentiated	4 (3.7)	2 (11.8)	0.0235
Moderately differentiated	11 (10.1)	4 (23.5)	
Poorly differentiated	19 (17.4)	5 (29.4)	

	gAS-E	gAS-Unk	p-value
Undifferentiated	0 (0.0)	0 (0.0)	
Other	0 (0.0)	0 (0.0)	
Unknown	75 (68.8)	6 (35.3)	
PD-L1 protein expression - n (%)			
< 1%	30 (27.5)	3 (17.6)	0.7960
>= 1% and < 50%	21 (19.3)	3 (17.6)	
>= 50%	30 (27.5)	5 (29.4)	
Unknown	28 (25.7)	6 (35.3)	
Histopathology type - n (%)			
Squamous	1 (0.9)	0 (0.0)	1.0000
Non-squamous	108 (99.1)	17 (100.0)	
Other	0 (0.0)	0 (0.0)	
Metastatic - n (%)			
Yes	105 (96.3)	17 (100.0)	1.0000
No	4 (3.7)	0 (0.0)	
Number of body sites of metastatic disease - n (%)			
0	4 (3.7)	0 (0.0)	0.3002
1	44 (40.4)	7 (41.2)	
2	28 (25.7)	2 (11.8)	
3	21 (19.3)	3 (17.6)	
> 3	12 (11.0)	5 (29.4)	
Liver metastasis - n (%)			
Yes	19 (17.4)	7 (41.2)	0.0469
No	90 (82.6)	10 (58.8)	
Brain metastasis - n (%)			
Yes	21 (19.3)	5 (29.4)	0.3429
No	88 (80.7)	12 (70.6)	
Bone metastasis - n (%)			
Yes	51 (46.8)	10 (58.8)	0.3558
No	58 (53.2)	7 (41.2)	

	gAS-E	gAS-Unk	p-value
Smoking history - n (%)			
Never	6 (5.5)	0 (0.0)	0.5504
Current	12 (11.0)	3 (17.6)	
Former	88 (80.7)	14 (82.4)	
Region - n (%)			
North America	67 (61.5)	12 (70.6)	0.5224
Europe	25 (22.9)	5 (29.4)	
Asia	12 (11.0)	0 (0.0)	
Rest of the world	5 (4.6)	0 (0.0)	
Best response to last prior line of therapy - n (%)			
Complete response	1 (0.9)	0 (0.0)	0.3204
Partial response	11 (10.1)	1 (5.9)	
Stable disease	28 (25.7)	5 (29.4)	
Progressive disease	43 (39.4)	5 (29.4)	
Unevaluable	0 (0.0)	1 (5.9)	
Unknown / not applicable / not done	22 (20.2)	5 (29.4)	

NA: Not Available; ECOG: Eastern Cooperative Oncology Group.

D. Safety and Effectiveness Results for the Guardant360 CDx Clinical Bridging Study for *KRAS* G12C Mutations

1. Safety Results

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

2. Effectiveness Results

a. ORR in Patients Positive by Guardant360 CDx for *KRAS* G12C Mutations

The efficacy of single-agent LUMAKRAS ™ (sotorasib) in both the primary sotorasib registration population (FAS) and in those subjects positive for *KRAS* G12C by Guardant360 CDx is shown in Table 17. The observed ORR based on IRR assessment for the 77 evaluable subjects (38%, 95% CI 27% –

49%) is similar to the ORR that for the entire primary sotorasib registration population (FAS, 36%, 95% CI 28% – 45%).

Table 17. ORR in the gCEAS and FAS Populations Assessed by Independent Radiological Review

Efficacy Parameter	gCEAS (n = 77)	FAS (n = 124)
Objective Response Rate, N (%) (95%CI)	29 (38) (27, 49)	45 (36) (28, 45)
Complete Response, N (%)	0 (0)	2 (2)
Partial Response, N (%)	29 (38)	43 (35)
Duration of Response		
Median [a], months (range)	7.1 (1.3, 8.4)	10.0 (1.3, 11.1)
Patient with DOR ≥ 6 months, %	42%	58%

[a]Estimated by the Kaplan-Meier method

b. Sensitivity Analysis

Sensitivity analyses were conducted to model the impact of the hypothetical Guardant360 CDx$^+$ tissue$^-$ population and patients without Guardant360 CDx results.

Sensitivity analysis for the unrepresented Guardant360 CDx$^+$ Tissue$^-$ subject population

The primary objective analysis above demonstrated sotorasib efficacy in the Guardant360 CDx$^+$ tissue$^+$ subset of the Guardant360 CDx intended use population. As subjects in the Amgen 20170543 clinical study were enrolled based on positive tissue testing for *KRAS* G12C, sensitivity analysis was assessed using matched tissue and plasma samples (procured from vendors and/or other clinical trial sources according to the selection criteria similar to the Amgen 20170543 clinical study). Sensitivity analysis modeling efficacy in the entire Guardant360 CDx$^+$ intended use population demonstrates robustness to the contribution of the unrepresented Guardant360 CDx$^+$ tissue$^-$ subjects, with estimated ORRs similar to the observed (Table 18 vs. Table 17, respectively) due to the high NPA of Guardant360 CDx relative to the *therascreen* KRAS RGQ PCR Kit using tissue. The lower limit of the 95% CI for the estimated ORRs across the modeled conditions (27.3%, Table 18) was greater than the size-adjusted benchmark ORR of 22%, which demonstrates statistically-significant sotorasib efficacy across the entire Guardant360 CDx intended use population, irrespective of sotorasib efficacy in the modeled hypothetical Guardant360 CDx$^+$ tissue$^-$ sub-population.

Table 18. Sensitivity Analysis for the Guardant360 CDx$^+$ Tissue$^-$ Population

	G360 CDx$^+$ Intended Use Population
Weighted ORR with postulated ORR equal to observed ORR	
Average weighted ORR - %	37.5
95% CI	(27.3, 48.1)
Weighted ORR with postulated ORR equal to 0	
Average weighted ORR - %	37.5
95% CI	(27.3, 48.1)

Sensitivity Analysis for FAS Subjects Without Valid Guardant360 CDx Results

The majority of the subjects in the primary sotorasib registration population 112/126 (88.9%) met the clinical bridging study inclusion criteria (gAS) and 109/126 (86.5%) subjects generated a valid Guardant360 CDx result (gCEAS or gAS–). To model the potential impact of the 17 subjects without Guardant360 CDx results, sensitivity analysis was performed based on 1000 simulations imputing Guardant360 CDx results for subjects without a valid Guardant360 CDx result in the bridging study using the P(Guardant360 CDx$^+$|Tissue$^+$) observed in the Guardant360 CDx evaluable analysis set. Table 19 shows that the modeled average ORR (36%, 95% CI 34% – 38%) with imputation for the missing population (gAS-Unk) is similar to the observed ORR in the gCEAS (38%, 95% CI 27% – 49%), demonstrating that the ORR observed in the clinical bridging study is robust to the potential impact of missing subjects.

Table 19. Sensitivity Analysis with Imputation for Subjects Without Valid Guardant360 CDx Results

	Simulated gCEAS
Objective response rate (ORR)	
Average number of overall responders – n (%)	32 (36)
95% CI	(34, 38)

E. Pediatric Extrapolation

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

F. Financial Disclosure

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

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

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

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

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

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

For the intended use of identifying NSCLC patients with *KRAS* G12C mutations for treatment with LUMAKRAS™ (sotorasib), the effectiveness of Guardant360 CDx was demonstrated through analytical studies using patient samples from the intended use population and a clinical bridging study using plasma from the Amgen 20170543 clinical study. The data from the analytical validation and clinical bridging studies support the reasonable assurance of safety and effectiveness of Guardant360 CDx when used in accordance with the indications for use. Data from the Amgen 20170543 clinical study show that patients with

KRAS G12C mutations received benefit from treatment with LUMAKRAS™ (sotorasib) and support the addition of the CDx indication to Guardant360 CDx.

B. Safety Conclusions

The risks of the device are based on data collected in the analytical studies conducted to support PMA approval as described above. Guardant360 CDx is an *in vitro* diagnostic test, which involves testing of cfDNA extracted from the plasma of whole blood routinely collected as part of the diagnosis and patient care.

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

C. Benefit-Risk Determination

The probable clinical benefit of Guardant360 CDx for the selection of NSCLC patients with *KRAS* G12C mutations for treatment with LUMAKRAS™ (sotorasib) was demonstrated in a retrospective analysis of efficacy and safety data obtained from the Phase 1/2, non-randomized, open-label Amgen 20170543 clinical study. The supporting clinical validation analyses demonstrate that the observed ORR based on IRR assessment for the 77 evaluable subjects that were positive by Guardant360 CDx for *KRAS* G12C was 38% (95% CI 27% – 49%), which was similar to the ORR observed in the primary sotorasib registration population (FAS, 36%, 95% CI 28% – 45%). This Guardant360 CDx selected patient population also exhibited an ORR that was greater than the size-adjusted benchmark ORR of 22% (prespecified acceptance criterion). Thus, this device identifies NSCLC patients with the *KRAS* G12C mutation, that have a meaningful clinical response to LUMAKRAS™ (sotorasib).

There is potential risk associated with the use of this device, mainly due to 1) false positives, false negatives, or failure to provide a result and 2) incorrect interpretation of test results by the user. The key risks of Guardant360 CDx are associated with the potential mismanagement of patients resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial which may lead to adverse events or may have delayed access to treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial drug. However, this risk is partially mitigated by reflex testing recommendation for negative results with an FDA-approved tissue test for the *KRAS* G12C mutation.

These risks of this assay are partially mitigated by the clinical and analytical studies for Guardant360 CDx detection of *KRAS* G12C mutations. An accuracy study of Guardant360 CDx for the detection of *KRAS* G12C mutations with an externally validated comparator method demonstrating a PPA of 96.2% and NPA of 98% and a LoB study demonstrating a false-positive rate of 0% also indicates a low likelihood of patient misassignment. Together, these results support the use of Guardant360 CDx as an aid in selecting NSCLC patients with *KRAS* G12C mutations for LUMAKRAS™ (sotorasib) treatment.

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

1. Patient Perspectives

   This submission either did not include specific information on patient perspectives or the information did not serve as part of the basis of the decision to approve or deny the PMA for this device.

In conclusion, the available data support the probable benefits of Guardant360 CDx use within the indications above outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the analytical and clinical studies support the use of Guardant360 CDx as an aid for the identification of *KRAS* G12C mutation-positive NSCLC patients for whom LUMAKRAS™ (sotorasib) may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order for the PMA (P200010/S002) on May 28, 2021. Additional study is requested as conditions of approval cited in the approval order are described below.

Guardant Health, Inc. must provide results from regression tests and associated software documentation for the report module software to confirm that the upgrade associated with *KRAS* G12C mutation reporting has no impact on the reporting of the other variants (CDx and tumor profiling) specified in the device intended use. Data from the study must be adequate to confirm the safety and effectiveness of the Guardant360 CDx device.

Guardant Health, Inc. must provide detailed protocols for the study and must include a detailed description of the data and sample sets including sample

size and tumor type to be tested, the complete testing protocol, acceptance criteria, and a data analysis plan, as applicable. These protocols must be submitted to FDA no later than 60 days after approval. 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.