GUARDANT360 CDx

Technical Information

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1 Intended 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 neoplasm. 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.

2 Contraindications

There are no known contraindications.

3 Warnings and Precautions

a. 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.
b. The test is not intended to replace germline testing or to provide information about cancer predisposition.
c. Somatic alterations in *ATM* and *CDK12* are not reported by the test as they are excluded from the test's reportable range.
d. 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).
e. 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.

4 Limitations

a. For *in vitro* diagnostic use.
b. For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
c. 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.

d. TAGRISSO efficacy has not been established in patients with *EGFR* exon 19 deletions < 0.08% MAF, in patients with *EGFR* L858R <0.09% MAF, and in patients with *EGFR* T790M < 0.03% MAF.
e. RYBREVANT efficacy has not been established in patients with *EGFR* exon 20 insertions < 0.02% MAF
f. LUMAKRAS efficacy has not been established in patients with *KRAS* G12C biomarkers < 0.11% MAF.
g. The test is not intended to be used for standalone diagnostic purposes.
h. The test is intended to be performed on specific serial number-controlled instruments by Guardant Health, Inc.
i. A negative result for any given variant does not preclude the presence of this variant in tumor tissue.
j. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care.
k. ctDNA shedding rate may be lower in patients with primary central nervous system (CNS) tumors.

5 Guardant360 CDx Overview

5.1 Test Summary and Explanation

Guardant360 CDx is a next generation sequencing-based test for the detection of genetic alterations in 55 genes frequently mutated in cancer. It is 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** of the Intended Use. 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 neoplasm.

The test report includes variants reported in the following categories (**Table 2**).

Table 2. 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.

5.2 Sample Collection and Test Ordering

To order Guardant360 CDx, the Test Requisition Form (TRF) provided with the Guardant360 CDx Blood Collection Kit must be fully completed and signed by the ordering physician or other authorized medical professional. Refer to the Guardant360 CDx Blood Collection Kit Instructions for Use for further details about collecting blood samples and shipping samples to the Guardant Health Clinical Laboratory.

To order the Guardant360 CDx Blood Collection Kit or obtain an electronic version of the TRF, contact the Guardant Health Client Services department (Tel: 855.698.8887, Fax: 888.974.4258, or Email: clientservices@guardanthealth.com).

5.3 Principles of the Procedure

Guardant360 CDx is performed by a single laboratory, the Guardant Health Clinical Laboratory, located in Redwood City, CA, USA. Guardant360 CDx is composed of the following major processes:

a. Whole Blood Collection and Shipping
b. Plasma Isolation and cfDNA Extraction
c. Library Preparation and Enrichment
d. DNA Sequencing
e. Data Analysis and Reporting

The Guardant360 CDx Blood Collection Kit is used by the ordering laboratories / physicians to collect whole blood specimens and ship them to the Guardant Health Clinical Laboratory. Whole blood is collected in the provided blood collection tubes, Streck Cell-Free DNA BCTs, which stabilize cfDNA and nucleated blood cells for shipping.

All other reagents, materials and equipment needed to perform the assay are used exclusively in the Guardant Health Clinical Laboratory.

Whole blood specimens are processed in the Guardant Health Clinical Laboratory within 7 days of blood collection. 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 is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture. The enriched libraries are then sequenced using next generation sequencing on the Illumina NextSeq 550 platform.

Sequencing data are then analyzed using a custom-developed bioinformatics pipeline designed to detect SNVs, indels, CNAs and fusions from cfDNA. Results (detected or not

detected) are presented in a results report. A not detected result from a plasma specimen for any given variant does not preclude the presence of this variant in tumor tissue.

The device is designed to detect pre-defined and *de novo* variants in the genes outlined in **Table 3**. Details on all variants reported can be found in the **Section 8** *Additional Guardant360 CDx Variant Details*.

Table 3. Genes Containing Alterations Reported by 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.

5.4 Reagent, Material, and Equipment Usage

Reagents, materials, and equipment needed to perform the test are used exclusively in the Guardant Health Clinical Laboratory. Guardant360 CDx is intended to be performed with the following instruments, to be identified by specific serial numbers, as needed.

a. Agilent Technologies 4200 TapeStation Instrument
b. Applied Biosystems Veriti 96-Well Thermal Cycler
c. Hamilton Company Microlab STAR
d. Hamilton Company Microlab STARlet
e. Illumina NextSeq 550 Sequencing System
f. Qiagen QIAsymphony SP Instrument

6 Summary of Performance Characteristics

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 include CDx variants as well as a broad range of representative alteration types (SNVs, indels, CNAs, and fusions) in various genomic contexts across a number of genes. 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 non-clinical studies. A contrived sample functional characterization 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 non-clinical studies. Fusion positive clinical samples were used to confirm the estimated limit of detection, analytical accuracy and precision.

6.1 Analytical Accuracy/Concordance

a. Concordance - Comparison to NGS Comparator Method #1

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 gene fusions which were not available from collection sets 1 and 2.

Of 359 patients, no samples failed QC on Guardant360 CDx, and three samples failed 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 4** for CDx alterations in samples from the intended use population, i.e., 176 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. 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 4**.

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 alterations 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.

The study demonstrated a PPA of 82.5% for indels, 91.4% for SNVs and >99% NPA for the entire reportable range, i.e., panel-wide, demonstrating the analytical accuracy of the device.

Table 4. Summary of Concordance Between Guardant360 CDx and NGS Comparator Method #1

Alteration Type	Guardant360 CDx(+), Comparator #1 (+)	Guardant360 CDx(+), Comparator #1 (-)	Guardant360 CDx(-), Comparator #1 (+)	Guardant360 CDx(-), Comparator #1 (-)	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%)
Category 2 Variants	9	0	0	76	5	17	100.0% (66.4%, 100.0%)	100.0% (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%)
Panel-Wide SNVs	428	48	40	13726844	38560	356	91.5% (88.5%, 93.8%)	99.9% (99.9%, 99.9%)
Panel-Wide Indels	118	19	25	15717238	44150	356	82.5% (75.3%, 88.4%)	99.9% (99.9%, 99.9%)

***** For Category 3, no number is given. 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.

b. Concordance – Comparison to NGS Comparator Method #2

The detection of *EGFR* exon 20 insertions by Guardant360 CDx was compared to results of another externally validated plasma-based NGS assay. NSCLC samples from 277 patients were collected for the study, including samples from all subjects tested in the associated clinical study with sufficient remnant material for testing with the comparator method. Four samples failed testing with the comparator assay due to sequencing failures, while one sample failed testing with Guardant360 CDx due to enrichment failure. PPA and NPA are reported in **Table 5 below**.

Of note, the comparator method used was less sensitive than Guardant360 CDx (LoD 0.5% vs. 0.3%), and 86% (24/28) of discordances observed were for variants with allelic fractions below the comparator LoD.

Table 5. Summary of Concordance Between Guardant360 CDx and NGS Comparator Method #2

Alteration Type	Guardant360 CDx(+), Comparator #2 (+)	Guardant360 CDx(+), Comparator #2 (-)	Guardant360 CDx(-), Comparator #2 (+)	Guardant360 CDx(-), Comparator #2 (-)	Patients (n)	PPA (95% CI)	NPA (95% CI)
EGFR exon 20 insertions	78	25	3	166	272	96.30% (89.56%, 99.23%)	86.91% (81.29%, 91.35%)

c. Concordance - Comparison to MassARRAY Comparator Method #3

An analytical accuracy study was performed with plasma clinical specimens (106 *KRAS* G12C mutation-positive patients and 107 *KRAS* G12C mutation-negative patients) from 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 was 96% PPA and 94% NPA. The discordance (10 samples) listed in **Table 6** occurs only in samples with circulating tumor amounts near or below the LoD, which results 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 MassARRAY Comparator Method #3

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 positive samples from the clincial 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% (93%, 100%)
GH-Biobank-Unselected (N=39)	3	0	0	36	100% (29%, 100%)	100% (90%, 100%)	100% (29%, 100%)	100% (90%, 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.

6.2 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 8**). 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 an orthogonal method to confirm MAF level. Detection rates of the 4 fusions, for each titration level, and for each of the two types of pools, are presented in **Table 8**.

Based on these analyses, the results 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 8. 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%)
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%, 100.0%)	100.0% (75.3%, 100.0%)

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%, 100.0%)	100.0% (76.8%, 100.0%)
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% (83.2%, 100.0%)	100.0% (75.3%, 100%)
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%, 100.0%)	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

6.3 Analytical Sensitivity

a. Limit of Blank (LoB)

The LoB was established by evaluating whole blood samples from healthy age-matched donor samples. 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 reportable range, as shown in **Table 9**. The false positive rate was zero for Category 1 (CDx) and Category 2 variants.

Table 9. 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 1: *EGFR* exon 20 insertions	0%	0 (0/240)
Category 1: *KRAS* G12C	0%	0 (0/240)
Category 2	0%	0 (0/240)
Panel-wide SNVs (38,560 bp)	<0.00005%	1.67% (4/240)
Panel-wide Indels (44,150 bp)	<0.00002%	0.83% (2/240)
Panel-wide CNAs (2 genes)	0.2%	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 5ng and 30ng 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 at least two reagent lots.

The LoDs of four (4) CDx alterations representing *EGFR* T790M, *EGFR* L858R, *EGFR* exon 19 deletions, and *EGFR* exon 20 insertions established using pools of cfDNA from clinical plasma samples from multiple cancer types are summarized in **Table 10**. The LoD was confirmed for these CDx variants using cfDNA sample pools from patients with NSCLC only; refer to **Table 12** below.

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 (**Table 11**). The established LoD was further confirmed in clincial samples to be 1.8% MAF at 5 ng DNA input and 0.5% MAF at 30 ng DNA input by testing 20 and 14 replicates, respectively, with 3 sets of reagent lots (**Table 10**). These confirmed LoD values were utilized in other performance studies (e.g., precision, guardbanding and interference). 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 6.5 below).

Table 10. Summary of LoDs for Alterations Associated with CDx Claims using Pools of cfDNA from Clinical Plasma Samples from Multiple Cancer Types

Alteration	Alteration Type	LoD (5 ng input)	LoD (30 ng input)
EGFR T790M	SNV	1.1% MAF	0.2% MAF
EGFR L858R	SNV	1.0% MAF	0.2% MAF
EGFR exon 19 deletion	Deletion (15 bp)	1.5% MAF	0.2% MAF
EGFR exon 20 insertions	Insertions (3 and 9 bp)	1.2% MAF* (0.8%-1.8%)	0.3% MAF
KRAS G12C	SNV	1.8% MAF	0.5% MAF

*Median MAF. MAF range shown in parenthesis.

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 11**.

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 11**).

Table 11. 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 E23fs	Deletion (2 bp)	2.6%	0.8%

BRCA2 S1982fs	Deletion (1 bp)	1.3%	0.4%
EGFR exon 20 insertion, A767_V769dup	Insertion (9 bp)	0.8%	0.2%
ERBB2 exon 20 insertion, A775_G776insYVMA	Insertion (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%)

Note: 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 1-1.5x LoD of the established LoD (refer to **Table 12**) 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, 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 6.5. 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 12.**

Table 12. 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, E746_A750del	1.5%*	Deletion (15bp)	NSCLC	20/20	100.0%
EGFR exon 19 deletion, A750_I759delinsPT	2.3%^	Deletion (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, A767_H769dup	1.4%	Insertion (9 bp)	NSCLC	41/42	97.6%
EGFR exon 20 insertion, H773dup	0.9%**	Insertion (3 bp)	NSCLC	41/42	97.6%
EGFR exon 20 insertion, N771_H773dup	1.8%**	Insertion (9 bp)	NSCLC	41/41	100%
EGFR exon 20 insertion, H773_V774insHPH	3.5%^	Insertion (9 bp)	NSCLC	22/22	100.0%
MET exon 14 skipping 7.116412041.AAGGTATATT TCAGTT>A	2.7%^	Deletion (15 bp)	NSCLC	20/20	100.0%
BRCA2 T3033fs	4.4%^	Indel (1 bp), homopolymer	NSCLC	21/21	100.0%
BRCA2 I605fs	5.0%^	Indel (1 bp), homopolymer	Prostate	20/20	100.0%
BRCA2 V1532fs	4.2%^	Indel (1 bp), homopolymer	Prostate	20/20	100.0%
STK11 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 LoD level in LoD Establishment Study. LoD was empirically established using NSCLC pools.
^ 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 are summarized in **Table 13** as median values.

Table 13. 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%

6.4 Analytical Specificity

a. Endogenous and Exogenous 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 from a total of 50 patients (at least ten patients per interfering substance), representing more than 13 cancer types. The 130 samples that passed QC checks included representative variants.

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 demonstrates 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 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.

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.

Additionally, to evaluate the potential impact of exogenous interfering substances on the performance of Guardant360 CDx, ten different representative variants were tested using clinical or cell line-derived cfDNA samples spiked with wash buffer (10% v/v) compared to a reference condition. Across a total of 25 reference and test samples passing post-sequencing QC, the qualitative detection rate ranged between 98.3% and 100%; per-sample NPA for both conditions was 100%.

In conclusion, no interference was found in albumin (60 g/L), conjugated bilirubin (342 µmol/L), unconjugated bilirubin (342 µmol/L), hemoglobin (2 g/L), *Staphylococcus epidermidis* (106 cfu), extraction wash buffer (10% v/v) or triglycerides (15 g/L).

b. In silico Analysis

Primer and probe specificity were addressed by mapping panel probes to the human genome. When mapped to the human genome (hg19) with decoy sequences, unplaced contigs, and representative microbial contaminants genomes, 97.6% of probes uniquely map to the genome (MAPQ ≥ 60). None of the primers or probes mapped to the representative microbial contaminant genomes.

6.5 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 precision. All studies were conducted exclusively with patient-derived samples; no cell line material was used.

a. Precision Across 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 of instrument sets, reagent lots, and operators over multiple days. This study was carried out on three distinct clinical sample pools from multiple cancer types, containing a total of 16 targeted alterations across the pools, prepared targeting 1-1.5x LoD at 5 ng cfDNA input, included variants associated with CDx claims and additional variants intended to demonstrate panel-wide validation. 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 (Guardant360 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 6.5.b. below. 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 14**.

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 14**). *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.

Across 480 alterations (150 SNVs, 150 indels, 60 CNAs, and 120 fusions), from 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%. Alteration-level repeatability and reproducibility showed high overall positive call rates (**Table 14**).

Table 14. Summary of Precision PPA Results

Alteration Class	Alteration	Number Positive / Number Expected	PPA (95% CI)
SNV	*EGFR* T790M	30/30	100.0% (88.4%, 100.0%)
SNV	*EGFR* L858R	30/30	100.0% (88.4%, 100.0%)
Indel	*EGFR* Exon 19 Del, E746_A750del	29/30	96.7% (82.8%, 99.9%)
SNV	*KRAS* G12V	30/30	100.0% (88.4%, 100.0%)
SNV	*NRAS* Q61R	30/30	100.0% (88.4%, 100.0%)
SNV	*BRAF* V600E	30/30	100.0% (88.4%, 100.0%)
Indel	*ERBB2* A775_G776insYVMA	30/30	100.0% (88.4%, 100.0%)
Indel	*EGFR* A767_V769dup	30/30	100.0% (88.4%-100.0%)
Indel	*BRCA1* E23fs	27/30	90.0% (73.5%-97.9%)
Indel	*BRCA2* S1982fs	30/30	100.0% (88.4%-100.0%)
CNA	*ERBB2*	30/30	100.0% (88.4%-100.0%)
CNA	*MET*	26/30	86.7% (69.3%-96.2%)
Fusion	*EML4-ALK*	30/30	100.0% (88.4%-100.0%)
Fusion	*TPM3-NTRK1*	30/30	100.0% (88.4%-100.0%)
Fusion	*TRIM33-RET*	30/30	100.0% (88.4%-100.0%)
Fusion	*ROS1-CCDC6*	28/30	93.3% (77.9%-99.2%)
SNV	Panel-wide	150/150	100.0% (97.6%-100.0%)
Indel	Panel-wide	146/150	97.3% (93.3%-99.3%)

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%.

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 6.3.b** above.

b. Precision for EGFR exon 20 Insertions from NSCLC cfDNA Clinical Sample Pools

A separate precision study evaluated three *EGFR* exon 20 insertions using NSCLC clinical sample pools. Precision was assessed and compared across six different unique reagent lot, instrument, and operator combinations over different start dates.

Variant source pools were prepared by diluting NSCLC patient cfDNA samples positive for selected *EGFR* exon 20 insertions with mutation-negative cfDNA derived from NSCLC clinical samples. Each insertion was tested across six precision combinations at 5 ng input at MAF levels ranging from 1.0x to 1.1x LoD.

PPA ranged from 97.6% to 100% across specific insertions and was 98.4% across all insertions and precision combinations (**Table 15**).

Table 15. Summary of Precision PPA Results for *EGFR* exon 20 Insertions

Alteration	Number Positive / Number Expected	PPA (95% CI)
EGFR exon 20 insertions	123/125	98.4% (94.3%, 99.8%)

c. 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, 1.3x LoD) and 30 ng (0.7% MAF, 1.4x LoD) cfDNA input amounts. For the 5ng and 30ng input amounts, seven (7) and three (3) replicates were tested, respectively, for each of six (6) precision combinations composed of three different reagent lots, two different instrument sets, and two different operator groups. In total, 42 replicates were tested at the 5ng input level and 18 replicates at the 30ng input level.
This study successfully verified the precision of Guardant360 CDx for detecting *KRAS* G12C mutation 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 16**). The acceptance criteria were met with a positive precision of 100% at both 5 and 30 ng cfDNA inputs.

Table 16. 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%]

d. 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 the 30 ng LoD MAFs, and 1.5x 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. 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 17**.

Table 17. 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 18**. 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 18**.

Table 18. 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 ANA was 99.9%. This statistic includes all called variant sites panel-wide, not just the eligible variants sites based on LoD in the source samples, so this statistic 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 19**. For each of these variants, there were 12 pairwise comparisons.

Table 19. 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% 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.

e. 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, *EGFR* exon 19 deletions, and *EGFR* exon 20 insertions) and category 2 variants.

Samples from healthy donors (*KRAS* G12C negatives), pre-screened by an externally validated orthogonal method, were reanalyzed specifically for *KRAS* G12C mutation 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.

6.6 Cross-Contamination/Carry-Over

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.

6.7 Guardbanding/ Robustness

The purpose of the guardbanding study was to evaluate cfDNA input at the minimum input amount (5 ng) and the maximum amount (30 ng), adapter volume tolerances for ligation steps, hybridization time tolerances in the enrichment process and wash buffer 2 temperature tolerances in the enrichment process (**Table 20**).

Table 20. 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°C	70°C	72°C

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 21**). The QDR was 100% with a QDR lower limit of the 95% confidence interval (LLCI) of 85.47%. For each tested guardbanding condition, all the LLCI were higher than 80%, meeting the acceptance criteria.

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

Table 21. Guardbanding Results Summary

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 20); 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.

6.8 Reagent Lot Interchangeability

Reagents lot interchangeability was assessed by testing two cfDNA sample pools containing 16 alterations, 9 variants in pool 1 and 7 variants in pool 2, in five replicates using two different lots of Guardant360 CDx Sample Preparation Kit in seven different lot combinations. For the sample replicates that proceeded to sequencing, all met the performance metrics. Kit Lot Interchangeability of Guardant360 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.

6.9 Stability

a. Reagent Stability

The stability of the Guardant360 CDx Sample Preparation Kit lots used in sample processing for Guardant360 CDx were evaluated in this study. Three lots of identical reagents were stored under the specified storage conditions for each box and then tested at defined time points using two cfDNA sample pools that contained in total 16 known variants, 9 variants in pool 1 and 7 variants in pool 2. Under the tested conditions, results from each time point, 3, 4, 7, 10, 13 and 19 months were compared against samples tested at day 0 (time point T_0). The Guardant360 SPK boxes were tested at each timepoint with five (5) replicates per each of the two unique sample pools at 5 ng cfDNA input.

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 time point. 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 showed 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. The qualitative detection rate, calculated from targeted sites, ranged between 95.0% and 100.0% by timepoint. 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.

Variant detection performance was stable for a claimed shelf life of 18 months.

b. Whole Blood Stability

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. The stability of whole blood used for Guardant360 CDx was evaluated by collecting 4 fresh whole blood samples from 16 cancer patients. 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) as follow:

- Reference Condition: Plasma processing 1 day after blood collection
- Condition 1: Summer Profile Storage: 4h at 22°C, 6h at 37°C, and 56h at 22°C, 6h at 37°C, plus remaining time at room temperature.
- Condition 2: Winter Profile Storage: 4h at 18°C, 6h at 0°C, 56h at 10°C, and 6h at 0°C plus remaining time at room temperature
- Condition 3: Room Temperature Storage: Storage at room temperature 18-25°C

After conditioning, plasma was isolated on the 8th day after blood collection and run on the Guardant360 CDx.

All 64 samples passed all QC and were included in analysis. All storage conditions demonstrated acceptable performance. 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.

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 100.0% for all conditions. 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.

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 each of 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°C) 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°C) 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°C) 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°C) 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 tubes 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. Samples were processed and run on Guardant360 CDx immediately after plasma isolation or after storage at -80°C ± 10°C for 46 days or 2-8°C for 24 hours. 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. Plasma from one BCT was processed through cfDNA extraction on the same day as a reference condition, 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; Condition 1), 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; Condition 2), 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 (Condition 3). Extracted cfDNA from each condition was stored at -20°C ± 5°C until further processing.

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. 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 0.9. Exon-level relative coverage demonstrated 92.8%-97.1% fraction of exons within 2σ of expected relative coverage.

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). NPA across the reportable range was 99.9%.

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

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. Samples were split into two extraction arms (with quantification either before, or after freezing) to establish stability of cfDNA under both measurement workflows.

Sixty-six (66) samples were processed for the reference and 2 conditions below.

- Reference condition A: Post-extraction quantitation: Quantitation, dilution, and library preparation post-extraction on the same day.
- Reference condition B: Quantitation, dilution, and library preparation post-extraction on the same day.
- Condition 1A: 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).
- Condition 1B: 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 2A: 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).
- Condition 2B: 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 3A: 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.
- Condition 3B: 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.

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 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.

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.

e. Intermediate Product Stability

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 22**.

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 22. 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. 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%.

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.

6.10 General Lab Equipment and Reagent Evaluation

a. cfDNA Extraction

The performance of the cfDNA extraction from plasma samples was evaluated on the QIAsymphony SP System. A retrospective analysis of clinical whole blood samples processed on the Guardant360 LDT implementation of the Guardant360 CDx device 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. The variation in QIAsymphony instrument and/or reagent lot explained <2.1% of variance in cfDNA extraction yield. Each combination of

QIAsymphony reagent kits (N=4) / instruments (N=7) resulted in successful extraction of ≥ 5ng cfDNA at a rate ≥ 94%, with a total success rate of 97.3%.

b. Other Instruments and Reagents

The other general lab instrument/reagent systems (4200 TapeStation, Microlab STAR, Microlab STARlet, NextSeq 550 Sequencer, 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 (**Tables 23**). Acceptable sequencing QC parameters were demonstrated across precision combinations (**Table 24**).

Table 23. Sequencer PPA and NPA Across Precision Combinations

Instrument #	PPA	95% CI	NPA	95% CI
1	98.1% (210/214)	[95.3%, 99.5%]	100% (40/40)	[91.2%, 100%]
2	98.1% (52/53)	[89.9%, 100%]	100% (10/10)	[69.2%, 100%]
3	98.1% (156/159)	[94.6%, 99.6%]	100% (30/30)	[88.4%, 100%]
4	96.3% (52/54)	[87.3%, 99.5%]	100% (10/10)	[69.2%, 100%]

Table 24. 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 Guardant360 CDx.

6.11 Pan-Cancer Analysis

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 refers to 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 25**).

Table 25. Sample Success Rate Across 23 Cancers

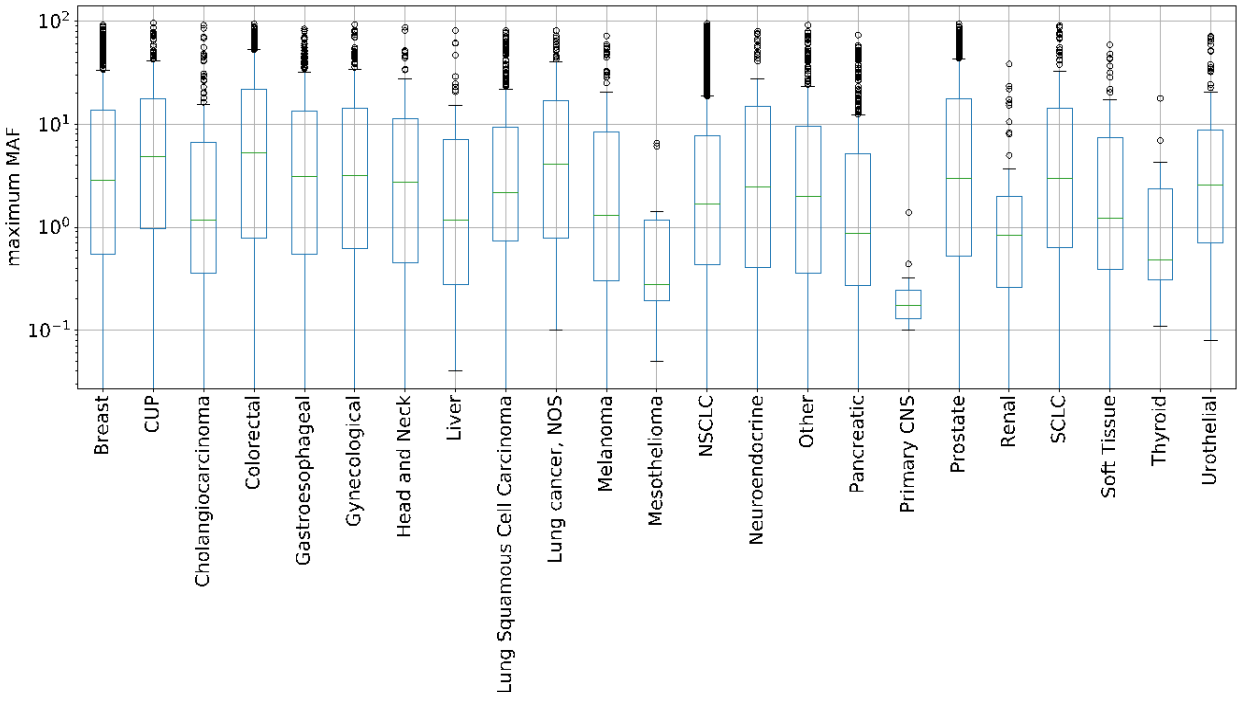
Category Data			Sample Preparation QC Data, % Pass			Patient Sample Sequencing QC Data, % Pass (median value)			Patient Outcome Metrics		
Cancer Category	Total Patients	First Tube Success	cfDNA Ex. Sample QC Pass %	Library Enrich. Sample QC Pass %	In process Contam-ination %	Coverage Exception	GC Bias	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)
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 Carcinoma	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)
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.

6.12 Concordance - Guardant360 CDx 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 26**. 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 26. 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% (98.49%,100%)
ERBB2 CNA	5	2	0	251	71.4% (29.04%,96.33%)	100% (98.54%,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 27** 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 27. 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%)

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 *EGFR* CDx variants.

6.13 Additional Studies

a. Blood Collection Tube Concordance

The purpose of this study was to establish concordance between the Streck Cell-Free DNA BCTs and BCTs 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 7 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 variant 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.

7 Summary of Primary Clinical Studies

Guardant360 CDx comprises three companion diagnostics claims as noted in **Table 1**:

1) To aid in the selection of patients with NSCLC whose tumors have *EGFR* exon 19 deletions, L858R mutations, and/or T790M mutations for osimertinib (TAGRISSO®) therapy
2) To aid in the selection of patients with NSCLC whose tumors have *EGFR* exon 20 insertions for amivantamab-vmjw (RYBREVANT™) therapy
3) To aid in the selection of patients with NSCLC whose tumors have *KRAS* G12C alterations for sotorasib (LUMAKRAS™) therapy

In support of the osimertinib CDx claim, Guardant Health performed two clinical bridging studies. In the first, 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 or L858R mutations for osimertinib therapy. Plasma from FLAURA patients negative for *EGFR* mutations by tissue testing was not available to represent the Guardant360-positive, tissue-negative portion of the Guardant360-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 but negative by tissue testing to evaluate the potential impact of this population on clinical efficacy. 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 osimertinib therapy based on a *EGFR* T790M mutation-detected result.

In support of the amivantamab-vmjw CDx claim, Guardant Health performed a clinical bridging study using banked plasma samples from the CHRYSALIS clinical study

(NCT02609776). The primary amivantamab-vmjw registration population comprises subjects from the CHRYSALIS clinical study with *EGFR* exon 20 insertions as determined by local test results, whose disease progressed on or after platinum-based chemotherapy, and who were treated with the recommended phase 2 dose (RP2D) of amivantamab-vmjw. Pre-treatment plasma samples from these subjects were tested with Guardant360 CDx. As the majority of subjects included in the primary amivantamab-vmjw registration population were enrolled based on positive local tissue testing for *EGFR* exon 20 insertions, sensitivity analysis to assess the possible influence of local test-negative, Guardant360 CDx plasma-positive patients (Guardant360 CDx^{+} local test^{-}) was performed using supplemental samples from the CHRYSALIS clinical study screen fail population and additional samples from the NILE Clinical Study.

In support of the sotorasib CDx claim, Guardant Health performed a clinical bridging study using banked samples from the Amgen 20170543 clinical study (NCT03600883). The subjects in the Amgen 20170543 clinical study were enrolled based on the presence of *KRAS* G12C in tissue specimens confirmed by Qiagen *therascreen* KRAS RGQ PCR test. 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) therapy based on the detection of *KRAS* G12C mutations. As subjects in the Amgen 20170543 clinical study were enrolled based on positive tissue testing for *KRAS* G12C, sensitivity analysis to assess the possible influence of tissue-negative, Guardant360 CDx plasma-positive subjects (Guardant360 CDx^{+} tissue^{-}) was performed using samples procured from other Amgen-sponsored clinical studies or vendors.

7.1 Guardant360 CDx Clinical Bridging Study for *EGFR* Exon 19 Deletions or L858R Mutations

FLAURA Clinical Study Design

The FLAURA clinical study was a phase III, double-blind, randomized study assessing the efficacy and safety of osimertinib versus standard of care (SoC) EGFR tyrosine kinase inhibitor (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 mutations in their tumor as determined by the cobas® EGFR Mutation Test at a central laboratory or testing at 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 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 or L858R mutations (Refer to Section 6.10.a. 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 (see below). Further a blood collection concordance study establishing the concordance between samples collected in Streck Cell-Free DNA BCTs and the BCT-CTAs was conducted to support the validity of the data generated by testing samples collected in BCT-CTAs (Refer to Section 6.12.a).

No plasma from FLAURA patients negative for *EGFR* mutations by tissue testing was available to represent the Guardant360-positive, tissue-negative portion of the Guardant360-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 but negative by tissue testing to evaluate the potential impact of this population on clinical efficacy.

a. *Bridging Study Inclusion and Exclusion Criteria*

- *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

b. *Follow-up Schedule*

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

c. *Clinical Endpoints*

The clinical endpoint used to assess osimertinib 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.

- *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.

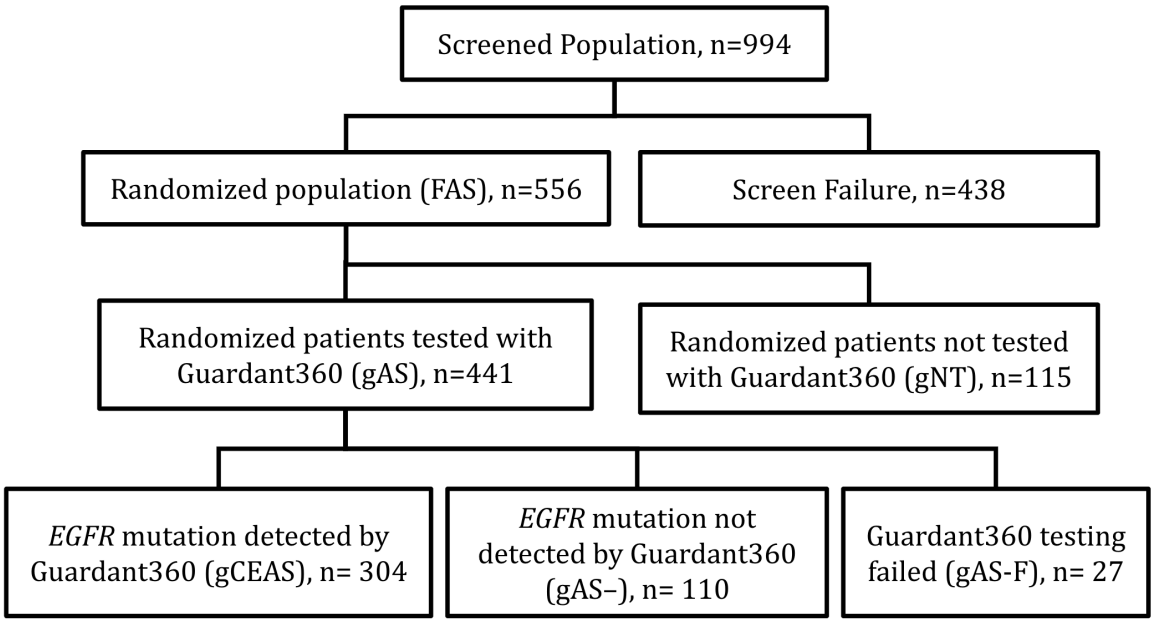
The possible influence of tissue-negative Guardant360 CDx-positive patients in the effectiveness of the Guardant360 CDx was assessed through a sensitivity analysis. As no plasma samples from FLAURA patients negative for *EGFR* mutations by tissue testing were available to represent the Guardant360 CDx-positive,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

Accountability of PMA Cohort

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 or L858R Mutations Bridging Study Patient Accountability and Analysis Set Definitions



Study Population Demographics and Baseline Parameters

Demographic and baseline clinical characteristics of patients enrolled in the FLAURA clinical study (FAS) were categorized relative to the Guardant360 CDx *EGFR* exon 19 deletions or L858R mutations bridging study populations as defined by Guardant360 results (gCEAS) and assessed for treatment arm balance. As shown in **Table 28**, 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 28. 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 29**).

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

Characteristics		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 Other	Adenocarci-noma	209 (95.4)	204 (91.9)	413 (93.7)	56 (93.3)	54 (98.2)	110 (95.7)	0.4185
	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.

Table 30 shows that demographic and baseline clinical characteristics of patients screened for the FLAURA and enrolled in the NILE clinical studies were well-balanced between the subgroups used in the supplementary Guardant360-positive, tissue-negative prevalence analysis. with the exception of race and smoking status.

Table 30. 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

Safety and Effectiveness Results

a. Safety Results

Data regarding the safety and efficacy 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 as these involved retrospective testing of banked specimens only.

b. Effectiveness Results

i. PFS in Patients Positive by Guardant360 for *EGFR* Exon 19 Deletions 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 31**. The observed PFS hazard ratio (HR) of 0.41 (95% CI 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.

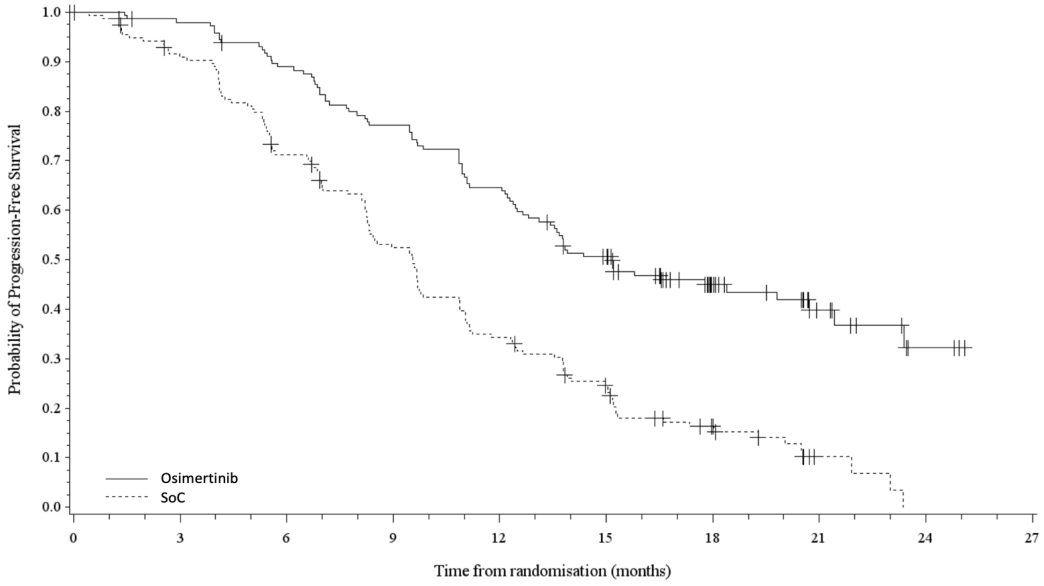
Kaplan-Meier analysis of PFS in the gCEAS is presented in **Figure 3**.

Table 31. 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	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, 057)	<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 32** 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 32. 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, 057
	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 33**. 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 33** for patients screened in FLAURA used a prevalence of 67%.

Table 33. 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	0.41	0.31, 0.54
			0.50	0.41	0.31, 0.54
			0.75	0.41	0.31, 0.54
			1.00	0.41	0.31, 0.54
gCEAS (observed and imputed)	0.99	0.97, 1.00	0.42	0.42	0.32, 0.54
			0.50	0.42	0.32, 0.54
			0.75	0.42	0.32, 0.54
			1.00	0.42	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 34** below) supporting the robustness of the study results.

iii. 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 from the FLAURA study is shown in **Table 34**.

Table 34. 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 Mutations**	**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 Mutations**	**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 35**. 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 35. Concordance Between Guardant360 and the cobas® EGFR Mutation Test Using Tissue in Samples from the NILE Clinical Study

***EGFR* Exon 19 Deletions or L858R Mutations**	**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.

7.2 Guardant360 CDx Clinical Bridging Study for *EGFR* T790M Mutations

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 AURA3 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 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 precisions 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 Section 6.10.a, Guardant360 CDx-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-CTA was conducted to support the validity of the data generated by testing samples collected in BCT-CTA (Refer to Section 6.12.b.).

a. Bridging Study Inclusion and Exclusion Criteria

- *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

b. Follow-up Schedule

The Guardant360 CDx *EGFR* T790M bridging study involved only retrospective testing of plasma samples; as such, additional patient follow-up was conducted.

c. 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.

- *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.

Accountability of PMA Cohort

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%).

Study Population Demographics and Baseline Parameters

Demographic and baseline clinical characteristics of patients enrolled in the AURA3 clinical study (FAS) were categorized relative to the Guardant360 CDx *EGFR* T790M bridging study populations as defined by Guardant360 results (gCEAS) and assessed for treatment arm balance. As shown in **Table 36**, 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.

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

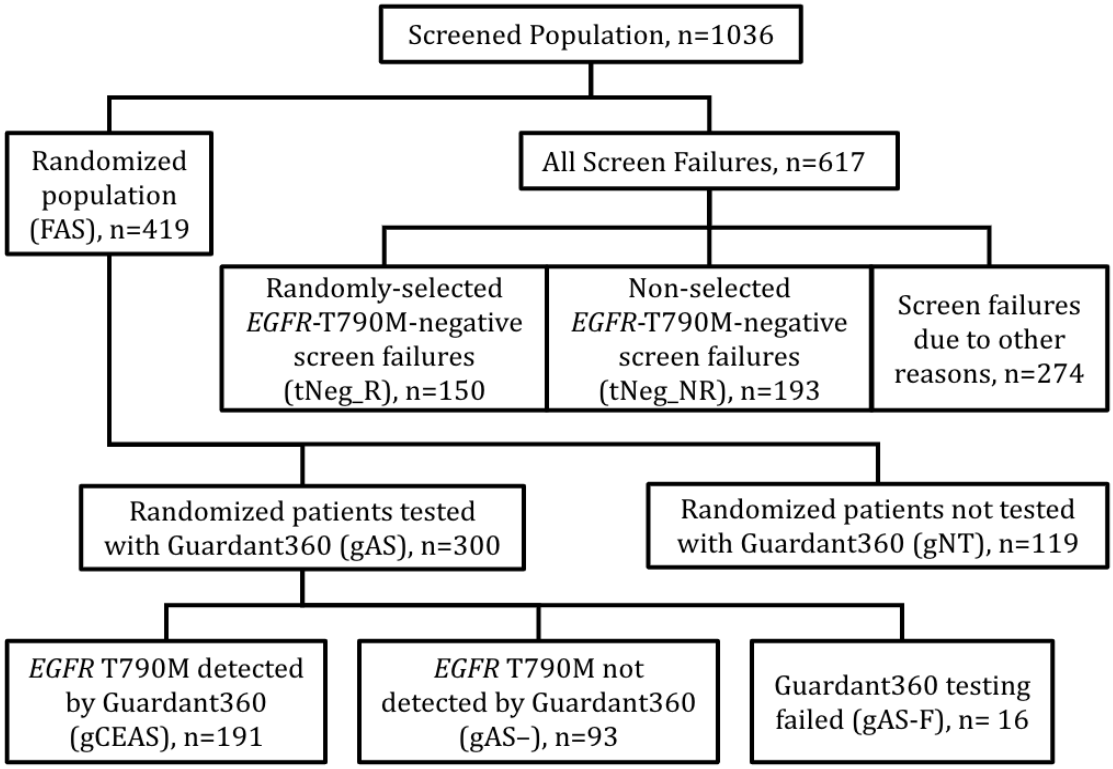


Table 36. Baseline Demographics and Clinical Characteristics

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 37**).

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 37. 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)	0.4657
	IV	174 (80.9)	62 (72.9)	236 (78.7)	51 (79.7)	47 (85.5)	98 (82.4)	
	Missing	2 (0.9)	0 (0)	2 (0.7)	0 (0)	0 (0)	0 (0)	
Overall disease classification	Metastatic	204 (94.9)	84 (98.8)	288 (96.0)	62 (96.9)	54 (98.2)	116 (97.5)	0.5712
	Locally advanced	11 (5.1)	1 (1.2)	12 (4.0)	2 (3.1)	1 (1.8)	3 (2.5)	
Histology type Other	Adeno-carcinoma	214 (99.5)	85 (100)	299 (9.7)	64 (100)	55 (100)	119 (100)	1.0000
	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.

Safety and Effectiveness Results

a. Safety

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 as these involved retrospective testing of banked specimens only.

b. Effectiveness Results

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

The efficacy of single-agent TAGRISSO relative to chemotherapy in patients positive for *EGFR* T790M mutations by Guardant360 (gCEAS) is shown in **Table 38**. 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.

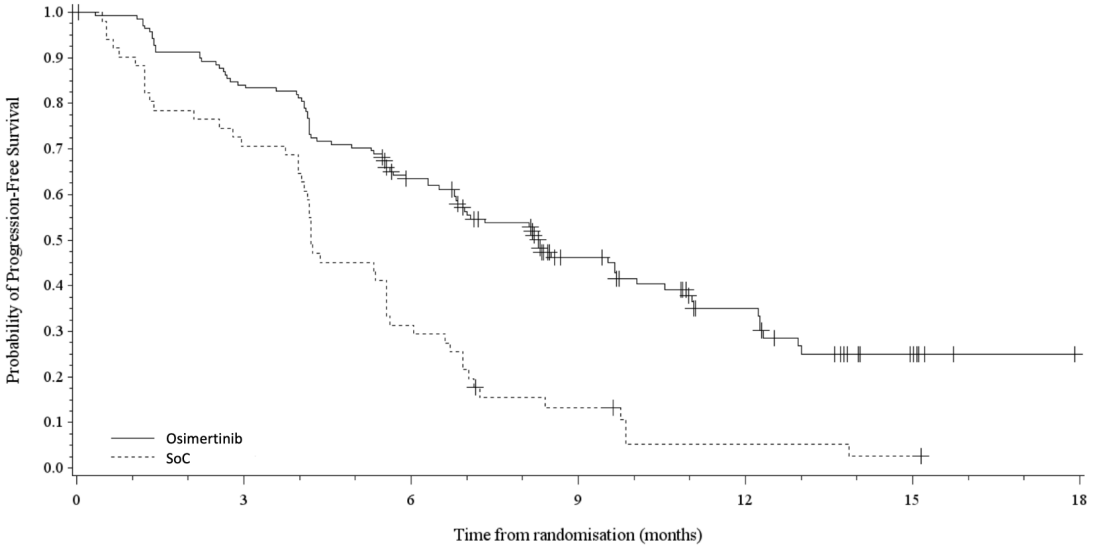
Kaplan-Meier analysis of PFS in the gCEAS is presented in **Figure 5**.

Table 38. 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 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 are 119 (300/419, 28%) randomized 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 39** 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 Guardant360 data have no meaningful impact on the robustness of the efficacy result observed in the AURA3 study.

Table 39. 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 40**, 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 40** for the patients screened in AURA3 used a prevalence of 55%.

Table 40. 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)	072	0.66, 0.77	0.34	0.34	0.22, 0.53
			0.50	0.38	0.27, 0.53
			0.75	0.43	0.30, 0.60
			1.00	0.46	0.33, 0.65
gCEAS (observed + imputed)	0.72	0.66, 0.77	0.35	0.36	0.24, 0.51
			0.50	0.39	0.29, 0.52
			0.75	0.43	0.32, 0.59
			1.00	0.47	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 + imputed).

iii. 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 41**.

Table 41. 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.

7.3 Guardant360 CDx Clinical Bridging Study for *EGFR* exon 20 Insertions

Diagnostic Study Design

This diagnostic study uses banked samples from the CHRYSALIS (Janssen EDI1001 or 61186372EDI1001) clinical study (NCT02609776) in the clinical bridging study. The primary amivantamab-vmjw registration population comprises 81 subjects from the CHRYSALIS clinical study with *EGFR* exon 20 insertions as determined by local test results, whose disease progressed on or after platinum-based chemotherapy, and who were treated with the recommended phase 2 dose (RP2D) of amivantamab-vmjw. The banked pre-treatment plasma samples from these subjects were retrospectively tested with Guardant360 CDx.

As the majority (75/81, 92.6%) of subjects included in the primary amivantamab-vmjw registration population were enrolled based on positive local tissue testing for *EGFR* exon 20 insertions, sensitivity analysis to assess the possible influence of local test-negative, Guardant360 plasma-positive patients (Guardant360 CDx^{+} local test^{-}) was performed using 83 valid results from 85 supplemental samples from the non-*EGFR* exon 20 insertion arms of the CHRYSALIS clinical study screen fail population and an additional 88 valid results from 92 samples from the NILE Clinical Study.

Primary Clinical Study Population

The primary amivantamab-vmjw registration population comprises *EGFR* exon 20 insertion mutation-positive subjects from the CHRYSALIS study whose disease progressed on or after platinum-based chemotherapy and who were treated with the RP2D of amivantamab-vmjw. Subjects must have received the first dose of amivantamab-vmjw as

monotherapy on or before 05 February 2020 and were to have undergone at least 3 scheduled post-baseline disease assessments or discontinued treatment for any reason, including disease progression and/or death, prior to the clinical data cut-off.

Pretreatment plasma samples were collected from subjects in Streck cfDNA BCTs and tested retrospectively using Guardant360 CDx after the completion of the CHRYSALIS study.

Supplemental Populations for Plasma-Tissue NPA Analysis

Since the primary amivantamab-vmjw registration population consists primarily of subjects positive for *EGFR* exon 20 insertions by local tissue testing, additional subjects were required to evaluate the local test-negative portion of the Guardant360 CDx+ intended use population. To this end, screen fail subjects from the non-*EGFR* exon 20 insertions cohorts of CHRYSALIS clinical study tested with both Guardant360 CDx and tissue-based NGS central testing as well as previously generated clinical sample data from subjects enrolled in the Noninvasive vs. Invasive Lung Evaluation (NILE) study (NCT03615443) were used.

Clinical Specimen Selection Criteria

All subjects enrolled in the primary clinical efficacy population for the primary amivantamab-vmjw registration population, were included in the diagnostic study efficacy cohort if the selection criteria below are met. Similarly, all subjects meeting the sensitivity analysis prevalence sub-study cohort selection criteria below are included.

Guardant360 CDx Diagnostic Study Efficacy Cohort Patient Inclusion Criteria

- Subject enrolled in the CHRYSALIS clinical study with informed consent for blood sample use for further research.
- Subject part of the primary amivantamab-vmjw registration population.
- Adequate pre-treatment plasma sample available for Guardant360 CDx testing or a previously generated Guardant360 CDx test result from the 01-LU-007 study

Guardant360 CDx Diagnostic Study Sensitivity Analysis Prevalence Sub-Study Cohort Patient Inclusion Criteria

Screen Fail Samples from the CHRYSALIS Clinical Study

- Subject failed screening for the CHRYSALIS clinical study with informed consent for blood sample use for further research.
- Pre-treatment plasma sample available for testing with Guardant360 CDx or a Guardant360 CDx test result previously generated under the Guardant Health 01-LU-007 protocol.
- Availability of previously generated CHRYSALIS clinical study central tissue testing results.

Samples from the NILE Clinical Study

- Subjects enrolled in the NILE clinical study with documented informed consent.
- A valid Guardant360 CDx test result previously generated from a pre-treatment plasma sample under the 01-LU-003 study.
- Previously generated valid test result from cobas EGFR Mutation Test v2 testing on tissue 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 requirements for the 01-LU-003 study.

Diagnostic study Primary Objective and Endpoint

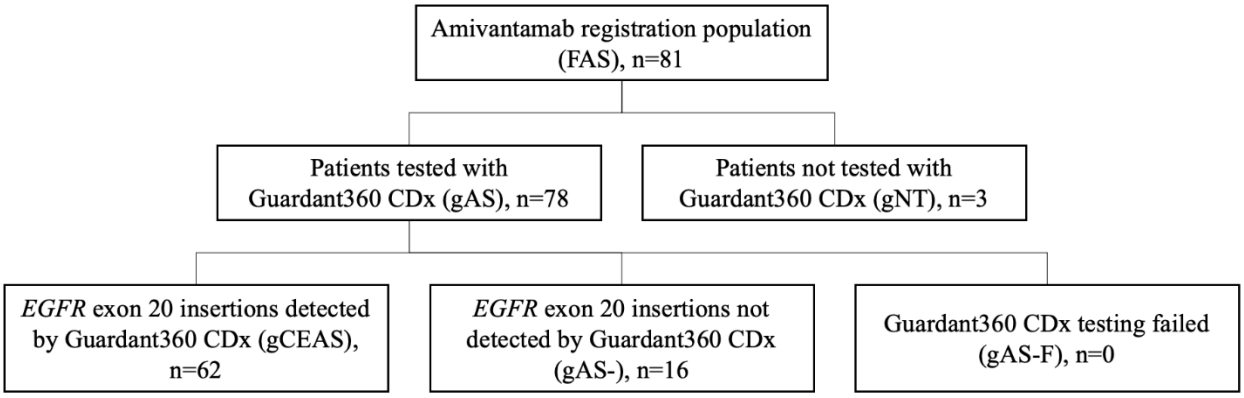
The primary objective of the diagnostic study is to demonstrate the comparability of single-agent amivantamab-vmjw efficacy in the primary amivantamab-vmjw registration population subjects who are positive for *EGFR* exon 20 insertions by Guardant360 CDx to the size-adjusted null hypothesis efficacy cited in the CHRYSALIS clinical study protocol. The primary endpoint is objective response rate (ORR) by RECIST 1.1 as assessed by blinded independent central review (BICR).

Sensitivity analyses were conducted to model the impact of the hypothetical Guardant360 CDx^{+} local $test^{-}$ population and subjects without Guardant360 CDx results.

Accountability of study subjects

The diagnostic study comprises 81 subjects of the primary amivantamab-vmjw registration population (**Figure 6**). Of the, 78 subjects (96%) with samples available for tested by the Guardant360 CDx, 62 subjects (79%) tested positive by the Guardant360 CDx were included in the primary objective analysis set, while 16 subjects (21%) tested negative, and 0 subjects (0%) failed testing. Three subjects (3.7% of the primary efficacy population) subjects did not have plasma samples for testing.

Figure 6. Guardant360 CDx Clinical Efficacy Analyses Subject Disposition



Diagnostic Study Efficacy Population Representativeness Demographics and Baseline Clinical Characteristics

Demographics and baseline clinical characteristics of subjects enrolled in the CHRYSALIS clinical study were categorized relative to the diagnostic study populations as defined by Guardant360 CDx results. As shown in **Table 42** and **Table 43**, the diagnostic study efficacy population (gCEAS) demographics and baseline clinical characteristics closely resemble those of the overall primary amivantamab-vmjw registration population (FAS).

To assess potential bias arising from plasma sample availability, demographic information and baseline clinical characteristics of the gAS and the gAS-Unk were compared, and the associated p value reported in **Table 42** and **Table 43**. No meaningful differences were observed.

Table 42. Comparison of Clinical Effectiveness Analysis Subgroup Demographics

	CHRYSALIS							
	FAS	**gAS**	**gNT**	**gCEAS**	**gAS-**	**gAS -F**	**gAS-F +gNT**	**p Value gAS vs gAS-Unk**
Analysis set:	81	78	3	62	16	-	3	
Age, years								
N	81	78	3	62	16	0	3	0.914
Mean (SD)	62.3 (9.96)	62.3 (10.04)	61.7 (9.29)	62.5 (10.03)	61.6 (10.40)	-	61.7 (9.29)	
Median	62.0	62.0	59.0	62.0	62.0	-	59.0	
Range	(42; 84)	(42; 84)	(54; 72)	(42; 84)	(46; 76)	-	(54; 72)	
<65	48 (59.3%)	46 (59.0%)	2 (66.7%)	38 (61.3%)	8 (50.0%)	-	2 (66.7%)	
>=65	33 (40.7%)	32 (41.0%)	1 (33.3%)	24 (38.7%)	8 (50.0%)	-	1 (33.3%)	
<75	74 (91.4%)	71 (91.0%)	3 (100.0%)	56 (90.3%)	15 (93.8%)	-	3 (100.0%)	
>=75	7 (8.6%)	7 (9.0%)	0	6 (9.7%)	1 (6.3%)	-	0	
Sex								
N	81	78	3	62	16	0	3	1.000
Female	48 (59.3%)	46 (59.0%)	2 (66.7%)	40 (64.5%)	6 (37.5%)	-	2 (66.7%)	
Male	33 (40.7%)	32 (41.0%)	1 (33.3%)	22 (35.5%)	10 (62.5%)	-	1 (33.3%)	
Race								
N	81	78	3	62	16	0	3	0.104
Asian	40 (49.4%)	39 (50.0%)	1 (33.3%)	34 (54.8%)	5 (31.3%)	-	1 (33.3%)	
Black or African America n	2 (2.5%)	1 (1.3%)	1 (33.3%)	1 (1.6%)	0	-	1 (33.3%)	
White	30 (37.0%)	29 (37.2%)	1 (33.3%)	21 (33.9%)	8 (50.0%)	-	1 (33.3%)	
Not reported	9 (11.1%)	9 (11.5%)	0	6 (9.7%)	3 (18.8%)	-	0	
Ethnicity								
N	81	78	3	62	16	0	3	1.000
Hispanic or Latino	3 (3.7%)	3 (3.8%)	0	3 (4.8%)	0	-	0	
Not Hispanic or Latino	68 (84.0%)	65 (83.3%)	3 (100.0%)	53 (85.5%)	12 (75.0%)	-	3 (100.0%)	
Not reported	10 (12.3%)	10 (12.8%)	0	6 (9.7%)	4 (25.0%)	-	0	
Weight, kg								
N	81	78	3	62	16	0	3	0.563

CHRYSALIS								
	FAS	**gAS**	**gNT**	**gCEAS**	**gAS-**	**gAS -F**	**gAS-F +gNT**	**p Value gAS vs gAS-Unk**
Mean (SD)	67.49 (16.784)	67.28 (16.407)	73.03 (29.258)	65.20 (16.149)	75.34 (15.297)	-	73.03 (29.258)	
Median	62.50	62.95	57.10	61.60	73.60	-	57.10	
Range	(35.4; 115.0)	(35.4; 115.0)	(55.2; 106.8)	(35.4; 106.2)	(52.0; 115.0)	-	(55.2; 106.8)	
Height, cm								
N	81	78	3	62	16	0	3	0.504
Mean (SD)	163.71 (9.020)	163.84 (9.044)	160.27 (9.295)	163.12 (9.406)	166.66 (7.034)	-	160.27 (9.295)	
Median	162.60	162.75	154.90	160.05	165.65	-	154.90	
Range	(144.5; 192.0)	(144.5; 192.0)	(154.9; 171.0)	(144.5; 192.0)	(150.0; 176.6)	-	(154.9; 171.0)	
Body mass index, kg/m^2								
N	81	78	3	62	16	0	3	0.320
Mean (SD)	24.993 (4.9047)	24.886 (4.8151)	27.776 (7.5866)	24.330 (4.7289)	27.043 (4.6727)	-	27.776 (7.5866)	
Median	24.250	24.508	23.798	23.455	25.858	-	23.798	
Range	(14.00; 36.87)	(14.00; 36.87)	(23.01; 36.52)	(14.00; 36.72)	(19.57; 36.87)	-	(23.01; 36.52)	
Underweight <18.5	4 (4.9%)	4 (5.1%)	0	4 (6.5%)	0	-	0	
Normal 18.5-<25	43 (53.1%)	41 (52.6%)	2 (66.7%)	35 (56.5%)	6 (37.5%)	-	2 (66.7%)	
Overweight 25-<30	21 (25.9%)	21 (26.9%)	0	16 (25.8%)	5 (31.3%)	-	0	
Obese >=30	13 (16.0%)	12 (15.4%)	1 (33.3%)	7 (11.3%)	5 (31.3%)	-	1 (33.3%)	
Local Test Type*								
N	81	78	3	62	16	0	3	0.803
NGS (Blood)	4 (4.9%)	4 (5.1%)	0	3 (4.8%)	1 (6.3%)	-	0	
NGS (Tissue)	34 (42.0%)	33 (42.3%)	1 (33.3%)	24 (38.7%)	9 (56.3%)	-	1 (33.3%)	
OTHER (Blood)	1 (1.2%)	1 (1.3%)	0	1 (1.6%)	0	-	0	
OTHER (Tissue)	7 (8.6%)	7 (9.0%)	0	7 (11.3%)	0	-	0	
PCR (Blood)	1 (1.2%)	1 (1.3%)	0	1 (1.6%)	0	-	0	
PCR (Tissue)	30 (37.0%)	28 (35.9%)	2 (66.7%)	23 (37.1%)	5 (31.3%)	-	2 (66.7%)	
UNKNOWN (Tissue)	4 (4.9%)	4 (5.1%)	0	3 (4.8%)	1 (6.3%)	-	0	

* Local test type as defined by the enrolling site.
FAS: Full Analysis Set, gAS: Guardant360 CDx analysis set, gNT: Guardant360 CDx not tested set, gCEAS: Guardant360 CDx primary clinical efficacy analysis set, gAS: Guardant360 CDx analysis set, gAS-F: Guardant360 CDx analysis set failed, gAS-Unk: Guardant360 CDx unknown set

Table 43. Comparison of Clinical Effectiveness Analysis Sub-Group Baseline Clinical Characteristics.

CHRYSALIS								
	FAS	**gAS**	**gNT**	**gCEAS**	**gAS-**	**gAS-F**	**gAS-Unk**	**p Value gAS vs gAS-Unk**
Analysis set:	81	78	3	62	16	-	3	
Initial diagnosis NSCLC subtype								
N	81	78	3	62	16	0	3	0.922
Adenocarcinoma	77 (95.1%)	74 (94.9%)	3 (100.0%)	59 (95.2%)	15 (93.8%)	-	3 (100.0%)	
Large cell carcinoma	0	0	0	0	0	-	0	
Squamous cell carcinoma	3 (3.7%)	3 (3.8%)	0	2 (3.2%)	1 (6.3%)	-	0	
Other	1 (1.2%)	1 (1.3%)	0	1 (1.6%)	0	-	0	
Not reported	0	0	0	0	0	-	0	
Histology grade at initial diagnosis								
N	81	78	3	62	16	0	3	0.708
Moderately differentiated	18 (22.2%)	17 (21.8%)	1 (33.3%)	16 (25.8%)	1 (6.3%)	-	1 (33.3%)	
Poorly differentiated	12 (14.8%)	11 (14.1%)	1 (33.3%)	8 (12.9%)	3 (18.8%)	-	1 (33.3%)	
Well differentiated	5 (6.2%)	5 (6.4%)	0	5 (8.1%)	0	-	0	
Other	46 (56.8%)	45 (57.7%)	1 (33.3%)	33 (53.2%)	12 (75.0%)	-	1 (33.3%)	
Not reported	0	0	0	0	0	-	0	
Cancer stage at initial diagnosis								
N	81	78	3	62	16	0	3	0.078
0	0	0	0	0	0	-	0	
IA	6 (7.4%)	6 (7.7%)	0	4 (6.5%)	2 (12.5%)	-	0	
IB	1 (1.2%)	1 (1.3%)	0	1 (1.6%)	0	-	0	

CHRYSALIS								
	FAS	**gAS**	**gNT**	**gCEAS**	**gAS-**	**gAS-F**	**gAS-Unk**	**p Value gAS vs gAS-Unk**
IIA	1 (1.2%)	1 (1.3%)	0	1 (1.6%)	0	-	0	
IIB	4 (4.9%)	3 (3.8%)	1 (33.3%)	3 (4.8%)	0	-	1 (33.3%)	
IIIA	4 (4.9%)	3 (3.8%)	1 (33.3%)	2 (3.2%)	1 (6.3%)	-	1 (33.3%)	
IIIB	4 (4.9%)	4 (5.1%)	0	3 (4.8%)	1 (6.3%)	-	0	
IV	61 (75.3%)	60 (76.9%)	1 (33.3%)	48 (77.4%)	12 (75.0%)	-	1 (33.3%)	
Not reported	0	0	0	0	0	-	0	
Location of metastasis [a]								
N	81	78	3	62	16	0	3	0.598
Bone	34 (42.0%)	33 (42.3%)	1 (33.3%)	30 (48.4%)	3 (18.8%)	-	1 (33.3%)	
Liver	7 (8.6%)	7 (9.0%)	0	5 (8.1%)	2 (12.5%)	-	0	
Brain	18 (22.2%)	17 (21.8%)	1 (33.3%)	14 (22.6%)	3 (18.8%)	-	1 (33.3%)	
Lymph Node	43 (53.1%)	43 (55.1%)	0	38 (61.3%)	5 (31.3%)	-	0	
Adrenal Gland	3 (3.7%)	3 (3.8%)	0	3 (4.8%)	0	-	0	
Other	45 (55.6%)	42 (53.8%)	3 (100.0%)	31 (50.0%)	11 (68.8%)	-	3 (100.0%)	
Not reported	0	0	0	0	0	-	0	
Time from initial diagnosis of cancer to first dose (months)								
N	81	78	3	62	16	0	3	0.881
Mean (SD)	22.905 (21.1901)	22.835 (21.3828)	24.717 (18.7773)	23.972 (22.8978)	18.427 (13.7407)	-	24.717 (18.7773)	
Median	17.018	16.986	26.021	16.789	18.431	-	26.021	
Range	(1.45; 130.10)	(1.45; 130.10)	(5.32; 42.81)	(2.86; 130.10)	(1.45; 45.37)	-	(5.32; 42.81)	

CHRYSALIS								
	FAS	**gAS**	**gNT**	**gCEAS**	**gAS-**	**gAS-F**	**gAS-Unk**	**p Value gAS vs gAS-Unk**
Time from metastatic disease diagnosis to first dose (months)								
N	81	78	3	62	16	0	3	0.401
Mean (SD)	18.071 (16.4424)	18.374 (16.6647)	10.185 (5.0347)	18.886 (17.4686)	16.388 (13.3918)	-	10.185 (5.0347)	
Median	14.160	14.883	9.856	14.883	14.850	-	9.856	
Range	(0.69; 116.40)	(0.69; 116.40)	(5.32; 15.38)	(0.69; 116.40)	(1.35; 45.37)	-	(5.32; 15.38)	
Number of prior lines of therapy								
N	81	78	3	62	16	0	3	0.614
Mean (SD)	2.3 (1.41)	2.2 (1.40)	2.7 (2.08)	2.3 (1.47)	1.9 (1.06)	-	2.7 (2.08)	
Median	2.0	2.0	2.0	2.0	2.0	-	2.0	
Range	(1; 7)	(1; 7)	(1; 5)	(1; 7)	(1; 4)	-	(1; 5)	
ECOG performance status								
N	81	78	3	62	16	0	3	0.980
0	26 (32.1%)	25 (32.1%)	1 (33.3%)	19 (30.6%)	6 (37.5%)	-	1 (33.3%)	
1	54 (66.7%)	52 (66.7%)	2 (66.7%)	42 (67.7%)	10 (62.5%)	-	2 (66.7%)	
2	1 (1.2%)	1 (1.3%)	0	1 (1.6%)	0	-	0	
>2	0	0	0	0	0	-	0	
Not reported	0	0	0	0	0	-	0	
History of smoking								
N	81	78	3	62	16	0	3	0.631
Yes	38 (46.9%)	37 (47.4%)	1 (33.3%)	25 (40.3%)	12 (75.0%)	-	1 (33.3%)	
No	43 (53.1%)	41 (52.6%)	2 (66.7%)	37 (59.7%)	4 (25.0%)	-	2 (66.7%)	
Unknown	0	0	0	0	0	-	0	

ECOG, Eastern Cooperative Oncology Group. a Subjects can be counted in more than one category. FAS: Full Analysis Set, gAS: Guardant360 CDx analysis set, gNT: Guardant360 CDx not tested set, gCEAS: Guardant360 CDx primary clinical efficacy analysis set, gAS: Guardant360 CDx analysis set, gAS-F: Guardant360 CDx analysis set failed, gAS-Unk: Guardant360 CDx unknown set

7.3.10. Sensitivity Analysis Prevalence Sub-Study Population Representativeness Demographics and Baseline Clinical Characteristics

Demographics and baseline clinical characteristics of CHRYSALIS screen fail subjects and NILE study subjects included in the Guardant360 CDx^{+} local test^{-} sensitivity analysis are reported in **Table 44** and **Table 45** alongside those for the primary amivantamab-vmjw registration population (FAS). Prevalence sub-study (AAAS-L, AAAS-C and AAAS-P) subjects were similar to the FAS with regards to demographics and baseline clinical characteristics.

Table 44. Demographics of the Prevalence Sub-Study Subjects and the FAS

	CHRYSALIS			
	FAS	**AAAS-L**	**AAAS-C**	**AAAS-P**
Analysis set:	81	97	83	88
Age, years				
N	81	97	83	88
Mean (SD)	62.3 (9.96)	62.2 (9.99)	58.7 (11.06)	67.4 (9.6)
Median	62.0	62.0	59.0	66.5
Range	(42; 84)	(41; 84)	(34; 83)	41 - 91
<65	48 (59.3%)	56 (57.7%)	55 (66.3%)	41 (46.59%)
>=65	33 (40.7%)	41 (42.3%)	28 (33.7%)	47 (53.41%)
<75	74 (91.4%)	89 (91.8%)	75 (90.4%)	69 (78.41%)
>=75	7 (8.6%)	8 (8.2%)	8 (9.6%)	19 (21.59%)
Sex				
N	81	97	83	88
Female	48 (59.3%)	60 (61.9%)	52 (62.7%)	53 (60.23%)
Male	33 (40.7%)	37 (38.1%)	31 (37.3%)	35 (39.77%)
Race				
N	81	97	83	88
American Indian or Alaska native	0	0	0	0
Asian	40 (49.4%)	48 (49.5%)	47 (56.6%)	5 (5.68%)

CHRYSALIS				
	FAS	**AAAS-L**	**AAAS-C**	**AAAS-P**
Black or African American	2 (2.5%)	1 (1.0%)	0	7 (7.95%)
Native Hawaiian or other Pacific Islander	0	0	0	0
White	30 (37.0%)	38 (39.2%)	29 (34.9%)	73 (82.95%)
Multiple	0	0	0	
Not reported	9 (11.1%)	10 (10.3%)	7 (8.4%)	3 (3.41%)
Ethnicity				
N	81	97	83	88
Hispanic or Latino	3 (3.7%)	4 (4.1%)	2 (2.4%)	10 (11.36%)
Not Hispanic or Latino	68 (84.0%)	82 (84.5%)	72 (86.7%)	78 (88.64%)
Not reported	10 (12.3%)	11 (11.3%)	9 (10.8%)	0
Weight, kg				
N	81	97	0	N/A
Mean (SD)	67.49 (16.784)	65.17 (15.9)	-	N/A
Median	62.50	62.1	-	N/A
Range	(35.4; 115.0)	(35.4; 115.0)	-	N/A
Height, cm				
N	81	97	0	N/A
Mean (SD)	163.71 (9.020)	163.5 (8.7)	-	N/A
Median	162.60	163.0	-	N/A
Range	(144.5; 192.0)	(144.5; 192.0)	-	N/A
Body mass index, kg/m^2				
N	81	97	0	N/A
Mean (SD)	24.993 (4.9047)	24.2 (4.7)	-	N/A
Median	24.250	23.9	-	N/A
Range	(14.00; 36.87)	(14.0; 36.9)	-	N/A
Underweight <18.5	4 (4.9%)	8 (8.2%)	-	N/A
Normal 18.5-<25	43 (53.1%)	55 (56.7%)	-	N/A
Overweight 25-<30	21 (25.9%)	22 (22.7%)	-	N/A

CHRYSALIS				
	FAS	**AAAS-L**	**AAAS-C**	**AAAS-P**
Obese >=30	13 (16.0%)	12 (12.4%)	-	N/A
Local Test Type*				
N	81	97	83	88
NGS (Blood)	4 (4.9%)	6 (6.2%)	0	
NGS (Tissue)	34 (42.0%)	37 (38.1%)	1 (1.2%)	
OTHER (Blood)	1 (1.2%)	2 (2.1%)	0	
OTHER (Tissue)	7 (8.6%)	10 (10.3%)	0	
PCR (Blood)	1 (1.2%)	1 (1.0%)	0	
PCR (Tissue)	30 (37.0%)	36 (37.1%)	2 (2.4%)	88
UNKNOWN (Tissue)	4 (4.9%)	4 (4.1%)	1 (1.2%)	
UNKNOWN (Unknown)	0	1 (1.0%)	79 (95.2%)	

N/A-Not available. *Local test type as defined by the enrolling site.
FAS: Full Analysis Set, AAAS-L: Assay agreement analysis set – Local testing,
AAAS-C: Assay agreement analysis set – Central NGS tissue testing,
AAAS-P: Assay agreement analysis set – PCR testing

Table 45. Baseline Clinical Characteristics of the Prevalence Sub-Study Subjects and the FAS

CHRYSALIS				
	FAS	**AAAS L**	**AAAS C**	**AAAS P**
Analysis set:	81	97	83	88
Initial diagnosis NSCLC subtype				
N	81	97	83	88
Adenocarcinoma	77 (95.1%)	92 (94.8%)	0	84 (95.45%)
Large cell carcinoma	0	0	0	3 (3.41%)
Squamous cell carcinoma	3 (3.7%)	3 (3.1%)	0	N/A
Other	1 (1.2%)	2 (2.1%)	0	1 (1.14%)
Not reported	0	0	83 (100.0%)	0

CHRYSALIS				
	FAS	**AAAS L**	**AAAS C**	**AAAS P**
Histology grade at initial diagnosis				
N	81	97	83	N/A
Moderately differentiated	18 (22.2%)	21 (21.6%)	0	N/A
Poorly differentiated	12 (14.8%)	17 (17.5%)	0	N/A
Well differentiated	5 (6.2%)	6 (6.2%)	0	N/A
Other	46 (56.8%)	53 (54.6%)	0	N/A
Not reported	0	0	83 (100.0%)	N/A
Cancer stage at initial diagnosis				
N	81	97	0	88
0	0	0	-	0
IA	6 (7.4%)	6 (6.2%)	-	4 (4.55%)
IB	1 (1.2%)	1 (1.0%)	-	0
IIA	1 (1.2%)	2 (2.1%)	-	3 (3.41%)
IIB	4 (4.9%)	3 (3.1%)	-	0
IIIA	4 (4.9%)	4 (4.1%)	-	6 (6.82%)
IIIB	4 (4.9%)	4 (4.1%)	-	3 (3.41%)
IV	61 (75.3%)	77 (79.4%)	-	72 (81.82%)
Not reported	0	0	-	0
Location of metastasis				
N	81	97	83	N/A
Bone	34 (42.0%)	44 (45.4%)	0	N/A
Liver	7 (8.6%)	12 (12.4%)	0	N/A
Brain	18 (22.2%)	24 (24.7%)	0	N/A
Lymph Node	43 (53.1%)	55 (56.7%)	0	N/A
Adrenal Gland	3 (3.7%)	5 (5.2%)	0	N/A
Other	45 (55.6%)	52 (53.6%)	0	N/A
Not reported	0	0	83 (100.0%)	N/A

	CHRYSALIS			
	FAS	**AAAS L**	**AAAS C**	**AAAS P**
Time from initial diagnosis of cancer to first dose (months)				
N	81	97	0	N/A
Mean (SD)	22.905 (21.1901)	22.051 (20.7520)	-	N/A
Median	17.018	16.624	-	N/A
Range	(1.45; 130.10)	(1.45; 130.10)	-	N/A
				N/A
Time from metastatic disease diagnosis to first dose (months)				
N	81	97	0	N/A
Mean (SD)	18.071 (16.4424)	17.870 (15.7044)	-	N/A
Median	14.160	14.489	-	N/A
Range	(0.69; 116.40)	(0.69; 116.40)	-	N/A
Number of prior lines of therapy				
N	81	97	83	88
Mean (SD)	2.3 (1.41)	2.1 (1.34)	2.8 (1.52)	0
Median	2.0	2.0	2.0	0
Range	(1; 7)	(1; 7)	(0; 7)	(0; 0)
ECOG performance status				
N	81	97	83	88
0	26 (32.1%)	27 (27.8%)	0	19 (21.59%)
1	54 (66.7%)	69 (71.1%)	0	59 (67.05%)
2	1 (1.2%)	1 (1.0%)	0	7 (7.95%)
>2	0	0	0	1 (1.14%)
Not reported	0	0	83 (100.0%)	2 (2.27%)

CHRYSALIS				
	FAS	**AAAS L**	**AAAS C**	**AAAS P**
History of smoking				
N	81	97	83	88
Yes	38 (46.9%)	42 (43.3%)	19 (22.9%)	66 (75.00%)
No	43 (53.1%)	55 (56.7%)	45 (54.2%)	19 (21.59%)
Unknown	0	0	19 (22.9%)	3 (3.41%)

N/A, Not available. [a] Subjects can be counted in more than one category.
FAS: Full Analysis Set, AAAS-L: Assay agreement analysis set – Local testing,
AAAS-C: Assay agreement analysis set – Central NGS tissue testing,
AAAS-P: Assay agreement analysis set – PCR testing

Diagnostic Study Primary Objective Analysis Results

The primary objective was assessed by comparing the efficacy of single-agent amivantamab-vmjw in subjects positive for *EGFR* exon 20 insertions by Guardant360 CDx to the benchmark efficacy cited in the CHRYSALIS study and modeling the impact of the hypothetical Guardant360 CDx-positive local test-negative population and subjects without Guardant360 CDx results.

Safety Results

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

Primary Efficacy Results

The ORR observed in the primary objective analysis set (gCEAS) of the diagnostic study by blinded independent central review was 38.7% (95% CI 26.6% – 51.9%, **Table 46**). The lower limit of the 95% CI of 26.6% establishes statistically significant amivantamab-vmjw efficacy relative to the size-adjusted benchmark ORR of 14% (unadjusted benchmark 15%) from the CHRYSALIS clinical study in the Guardant360 CDx-positive, local test-positive portion of the intended use population and satisfies the prespecified efficacy acceptance criterion. The gCEAS ORR point estimate was also similar to the FAS ORR of 39.5% (95% CI 28.8% – 51.0%, **Table 46**).

Table 46. Summary of ORR in the gCEAS and FAS by BICR

	CHRYSALIS	
	gCEAS	FAS
Analysis set: Efficacy	**62**	**81**
Best overall response		
N	62	81
Complete response (CR)	2 (3.2%)	3 (3.7%)
Partial response (PR)	22 (35.5%)	29 (35.8%)
Stable disease (SD)	29 (46.8%)	39 (48.1%)
Progressive disease (PD)	7 (11.3%)	8 (9.9%)
Not evaluable/unknown	2 (3.2%)	2 (2.5%)
Overall response rate (Confirmed CR + Confirmed PR)	24 (38.7%)	32 (39.5%)
95% CI	(26.6%, 51.9%)	(28.8%, 51.0%)
Clinical benefit rate [a] (Confirmed CR + Confirmed PR + SD)	43 (69.4%)	60 (74.1%)
95% CI	(56.3%, 80.4%)	(63.1%, 83.2%)

Sensitivity Analyses for Primary Efficacy Objective for the Unrepresented Guardant360 CDx^{+} Local test^{-} Patient Population

The primary objective analysis above demonstrated amivantamab-vmjw efficacy in the Guardant360-positive, local test-positive subset of the Guardant360 CDx intended use population. The sensitivity analysis was done using the lower bound estimate of the 95% CI for the Pr(local test+|CDx+), which was 95.6%. Sensitivity analysis modeling efficacy across the entire Guardant360 CDx intended use population using BICR ORR demonstrates robustness to the contribution of the unrepresented Guardant360 CDx-positive, local test-negative subjects, with estimated ORRs for the overall Guardant360 CDx intended use population highly similar to those observed for both the gCEAS and FAS due to the low observed prevalence (0%) of the Guardant360 CDx-positive, local test-negative population. Moreover, the lower limits of the 95% CI for the estimated ORRs across all modeled conditions exceeded the size-adjusted benchmark ORR of 14%, which demonstrates statistically-significant amivantamab-vmjw efficacy across the entire Guardant360 CDx intended use population, irrespective of amivantamab-vmjw efficacy in the modeled hypothetical Guardant360 CDx-positive, local test-negative sub-population.

Secondary Objective Analyses

Agreement Between Guardant360 CDx and CHRYSALIS Enrollment Testing

Agreement between Guardant360 CDx and predominantly tissue testing in the total AAAS population (combined AAAS-L, AAAS-C and AAAS-P) is shown in **Table 47**. The Guardant360 CDx diagnostic study assay agreement analysis originally included 268 patients tested with Guardant360 CDx and other test results from both the CHRYSALIS and NILE clinical studies. The agreement analysis set included 97 patients with local test results (9 with plasma testing results, 87 with tissue testing results, 1 with test results using an unknown analyte), 83 screen-fail patients with central tissue test results from other cohorts of CHRYSALIS, and 88 with cobas® EGFR Mutation PCR tissue test results from the NILE study. The additional 19 samples (19/97) included in the positive agreement analysis had the same inclusion criteria as the primary registration population except that these began treatment after the clinical cutoff date and therefore did not have 3 post-baseline disease assessment at the clinical cutoff. The negative agreement analysis cohort did not include samples from the primary registration population, but the 83 samples were screen fails from other arms of the clinical study (non-*EGFR* exon 20 insertions arms of CHRYSALIS). Of the 83 screen-fail samples and the 88 samples from the NILE study, 4 and 3 samples, respectively, had *EGFR* exon 20 insertion mutations identified; and, therefore excluded from the negative agreement analysis. The remaining 164 samples were used for negative agreement analysis. The final number of samples used in the agreement analysis was 261.

Central testing for the screen fail samples utilized two different tissue-based NGS tests (69% with FoundationOne® CDx and 31% with Oncomine Dx Target Test) while samples from the NILE study were selected using the tissue-based PCR cobas® EGFR Mutation Test. Overall, the combination of the NILE clinical study and CHRYSALIS non-registration cohorts closely represents the local testing distribution used to enroll the registration population, both in terms of general test methodology (i.e. the registration population 40% PCR, 55% NGS; the supplemental cohorts 51% PCR, 49% NGS) and specific test methodology (i.e. the registration population enrolled by NGS with 35% Oncomine Dx Target Test, 65% FoundationOne® CDx; the supplemental cohorts with 31% and 69% respectively).Guardant360 CDx demonstrates high NPA (100%, 95% CI 97.7% – 100%) and relatively high PPA (80.4%, 95% CI 71.4% – 87.1%) relative to local testing results.

Table 47. Unadjusted Agreement Between CHRYSALIS Enrollment Testing, CHRYSALIS Central Testing, or cobas EGFR Testing and Guardant360 CDx (AAAS)

	CHRYSALIS Enrollment Testing, CHRYSALIS Central Testing, or cobas *EGFR* Testing		
	***EGFR* exon 20 insertion +**	***EGFR* exon 20 insertion -**	**Total**
Guardant360 CDx			
EGFR exon 20 insertion +	78	0	78
EGFR exon 20 insertion -	19	164	183
Total	97	164	261
PPA (95% CI)		80.4% (71.4% - 87.1%)	
NPA (95% CI)		100.0% (97.7% - 100.0%)	

Due to the enrichment of the AAAS-L population for subjects positive for *EGFR* exon 20 insertions, adjusted agreement was assessed using the PPV = P(local test^{+} | Guardant360 CDx^{+}) and NPV = P(local test^{-} | Guardant360 CDx^{-}) for the total AAAS population (combined AAAS-L, AAAS-C and AAAS-P). In this analysis, Guardant360 CDx demonstrated high adjusted PPV of 100% (95% CI, 95.6% - 100%) and NPV of 99.6% (95% CI, 99.5% - 99.8%) relative to local testing. The prevalence estimate P(local test+) used in the adjusted agreement was 1.8%.

7.4 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 mutation 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 KRAS Bridging Study Design for KRAS G12C Mutation

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 mutations

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.

a. Clinical Bridging Study Inclusion and Exclusion Criteria

All subjects in the primary sotorasib registration population were included in the diagnostic study efficacy cohort if the selection criteria below were met. Similarly, all subjects meeting the sensitivity analysis prevalence sub-study cohort selection criteria below are included.

- *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*

Additional subjects were included in the sensitivity analysis prevalence sub-study if the selection criteria below were met.

- 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.

b. 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.

c. 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 radiographic review (IRR). The Guardant360 CDx bridging study for NSCLC patients with a *KRAS* G12C mutation uses the same clinical endpoint for its primary objective.

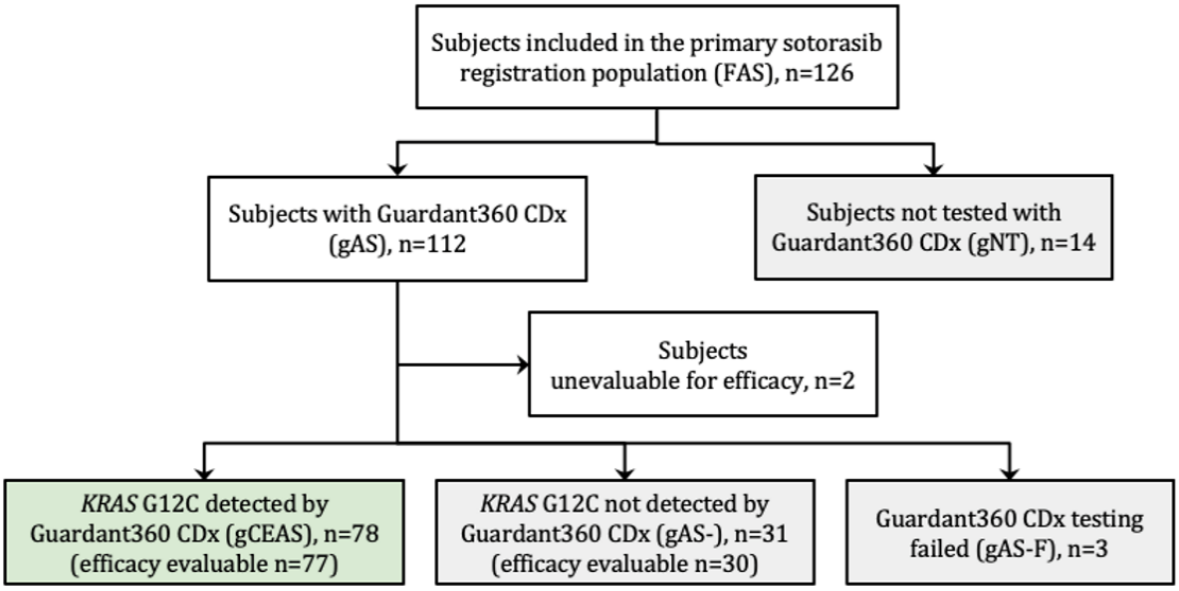
d. Diagnostic Objective and Endpoints

The primary objective of the clinical bridging study is to demonstrate the safety and effectiveness of Guardant360 CDx for the selection of 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.

Accountability of the PMA Cohort for the Guardant360 CDx Clinical Bridging Study for KRAS G12C Mutation

The Guardant360 CDx clinical bridging study included 112 of the total 126 (89%) patients in the Amgen 20170543 registration population (**Figure 7**). 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).

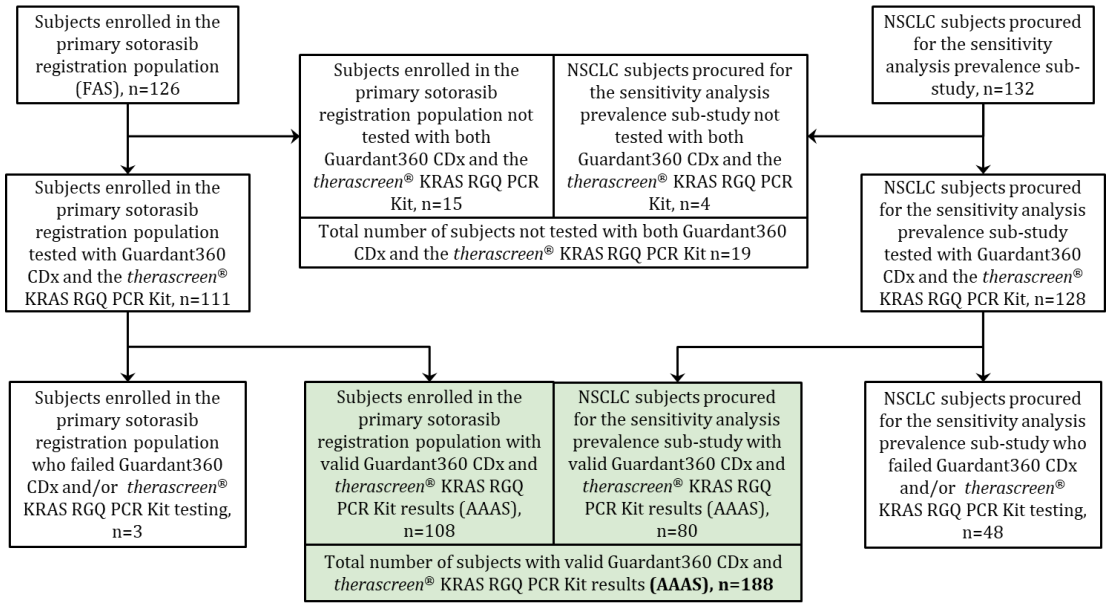
Figure 7. Guardant360 CDx *KRAS* G12C Mutation Bridging Study Efficacy Analysis Patient Accountability and Analysis Set Definitions



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 and the sensitivity analysis prevalence sub-study group (**Figure 8**).

Figure 8. 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 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 48** 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 7**).

For the concordance analysis (**Table 48**), 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 7**) 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 7** and **Table 48**).

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 48**).

Table 48. 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% – 100%)		

Study Population Demographics and Baseline Clinical 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.

As shown in **Table 49** and **Table 50**, 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 49. 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)
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 50. 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

	FAS	gCEAS	gAS	gAS-UNK
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)
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)

	FAS	gCEAS	gAS	gAS-UNK
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)
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)

	FAS	gCEAS	gAS	gAS-UNK
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 value reported in **Table 51** and **Table 52**. No meaningful differences were observed.

Table 51. 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)	

	gAS-E	gAS-Unk	p-value
>= 85 years	0 (0.0)	0 (0.0)	

Table 52. Comparison of Baseline Clinical Characteristics Between gAS-E and gAS-Unk

	gAS-E	gAS-Unk	p-value
ECOG status at baseline[a] - 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)[d]			
Mean	71.57	67.92	0.4158
Height (cm)[d]			
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[b,e] - 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[c]	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)	

	gAS-E	gAS-Unk	p-value
Poorly differentiated	19 (17.4)	5 (29.4)	
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)	
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)	

	gAS-E	gAS-Unk	p-value
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.

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

a. Safety Results

Data regarding the safety and efficacy 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 these claims as these involved retrospective testing of banked specimens only.

b. Effectiveness Results

i. ORR in Patients 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 53**. The observed ORR (38%, 95% CI 27% – 49%) is similar to that for the full primary sotorasib registration population (FAS, 36%, 95% CI 28% – 45%).

Table 53. 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 Kaplan-Meier method

ii. 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 highly similar to the observed (**Table 54** vs. **Table 53**, 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 54**) is 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 54. Sensitivity Analysis for the Guardant360 CDx$^+$ Tissue$^-$ Population

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

G360 CDx: Guardant360 CDx.

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 Guardant360CDx 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 55** 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 55. Sensitivity Analysis with Imputation for Subjects Without Valid Guardant360 CDx Results

	Simulated gCEAS
Objective response rate (ORR)	
Average number of overall responders – n (%)	32 (35.8)
95% CI	(34, 38)

Diagnostic Study Conclusions

The diagnostic study met the prespecified acceptance criterion associated with its primary objective. Clinically relevant drug efficacy was established by demonstrating that the ORR for subjects from the primary sotorasib registration population positive by Guardant360 CDx for *KRAS* G12C mutations (gCEAS, observed ORR 38%, 95% CI 27% – 49%) was superior to the prespecified benchmark ORR of 22% and was highly similar to that of the total primary sotorasib registration population (FAS, observed ORR 36%, 95% CI 28% – 45%).

Sensitivity analysis for the hypothetical Guardant360 CDx^{+} tissue^{-} population and imputation analysis for subjects without valid Guardant360 CDx results demonstrated robustness of the observed ORR to potential effects from these populations.

Guardant360 CDx and the *therascreen* KRAS RGQ PCR Kit using tissue were highly concordant in the detection of *KRAS* G12C mutations.

8 Additional Guardant360 CDx Variant Details

Table 56. Guardant360 CDx Reportable Alterations Based on cDNA and Amino Acid Changes

Gene (Transcript ID)	Reportable cDNA and Amino Acid Changes
AKT1 (NM_001014432)	E17K, R69_C77dup
ALK (NM_004304)	V1123S; T1151M; L1152P; L1152R; L1152V; C1156T; C1156Y; L1156Y; I1171N; I1171S; I1171T; F1174C; F1174L; F1174V; F1174I; F1174X; F1175C; F1175L; V1180L; L1196M; L1196Q; L1198F; G1202R; G1202del; D1203N; S1206C; S1206F; S1206Y; E1210K; D1225N; E1242K; F1245C; G1269A; R1275Q; P43A; R557C
APC (NM_001127511)	c.1312+1G>A; c.1312+1G>T; c.1409-1G>A; c.1548+1G>C; c.1744-1G>A; c.532-1G>A; c.730-1G>A; c.834+1G>A; c.834+2T>C; c.835-1G>A

Gene (Transcript ID)	Reportable cDNA and Amino Acid Changes
	Y1000*; N1026S; K1030*; Y1031*; Q1045*; W1049*; I1055fs; K1061*; Q1062fs; R1066fs; S1068*; E1080*; S1104*; E1111*; R1114*; G1120E; Q1123*; N1142fs; E1149*; E1156*; E1156fs; K1165*; E1168*; Q1175*; K1182*; Y1183*; K1192*; S1196*; Q1204*; E1209*; S1213fs; Q1244*; Q1260fs; S1281*; S1282*; E1286*; I1287fs; E1288*; G1288*; G1288fs; Q1291*; Q1294*; Q1294fs; E1295*; E1295fs; A1296fs; S1298fs; T1301fs; L1302fs; Q1303*; I1304fs; E1306*; E1306fs; I1307fs; E1309*; E1309fs; K1310*; K1310fs; I1311fs; G1312*; G1312fs; R1314fs; S1315*; E1317*; P1319fs; E1322*; E1322fs; S1327*; Q1328*; R1331*; R1331fs; Q1338*; Q1338fs; L1342fs; E1345*; S1346*; S1346fs; Q1349*; V1352fs; E1353*; E1353fs; S1355fs; S1356*; G1357*; Q1360*; S1364fs; G1365fs; Q1367*; K1370*; K1370fs; E1374*; Y1376*; Y1376fs; Q1378*; E1379*; M1383fs; R1386*; C1387*; S1392*; D1394fs; S1395C; F1396fs; E1397*; R1399fs; S1400L; S1400fs; A1402V; Q1406*; S1407fs; E1408*; Q1411*; S1411fs; V1414*; V1414fs; S1415fs; I1417fs; I1418fs; S1421fs; D1422fs; L1423fs; P1424fs; P1427fs; Q1429*; T1430fs; M1431fs; S1434fs; R1435fs; T1438fs; P1439fs; P1440fs; P1441fs; P1442fs; P1443fs; Q1444*; T1445fs; Q1447*; K1449*; K1449fs; R1450*; R1450fs; E1451*; V1452fs; N1455fs; A1457fs; E1461*; E1464fs; S1465fs; G1466R; Q1469fs; V1472fs; Q1477*; V1479fs; Q1480*; A1485fs; D1486fs; T1487fs; L1488fs; L1489fs; H1490fs; F1491fs; A1492fs; T1493fs; E1494fs; S1495fs; T1496fs; D1498fs; S1501fs; E1513*; F1515fs; D1519fs; E1521*; Q1529*; E1530*; N1531fs; E1536*; E1538*; E1538fs; S1539*; E1544*; S1545*; N1546fs; E1547*; N1548fs; Q1549*; E1550*; E1552*; E1552fs; A1553fs; E1554*; T1556fs; K1561fs; L1564*; S1567*; E1573*; E1576*; E1576fs; C1578fs; I1579fs; K1593fs; P1594fs; Q1621*; D1636fs; R1687*; D170fs; L1713fs; P173fs; N1792fs; R1858*; A1879fs; R1920*; A199V; H2063fs; S21*; E211*; R213*; S2140*; R216*; R2166Q; V2194fs; R2204*; Q222*; R2237*; E225*; R230C; S2307L; S2310*; R232*; G2332fs; Q236*; T2382fs; S2441*; Q247*; W2504*; S2555*; W2564*; R259W; I2615fs; E2619*; R2714C; H2770D; S280*; R283*; A290T; H298fs; N30fs; R302*; R332*; R348*; C352*; R405*; Q412*; W421*; Q424*; N436fs; V452fs; S457fs; Q473*; Q480*; R499*; Q532*; K534*; L540*; L548*; L548fs; W553*; R554*; R564*; E574*; K581fs; E582*; E582fs; S583*; L585fs; S587fs; W593*; S596*; L616fs; G618fs; Y622*; Y622fs; N627fs; S634fs; R640G; E658*; L665fs; K670*; W685*; A703fs; G721*; S747*; Q757*; Q767*; S770*; E771*; F773fs; L779*; D78fs; K782*; R786C; Q789*; Y796*; Y799fs; R805*; F814fs; L822fs; Y825fs; L826fs; P832fs; S837*; S843fs; D849fs; R854fs; E855*; E855fs; N869fs; R876*; V915fs; E918*; Y935*; Y935fs; N936fs; S940*; E941*; N942fs; S943*; C947fs; K953*; R976fs; G977fs; Q978*; E984*; E991*; K993*; Y997fs; Q999*
AR (NM_000044)	A270T; R630Q; Q641*; L702H; V716M; W742C; M750L; G796R; F814V; E873Q; H875Q; H875Y; T878A; T878S; M887I; S889G; D891H; M896V
ARAF (NM_001654)	S214A; S214C; S214F; S214Y; S214P

Gene (Transcript ID)	Reportable cDNA and Amino Acid Changes
BRAF (NM_004333)	S365L; R444W; R462E; R462I; I463S; G464V; G466V; G466A; G466E; G466R; S467L; F468C; G469A; G469E; G469L; G469V; G469R; G469S; V471F; L485F; K499E; E501K; L505H; L525R; N581H; N581S; N581T; N581Y; N581K; D587A; D587E; I592M; I592V; D594E; D594N; D594A; D594G; D594H; D594V; D594Y; F595S; G596C; G596D; G596R; G596S; G596V; L597Q; L597R; L597S; L597V; T599R; V600D; V600E; V600G; V600K; V600M; V600R; V600A; V600L; K601E; K601N; K601Q; K601R; S605N
BRCA1 (NM_007294)	M?; M1R; S1164I; Q1395Q; L1407P; K1487R; R1495K; R1495M; R1495T; E1559K; E1559Q; M1652K; V1653M; S1655F; G1656D; L1657P; E1660G; T1685A; T1685I; H1686Q; H1686R; M1689R; M1689T; T1691I; T1691K; D1692H; D1692Y; D1692N; V1696L; C1697R; R1699L; R1699Q; R1699W; T1700A; K1702E; Y1703H; Y1703S; F1704S; L1705P; G1706E; G1706R; A1708E; A1708V; V1713A; V1714G; S1715C; S1715N; S1715R; W1718C; W1718L; W1718S; S1722F; F1734L; F1734S; V1736A; V1736D; V1736G; G1738R; G1738E; D1739E; D1739G; D1739V; D1739Y; V1741G; G1743R; H1746N; P1749R; R1751P; A1752P; A1752V; R1753T; Q1756C; F1761I; F1761S; G1763V; L1764P; I1766S; G1770V; T1773I; M1775K; M1775R; M1775E; L1780P; C1787S; G1788V; G1788D; A1789T; M18T; G1803A; I1807S; V1809F; V1810G; Q1811R; P1812A; W1815*; E1817*; A1823T; V1833E; V1833M; R1835P; E1836K; W1837C; W1837G; W1837R; V1838E; S1841A; S1841N; S1841R; A1843P; A1843T; Y1853C; L1854P; L22S; C24R; C27A; E33A; T37R; T37K; C39Y; C39R; H41R; C44Y; C44F; C44S; C47G; C61G; A622V; C64G; C64W; C64Y; R71G; R71K; R71T; C1787_G1788delinsSD
BRCA2 (NM_000059)	M1?; A1393V; S142I; V159M; G173C; R174C; D191G; S196N; S206C; V211I; V211L; E2258K; R2336C; R2336H; R2336P; R2336L; P2532L; R2602T; W2626C; I2627F; L2647P; L2653P; R2659K; R2659T; E2663V; S2670L; I2675V; S2695L; T2722R; D2723A; D2723G; D2723H; G2748D; R2784W; N2829R; R2842C; E2918E; E3002K; P3039P; R3052W; D3095E; E3167E; E3342K
CCND1 (NM_053056)	P287H; T286A; T286I; P287L; P287A; P287S; P287T
CDK4 (NM_000075)	K22M; K22A; R24H; R24L; R24S; R24C
CDK6 (NM_001259)	R87Q
CDKN2A (NM_058195, NM_000077)	E10*; G101W; D108G; D108H; D108N; D108V; D108Y; W110*; P114H; P114L; P114T; S12*; E120*; G125R; A128D; Y129*; W15*; G23D; R24P; E27del; V28_E33del; R29_A34del; L32_L37del; G35_A36del; G35del; A36_N39delinsD; L37_Y44delinsVR; N39_N42del; Y44*; P48L; Q50*; Q50H; M53I; R58*; V59G; A60T; E61*; G67S; E69*; E69A; N71S; D74N; D74Y; D74A; G75V; R80*; R80Q; P81L; G83V; H83Q; H83R; H83Y; H83N; D84H; D84N; D84A; D84Y; R87W; E88*; E88K; A97G; A97V; R98L; H98P
CTNNB1 (NM_001904)	D32A; D32G; D32H; D32N; D32V; D32Y; S33A; S33C; S33F; S33P; S33T; S33Y; G34E; G34R; G34V; G34A; S37A; S37C; S37F; S37P; S37Y; T41A; T41I; T41N; S45C; S45F; S45P; S45Y; S45A

Gene (Transcript ID)	Reportable cDNA and Amino Acid Changes
EGFR (NM_005228)	Y1069C; R108G; R108K; E114K; R222C; S229C; R252P; T263P; A289D; A289T; A289V; R324L; R324C; E330K; V441D; V441G; R451C; S464L; G465E; G465R; K467T; I491M; I491R; S492G; S492R; P546S; D587H; P596L; G598A; G598V; C624Y; T638M; S645C; R671C; Q684H; P691S; L692F; L703P; L703V; E709A; E709G; E709K; E709Q; E709V; T710A; L718Q; L718V; G719A; G719C; G719D; G719R; G719S; S720P; A722V; F723L; G724S; T725M; V726M; Y727H; W731*; W731L; P733L; E734K; E734Q; G735S; V742A; K745R; E746G; E746K; E746Q; E746V; L747P; L747F; L747S; L747V; E749Q; A750P; A750E; T751I; S752Y; P753S; E758G; D761N; D761Y; V765A; S768I; V769M; V769L; N771D; H773L; H773Y; V774A; V774M; R776H; R776C; R776G; T783A; S784F; T785A; T790M; L792F; L792H; L792R; L792V; L792X; G796D; G796R; G796S; G796A; C797S; C797Y; C797G; C797D; C797W; Y801H; V802F; E804G; K806A; G810S; S811F; N826S; N826Y; R831H; L833V; V834L; H835L; R836C; D837N; L838P; L838V; L844V; V851I; T854S; T854A; T854I; G857E; L858R; L858M; L858Q; A859T; L861Q; L861R; L861F; L861P; A864V; A864T; E868G; H870R; A871G; E884K; Y891D
ERBB2 (NM_004448)	E265K; G279A; G279E; S280F; S280Y; G292R; G309A; G309E; S310F; S310Y; E321G; S653C; V659E; G660D; R678W; R678Q; L726F; L726I; T733I; D739Y; G746S; L755A; L755P; L755R; L755S; L755F; L755M; L755W; L755V; V762L; V762M; I767F; I767M; D769H; D769V; D769Y; D769N; L770P; V773A; G776D; G776S; G776V; V777A; V777L; V777M; P780L; V794M; T798I; T798M; D808N; D821N; N827S; V842I; N857S; T862A; L866M; L869R; H878Y; E884K
ESR1 (NM_001122742)	K303R; E380Q; V392I; S436P; S463P; L469V; R503W; V534E; P535H; L536H; L536P; L536R; L536Q; L536G; L536K; Y537S; Y537C; Y537D; Y537H; Y537N; D538G; D538E; T594R
FGFR1 (NM_023110)	S125L; P252T; M515V; N544K; N546D; N546K; N577K; K656N; K656E; K687E
FGFR2 (NM_000141)	D101Y; R203C; S252L; S252W; P253R; T268dup; F276C; K310R; S320C; C342Y; S354C; D374G; Y375C; C382R; C382Y; Y382H; C383Y; T524A; M536I; M537I; M538I; I547V; I548L; N549H; N549K; N550K; V564F; E565A; N638T; N639K; K658E; K658N; K659E; K659M; K659N; K660E; E731K
FGFR3 (NM_000142)	R248C; S249C; E322K; G370C; Y373C; Y375C; G380R; Y648S; K650E; K650M; K650N; K650Q; K650R; K650T; Y650F; G699C
GNA11 (NM_002067)	R183C; Q209L; Q209P
GNAQ (NM_002072)	R183Q; Q209L; Q209P; Q209R; T96S
HNF1A (NM_000545)	P291fs; G292fs
HRAS (NM_005343)	K117N; K117R; G12C; G12R; G12V; G12D; G12S; G12A; G13dup; G13R; G13V; G13C; G13D; A146T; A146V; A59G; A59T; Q61K; Q61L; Q61R; Q61H
IDH1 (NM_005896)	R132C
IDH2 (NM_002168)	R172G; R172K; R172M; R172S

Gene (Transcript ID)	Reportable cDNA and Amino Acid Changes
KIT (NM_000222)	C443Y; N463S; E490K; F504L; N505I; D52N; D52G; F522C; V530I; K550N; Y553N; Y553C; W557G; W557R; W557C; W557S; K558N; K558E; K558Q; K558P; V559C; V559D; V559G; V560D; V560G; V560A; V560E; N566D; V569G; Y570H; D572A; L576P; Y578C; Y578S; R634W; E635K; L641P; K642E; K642N; K642Q; V643A; L647P; I653T; V654A; V654E; N655K; N655S; N655T; T670E; T670I; N680K; H697Y; S709F; D716N; S746A; L783V; R804W; C809G; D816; D814V; D816F; D816H; D816V; D816Y; D816A; D816E; D816G; D816N; D820A; D820E; D820G; D820Y; D820H; D820V; D820N; S821F; N822H; N822I; N822K; N822Y; N822T; Y823D; V825A; A829P; P838L; I841V; S864F
KRAS (NM_004985)	G10dup; A11_G12dup; N116H; K117N; K117F; K117R; D119N; D119H; G12A; G12C; G12D; G12F; G12R; G12S; G12V; G12E; G12I; G12L; G12W; G12_G13dup; G13A; G13C; G13D; G13E; G13G; G13R; G13S; G13V; G13H; G13dup; G12_G13insAG; V14I; V14L; A146P; A146T; A146V; A146S; A18D; L19F; Q22E; Q22K; Q22R; Q22L; I24N; D33E; P34L; P34R; I36M; K5N; K5E; T50I; T58I; A59E; A59G; A59T; G60R; G60D; Q61H; Q61K; Q61L; Q61R; Q61E; Q61P; E62K; S65N; S65I; Y71H; Y71C; T74P; R97K
MAP2K1 (NM_002755)	I111N; I111S; I111A; I111P; I111R; H119P; E120D; C121R; C121S; P124L; P124S; P124Q; G128D; G128V; E203K; V211D; L215P; P264S; N382H; F53C; F53I; F53L; F53V; F53Y; F53S; Q56P; K57N; K57E; K57T; D67N; I99T
MAP2K2 (NM_030662)	C125S; P128Q; P128R; Y134H; Y134C; V215E; F57C; F57L; F57V; Q60P
MET (NM_000245)	Y1003C; Y1003F; Y1003N; P1009S; D1010H; D1010N; D1010Y; Y1021C; Y1021F; Y1021N; V1070A; V1070E; V1070R; V1088A; V1088E; V1088R; V1092I; V1092L; H1094L; H1094R; H1094Y; H1106D; V1110I; V1110L; H1112Y; H1112L; H1112R; N1118Y; H1124D; M1131T; M1149T; G1163R; T1173I; G1181R; V1188L; T1191I; L1195V; L1195F; V1206L; L1213V; F1218I; V1220I; D1228H; D1228N; Y1230C; Y1230H; Y1230S; Y1230F; Y1230N; Y1235D; Y1235H; V1238I; D1246H; D1246N; D1246V; Y1248C; Y1248H; Y1248S; Y1248D; M1250T; Y1253D; Y1253H; K1262R; M1268I; M1268T
MTOR (NM_004958)	L1433S; K1452N; W1456G; W1456R; A1459P; L1460P; C1483F; C1483W; C1483Y; E1799K; F1888L; F1888I; F1888V; T1977K; T1977I; T1977R; E2014K; S2215F; S2215T; S2215Y; L2230V; L2427P; L2427Q; I2500F; I2500M
NFE2L2 (NM_006164)	W24C; W24R; W24S; I28T; D29H; D29N; D29Y; L30F; L30P; G31A; G31R; G31V; V32G; R34G; R34Q; E63Q; E63V; D77G; D77H; E79D; E79K; E79Q; T80K; T80A; T80R; G81S; G81V; G81D; G81R; E82D; E82A; E82G; E82V
NRAS (NM_002524)	K117R; G12A; G12C; G12D; G12S; G12V; G12R; G12L; G13D; G13A; G13C; G13R; G13S; G13V; A146T; K170N; A18T; Q22K; D33E; K5N; T50I; T58I; A59G; A59T; G60E; Q61H; Q61K; Q61P; Q61R; Q61*; Q61E; Q61L; S65R
NTRK1 (NM_002529)	R342Q; T434M; L564H; V573M; R583P; F589L; G595R; G595L; A608D; F646I; G667S; G667C; D679G; R692C; R692H
NTRK3 (NM_001012338)	G623R; G696A
PDGFRA (NM_006206)	E229K; L275F; Y288C; V469A; V536E; V536M; Y555C; E556K; V561A; V561D; E563K; D568N; P577S; Q579R; A633T; H650Q; V658A; N659K; N659R; N659S; R748G; R841K; D842I; D842V; H845Y; D846Y; N848K; Y849C; Y849S; G853D; V859M

Gene (Transcript ID)	Reportable cDNA and Amino Acid Changes
PIK3CA (NM_006218)	Y1021C; Y1021H; T1025A; T1025S; D1029Y; P104L; M1043I; M1043L; M1043T; M1043V; N1044K; N1044Y; H1047L; H1047Q; H1047R; H1047Y; G1049R; G1049S; G106D; G106R; G106V; N1068Kfs; *1069fs; R108H; E110K; K111E; K111N; K111R; G118D; V344G; V344M; V344A; N345H; N345K; N345S; N345T; N345I; D350G; E365K; C378R; C378Y; R38C; R38G; R38H; R38L; R38S; E39K; E418K; C420G; C420R; P449T; E453A; E453D; E453K; E453Q; P539R; E542A; E542G; E542K; E542Q; E542V; E545A; E545D; E545G; E545K; E545Q; E545V; Q546H; Q546K; Q546L; Q546P; Q546R; Q546E; D549N; D578G; E579K; C604R; H701P; E726A; E726K; E81K; R88Q; C901F; G914R; R93Q; R93W
RAF1 (NM_002880)	R143Q; R143W; S257L; S257W; S259A; S259F; S259P; T260R; P261L; P261R; N262K; V263A; W368S; L397M; S427G; I448V; L613V; R73Q
RET (NM_020975)	A373V; Y606C; C618Y; P628_L633del; P628_L633delinsH; L629_D631delinsH; C630_D631del; D631_L633delinsE; D631_L633delinsA; D631_L633delinsV; E632_L633del; E632_T636delinsSS; L730I; L730V; E732K; V738A; V778I; V804E; V804L; V804M; Y806C; Y806N; A807V; G810A; G810S; G810R; R833C; I852M; V871I; R873W; A883F; S904F; M918T; S922F; G949R; F998V;
RHEB (NM_005614)	Y35N; Y35C; Y35H
ROS1 (NM_002944)	A1921G; L1951R; E1974K; V1979A; V1979M; 1981Tins; L1982F; L1982V; S1986F; S1986Y; E1990G; F1994L; M2001T; K2003I; F2004C; F2004I; F2004V; I2009L; L2028; E2020K; F2024C; F2024V; L2026M; L2026R; D2033; G2032R; D2033N; F2075C; F2075I; F2075V; V2089M; G2101A; N2112K; D2113G; R2116K; W2127*; M2128T; M2134I; L2155S; L2223*; N2224K
SMAD4 (NM_005359)	Q245*; E330A; E330G; E330K; D351G; D351H; D351N; D351Y; P356L; P356R; P356S; G358*; R361C; R361H; R361P; R361S; R361G; G386A; G386C; G386V; Y412*; R445*; D493N; D493A; D493H; R515*; W524C; W524L; W524R; D537E; D537H; D537V
SMO (NM_005631)	T241M; W281L; V321A; V321M; A324T; I408V; L412F; D473H; D473N; D473Y; G497W; S533N; W535R; W535L; R562Q
TERT (NM_198253)	c.-124C>T; c.-146C>T; c.-57A>C; c.-45G>T; c.-236G>A; c.-124C>A; c.-138C>T; c.-139C>T; c.-1G>A; c.-54C>A

Table 57. Guardant360 CDx Reportable Alterations Based on Exons and Codons

Gene (Transcript ID)	Alteration Type	Exon	Codon
BRAF (NM_004333)	Indel	12; 15	-
EGFR (NM_005228)	SNV	-	436; 441; 442; 451; 464; 465; 466; 489; 491; 492; 497; 498
EGFR (NM_005228)	Indel	18; 19; 20	-
ERBB2 (NM_004448)	Indel	19; 20	-
ESR1 (NM_001122742)	Indel	8; 10	-
KIT (NM_000222)	Indel	All in-frame, excluding splice site	-
MET (NM_000245)	SNV, Indel	14	-

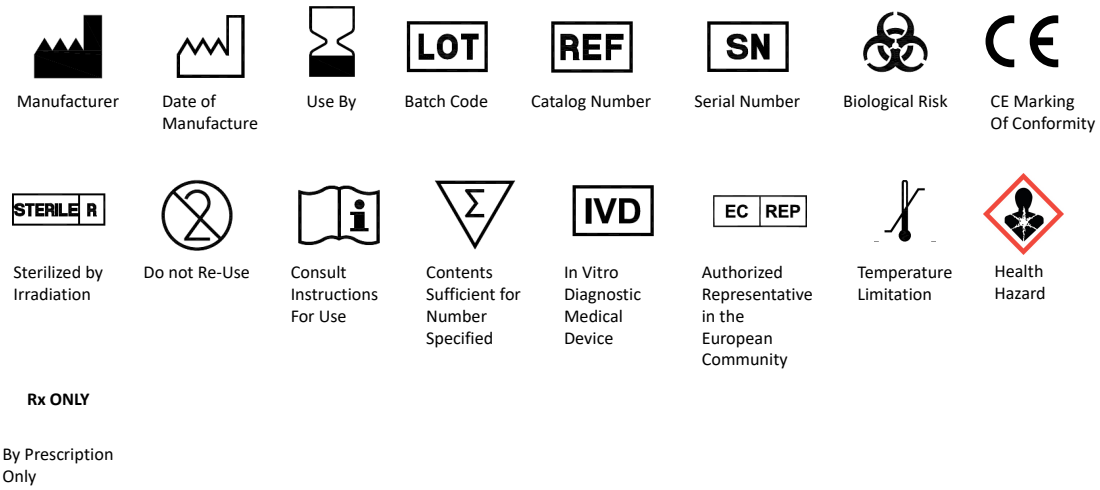
Gene (Transcript ID)	Alteration Type	Exon	Codon
MET (NM_000245)	SNV	19	-
MYC (NM_002467)	SNV	-	74, 161, 251
NFE2L2 (NM_006164)	SNV	-	24, 26, 27, 28, 29, 30, 31,32, 34, 77, 79, 80, 81, 82
PDGFRA (NM_006206)	Indel	All in-frame, excluding splice site	-
PIK3CA (NM_006218)	Indel	2; 8	-
ROS1 (NM_002944)	Indel	37	-

Table 58. Guardant360 CDx Reportable Alterations Based on Loss of Function

Gene (Transcript ID)	Reportable cDNA and Amino Acid Changes
BRCA1 (NM_007294)	Loss of function alterations found in all exons.
BRCA2 (NM_000059)	Loss of function alterations found in all exons.
CDH1 (NM_004360)	Loss of function alterations found in exons 3, 8, and 9.
GATA3 (NM_001002295)	Loss of function alterations found in exons 5 and 6.
MLH1 (NM_000249)	Loss of function alterations found in exon 12.
NF1 (NM_001042492)	Loss of function alterations found in exons 11 and 29.
PTEN (NM_000314)	Loss of function alterations found in all exons.
STK11 (NM_000455)	Loss of function alterations found in all exons.
TSC1 (NM_000368)	Loss of function alterations found in exons 15 and 23.
VHL (NM_000551)	Loss of function alterations found in all exons.

9 Additional Information

9.1 Symbols



Manufacturer | Date of Manufacture | Use By | Batch Code | Catalog Number | Serial Number | Biological Risk | CE Marking Of Conformity

Sterilized by Irradiation | Do not Re-Use | Consult Instructions For Use | Contents Sufficient for Number Specified | In Vitro Diagnostic Medical Device | Authorized Representative in the European Community | Temperature Limitation | Health Hazard

By Prescription Only

10 References

Meijuan Li. Statistical consideration and challenges in bridging study of personalized medicine. *J. Biopharma Stat.* (2015); 25: 397-407.

Last Name, First Name (Accession ID)
Patient MRN: NNNNNN | DOB: MMM-DD-YYYY | Sex: [Male/Female]
Diagnosis: [Cancer Type]

GUARDANT360 CDx

REPORTING

Report Date:	MMM-DD-YYYY
Receipt Date:	MMM-DD-YYYY
Collection Date:	MMM-DD-YYYY
Specimen:	Blood
Status:	[Status]

PHYSICIAN
First and Last Name
Site Name
Site Address
Ph: (xxx) xxx-xxxx | Fax: (xxx) xxx-xxxx
Additional Recipient: First and Last Name

Companion Diagnostic

Biomarker	Status	Additional Information
[Insert biomarker as appropiate]		
[Dynamic] {1, 2, 3} placed after *EGFR* exon 19 deletion, L858R, and/or T790M		

[Dynamic] [(1)]The MAF for EGFR exon 19 detection for this patient is <0.08%. Please refer below to Limitations section.
[Dynamic] [(2)]The MAF for EGFR L858R for this patient is <0.09%. Please refer below to Limitations section.
[Dynamic] [(3)]The MAF for EGFR T790M for this patient is <0.03%. Please refer below to Limitations section.

Other Biomarkers Identified

Results reported in this section are not prescriptive or conclusive for labeled use of any specific therapeutic product. See professional services section for additional information.

ctDNA Biomarkers with Strong Evidence of Clinical Significance in ctDNA †

Biomarker	Status	Additional Information
[Insert alteration as appropiate]		

† Please refer below to Performance Characteristics and Definitions sections for descriptions of categories.

Biomarkers with Evidence of Clinical Significance in Tissue and ctDNA †

Biomarker	Status	Additional Information
[Insert alteration as appropiate]		

† Please refer below to Performance Characteristics and Definitions sections for descriptions of categories.

Other Biomarkers with Potential Clinical Significance
Clinical significance has not yet been established for biomarkers in this section. See the professional services section for additional information.
- [Insert biomarker as appropiate]

Last Name, First Name (Accession ID)

Patient MRN: NNNNNN | DOB: MMM-DD-YYYY | Sex: [Male/Female]
Diagnosis: [Cancer Type]

Intended Use

Guardant360® CDx is a qualitative next generation sequencing-based in vitro diagostic 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 neoplasm. 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.

Warnings and Precautions

- 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.

Limitations

- For *in vitro* diagnostic use.
- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- 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.
- TAGRISSO® efficacy has not been established in patients with *EGFR* exon 19 deletions < 0.08% MAF, in patients with *EGFR* L858R <0.09% MAF, and in patients with *EGFR* T790M < 0.03% MAF.
- The test is not intended to be used for standalone diagnostic purposes.
- RYBREVANT™ efficacy has not been established in patients with *EGFR* exon 20 insertions < 0.02% MAF.
- LUMAKRAS™ efficacy has not been established in patients with *KRAS* G12C biomarkers < 0.11% MAF.
- The test is intended to be performed on specific serial number-controlled instruments by Guardant Health, Inc.
- A negative result for any given variant does not preclude the presence of this variant in tumor tissue.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care.
- ctDNA shedding rate may be lower in patients with primary central nervous system (CNS) tumors.

Performance Characteristics

Please refer to product label, www.guardant360cdx.com/technicalinfo. Clinical Performance has not been established for biomarkers in categories 2, 3A, 3B, and 4. Guardant360 CDx is indicated to report the following 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, TSC, VHL).* Indels *(ALK, AKT1, 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*), Fusion *(ALK , NTRK1, RET, ROS1)*, and Amplifications *(ERBB2, MET).*

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

Definition of Categories
The test report includes genomic finding reported in the following categories:

Category	Prescriptive use for Therapeutic Product	Clinical Performance	Analytical Performance	Comments
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* preclinical models, for which Guardant360 CDx has demonstrated minimum analytical performance.

Testing performed at: Guardant Health
Laboratory Director: [First and Last Name] | CLIA ID: [XXXXXX] | CAP #: [XXXXXX] | 505 Penobscot Drive Redwood City, CA, 94063, USA

Bruce, Wayne (A0123456)
Patient MRN: 987654321 | DOB: JAN-01-1976 | Sex: Male
Diagnosis: Non Small Cell Lung Cancer (NSCLC)

GUARDANT360 CDx

REPORTING

Report Date:	MAR-20-2017
Receipt Date:	MAR-04-2017
Collection Date:	MAR-03-2017
Specimen:	Blood
Status:	FINAL

PHYSICIAN

Dougie Houser
Center for People Who are Sick and Want to Get Better
123 Four St., Metropolis, NY, 12345, USA
Ph: (808) 555-1234 | Fax: N/A
Additional Recipient: N/A

Companion Diagnostic

Biomarker	Status	Additional Information
KRAS G12C	**DETECTED**	*KRAS* G12C LUMAKRAS™ (sotorasib) is FDA approved for this indication
EGFR T790M	NOT DETECTED	
EGFR L858R	NOT DETECTED	
EGFR exon 19 Deletions	NOT DETECTED	
EGFR exon 20 Insertions	NOT DETECTED	

Last Name, First Name (Accession ID)

Patient MRN: NNNNNN | DOB: MMM-DD-YYYY | Sex: [Male/Female]
Diagnosis: [Cancer Type]

GUARDANT360 CDx

Intended Use

Guardant360® CDx is a qualitative next generation sequencing-based in vitro diagostic 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 neoplasm. 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.

Warnings and Precautions

- 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.

Limitations

- For *in vitro* diagnostic use.
- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- 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.
- TAGRISSO® efficacy has not been established in patients with *EGFR* exon 19 deletions < 0.08% MAF, in patients with *EGFR* L858R <0.09% MAF, and in patients with *EGFR* T790M < 0.03% MAF.
- The test is not intended to be used for standalone diagnostic purposes.
- RYBREVANT™ efficacy has not been established in patients with *EGFR* exon 20 insertions < 0.02% MAF.
- LUMAKRAS™ efficacy has not been established in patients with *KRAS* G12C biomarkers < 0.11% MAF.
- The test is intended to be performed on specific serial number-controlled instruments by Guardant Health, Inc.
- A negative result for any given variant does not preclude the presence of this variant in tumor tissue.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care.
- ctDNA shedding rate may be lower in patients with primary central nervous system (CNS) tumors.

Performance Characteristics

Please refer to product label, www.guardant360cdx.com/technicalinfo. Clinical Performance has not been established for biomarkers in categories 2, 3A, 3B, and 4. Guardant360 CDx is indicated to report the following 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, TSC, VHL).* Indels *(ALK, AKT1, 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*), Fusion *(ALK , NTRK1, RET, ROS1)*, and Amplifications *(ERBB2, MET).*

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

Definition of Categories
The test report includes genomic finding reported in the following categories:

Category	Prescriptive use for Therapeutic Product	Clinical Performance	Analytical Performance	Comments
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* preclinical models, for which Guardant360 CDx has demonstrated minimum analytical performance.

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