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 patients who may benefit from treatment with the therapies listed in **Table 1** in accordance with the approved therapeutic product labeling.

Table 1. Companion Diagnostic Indications

Indication	Biomarker	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions, L858R, and T790M*	TAGRISSO® (osimertinib)
	EGFR exon 20 insertions	RYBREVANT® (amivantamab-vmjw)
	ERBB2/HER2 activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
	KRAS G12C	LUMAKRAS™ (sotorasib)
Breast cancer	*ESR1* missense mutations between codons 310 and 547	ORSERDU™ (elacestrant)
	ESR1 E380, V422del, S463, L469, L536, Y537, and D538 mutations	INLURIYO™ (imlunestrant)
Colorectal cancer	*BRAF* V600E	BRAFTOVI® (encorafenib) in combination with ERBITUX® (cetuximab)

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

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

Additionally, the test is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with any solid malignant 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

- 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.

4. 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.
- 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.
- ENHERTU efficacy has not been established in patients with *ERBB2* exon 20 insertions < 0.03% MAF and in patients with *ERBB2* SNVs < 0.23% MAF.
- ORSERDU efficacy has not been established in patients with *ESR1* missense mutations < 0.03% MAF.
- INLURIYO efficacy has not been established in patients with *ESR1* V422 deletion < 0.04% MAF and in patients with *ESR1* SNVs < 0.02% MAF.
- BRAFTOVI + ERBITUX efficacy has not been established in patients with *BRAF* V600E mutation < 0.04% MAF.
- The test is not intended to be used for standalone diagnostic purposes.

- 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.

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 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	Guardant360 CDx			Comments
	Prescriptive use for a Therapeutic Product	Clinical Performance	Analytical 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:

- Whole Blood Collection and Shipping
- Plasma Isolation and cfDNA Extraction
- Library Preparation and Enrichment
- DNA Sequencing
- 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, up to 30 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 for all samples that pass QC, including minimal unique molecule coverage that ensures sufficient input material was successfully processed through the assay. This QC is applied to all samples with cfDNA input spanning the input range up to 30ng. 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 **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.

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

- 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 439 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. 412 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. Collection set four consisted of 53 samples from the Guardant Health biobank based on Guardant360 LDT results to include additional Category 3 variants.

Of 412 patients, two samples failed QC on Guardant360 CDx, and three samples failed with the comparator NGS assay. In total, 407 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 (188), gastrointestinal (82), colon (24), breast (48), 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 (8), and other (6). 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.*, 188 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 sets 3 and 4 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 eighteen (18) 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 95.0% PPA and 100% NPA for the Category 2 variants.

Positive agreement rates for clinical Categories 3 and 4 variants resulted in 92.8% PPA and 77.7% PPA, respectively. Variants in clinical category 3 and 4 showed 99.8% and 99.9% NPA.

MET amplifications had a PPA of 57.7%, 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 73.2% for indels, 87.2% 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	19	0	1	220	5	48	95.0% (75.1%, 99.9%)	100.0% (98.3 %, 100.0%)
Category 3 Variants	207	22	16	10220	86	N/A*	92.8% (88.6%, 95.8%)	99.8% (99.7%, 99.9%)
Category 4 Variants	404	92	116	155269	383	407	77.7% (73.9%, 81.2%)	99.9% (99.9%, 100.0%)
MET CNAs	15	3	11	378	1	407	57.7% (36.9%, 76.7%)	99.2% (97.7%, 99.8%)
ERBB2 CNAs	26	1	2	378	1	407	92.9% (76.5%, 99.1%)	99.7% (98.5%, 100.0%)
NTRK1 Fusions	6	0	0	401	1	407	100.0% (54.0%, 100.0%)	100.0% (99.1%, 100.0%)
RET Fusions	14	3	1	389	1	407	93.3% (68.1%, 99.8%)	99.2% (97.8%, 99.8%)

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)
ALK Fusions	10	2	0	395	1	407	100.0% (69.2%, 100.0%)	99.5% (98.2%, 99.9%)
ROS1 Fusions	11	0	0	396	1	407	100.0% (71.5%, 100.0%)	100.0% (99.1%, 100.0%)
Panel-Wide SNVs	497	76	73	125117	309	407	87.2% (84.2%, 89.8%)	99.9% (99.9%, 100.0%)
Panel-Wide Indels	131	35	48	64092	158	407	73.2% (66.1%, 79.5%)	100.0% (99.9%, 100.0%)

* 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 and *ERBB2* activating mutations (SNVs and 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 on *EGFR* exon 20 insertions 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 92% (24/26) of discordances observed were for variants with allelic fractions below the comparator LoD.

NSCLC samples from 205 patients were tested for the study on *ERBB2* activating mutations (SNVs and exon 20 insertions), including samples from all available subjects tested in the associated clinical study with sufficient remnant material for testing with the comparator method. No samples failed testing on the comparator, while two samples failed testing on Guardant360 CDx and were excluded from the subsequent analysis. PPA and NPA are reported in **Table 5** below.

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	80	25	1	166	272	98.76% (93.31%, 99.96%)	86.91% (81.29%, 91.35%)
ERBB2 activating mutations (SNVs and exon 20 insertions)	85	10	1	107	203	98.8% (93.7%, 100.0%)	91.5% (84.8%, 95.8%]

c. Concordance - Comparison to Mass Spectrometry-Based 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.

An analytical accuracy study was performed for *ESR1* mutations with 259 samples from patients in the RAD1901-308 clinical study selected without reference to biomarker status. All samples were tested by both Guardant360 CDx and the externally validated comparator method. Eligible *ESR1* mutations were detected in 141 out of 254 samples (55.5%) for Guardant360 CDx (which excluded one QC failure and two pairs of duplicated samples), and 124 out of 254 samples (48.8%) for the comparator method. The analyses only included non-duplicated samples that passed QC on both platforms (N=254). **Table 6** summarizes the sample-level agreement between Guardant360 CDx and the comparator method.

Table 6. Summary of Concordance Between Guardant360 CDx and 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)
ESR1 mutations	121	20	3	110	254	98% (93%, 99%)	85% (77%, 90%)	86% (79%, 91%)	97% (93%, 99%)
KRAS G12C	102	6	4	101	213	96% (91%, 99%)	94% (88%, 98%)	94% (88%, 98%)	96% (91%, 99%)

Due to poor sensitivity of the mass-spectrometry based comparator for *ESR1* V422del, positive agreement was additionally assessed with an externally validated NGS method. PPA of *ESR1* V422del with this comparator for samples was 90% (9/10, 95% CI 55.5% – 99.7%) at analyte levels observed in the intended use population (0.1-3% MAF).

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

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

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

d. Concordance - Comparison to NGS Comparator Method #4

The detection of alterations by Guardant360 CDx was compared to results of an externally validated NGS Comparator Method #4.

The detection of *BRAF* V600E was tested using 219 samples from patients with colorectal cancer. Of the 219 tested samples, 213 (98.2%) samples passed Guardant360 CDx QC and 209 (95.4%) samples passed comparator QC; 208 (95.0%) passed QC on both Guardant360 CDx and the comparator. *BRAF* V600E was detected in 103 out of 208 samples (49.5%) with Guardant360 CDx and 102 out of 208 samples (49.0%) with the comparator. Since the samples were selected from different sources based on different assays, the unadjusted agreements in Table 8 may be subject to potential bias. **Table 8**

summarizes the sample-level agreement between Guardant360 CDx and the comparator for the detection of *BRAF* V600E.

Table 8. Summary of Concordance Between Guardant360 CDx and Comparator Method #4

Alteration Type	Guardant360 CDx (+), Comparator (+)	Guardant360 CDx (+), Comparator (-)	Guardant360 CDx (-), Comparator (+)	Guardant360 CDx (-), Comparator (-)	Patients (n)	PPA (95% CI)	NPA (95% CI)
BRAF V600E	101	2	1	104	208	99.0% (94.7%, 100.0%)	98.1% (93.4%, 99.8%)

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 9**). Cell line cfDNA sample pools were tested across 13-20 replicates, 13 replicates for level 6, 14 replicates for level 2, and 20 replicates for the other levels at 5 ng cfDNA input. Clinical cfDNA sample pools from multiple cancer types were tested with 14 replicates at 5 ng cfDNA input. Both cell line and clinical cfDNA sample pools were tested with 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 9**.

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 9. Fusion Detection Rate in the CSFC study

Fusion	Sample Type	Detection Rate (95% confidence interval)					
		Level 1 Target MAF 0.07%	Level 2 Target MAF 0.175%	Level 3 Target MAF 0.35%	Level 4 Target MAF 0.7%	Level 5 Target MAF 1.4%	Level 6 Target MAF 1.8%
EML4-ALK	Cell line	5.0% (0.1%, 24.9%)	28.6% (8.4%, 58.1%)	50.0% (27.2%, 72.8%)	90.0% (68.3%, 98.8%)	100.0% (83.2%, 100.0%)	100.0% (75.3%, 100%)
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%)

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%
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 10**. The false positive rate was zero for Category 1 (CDx) and Category 2 variants.

Table 10. LoB Study Summary Results

Category	Per Position False Positive Rate	Per Sample False Positive Rate
Category 1: *EGFR* L858R	0%	0 (0/240)
Category 1: *EGFR* T790M	0%	0 (0/240)
Category 1: *EGFR* exon 19 deletions	0%	0 (0/240)
Category 1: *EGFR* exon 20 insertions	0%	0 (0/240)
Category 1: *ERBB2* activating mutations (SNVs and exon 20 insertions)	0%	0 (0/240)
Category 1: *ESR1* mutations	0%	0 (0/240)
Category 1: *KRAS* G12C	0%	0 (0/240)
Category 1: *BRAF* V600E	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 number 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 11**. The LoD was confirmed for these CDx variants using cfDNA sample pools from patients with NSCLC only; refer to **Table 13** below.

The LoDs for *ERBB2* activating mutations (SNVs and exon 20 insertions) were established using pools of cfDNA from clinical plasma samples from NSCLC patients. The LoD for *ERBB2* activating SNV mutations was established to be 1.3% MAF at 5 ng cfDNA input and 0.3% MAF at 30 ng cfDNA input (**Table 11**). The LoDs for *ERBB2* activating exon 20 insertions were established to be 1.3% and 1.0% MAFs at 5 ng cfDNA input for insertion sizes of 9 bp and 12 bp, respectively. The LoD for *ERBB2* activating exon 20 insertion of 12 bp at 30 ng cfDNA input was established to be 0.4% MAF. The *ERBB2* activating exon 20 insertion of 9 bp at 30 ng cfDNA input was not determined as all dilutions tested down to 0.1% MAF were detected at 100%.

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 12**). The established LoD was further confirmed in clinical 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 11**). These confirmed LoD values were utilized in other performance studies (*e.g.*, precision, guard banding 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 Precision**).

The LoD for *ESR1* mutations was established using sample pools prepared from *ESR1* mutation-positive breast cancer samples and is summarized in **Table 11**.

Table 11. Summary of LoDs for Alterations Associated with CDx Claims using Pools of cfDNA from Clinical Plasma Samples

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, 6, 9, and 12 bp)	1.4% MAF* (0.8%-1.8%)	0.3% MAF
ERBB2 SNVs	SNV	1.3% MAF* (1.0%-1.8%)	0.3% MAF* (0.2%-0.5%)
ERBB2 exon 20 insertions	Insertion (9 bp)	1.3 % MAF	ND
	Insertion (12 bp)	1.0 % MAF	0.4% MAF

Alteration	Alteration Type	LoD (5 ng input)	LoD (30 ng input)
ESR1 missense mutations	SNV	1.1% MAF^	0.3% MAF^
KRAS G12C	SNV	1.8% MAF	0.5% MAF

* Mean MAF. MAF range shown in parenthesis. ND: Not determined; all dilutions down to 0.1% MAF were detected at 100%.
^ The MAF values were established for prevalent *ESR1* mutations (E380Q, Y537S, and D538G).

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

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

The higher of the LoD values established using cell lines and confirmed using clinical samples were used to claim the LoD performance levels of the test for fusions at 5 ng (**Table 12**).

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

Alteration	Alteration Type	LoD, 5 ng (MAF/CN)	LoD, 30 ng (MAF/CN)
BRAF V600E	SNV	1.8%	0.2%
KRAS G12V	SNV	1.5%	0.5%
NRAS Q61R	SNV	3.0%	0.8%
ESR1 E380Q	SNV	1.0%	0.3%
ESR1 Y537S	SNV	1.0%	0.3%
ESR1 D538G	SNV	1.1%	0.2%
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%
EGFR exon 20 insertion, A767_V769dup*	Insertion (9 bp)	1.4%	0.3%
EGFR exon 20 insertion, H773dup*	Insertion (3 bp)	0.9%	NA
EGFR exon 20 insertion, N771_ H773dup*	Insertion (9 bp)	1.8%	0.3%
EGFR exon 20 insertion, P772_H773dup*	Insertion (6 bp)	1.5%	NA
EGFR exon 20 insertion, P772_H773insQANP*	Insertion (12 bp)	1.8%	NA
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%)

Alteration	Alteration Type	LoD, 5 ng (MAF/CN)	LoD, 30 ng (MAF/CN)
ROS1	Fusion	1.9% (1.2%)	(0.2%)
ALK	Fusion	1.4% (1.5%)	(0.2%)

Note: *NSCLC sample pool background. 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 13**) 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 Precision** below for additional studies demonstrating assay precision starting from cfDNA extraction, and with additional mutation positive and negative samples) samples were tested across three precision combinations that evaluated three operator groups, three instrument combinations, and three SPK reagent lots over at least three different start dates.

The higher of the LoD values established using clinical sample pools from cancer patients and confirmed using clinical samples exclusively from the relevant cancer type source material were used to claim LoD performance of the test at 5 ng input as summarized in **Table 13**.

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

Alteration	MAF	Alteration Type	Cancer Type	Number Positive / Number Expected	PPA
BRAF V600E	2.7%	SNV	Colon	24/24	100.0%
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%
ESR1 E380Q	1.0%**	SNV	Breast	24/24	100.0%
ESR1 Y537S	1.0%**	SNV	Breast	23/24	95.8%
ESR1 D538G	1.1%**	SNV	Breast	23/24	95.8%
ESR1 G442A	2.3%^	SNV	Breast	24/24	100.0%
ESR1 S436P	2.8%^	SNV	Breast	24/24	100.0%
ESR1 V422del	3.4%^	Deletion (3 bp)	Breast	24/24	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%

Alteration	MAF	Alteration Type	Cancer Type	Number Positive / Number Expected	PPA
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 or breast cancer 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 14** as median values.

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

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

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 an exogenous interfering substance 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 were 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* (10^6 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.g Precision from Plasma Evaluation of Extraction Precision and Precision of Downstream Steps.** 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 15**.

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 15**). *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 15**).

Table 15. 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%)

Alteration Class	Alteration	Number Positive / Number Expected	PPA (95% CI)
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%)

* PPA for *ERBB2* and *MET* CNAs after reanalyzing the data with BIP version 3.5.5 are 93.3% (77.9%, 99.2%) and 76.7% (57.7%, 90.1%), respectively. These discrepancies are attributed to CNV caller retraining with BIP version 3.5.5, resulting in an adjusted copy number baseline across *MET* CNAs, and do not impact the safety and the effectiveness of the device.

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 Limit of Detection (LoD)**.

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 16**).

Table 16. 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 ERBB2 Activating Mutations (SNVs and Exon 20 Insertions) from NSCLC cfDNA Clinical Sample Pools

A precision study evaluated five *ERBB2* activating mutations (SNVs and 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 *ERBB2* activating mutations (SNVs and exon 20 insertions) with mutation-negative cfDNA derived from NSCLC clinical samples. Each variant was tested across six precision combinations at 5 ng input at MAF levels ranging from 1.0x to 1.4x LoD.

PPA ranged from 95.7% to 100% across specific variants and was 99.2% across all variants and precision combinations (**Table 17**).

Table 17. Summary of Precision PPA Results for *ERBB2* Activating Mutations (SNVs and Exon 20 Insertions)

Alteration	Number Positive / Number Expected	PPA (95% CI)
ERBB2 SNVs	70 / 71	98.6% (92.4%, 100.0%)
ERBB2 exon 20 insertions	47 / 47	100% (92.5%, 100.0%)

d. 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 18**). The acceptance criteria were met with a positive precision of 100% at both 5 and 30 ng cfDNA inputs.

Table 18. Summary of Precision Results for *KRAS* G12C

Input Amount	Concordant / Expected Positives	PPA (95% CI)
5 ng	42/42	100% (91.6% - 100.0%)
30 ng	18/18	100% (81.5% - 100.0%)

e. Precision for ESR1 mutations

Precision of *ESR1* mutations on Guardant360 CDx was analyzed for *ESR1* H356D, E380Q, G442A, S463P, Y537S, and D538G at 5 ng cfDNA input using breast cancer patient samples. Each mutation was tested at 1-3X LoD, which was established for prevalent *ESR1* mutations (E380Q, Y537S, and D538G), with 24 replicates across 6 unique reagent lot-instrument-operator combinations, which are the main sources of variability in an automated assay (**Table 19**).

Table 19. Summary of Precision Results for *ESR1* Mutations

ESR1 Mutation	Observed MAF%	Relative LoD Level[1]	Number Positive/ Number Expected	PPA (95% CI)
E380Q	1.0	1.0x	24/24	100% (85.8%-100%)
Y537S	1.0	1.0x	23/24	95.8% (78.9% - 99.9%)
D538G	1.1	1.0x	23/24	95.8% (78.9% - 99.9%)
H356D	2.1[2]	2.0x	20/24	83.3% (62.6% - 95.3%)[3]
H356D	3.1[2]	2.9x	22/24	91.7% (73.0% - 99.0%)[3]

ESR1 Mutation	Observed MAF%	Relative LoD Level[1]	Number Positive/ Number Expected	PPA (95% CI)
G442A	2.3	2.1x	24/24	100% (85.8% - 100%)
S463P	2.8	2.6x	24/24	100% (85.8% - 100%)
V422del	3.4	1.3x	24/24	100% (85.8% - 100%)

[1] Compared to the established LoD for the prevalent *ESR1* missense mutations. For V422del, compared to the panel-wide indel LoD.
[2] Note that the observed MAF is the average variant MAF from all samples with a reported variant (i.e., excluding dropouts).
[3] PPA for *ESR1* H356D mutations after reanalyzing the data with BIP version 3.5.5 at 2.1% and 3.1% MAF are 95.8% (78.9% - 99.9%) and 100% (85.8% - 100%), respectively. These changes reflect improvements in *ESR1* H356D precision due to changes made to the BIP software and do not impact the safety and effectiveness of the device.

f. Precision for BRAF V600E mutations

The purpose of the precision study was to verify the positive precision of Guardant360 CDx for *BRAF* V600E using CRC patient samples. *BRAF* V600E positive cfDNA from CRC patients was diluted in a background of *BRAF* V600E negative CRC patient cfDNA to target MAFs either within 1.0x-1.5x or 2.0x-3.0x of the established LoD. The samples were tested on Guardant360 CDx at 5 ng cfDNA input with 24 replicates across 6 unique reagent lot-instrument-operator precision combinations of fully crossed instruments (2) and reagent lots (3) over at least three different start dates.

Positive precision was analyzed at both 1.0x-1.5x and 2.0x-3.0x the established LoD through the assessment of PPA (# positives passing QC / # tested passing QC) across all precision combinations. One sample with MAF at 2.0x-3.0x LoD failed enrichment QC and was excluded from sequencing and subsequent analysis. All other samples passed QC. At an observed MAF of 2.7%, which is 1.5x the established LoD of Guardant360 CDx for *BRAF* V600E, the PPA was 100% [24/24] (**Table 20**). At an observed MAF of 5.3%, which is 2.9x the established LoD of Guardant360 CDx for *BRAF* V600E, the PPA was 100% [24/24] (**Table 20**).

Table 20. Summary of *BRAF* V600E Positive Precision Results

Mutation	Observed MAF%	Relative LoD Level	Number Positive/ Number Expected	PPA (95% CI)
BRAF V600E	2.7%	1.5X	24/24	100% (85.8% - 100.0%)
	5.3%	2.9X	23/23	100% (85.2% - 100.0%

The PPA at both MAF levels were ≥ 95%. The results demonstrate the positive precision of Guardant360 CDx in CRC patient specimens at both near (1.0x-1.5x) and above (2.0x-3.0x) LoD.

g. 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 21**.

Table 20. 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 four together are shown in **Table 22**. 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 22**.

Table 21. 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%
CNA	15	13	2	92.9%*
Fusion	12	12	0	100%

Variant Type	Variant Lot Comparisons	Concordant (C)	Discordant (D)	APA
ALL	212	201	11	97.3%

* APA for CNAs after reanalyzing the data with BIP version 3.5.5 is 100%. The change reflects improvements in CNA precision due to changes made to the BIP software and does not impact the safety and effectiveness of the device.

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 23**. For each of these variants, there were 12 pairwise comparisons.

Table 22. 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.

h. 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. Guard Banding/ Robustness

The purpose of the guard banding 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 24**).

Table 23. Guard Banding Study Overview

Guard Banding 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 25**). The QDR was 100% with a QDR lower limit of the 95% confidence interval (LLCI) of 85.47%. For each tested guard banding 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 24. Guard Banding Results Summary

Guard Banding 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 24**); QDR: qualitative detection rate.

These results demonstrate the robustness of Guardant360 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 T0). 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.

An additional study was conducted with whole blood samples collected in four BCTs from 11 breast cancer patients subjected to the same reference, summer profile, winter profile, and room temperature conditions described above, and plasma was isolated on the eighth day. In total, 43 out of 44 samples passed all sequencing QC metrics. All 4 samples from one patient were excluded from analysis due to the reference condition not containing sufficient cfDNA input. After removing these samples, a total of 10 patient groups were evaluable for the winter and room temperature storage conditions and 9 patient groups were evaluable for the summer storage condition.

The fold change of median NSC in storage condition over the reference condition or time zero ranged between 0.87 and 1.00. The 90% two-sided Clopper-Pearson exact binomial lower confidence limit for the fraction of genomic targeted exonic regions with relative exon-level NSC within 2σ of that for the reference condition, where σ = 0.204, ranged from 98.3% to 98.7%. These data indicate that whole blood samples collected from breast cancer patients are stable under the shipping and storage conditions tested.

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 0.99. 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.

Additionally, the stability of plasma isolated from breast cancer patients was studied using whole blood specimens collected from 22 donors. For the reference condition, cfDNA was extracted after plasma isolation within 48 hours of delivery. For the test storage condition, plasma was stored at -80°C ± 10°C for ≥ 45 days before cfDNA extraction. All 44 samples passed their respective in-process and post sequencing QC metrics leading to 22 evaluable sample pairs.

The fold change of median NSC in storage condition over the reference condition was 0.94. The 90% two-sided Clopper-Pearson exact binomial lower confidence limit for the fraction of genomic targeted exonic regions with relative exon-level NSC within 2σ of that for the reference condition, where σ = 0.204, was 98.1%. PPA and NPA across all reference-positive and reference-negative positions among the paired samples in a reference-storage condition were 88.4% and 100.0%, respectively. The results confirm that storing plasma at -80°C for over 45 days preserves the sample quality of breast cancer samples.

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.

An additional study was conducted to demonstrate the sample stability for cfDNA extracted from plasma specimens of breast cancer patients. The study samples were derived from the second plasma aliquot belonging to 28 previously reported breast cancer patient samples. After extraction and sequencing of the second plasma aliquot, the remnant cfDNA was stored at 20°C ± 5°C plus 1 freeze/thaw for 46 days. After storage, an equivalent input of cfDNA was processed through the Guardant360 CDx workflow. After sequencing the stored sample, the sample-level molecule recovery, exon-level molecule recovery, and variant call concordance were compared between the original (reference) and stored samples to evaluate stability. In total, 55 out of 56 samples tested for the study passed all QC metrics, resulting in 27 evaluable sample pairs.

The fold change of median NSC in storage condition over the reference condition was 1.05. The 95% two-sided Clopper-Pearson exact binomial lower confidence limit for the fraction of genomic targeted exonic regions with relative exon-level NSC within 2σ of that for the reference condition, where σ = 0.108, was 90.3%. PPA and NPA across all reference-positive and reference-negative positions among the paired samples in a reference-storage condition were 89.6% and 100.0%, respectively. The results confirm that storing cfDNA at -20°C ± 5°C for over 45 days preserves the sample quality of breast cancer samples.

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 26**.

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 25. 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 (**Table 27**). Acceptable sequencing QC parameters were demonstrated across precision combinations (**Table 28**).

Table 26. 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 27. 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), liver, prostate, rectal, stomach, 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 29**).

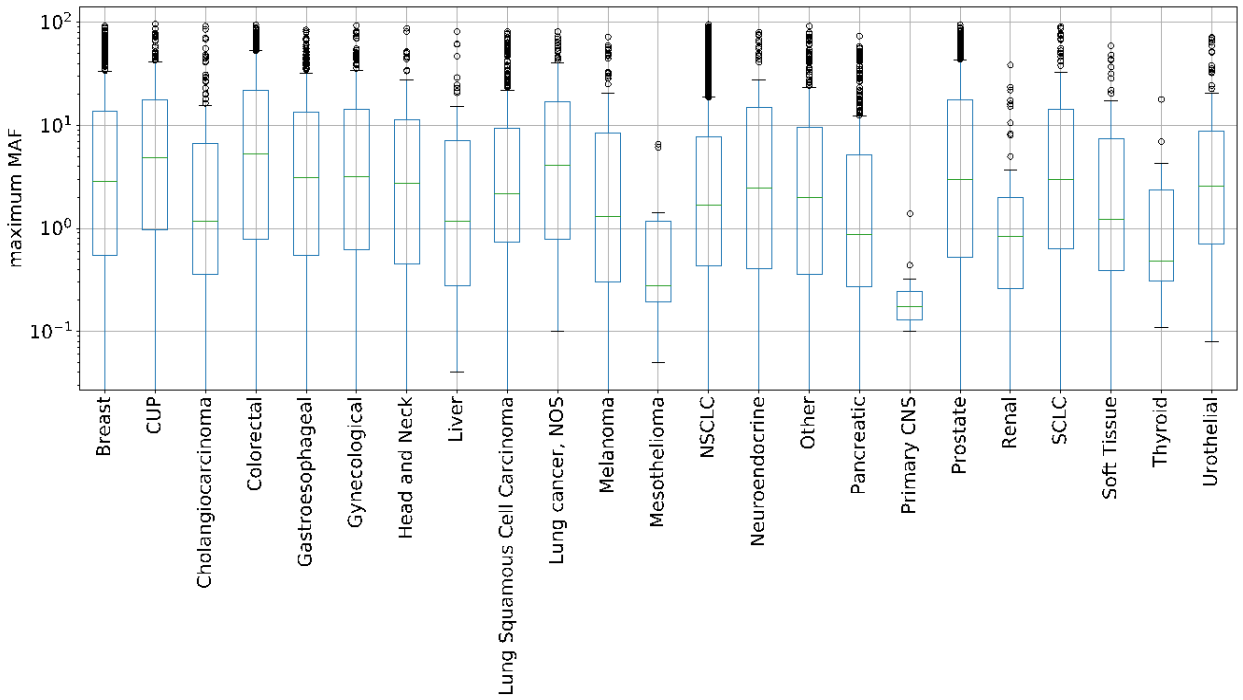
Table 28. Sample Success Rate across 23 Cancers

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)
Gastroesophageal	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)
Gynecological	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)
Mesothelioma	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)
Neuroendocrine	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)

Category Data			Sample Preparation QC Data, % Pass			Patient Sample Sequencing QC Data, % Pass (median value)			Patient Outcome Metrics		
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.



Note that Y-axis represents % Maximum MAF

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 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 30**. 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 30. 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 31** 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 29. 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. Summary of Primary Clinical Studies**).

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 these 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
4. To aid in the selection of patients with NSCLC whose tumors have *ERBB2* activating mutations (SNVs and exon 20 insertions) for fam-trastuzumab deruxtecan-nxki (ENHERTU®) therapy
5. To aid in the selection of patients with breast cancer whose tumors have *ESR1* missense mutations between codons 310 and 547 for elacestrant (ORSERDU™) therapy
6. To aid in the selection of patients with breast cancer whose tumors have *ESR1* mutations for INLURIYO (imlunestrant) therapy
7. To aid in the selection of patients with colorectal cancer whose tumors have *BRAF* V600E mutation for encorafenib (BRAFTOVI®) 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 an *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.

In support of the fam-trastuzumab deruxtecan-nxki (ENHERTU®) CDx claim, Guardant Health performed a clinical bridging study using banked samples from the Daiichi Sankyo DS8201-A-U204 clinical study (NCT03505710). The subjects in the DS8201-A-U204 clinical study were enrolled based on the presence of *ERBB2* activating mutations (SNVs and exon 20 insertions) in tissue specimens. A clinical bridging study using pre-treatment plasma samples and clinical outcome data from patients enrolled in the DS8201-A-U204 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 ENHERTU® therapy based on the detection of *ERBB2* activating mutations (SNVs and exon 20 insertions). As subjects in the DS8201-A-U204 clinical study were enrolled based on positive tissue testing for *ERBB2* activating mutations (SNVs and exon 20 insertions), sensitivity analysis to assess the possible influence of tissue-negative, Guardant360 CDx plasma-positive subjects (Guardant360 CDx^{+} tissue^{-}) was performed using samples procured from commercial vendors.

In support of the elacestrant CDx claim, Guardant Health prospectively tested samples from the Radius RAD1901-308 clinical study (NCT03778931) and eligible subjects were randomized in a 1:1 ratio to either elacestrant or standard of care (SOC) consisting of fulvestrant or an aromatase inhibitor and stratified by mutation status of *ESR1* using Guardant360 CDx and other criteria described in the clinical study protocol. Subjects from the primary RAD1901-308 registration population positive for *ESR1* missense mutations by Guardant360 CDx were included in the diagnostic study primary clinical efficacy cohort to assess the clinical validity of Guardant360 CDx to aid in the selection of breast cancer patients with *ESR1* missense mutations for ORSERDU™ (elacestrant) therapy.

In support of the INLURIYO (imlunestrant) CDx claim, Guardant Health performed a clinical study using pre-treatment plasma specimen from patients enrolled into the EMBER-3 (NCT04975308) clinical study. Subjects from the primary EMBER-3 registration population (Arm A and Arm B) with *ESR1* mutations detected by Guardant360 CDx were included in the diagnostic study primary clinical efficacy cohort to assess the clinical validity of Guardant360 CDx to aid in the selection of breast cancer patients with *ESR1* mutations for INLURIYO therapy.

In support of the encorafenib CDx claim, Guardant Health performed a clinical bridging study using pre-treatment plasma samples from metastatic colorectal cancer (mCRC) patients with *BRAF* V600E mutation enrolled in the phase 3 portion of BREAKWATER clinical study (NCT04607421). The diagnostic study supports the clinical validity of Guardant360 CDx to aid in selection of mCRC patients with *BRAF* V600E mutation for treatment with encorafenib.

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 6.12 Concordance - Guardant360 CDx Comparison to Guardant360 LDT** 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 6.13.a Blood Collection Tube Concordance**).

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 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.

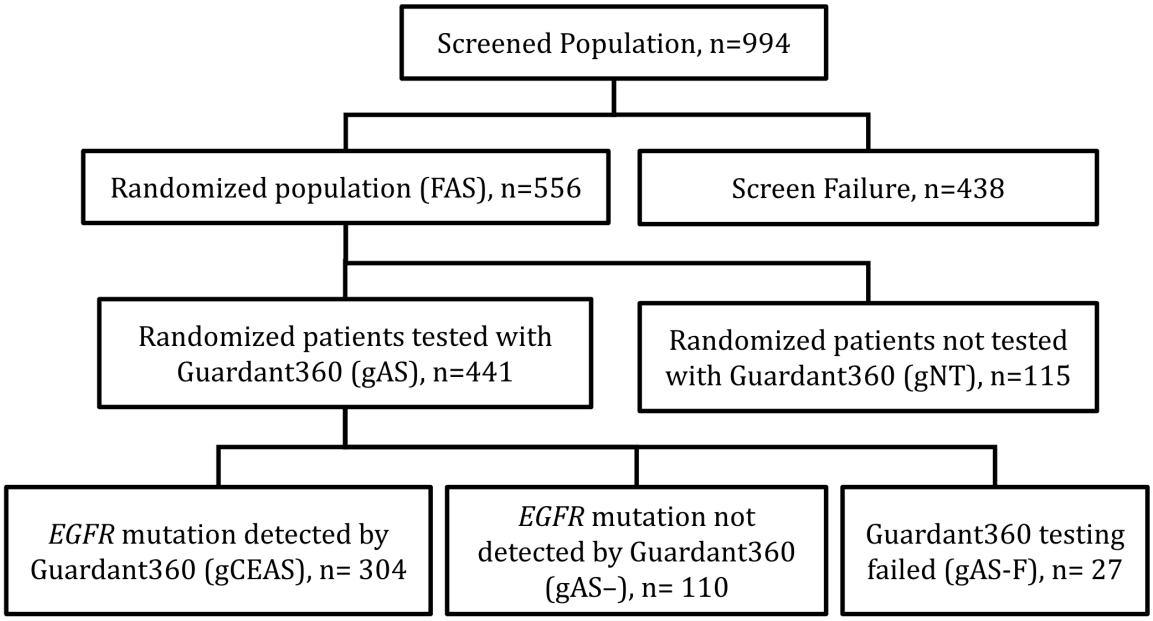


Figure 2. Guardant360 CDx *EGFR* Exon 19 Deletions or L858R Mutations Bridging Study Patient Accountability and Analysis Set Definitions

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 32**, 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 30. 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)

Characteristic		gCEAS		FAS	
		TAGRISSO (n=146)	***EGFR* TKI (gefitinib or erlotinib) (n=158)**	**TAGRISSO (n=279)**	***EGFR* TKI (gefitinib or erlotinib) (n=277)**
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 33**).

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 31. 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 34 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 32. Supplementary Guardant360-Positive, Tissue-Negative Prevalence Analysis Subgroup Demographics and Baseline Clinical Characteristics

Characteristic		FLAURA Patients			NILE Patients
		FAS (n=556)	Screen Failure (n=438)	Total (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

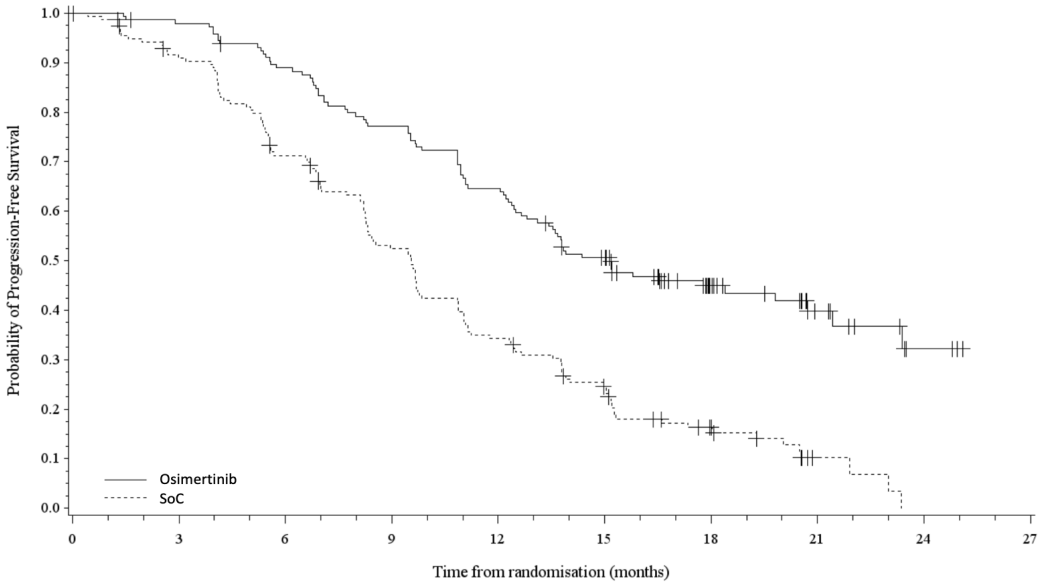
The efficacy of single-agent TAGRISSO relative to *EGFR* TKI therapy in patients randomized in FLAURA positive for *EGFR* exon 19 deletions or L858R mutations by tissue and by Guardant360 (gCEAS) is shown in **Table 35**. The observed PFS hazard ratio (HR) of 0.41 (95% CI 0.31, 0.54) is similar to that 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 33. 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.



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

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

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 36** 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 34. 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	0.32, 0.54
	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 (0.42, 95% confidence 0.32, 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 for tissue-, G360+), and the analysis results are presented in **Table 37**. 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 37** for patients screened in FLAURA used a prevalence of 67%.

Table 35. 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 38** 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 38**.

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

***EGFR* Exon 19 Deletions**	**cobas® *EGFR* Mutation Test Using Tissue**			
	Positive	**Negative**	**Failed**	**Total**
Guardant360				
Positive	185	1	2	188
Negative	53	141	3	197
Failed	14	12	1	27
Total	252	154	6	412
PPA (95% CI) [a]	77.7% [71.9%, 82.9%]			
NPA (95% CI) [a]	99.3% [96.1%, 100.0%]			

***EGFR* Exon 19 Deletions**	**cobas® *EGFR* Mutation Test Using Tissue**			
***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 39**. Of note, the single sample that tested positive for by Guardant360 CDx but negative by the cobas® *EGFR* Mutation Test using tissue comprised an uncommon *EGFR* exon 19 deletion, p.T751_I759delinsN, which is not targeted by the cobas® *EGFR* Mutation Test.

Table 37. Concordance 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 6.12 Concordance - Guardant360 CDx Comparison to Guardant360 LDT**). 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 6.13.a Blood Collection Tube Concordance**).

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, no 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 40**, 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.

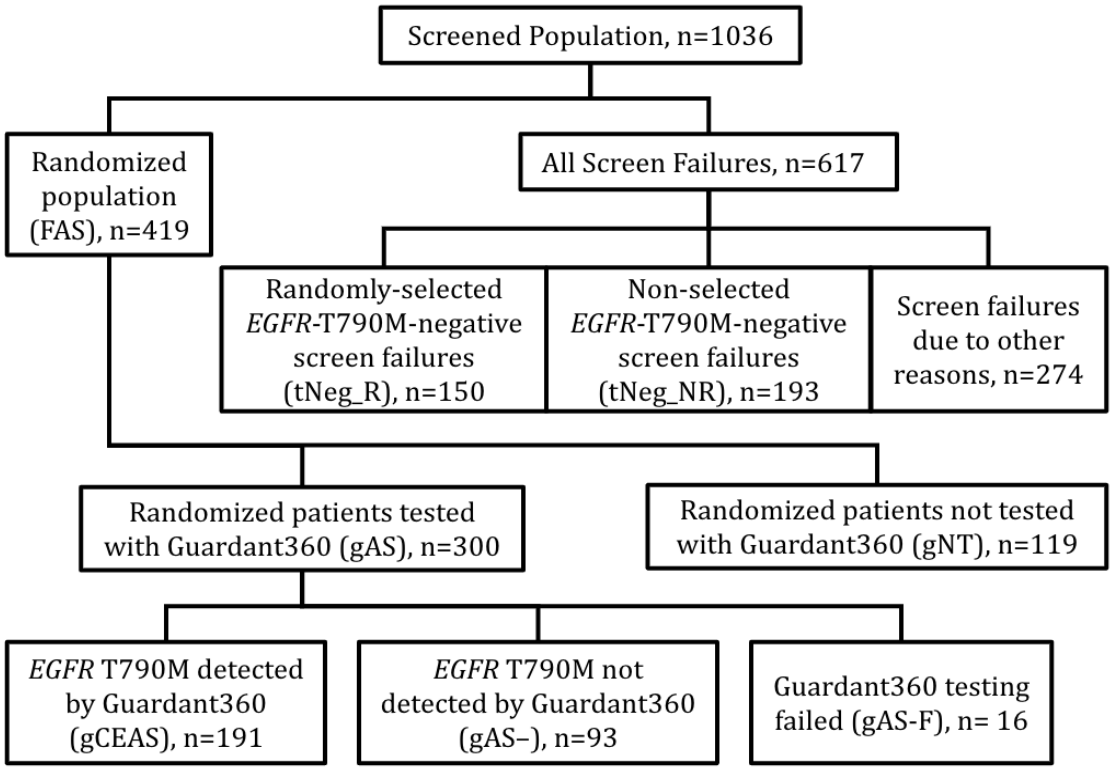


Figure 4. Guardant360 CDx *EGFR* T790M Bridging Study Patient Accountability and Analysis Set Definitions

Table 40. Baseline Demographics and Clinical Characteristics

Characteristic		gCEAS		FAS	
		TAGRISSO (n=138)	Chemotherapy (n=53)	TAGRISSO (n=279)	Chemotherapy (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)

Characteristic		gCEAS		FAS	
		TAGRISSO (n=138)	Chemotherapy (n=53)	TAGRISSO (n=279)	Chemotherapy (n=140)
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 41**).

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 38. 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	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 42**. 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 39. 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

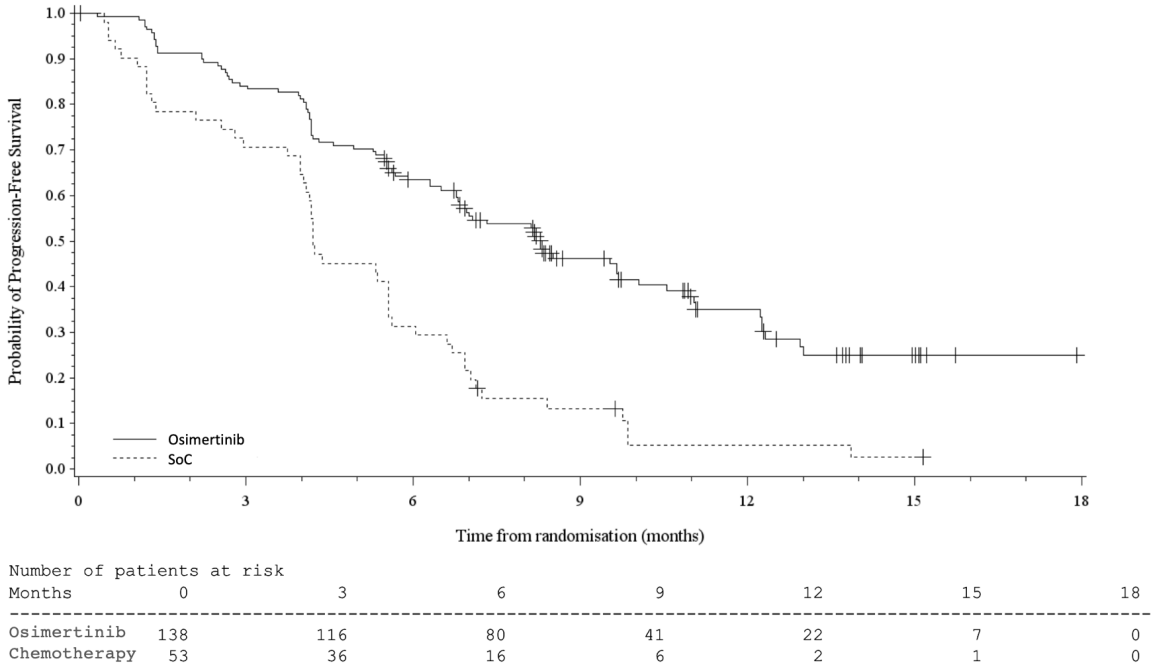


Figure 5. Kaplan-Meier Plot of Investigator-Assessed PFS for gCEAS

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 43** 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 40. 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 44**, 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 44** for the patients screened in AURA3 used a prevalence of 55%.

Table 41. Sensitivity Analysis for Investigator-Assessed PFS (Guardant360 positive irrespective of tissue result)

	Estimated P(Tissue+|Guardant360+) with 95% CI		Estimated HR (Guardant360+) with 95% CI		
	PPV Point Estimate	**95% CI**	**Assumed HR (Tissue- and Guardant360+)**	**Estimated HR**	**95% CI**
gCEAS (observed)	0.72	0.66, 0.77	0.34	0.34	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

	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 + 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 45**.

Table 42. 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 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, 64 subjects (82%) tested positive by the Guardant360 CDx were included in the primary objective analysis set, while 14 subjects (18%) 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.

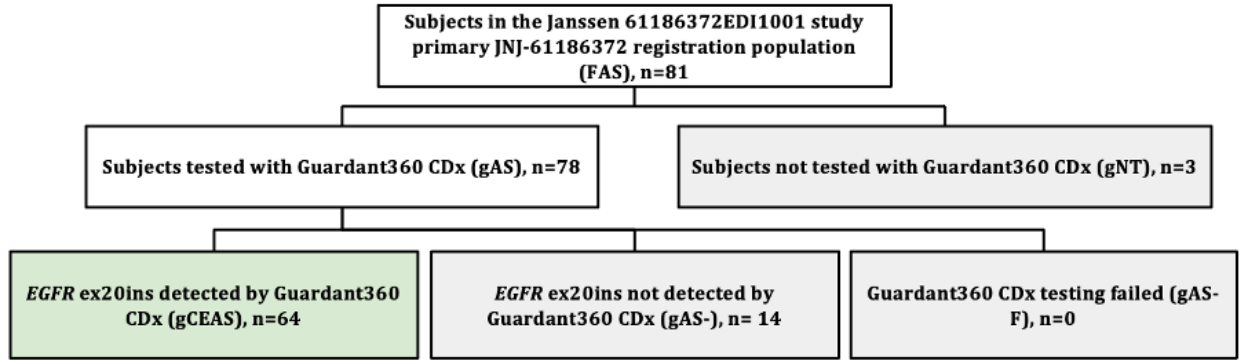


Figure 6. Guardant360 CDx Clinical Efficacy Analyses Subject Disposition

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 46** and **Table 47**, 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 46** and **Table 47**. No meaningful differences were observed.

Table 43. 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	64	14	-	3	
Age, years								
N	81	78	3	64	14	0	3	0.914
Mean (SD)	62.3 (9.96)	62.3 (10.04)	61.7 (9.29)	62.1 (10.13)	63.2 (9.94)	-	61.7 (9.29)	
Median	62.0	62.0	59.0	61.5	66.5	-	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%)	40 (62.5%)	6 (57.1%)	-	2 (66.7%)	
>=65	33 (40.7%)	32 (41.0%)	1 (33.3%)	24 (37.5%)	8 (57.1%)	-	1 (33.3%)	
<75	74 (91.4%)	71 (91.0%)	3 (100.0%)	58 (90.6%)	13 (92.9%)	-	3 (100.0%)	
>=75	7 (8.6%)	7 (9.0%)	0	6 (9.4%)	1 (7.1%)	-	0	
Sex								
N	81	78	3	64	14	0	3	1.000
Female	48 (59.3%)	46 (59.0%)	2 (66.7%)	40 (62.5%)	6 (42.9%)	-	2 (66.7%)	
Male	33 (40.7%)	32 (41.0%)	1 (33.3%)	24 (37.5%)	8 (57.1%)	-	1 (33.3%)	
Race								
N	81	78	3	64	14	0	3	0.104
Asian	40 (49.4%)	39 (50.0%)	1 (33.3%)	36 (56.3%)	3 (21.4%)	-	1 (33.3%)	
Black or African American	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 (32.8%)	8 (57.1%)	-	1 (33.3%)	
Not reported	9 (11.1%)	9 (11.5%)	0	6 (9.4%)	3 (21.4%)	-	0	
Ethnicity								
N	81	78	3	64	14	0	3	1.000
Hispanic or Latino	3 (3.7%)	3 (3.8%)	0	3 (4.7%)	0	-	0	
Not Hispanic or Latino	68 (84.0%)	65 (83.3%)	3 (100.0%)	55 (85.9%)	10 (71.4%)	-	3 (100.0%)	
Not reported	10 (12.3%)	10 (12.8%)	0	6 (9.4%)	4 (28.6%)	-	0	

CHRYSALIS	FAS	gAS	gNT	gCEAS	gAS-	gAS-F	gAS-F +gNT	p Value gAS vs gAS-Unk
Weight, kg								
N	81	78	3	64	14	0	3	0.563
Mean (SD)	67.49 (16.784)	67.28 (16.407)	73.03 (29.258)	65.32 (16.033)	76.24 (15.596)	-	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	64	14	0	3	0.504
Mean (SD)	163.71 (9.020)	163.84 (9.044)	160.27 (9.295)	163.16 (9.260)	166.97 (7.491)	-	160.27 (9.295)	
Median	162.60	162.75	154.90	160.55	166.70	-	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	64	14	0	3	0.320
Mean (SD)	24.993 (4.9047)	24.886 (4.8151)	27.776 (7.5866)	24.368 (4.7270)	27.254 (4.6572)	-	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 (6.3%)	0	-	0	
Normal 18.5-<25	43 (53.1%)	41 (52.6%)	2 (66.7%)	36 (56.3%)	5 (35.7%)	-	2 (66.7%)	
Overweight 25-<30	21 (25.9%)	21 (26.9%)	0	16 (25.0%)	5 (35.7%)	-	0	
Obese >=30	13 (16.0%)	12 (15.4%)	1 (33.3%)	8 (12.5%)	4 (28.6%)	-	1 (33.3%)	
Local Test Type*								
N	81	78	3	64	14	0	3	0.803
NGS (Blood)	4 (4.9%)	4 (5.1%)	0	3 (4.7%)	1 (7.1%)	-	0	
NGS (Tissue)	34 (42.0%)	33 (42.3%)	1 (33.3%)	24 (37.5%)	9 (64.3%)	-	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 (10.9%)	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%)	25 (39.1%)	3 (21.4%)	-	2 (66.7%)	
UNKNOWN (Tissue)	4 (4.9%)	4 (5.1%)	0	3 (4.7%)	1 (7.1%)	-	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 44. 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	64	14	-	3	
Initial diagnosis NSCLC subtype								
N	81	78	3	64	14	0	3	0.922
Adenocarcinoma	77 (95.1%)	74 (94.9%)	3 (100.0%)	61 (95.3%)	13 (92.9%)	-	3 (100.0%)	
Large cell carcinoma	0	0	0	0	0	-	0	
Squamous cell carcinoma	3 (3.7%)	3 (3.8%)	0	2 (3.1%)	1 (7.1%)	-	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	64	14	0	3	0.708
Moderately differentiated	18 (22.2%)	17 (21.8%)	1 (33.3%)	16 (25.0%)	1 (7.1%)	-	1 (33.3%)	
Poorly differentiated	12 (14.8%)	11 (14.1%)	1 (33.3%)	8 (12.5%)	3 (21.4%)	-	1 (33.3%)	
Well differentiated	5 (6.2%)	5 (6.4%)	0	5 (7.8%)	0	-	0	
Other	46 (56.8%)	45 (57.7%)	1 (33.3%)	35 (54.7%)	10 (71.4%)	-	1 (33.3%)	
Not reported	0	0	0	0	0	-	0	
Cancer stage at initial diagnosis								
N	81	78	3	64	14	0	3	0.078
0	0	0	0	0	0	-	0	
IA	6 (7.4%)	6 (7.7%)	0	4 (6.3%)	2 (14.3%)	-	0	
IB	1 (1.2%)	1 (1.3%)	0	1 (1.6%)	0	-	0	
IIA	1 (1.2%)	1 (1.3%)	0	1 (1.6%)	0	-	0	
IIB	4 (4.9%)	3 (3.8%)	1 (33.3%)	3 (4.7%)	0	-	1 (33.3%)	
IIIA	4 (4.9%)	3 (3.8%)	1 (33.3%)	2 (3.1%)	1 (7.1%)	-	1 (33.3%)	
IIIB	4 (4.9%)	4 (5.1%)	0	3 (4.7%)	1 (7.1%)	-	0	
IV	61 (75.3%)	60 (76.9%)	1 (33.3%)	50 (78.1%)	10 (71.4%)	-	1 (33.3%)	
Not reported	0	0	0	0	0	-	0	

CHRYSALIS	FAS	gAS	gNT	gCEAS	gAS-	gAS-F	gAS-Unk	p Value gAS vs gAS-Unk
Location of metastasis [a]								
N	81	78	3	64	14	0	3	0.598
Bone	34 (42.0%)	33 (42.3%)	1 (33.3%)	30 (46.9%)	3 (21.4%)	-	1 (33.3%)	
Liver	7 (8.6%)	7 (9.0%)	0	5 (7.8%)	2 (14.3%)	-	0	
Brain	18 (22.2%)	17 (21.8%)	1 (33.3%)	15 (23.4%)	2 (14.3%)	-	1 (33.3%)	
Lymph Node	43 (53.1%)	43 (55.1%)	0	39 (60.9%)	4 (28.6%)	-	0	
Adrenal Gland	3 (3.7%)	3 (3.8%)	0	3 (4.7%)	0	-	0	
Other	45 (55.6%)	42 (53.8%)	3 (100.0%)	31 (48.4%)	11 (78.6%)	-	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	64	14	0	3	0.881
Mean (SD)	22.905 (21.1901)	22.835 (21.3828)	24.717 (18.7773)	23.668 (22.6295)	19.025 (14.4020)	-	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)	
Time from metastatic disease diagnosis to first dose (months)								
N	81	78	3	64	14	0	3	0.401
Mean (SD)	18.071 (16.4424)	18.374 (16.6647)	10.185 (5.0347)	18.741 (17.2524)	16.695 (14.0984)	-	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	64	14	0	3	0.614
Mean (SD)	2.3 (1.41)	2.2 (1.40)	2.7 (2.08)	2.3 (1.45)	2.0 (1.11)	-	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	64	14	0	3	0.980
0	26 (32.1%)	25 (32.1%)	1 (33.3%)	20 (31.3%)	5 (35.7%)	-	1 (33.3%)	
1	54 (66.7%)	52 (66.7%)	2 (66.7%)	43 (67.2%)	9 (64.3%)	-	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	

CHRYSALIS	FAS	gAS	gNT	gCEAS	gAS-	gAS-F	gAS-Unk	p Value gAS vs gAS-Unk
History of smoking								
N	81	78	3	64	14	0	3	0.631
Yes	38 (46.9%)	37 (47.4%)	1 (33.3%)	27 (42.2%)	10 (71.4%)	-	1 (33.3%)	
No	43 (53.1%)	41 (52.6%)	2 (66.7%)	37 (57.8%)	4 (28.6%)	-	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

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 48** and **Table 49** 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 45. 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%)
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%)

CHRYSALIS	FAS	AAAS-L	AAAS-C	AAAS-P
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 (15.862)	-	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)	163.47 (8.729)	-	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)	24.231 (4.7206)	-	N/A
Median	24.250	23.946	-	N/A
Range	(14.00; 36.87)	(14.00; 36.87)	-	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
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 46. 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
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
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)

CHRYSALIS	FAS	AAAS L	AAAS C	AAAS P
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%)
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 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 39.1% (95% CI 27.1% – 52.1%, **Table 50**). The lower limit of the 95% CI of 27.1% 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 50**).

Table 47. Summary of ORR in the gCEAS and FAS by BICR

CHRYSALIS	gCEAS	FAS
Analysis set: Efficacy	**64**	**81**
Best overall response		
N	64	81
Complete response (CR)	2 (3.1%)	3 (3.7%)
Partial response (PR)	23 (35.9%)	29 (35.8%)
Stable disease (SD)	30 (46.9%)	39 (48.1%)
Progressive disease (PD)	7 (10.9%)	8 (9.9%)
Not evaluable/unknown	2 (3.1%)	2 (2.5%)

CHRYSALIS	gCEAS	FAS
Overall response rate (Confirmed CR + Confirmed PR)	25 (39.1%)	32 (39.5%)
95% CI	(27.1%, 52.1%)	(28.8%, 51.0%)
Clinical benefit rate [a] (Confirmed CR + Confirmed PR + SD)	44 (68.8%)	60 (74.1%)
95% CI	(55.9%, 79.8%)	(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 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 51**. 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 16 samples (16/97) 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 268.

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 (83.7%, 95% CI 75.4% – 89.5%) relative to local testing results.

Table 48. 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 +	87	0	87
EGFR exon 20 insertion -	17	164	181
Total	104	164	268
PPA (95% CI)		83.7% (75.4% - 89.5%)	
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, 95.8% - 100%) and NPV of 99.7% (95% CI, 99.6% - 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).

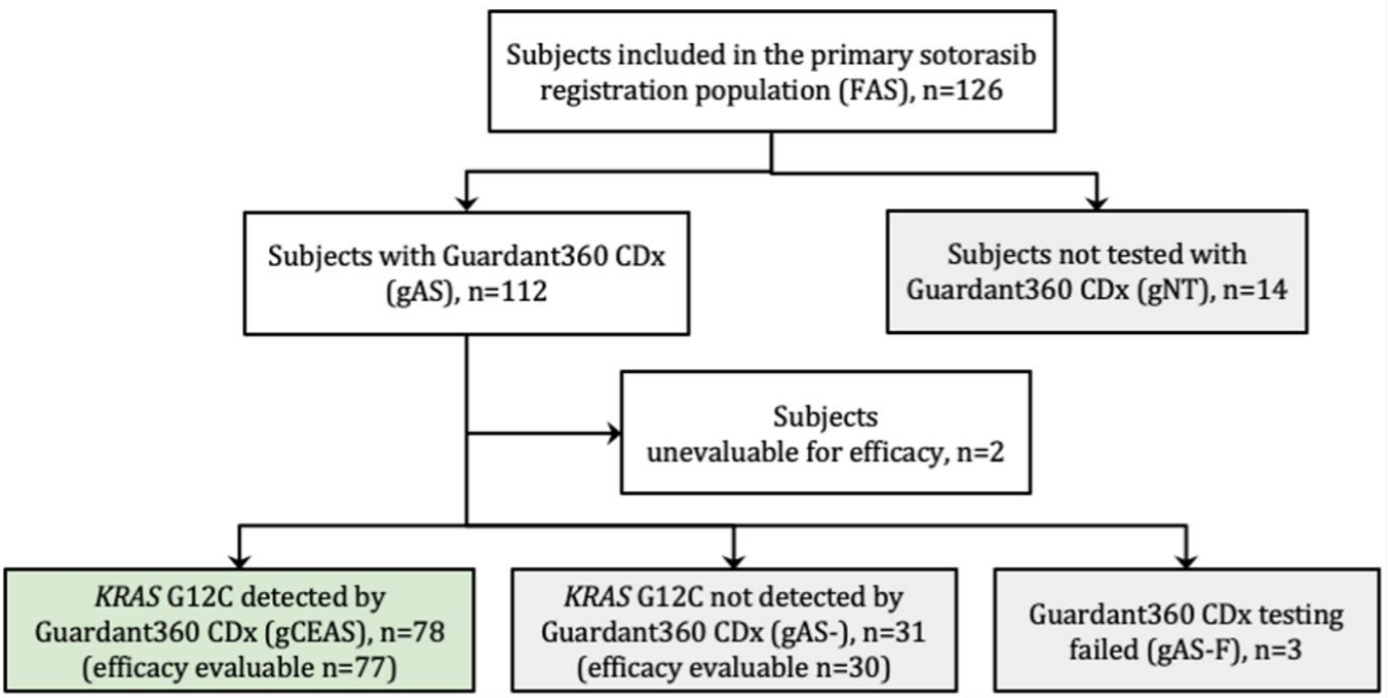


Figure 7. Guardant360 CDx *KRAS* G12C Mutation Bridging Study Efficacy Analysis Patient Accountability and Analysis Set Definitions

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**).

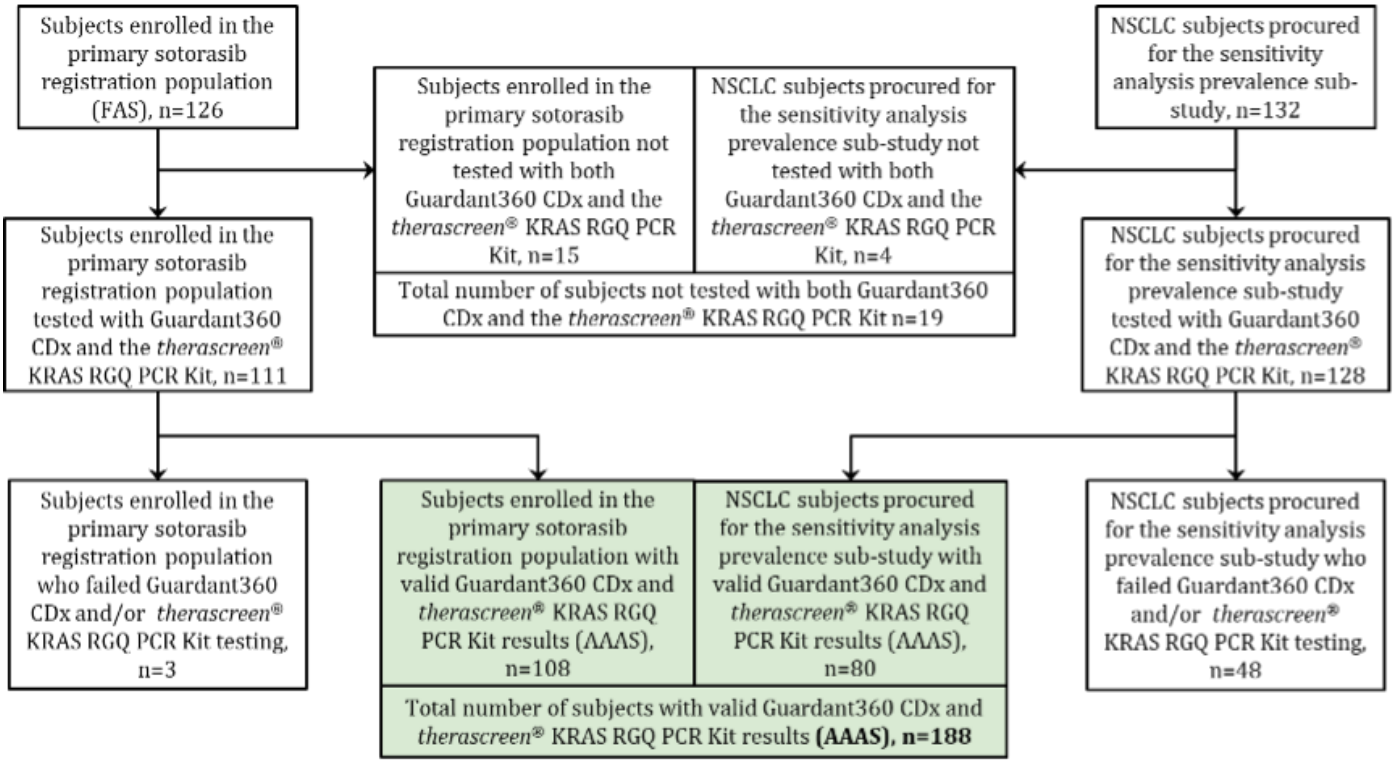


Figure 8. Guardant360 CDx *KRAS* G12C Assay Agreement Analysis Patient Accountability and Analysis Set Definitions

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 52** 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 52**), 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 52**).

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 52**).

Table 49. 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) (%)	82 (70.1)	0 (0.0)	82 (43.4)
Guardant360 CDx Negative (n) (%)	35 (29.9)	72 (100.0)	107 (56.6)
Total	117	72	181
Positive Percent Agreement (95% CI)	70.1% (60.9% – 78.2%)		
Negative Percent Agreement (95% CI)	100% (95.0% – 100.0%)		

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 53** and **Table 54**, 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 50. 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)

	FAS	gCEAS	gAS	gAS-UNK
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 51. Baseline Clinical Characteristics of the FAS and Sub-Groups

	FAS	gCEAS	gAS	gAS-UNK
ECOG status at baseline - n (%)				
0	38 (30.2)	20 (25.6)	35 (31.3)	5 (29.4)
1	88 (69.8)	58 (74.4)	77 (68.8)	12 (70.6)
2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Weight (kg)				
n	126	78	112	17
Mean	71.08	71.18	71.35	67.92
SD	17.14	17.38	17.06	18.30
Median	70.65	70.15	71.00	70.00
Q1, Q3	58, 83	58, 83	58, 83	57, 82
Min, Max	37, 123	37, 123	37, 123	40, 108
Height (cm)				
n	123	77	110	16
Mean	168	168	168	168
SD	9.2	8.9	8.9	11.6
Median	169	168	169	168
Q1, Q3	161, 175	161, 175	161, 175	156, 175
Min, Max	146, 188	151, 188	151, 188	146, 183
Prior line of anti-cancer therapy - n (%)				
0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1	54 (42.9)	33 (42.3)	48 (42.9)	8 (47.1)
2	44 (34.9)	28 (35.9)	38 (33.9)	7 (41.2)
3	28 (22.2)	17 (21.8)	26 (23.2)	2 (11.8)
≥ 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Median (number of prior lines)	2	2	2	2

	FAS	gCEAS	gAS	gAS-UNK
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)
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)

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

To assess potential bias arising from plasma sample availability, baseline demographic information and baseline clinical disease characteristics of subjects with a valid Guardant360 CDx result (gAS-E) and those without (gAS-Unk) were compared and the associated p value reported in **Table 55** and **Table 56**. No meaningful differences were observed.

Table 52. Comparison of Baseline Demographics between gAS-E and gAS-Unk

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

Table 53. 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)	

	gAS-E	gAS-Unk	p-value
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)	
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)	

	gAS-E	gAS-Unk	p-value
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)	
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 57**. 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 54. 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 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 58** vs. **Table 57**, 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 58**) 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 Guardant360 CDx$^+$ tissue$^-$ sub-population.

Table 55. Sensitivity Analysis for the Guardant360 CDx$^+$ Tissue$^-$ Population

	Guardant360 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)

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 59** 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 56. 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 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.

7.5. Guardant360 CDx Clinical Bridging Study for *ERBB2* Activating Mutations (SNVs and Exon 20 Insertions)

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

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

Guardant360 CDx Bridging Study Design for *ERBB2* Activating Mutations (SNVs and Exon 20 Insertions)

Pre-treatment plasma samples from 89 DS8201-A-U204 clinical study subjects from Cohort 2 (89/91, 97.8% of the DS8201-A-U204 clinical study population) were tested with Guardant360 CDx. The DS8201-A-U204 clinical study did not include patients negative for *ERBB2* activating mutations (SNVs and exon 20 insertions) and therefore did not represent the Guardant360 CDx-positive, tissue-based CTA-negative (Guardant360 CDx$^+$ CTA$^-$) subgroup of the Guardant360 CDx intended use population. As such, supplemental matched tissue and plasma samples were commercially procured from vendors using subject sample selection criteria similar to those of the DS8201-A-U204 clinical study, and a sensitivity analysis was performed to evaluate the potential impact of the Guardant360 CDx$^+$ CTA$^-$ population on the efficacy in the Guardant360 CDx intended use population.

a. Clinical Bridging Study Inclusion and Exclusion Criteria

All subjects in the DS8201-A-U204 clinical study 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 are included.

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

b. Clinical Endpoints

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

c. Diagnostic Objective and Endpoints

The primary objective of the clinical bridging study is to demonstrate the clinical validity of Guardant360 CDx for the selection of NSCLC subjects with *ERBB2* activating mutations (SNVs and exon 20 insertions) detected in plasma for treatment with ENHERTU. The primary endpoint is ORR by RECIST version 1.1 as assessed by ICR. A sensitivity analysis was conducted to model the impact of the Guardant360 CDx^{+} CTA^{-} population.

Accountability of the PMA Cohort for the Guardant360 CDx Clinical Bridging Study for *ERBB2* Activating Mutations (SNVs and Exon 20 Insertions)

The Guardant360 CDx clinical bridging study included 89 (gAS; 97.8%) of the 91 subjects (FAS) enrolled in Cohort 2 of the DS8201-A-U204 (**Figure 9**). Of these, 81 subjects (gCEAS, 89%) were tested positive by Guardant360 CDx and were included in the primary objective analysis set (gCEAS), while 8 (gAS-, 8.8%) were negative. Of the 91 subjects enrolled in the DS8201-A-U204, 2 (gNT, 2.2%) were not tested because plasma was unavailable. No samples failed testing by Guardant360 CDx.

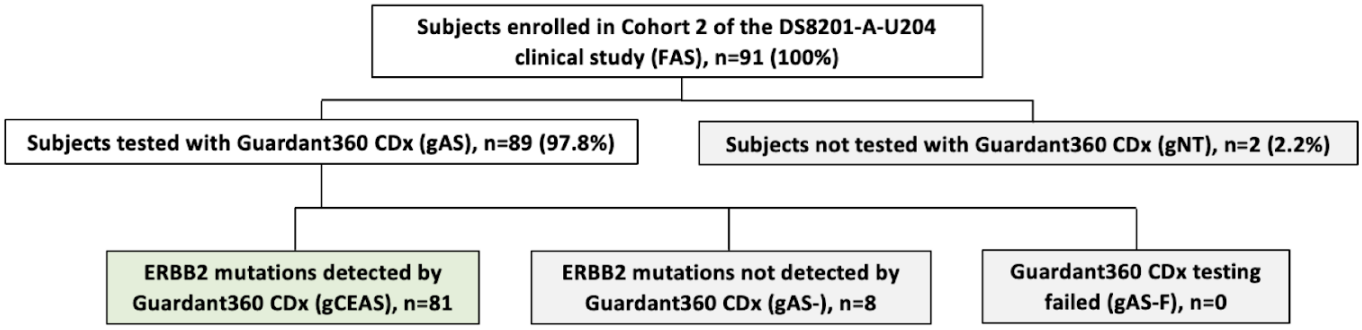


Figure 9. Guardant360 CDx *ERBB2* Activating Mutation Bridging Study Efficacy Analysis Subject Accountability and Analysis Set Definitions

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

The sensitivity analysis prevalence sub-study set included 169 subjects with matched plasma and tissue (**Figure 10**). Of those 169, 58 subjects (34.3%) failed or were not tested by either Guardant360 CDx and/or tissue-based CTA testing. This is comprised of 36 samples that failed CTA testing but have Guardant360 CDx results; 17 samples that failed Guardant360 CDx testing but have CTA results; 2 samples that failed both CTA and Guardant360 CDx testing; and 3 samples that were unable to be tested by Guardant360 CDx and/or CTA. This resulted in 111 subjects with valid Guardant360 CDx and tissue CTA results. Of these, one subject was Guardant360 CDx^{+} CTA^{+}, no subjects were Guardant360 CDx^{+} CTA^{-} or Guardant360 CDx^{-} CTA^{+}, and 110 subjects were Guardant360 CDx^{-} CTA^{-}.

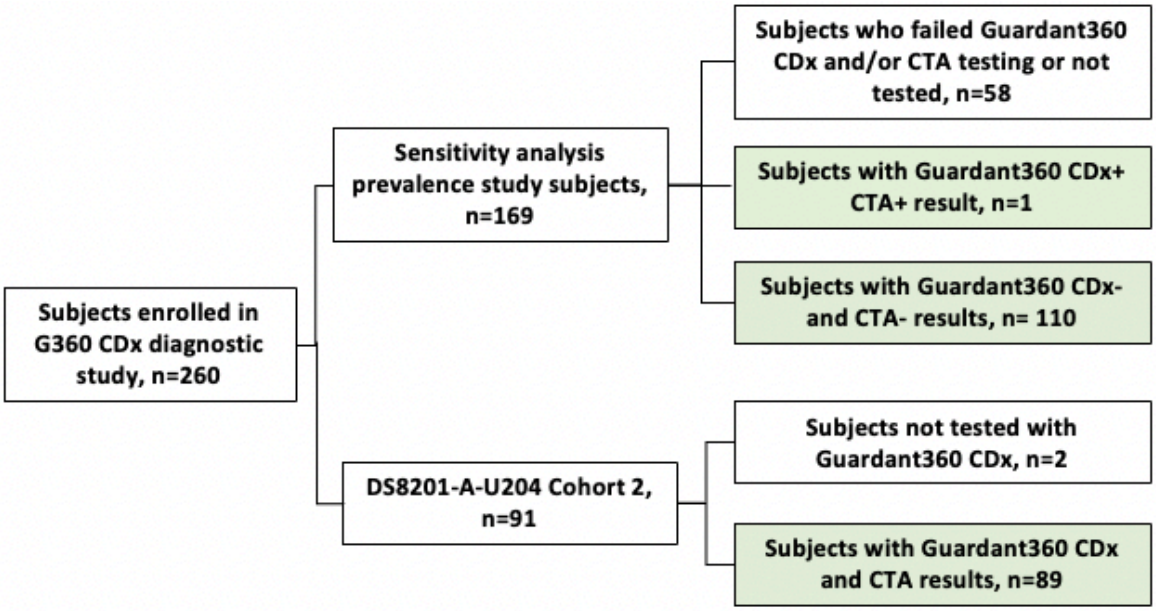


Figure 10. Guardant360 CDx *ERBB2* Sensitivity Analysis Prevalence Sub-Study Subject Accountability

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

Concordance between Guardant360 CDx and Tissue Testing

Concordance between Guardant360 CDx and tissue-based CTA testing using matched plasma and tissue samples from Cohort 2 of the DS8201-A-U204 clinical study, along with the sensitivity analysis prevalence sub-study group, is shown in **Table 60** below. While all samples from the primary ENHERTU registration population were positive for *ERBB2* activating mutations (SNVs and exon 20 insertions) by tissue testing as a condition of their enrollment in the clinical study, the sensitivity analysis prevalence sub-study subjects were recruited in an effort to represent the *ERBB2*-negative population.

For the concordance analysis (**Table 60**), when assessing the positive percent agreement (PPA), the 89 tissue-positive subjects from the primary ENHERTU registration population were included. In addition, the 111 subjects from the sensitivity analysis prevalence sub-study with valid results were included as described in **Figure 10** above.

Table 60. Concordance Between Guardant360 CDx and Tissue-based CTA

	CTA Positive, n	CTA Negative, n	Total
Guardant360 CDx Positive, n	82	0	82
Guardant360 CDx Negative, n	8	110	118
Total	90	110	200
Positive Percent Agreement [95% CI[1]]	91.1% (82/90) [83.2% - 96.1%]		
Negative Percent Agreement [95% CI[1]]	100% (110/110) [96.7% - 100.0%]		

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

Study Population Demographics and Baseline Clinical Parameters for the Guardant360 CDx Clinical Bridging Study for *ERBB2* Activating Mutations (SNVs and Exon 20 Insertions)

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

As shown in **Table 61** and **Table 62**, the diagnostic study efficacy population (gCEAS) demographics and the baseline clinical characteristics closely resemble those of the overall DS8201-A-U204 DESTINY Lung 01 diagnostic clinical study population (FAS). Demographic and baseline clinical characteristics of the additional sub-group populations were also comparable to FAS and gCEAS.

Table 57. Baseline Demographics of the Clinical Effectiveness Analysis FAS and Sub-Groups

	gCEAS N=81	gAS- N=8	gAS N=89	gAS-Unk N=2	Total (FAS) N=91
Age (years)					
N	81	8	89	2	91
Mean	59.8	65.9	60.4	55.5	60.3
SD	11.26	14.74	11.64	28.99	11.94
Median	60	62.5	60	55.5	60
Min, Max	29, 79	48, 88	29, 88	35, 76	29, 88
Sex – n (%)					
Female	52 (64.2)	6 (75.0)	58 (65.2)	2 (100.0)	60 (65.9)
Male	29 (35.8)	2 (25.0)	31 (34.8)	0	31 (34.1)
Race – n (%)					
White	34 (42.0)	5 (62.5)	39 (43.8)	1 (50.0)	40 (44.0)
Black or African American	1 (1.2)	0	1 (1.1)	0	1 (1.1)
Asian	28 (34.6)	3 (37.5)	31 (34.8)	0	31 (34.1)
American Indian or Alaska Native	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Hispanic	0	0	0	0	0
Other	18 (22.2)	0	18 (20.2)	1 (50.0)	19 (20.9)
Missing/Unknown	0	0	0	0	0
Ethnicity – n (%)					
Hispanic or Latino	2 (2.5)	0	2 (2.2)	0	2 (2.2)
Not Hispanic or Latino	60 (74.1)	7 (87.5)	67 (75.3)	1 (50.0)	68 (74.7)
Not Applicable	19 (23.5)	1 (12.5)	20 (22.5)	1 (50.0)	21 (23.1)
ECOG Score – n (%)					
0	20 (24.7)	2 (25.0)	22 (24.7)	1 (50.0)	23 (25.3)
1	61 (75.3)	6 (75.0)	67 (75.3)	1 (50.0)	68 (74.7)

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

Table 58. Baseline Clinical Characteristics of the Clinical Effectiveness Analysis FAS and Sub-Groups

	gCEAS N=81	gAS- N=8	gAS N=89	gNT N=2	Total (FAS) N=91
Histology – n (%)					
Adenocarcinoma	81 (100.0)	8 (100.0)	89 (100.0)	2 (100.0)	91 (100.0)
Large Cell	0	0	0	0	0
Other	0	0	0	0	0
Tumor Stage at Study Entry – n (%)					
I-II	0	0	0	0	0
IIIA	1 (1.2)	1 (12.5)	2 (2.2)	0	2 (2.2)

	gCEAS N=81	gAS- N=8	gAS N=89	gNT N=2	Total (FAS) N=91
IIIB	2 (2.5)	0	2 (2.2)	0	2 (2.2)
IIIC	1 (1.2)	0	1 (1.1)	0	1 (1.1)
IV	18 (22.2)	1 (12.5)	19 (21.3)	1 (50.0)	20 (22.0)
IVA	19 (23.5)	3 (37.5)	22 (24.7)	1 (50.0)	23 (25.3)
IVB	40 (49.4)	3 (37.5)	43 (48.3)	0	43 (47.3)

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

Safety and Effectiveness Results for the Guardant360 CDx Clinical Bridging Study for *ERBB2* Activating Mutations (SNVs and Exon 20 Insertions)

a. Safety Results

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

Data regarding the safety of ENHERTU therapy are presented in the original drug approval. Refer to the ENHERTU 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 *ERBB2* Activating Mutations (SNVs and Exon 20 Insertions)

The efficacy of fam-trastuzumab deruxtecan-nxki (ENHERTU®) was evaluated in DS8201-A-U204 (DESTINY Lung 01, n=91) and DS8201-A-U206 (DESTINY Lung 02, n=52) studies. The efficacy of ENHERTU in both study populations (DESTINY Lung 01 and DESTINY Lung 02) and subjects in the diagnostic study positive for *ERBB2* activating mutations (SNVs and exon 20 insertions) by Guardant360 CDx (gCEAS) is shown in **Table 63**. The observed gCEAS ORR (58.0%, 95% CI 46.5% - 68.9%) based on the DESTINY Lung 01 study population is similar to the ORR (57.7%, 95% CI: 43.2% - 71.3%) from the ENHERTU efficacy population (DESTINY Lung 02). The lower limit of the 95% CI exceeds the benchmark ORR of 30% from the DS8201-A-U204 and DS8201-A-U206 clinical studies. The duration of response (DOR) for Guardant360 clinical efficacy population (gCEAS) was 9.25 months (95% CI: 5.7, 18.2).

Table 59. ORR in the gCEAS and ENHERTU Study Populations Assessed by Independent Central Review

	gCEAS (n=81)	DESTINY Lung 01 (n=91) - 6.4 mg/kg	*DESTINY Lung 02 (n=52)- 5.4 mg/kg
Objective Response Rate, n (%) (95% CI)	47 (58.0) (46.5, 68.9)	50 (54.9) (44.2, 65.4)	30 (57.7) (43.2, 71.3)
Complete response (CR)	1 (1.2)	1 (1.1)	1 (1.9)
Partial response (PR)	46 (56.8)	49 (53.8)	29 (55.8)

	gCEAS (n=81)	DESTINY Lung 01 (n=91) - 6.4 mg/kg	*DESTINY Lung 02 (n=52)- 5.4 mg/kg
Duration of Response (DOR)			
Median[a], months (95% CI)	9.3 (5.7, 18.2)	9.3 (5.7, 14.7)	8.7 (7.1, NE)

*This is the primary efficacy population for the approval of fam-trastuzumab deruxtecan-nxki (ENHERTU®). [a]Estimated by the Kaplan-Meier Method. NE = not estimable, CI= confidence interval
The 95% CI is calculated using the Exact (Clopper-Pearson) method.

ii. Sensitivity Analysis

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

Sensitivity Analysis for the Unrepresented Guardant360 CDx$^+$ CTA$^-$ Subject Population

The primary objective analysis described above demonstrated ENHERTU efficacy in the Guardant360 CDx$^+$ CTA$^+$ subset of the Guardant360 CDx intended use population. As subjects in the DS8201-A-U204 clinical study were enrolled based on positive tissue testing for *ERBB2* activating mutations (SNVs and exon 20 insertions), a sensitivity analysis was assessed using matched tissue and plasma samples (procured from vendors according to the selection criteria similar to the DS8201-A-U204 clinical study). The sensitivity analysis modeling efficacy in the entire Guardant360 CDx intended use population demonstrates robustness to the contribution of the unrepresented Guardant360 CDx$^+$ CTA$^-$ subjects, with estimated ORRs highly similar to the observed (**Table 63** vs. **Table 64**) due to the high NPA (100%) of Guardant360 CDx relative to tissue testing. The lower limit of the 95% CI for the estimated ORRs across the modeled conditions (**Table 64**) is greater than the benchmark ORR of 30% in the clinical study, which demonstrates ENHERTU efficacy across the entire Guardant360 CDx intended use population, irrespective of efficacy in the modeled Guardant360 CDx$^+$ CTA$^-$ population.

Table 60. Sensitivity Analysis for the Guardant360 CDx$^+$ CTA$^-$ Population

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

Point estimate, variances and confidence intervals are from bootstrapping with a seed of 12345 and 10,000 replicates.

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 DS8201-A-U204 clinical study population positive by Guardant360 CDx for *ERBB2* activating mutations (gCEAS, observed ORR 58.0%, 95% CI 46.5% - 68.9%) exceeded the prespecified benchmark ORR of 30% and was highly similar to that of the total DS8201-A-U204 clinical study population (FAS, observed ORR 54.9%, 95% CI 44.2% - 65.4%).

Sensitivity analysis for the Guardant360 CDx$^+$ CTA$^-$ population, demonstrated robustness of the observed ORR to potential effect from this unevaluated population. Additionally, Guardant360 CDx

and comparator tissue testing were highly concordant (PPA 91.1%; NPA 100%) in the detection of *ERBB2* activating mutations (SNVs and exon 20 insertions).

Thus, ENHERTU demonstrated clinically meaningful efficacy in the Guardant360 CDx+ intended use population which is comparable to that observed in the ENHERTU sBLA efficacy population. This supports the clinical validity of Guardant360 CDx to aid in the selection of patients with NSCLC whose tumors have *ERBB2* activating mutations (SNVs and exon 20 insertions) detected in plasma for ENHERTU therapy.

7.6. Guardant360 CDx Clinical Study for *ESR1* Mutations for the ORSERDU (elacestrant) Therapy

RAD1901-308 [EMERALD (NCT03778931)] Clinical Study Design

The RAD1901-308 clinical study is an international, multicenter, randomized, open-label, active-controlled, event-driven, Phase 3 clinical study comparing the efficacy and safety of ORSERDU™ (elacestrant) to the SOC options of fulvestrant or an aromatase inhibitor (AI) in post-menopausal women and men with ER+/HER2- metastatic breast cancer (mBC). Eligible subjects were randomized in a 1:1 ratio to either ORSERDU™ (elacestrant) or SOC and stratified by mutation status of *ESR1* using Guardant360 CDx and other criteria described in the clinical study protocol.

Guardant360 CDx Clinical Study Design for *ESR1* Missense Mutations

To demonstrate the clinical validity of Guardant360 CDx for the selection of ER+/HER2- mBC patients with *ESR1* missense mutations for treatment with ORSERDU™ (elacestrant), the primary diagnostic study objective (PFS) was assessed by comparing the efficacy of single-agent ORSERDU™ (elacestrant) relative to SOC in subjects positive for *ESR1* missense mutations by Guardant360 CDx. Subjects from the primary RAD1901-308 registration population positive for *ESR1* missense mutations by Guardant360 CDx were included in the diagnostic study primary clinical efficacy cohort

a. Clinical Study Inclusion and Exclusion Criteria

Subjects in the primary RAD1901-308 registration population were included in the diagnostic study efficacy cohort if the selection criteria below were met.

- Male or postmenopausal female
- Histologically- or cytologically-proven adenocarcinoma of the breast with evidence of either locally advanced disease not amenable to resection or radiation therapy with curative intent or metastatic disease not amenable to curative therapy
- Must be appropriate candidates for endocrine monotherapy
- Must have ER+ and HER2- tumor status confirmed per local laboratory testing
- Must have previously received at least 1 and no more than 2 lines of endocrine therapy, either as monotherapy or as a combination therapy with another agent; prior treatment with a CDK4/6 inhibitor in combination with either fulvestrant or an AI; and no more than 1 line of cytotoxic chemotherapy in the mBC setting
- Measurable disease or non-measurable bone-only disease.

b. Follow-up Schedule

The Guardant360 CDx diagnostic study involved only testing and analysis of plasma samples; as such, no additional patient follow-up was conducted in regard to the diagnostic study.

c. Clinical Endpoints

The clinical endpoint used to assess ORSERDU™ (elacestrant) efficacy in the RAD1901-308 clinical study primary objective was PFS by RECIST version 1.1 as assessed by independent central review (ICR) or death from any cause.

d. Diagnostic Objective and Endpoints

The diagnostic study objective was to demonstrate the safety and effectiveness of Guardant360 CDx as a companion diagnostic to aid in the selection of breast cancer patients with *ESR1* missense mutations for ORSERDU™ (elacestrant) therapy was a co-development study utilizing plasma samples and clinical outcome data from the RAD1901-308 clinical study.

The objective was assessed by comparing the efficacy of ORSERDU™ (elacestrant) to that of SOC therapy (fulvestrant or an aromatase inhibitor) in patients that are positive for *ESR1* missense mutations by Guardant360 CDx. The primary endpoint is the same as that used for the clinical study, PFS by RECIST 1.1 as assessed by ICR.

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

The RAD1901-308 clinical study registration population (FAS) included 478 subjects, 228 of which had *ESR1* missense mutations detected by Guardant360 CDx (*ESR1*-mut), and 249 of which did not have an *ESR1* missense mutation detected by Guardant360 CDx (*ESR1*-mut-nd) (**Figure 11**). Note, one subject was enrolled into the registrational population based on a successful Guardant360 CTA test result but was excluded from the diagnostic study efficacy analysis due to QC failure on reanalysis with the final Guardant360 CDx bioinformatics software.

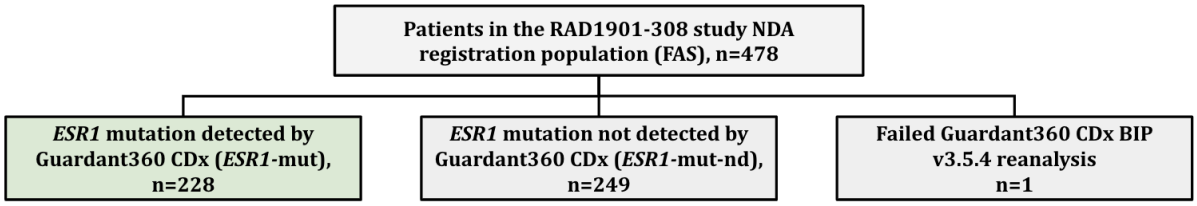


Figure 11. Guardant360 CDx *ESR1* Mutation Efficacy Analysis Patient Accountability and Analysis Set Definitions

Note: Primary clinical efficacy population (*ESR1*-mut) shaded in green. Excluded or secondary clinical efficacy populations (*ESR1*-mut-nd and FAS) shaded in gray.

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

Demographics and baseline clinical characteristics of subjects enrolled in the RAD1901-308 clinical study were categorized relative to the diagnostic study populations as defined by Guardant360 CDx results.

As shown in **Table 65** and **Table 66**, the diagnostic study primary efficacy population (*ESR1*-mut) and the *ESR1*-mut-nd population are well balanced. The ORSERDU™ (elacestrant) and SOC treatment arms are also well balanced.

Table 61. Baseline Demographics of the FAS and Sub-Groups

	Elacestrant		SOC		Total	
	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**
Analysis set:	**115**	**124**	**113**	**125**	**228**	**249**
Age (years), n (missing)	**115 (0)**	**124 (0)**	**113 (0)**	**125 (0)**	**228 (0)**	**249 (0)**
Mean	62.7	62.4	62.0	64.4	62.4	63.4
SD	12.25	11.91	11.74	10.03	11.98	11.03
Median	64.0	63.0	63.0	64.0	63.0	64.0
Min	28	24	32	41	28	24
Max	89	84	83	82	89	84
Age (years), n (%), n (missing)	**115 (0)**	**124 (0)**	**113 (0)**	**125 (0)**	**228 (0)**	**249 (0)**
>=18 - <50	15 (13.0)	18 (14.5)	19(16.8)	10 (8.0)	34 (14.9)	28 (11.2)
>=50 - <65	47 (40.9)	55 (44.4)	43 (38.1)	55 (44.0)	90 (39.5)	110 (44.2)
>=65 - <75	36 (31.3)	28 (22.6)	34 (30.1)	31 (24.8)	70 (30.7)	59 (23.7)
>=75	17 (14.8)	23 (18.5)	17 (15.0)	29 (23.2)	34 (14.9)	52 (20.9)
<65	62 (53.9)	73 (58.9)	62 (54.9)	65 (52.0)	124 (54.4)	138 (55.4)
>=65	53 (46.1)	51 (41.1)	51 (45.1)	60 (48.0)	104 (45.6)	111 (44.6)
Race n (%)[1], n (missing)	**94 (21)**	**96 (28)**	**92 (21)**	**102 (23)**	**186 (42)**	**198 (51)**
American Indian or Alaska Native	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Asian	5 (5.3)	11 (11.5)	8 (8.7)	8 (7.8)	13 (7.0)	19 (9.6)
Black or African American	4 (4.3)	1 (1.0)	4 (4.3)	3 (2.9)	8 (4.3)	4 (2.0)
Native Hawaiian or Other Pacific Islander	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
White/Caucasian	84 (89.4)	84 (87.5)	80 (87.0)	90 (88.2)	164 (88.2)	174 (87.9)
Other	1 (1.1)	0 (0.0)	0 (0.0)	1 (1.0)	1 (0.5)	1 (0.5)
Gender n (%), n (missing)	**115 (0)**	**124 (0)**	**113 (0)**	**125 (0)**	**228 (0)**	**249 (0)**
Male	0 (0.0)	6 (4.8)	0 (0)	1 (0.8)	0 (0.0)	7 (2.8)
Female	115 (100.0)	118 (95.2)	113 (100.0)	124 (99.2)	228 (100.0)	242 (97.2)
Ethnicity, n (%), n (missing)	**115 (0)**	**124 (0)**	**113 (0)**	**125 (0)**	**228 (0)**	**249 (0)**
Hispanic or Latino	10 (8.7)	9 (7.3)	10 (8.8)	8 (6.4)	20(8.8)	17 (6.8)
Non-Hispanic or Latino	92 (80.0)	102 (82.3)	88 (77.9)	102(81.6)	180 (78.9)	204 (81.9)
Unknown	13 (11.3)	13 (10.5)	15 (13.3)	15 (12.0)	28 (12.3)	28 (11.2)
Height, (cm), n (missing)	**113 (2)**	**123 (1)**	**112 (1)**	**124 (1)**	**225 (3)**	**247 (2)**
Mean	161.98	162.62	160.65	161.24	161.27	161.93
SD	7.454	8.230	6.482	7.743	6.998	8.003
Median	160.00	161.00	160.40	162.00	160.30	162.00
Min	143.0	144.8	145.0	142.0	143.0	142.0
Max	183.0	190.0	173.0	183.0	183.0	190.0

	Elacestrant		SOC		Total	
	ESR1-mut	*ESR1*-mut-nd	*ESR1*-mut	*ESR1*-mut-nd	*ESR1*-mut	*ESR1*-mut-nd
Weight (kg), n (missing)	**115 (0)**	**124 (0)**	**113 (0)**	**125 (0)**	**228(0)**	**249 (0)**
Mean	73.41	72.04	71.87	72.83	72.65	72.43
SD	17.145	15.092	16.455	16.443	16.787	15.758
Median	69.00	70.00	69.10	72.00	69.05	70.45
Min	42.0	44.0	44.0	42.0	42.0	42.0
Max	135.0	125.7	124.0	132.3	135.0	132.3
BMI (kg/m2), n (missing)	**113 (2)**	**123 (1)**	**112 (1)**	**124 (1)**	**225 (3)**	**247 (2)**
Mean	28.07	27.13	27.88	27.95	27.97	27.55
SD	6.058	4.901	6.012	5.752	6.023	5.350
Median	26.30	27.03	27.41	26.75	26.48	26.85
Min	17.5	18.2	16.9	16.5	16.9	16.5
Max	52.7	40.9	45.1	47.8	52.7	47.8
ECOG Performance Status n (%), n (missing)	**115 (0)**	**124 (0)**	**113 (0)**	**125 (0)**	**228 (0)**	**249 (0)**
0	67 (58.3)	76 (61.3)	62 (54.9)	73 (58.4)	129 (56.6)	149 (59.8)
1	48 (41.7)	48 (38.7)	51 (45.1)	51 (40.8)	99(43.4)	99 (39.8)
>1	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.4)

SD = Standard Deviation, Min = Minimum, Max = Maximum, BMI = Body Mass Index, ECOG = Eastern Cooperative Oncology Group

[1] Subjects may select more than 1 race.

Table 62. Baseline Clinical Characteristics of the FAS and Sub-Groups

	Elacestrant				SOC				Total			
	ESR1-mut		*ESR1*-mut-nd		*ESR1*-mut		*ESR1*-mut-nd		*ESR1*-mut		*ESR1*-mut-nd	
Years Since Initial Diagnosis												
N (missing)	115 (0)		124 (0)		113 (0)		125 (0)		228 (0)		249 (0)	
Mean	7.49		8.63		8.41		8.90		7.95		8.77	
SD	6.527		6.372		6.985		7.742		6.759		7.080	
Median	4.92		6.76		5.75		6.42		5.42		6.63	
Min	0.2		0.7		0.9		0.5		0.2		0.5	
Max	28.4		32.2		31.0		40.1		31.0		40.1	
Stage at Initial Diagnosis, n (%)												
I	15	(13.0)	20	(16.1)	11	(9.7)	18	(14.4)	26	(11.4)	38	(15.3)
II	27	(23.5)	53	(42.7)	39	(34.5)	42	(33.6)	66	(28.9)	95	(38.2)
III	1	(0.9)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.4)	0	(0.0)
IIIA	5	(4.3)	14	(11.3)	6	(5.3)	14	(11.2)	11	(4.8)	28	(11.2)
IIIB	4	(3.5)	3	(2.4)	0	(0.0)	3	(2.4)	4	(1.8)	6	(2.4)
IIIC	4	(3.5)	7	(5.6)	6	(5.3)	1	(0.8)	10	(4.4)	8	(3.2)
IV	42	(36.5)	20	(16.1)	38	(33.6)	38	(30.4)	80	(35.1)	58	(23.3)
Unknown	17	(14.8)	7	(5.6)	12	(10.6)	9	(7.2)	29	(12.7)	16	(6.4)
T Stage at Initial Diagnosis, n (%)												
T1	18	(15.7)	29	(23.4)	20	(17.7)	23	(18.4)	38	(16.7)	52	(20.9)
T2	29	(25.2)	48	(38.7)	40	(35.4)	49	(39.2)	69	(30.3)	97	(39.0)
T3	13	(11.3)	18	(14.5)	6	(5.3)	11	(8.8)	19	(8.3)	29	(11.6)
T4	11	(9.6)	7	(5.6)	10	(8.8)	13	(10.4)	21	(9.2)	20	(8.0)
Unknown	3	(2.6)	2	(1.6)	5	(4.4)	2	(1.6)	8	(3.5)	4	(1.6)

	Elacestrant				SOC				Total			
	ESR1-mut		*ESR1*-mut-nd		*ESR1*-mut		*ESR1*-mut-nd		*ESR1*-mut		*ESR1*-mut-nd	
N Stage at Initial Diagnosis, n (%)												
N0	16	(13.9)	35	(28.2)	15	(13.3)	38	(30.4)	31	(13.6)	73	(29.3)
N1	34	(29.6)	45	(36.3)	37	(32.7)	31	(24.8)	71	(31.1)	76	(30.5)
N2	14	(12.2)	14	(11.3)	10	(8.8)	14	(11.2)	24	(10.5)	28	(11.2)
N3	9	(7.8)	7	(5.6)	11	(9.7)	11	(8.8)	20	(8.8)	18	(7.2)
Unknown	1	(0.9)	3	(2.4)	8	(7.1)	4	(3.2)	9	(3.9)	7	(2.8)
M Stage at Initial Diagnosis, n (%)												
M0	41	(35.7)	82	(66.1)	51	(45.1)	67	(53.6)	92	(40.4)	149	(59.8)
M1	27	(23.5)	15	(12.1)	26	(23.0)	27	(21.6)	53	(23.2)	42	(16.9)
Unknown	6	(5.2)	7	(5.6)	4	(3.5)	4	(3.2)	10	(4.4)	11	(4.4)
Stage at Baseline, n (%)												
IIA	1	(0.9)	0	(0.0)	0	(0.0)	1	(0.8)	1	(0.4)	1	(0.4)
IIIA	0	(0.0)	2	(1.6)	0	(0.0)	0	(0.0)	0	(0.0)	2	(0.8)
IIIC	0	(0.0)	1	(0.8)	1	(0.9)	0	(0.0)	1	(0.4)	1	(0.4)
IV	8	(7.0)	4	(3.2)	7	(6.2)	11	(8.8)	15	(6.6)	15	(6.0)
IVA	1	(0.9)	2	(1.6)	2	(1.8)	1	(0.8)	3	(1.3)	3	(1.2)
IVB	1	(0.9)	2	(1.6)	1	(0.9)	2	(1.6)	2	(0.9)	4	(1.6)
IVC	0	(0.0)	1	(0.8)	1	(0.9)	0	(0.0)	1	(0.4)	1	(0.4)
Unknown	91	(79.1)	103	(83.1)	88	(77.9)	103	(82.4)	179	(78.5)	206	(82.7)
T Stage at Baseline, n (%)												
T1	2	(1.7)	6	(4.8)	2	(1.8)	3	(2.4)	4	(1.8)	9	(3.6)
T2	6	(5.2)	7	(5.6)	8	(7.1)	7	(5.6)	14	(6.1)	14	(5.6)
T3	3	(2.6)	3	(2.4)	0	(0.0)	4	(3.2)	3	(1.3)	7	(2.8)
T4	8	(7.0)	4	(3.2)	4	(3.5)	7	(5.6)	12	(5.3)	11	(4.4)
Unknown	24	(20.9)	30	(24.2)	25	(22.1)	29	(23.2)	49	(21.5)	59	(23.7)
N Stage at Baseline, n (%)												
N0	8	(7.0)	6	(4.8)	3	(2.7)	9	(7.2)	11	(4.8)	15	(6.0)
N1	4	(3.5)	10	(8.1)	6	(5.3)	7	(5.6)	10	(4.4)	17	(6.8)
N2	4	(3.5)	3	(2.4)	3	(2.7)	4	(3.2)	7	(3.1)	7	(2.8)
N3	3	(2.6)	3	(2.4)	1	(0.9)	4	(3.2)	4	(1.8)	7	(2.8)
Unknown	24	(20.9)	28	(22.6)	27	(23.9)	27	(21.6)	51	(22.4)	55	(22.1)
M Stage at Baseline, n (%)												
M0	3	(2.6)	5	(4.0)	0	(0.0)	6	(4.8)	3	(1.3)	11	(4.4)
M1	27	(23.5)	33	(26.6)	25	(22.1)	37	(29.6)	52	(22.8)	70	(28.1)
Unknown	13	(11.3)	13	(10.5)	14	(12.4)	10	(8.0)	27	(11.8)	23	(9.2)
Sites of Disease, n (%)												
Breast	24	(20.9)	15	(12.1)	21	(18.6)	28	(22.4)	45	(19.7)	43	(17.3)
Bone	101	(87.8)	91	(73.4)	93	(82.3)	91	(72.8)	194	(85.1)	182	(73.1)
Bone only	14	(12.2)	24	(19.4)	14	(12.4)	15	(12.0)	28	(12.3)	39	(15.7)
Lymph Nodes	34	(29.6)	34	(27.4)	27	(23.9)	41	(32.8)	61	(26.8)	75	(30.1)
Visceral[1]	81	(70.4)	82	(66.1)	83	(73.5)	85	(68.0)	164	(71.9)	167	(67.1)
Brain	3	(2.6)	1	(0.8)	2	(1.8)	1	(0.8)	5	(2.2)	2	(0.8)
Liver	60	(52.2)	62	(50.0)	64	(56.6)	49	(39.2)	124	(54.4)	111	(44.6)
Lung	27	(23.5)	29	(23.4)	31	(27.4)	37	(29.6)	58	(25.4)	66	(26.5)
Abdominal Cavity	2	(1.7)	3	(2.4)	1	(0.9)	4	(3.2)	3	(1.3)	7	(2.8)
Adrenal Gland	5	(4.3)	3	(2.4)	5	(4.4)	4	(3.2)	10	(4.4)	7	(2.8)
Cervix Uteri	0	(0.0)	0	(0.0)	1	(0.9)	0	(0.0)	1	(0.4)	0	(0.0)
Chest Wall	0	(0.0)	3	(2.4)	3	(2.7)	8	(6.4)	3	(1.3)	11	(4.4)
Esophagus	0	(0.0)	1	(0.8)	0	(0.0)	1	(0.8)	0	(0.0)	2	(0.8)
Head And Neck	1	(0.9)	1	(0.8)	0	(0.0)	0	(0.0)	1	(0.4)	1	(0.4)
Intestine	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.8)	0	(0.0)	1	(0.4)
Kidney	1	(0.9)	0	(0.0)	0	(0.0)	2	(1.6)	1	(0.4)	2	(0.8)
Mediastinum	6	(5.2)	3	(2.4)	1	(0.9)	2	(1.6)	7	(3.1)	5	(2.0)

	Elacestrant				SOC				Total			
	***ESR1*-mut**		***ESR1*-mut-nd**		***ESR1*-mut**		***ESR1*-mut-nd**		***ESR1*-mut**		***ESR1*-mut-nd**	
Other	1	(0.9)	4	(3.2)	1	(0.9)	4	(3.2)	2	(0.9)	8	(3.2)
Pancreas	1	(0.9)	2	(1.6)	0	(0.0)	1	(0.8)	1	(0.4)	3	(1.2)
Pericardium	1	(0.9)	0	(0.0)	0	(0.0)	1	(0.8)	1	(0.4)	1	(0.4)
Skin	6	(5.2)	3	(2.4)	5	(4.4)	4	(3.2)	11	(4.8)	7	(2.8)
Soft Tissue	5	(4.3)	1	(0.8)	3	(2.7)	2	(1.6)	8	(3.5)	3	(1.2)
Spleen	1	(0.9)	1	(0.8)	0	(0.0)	0	(0.0)	1	(0.4)	1	(0.4)
Stomach	1	(0.9)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.4)	0	(0.0)
Thyroid Gland	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.8)	0	(0.0)	1	(0.4)
Number of Metastatic Sites, n (%)												
0	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
1	16	(13.9)	35	(28.2)	19	(16.8)	27	(21.6)	35	(15.4)	62	(24.9)
2	43	(37.4)	31	(25.0)	34	(30.1)	38	(30.4)	77	(33.8)	69	(27.7)
>=3	44	(38.3)	34	(27.4)	41	(36.3)	41	(32.8)	85	(37.3)	75	(30.1)

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

Safety and Effectiveness Results for the Guardant360 CDx Clinical Study for *ESR1* Mutations

a. Safety Results

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

b. Effectiveness Results

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

The PFS HR observed in the *ESR1*-mut population treated with ORSERDU™ (elacestrant) vs. SOC was 0.546, 95% CI 0.387 – 0.768, p=0.0005 (**Figure 12**), which met the diagnostic study acceptance criterion. Similar results were seen in the sensitivity analysis using an unstratified Cox Proportional Hazard model with an observed HR of 0.531, 95% CI 0.378 - 0.742, p=0.0002. Demonstration of clinical efficacy in the *ESR1*-mut population is further supported by clear separation of the treatment arms in the Kaplan-Meier plot of PFS.

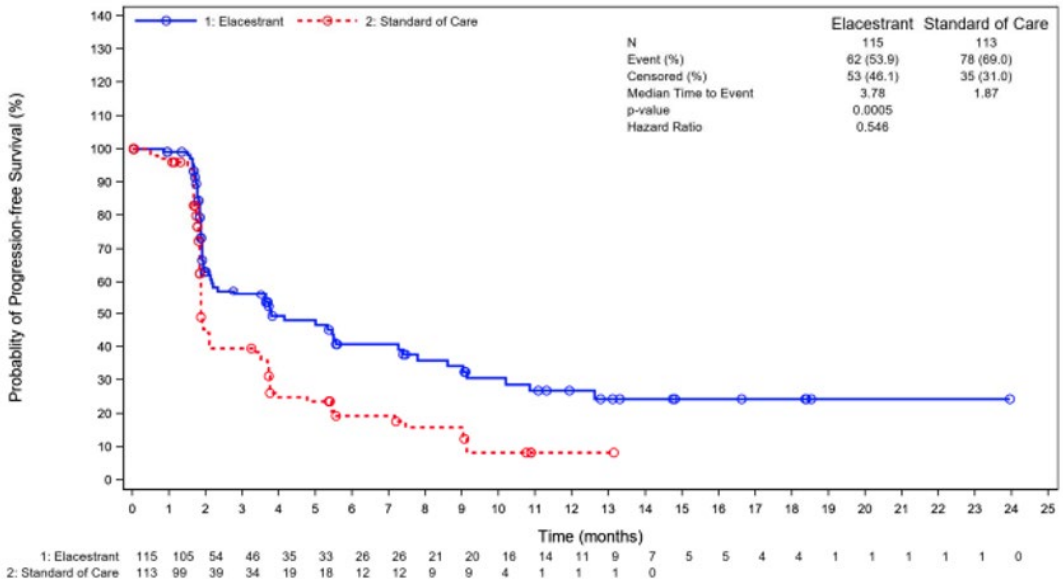


Figure 12. Progression-Free Survival for Elacestrant versus SOC in *ESR1*-mut Subjects

The median PFS in the *ESR1*-mut population treated with ORSERDU™ (elacestrant) was 3.78 months (95% CI 2.17 – 7.26) vs. SOC 1.87 months (95% CI 1.87 – 2.14) (**Table 67**).

Table 63. Efficacy Results for EMERALD (Patients with *ESR1* Missense Mutations)

	ORSERDU (N = 115)	SOC (Fulvestrant or an Aromatase Inhibitor) (N=113)
Progression-free Survival (PFS)[a]		
Number of PFS Events, n (%)	62 (53.9)	78 (69.0)
Median PFS months[b] (95% CI)	3.78 (2.17, 7.26)	1.87 (1.87, 2.14)
Hazard ratio[c] (95% CI)	0.55 (0.39, 0.77)	
p-value[d] (stratified log-rank)	0.0005	
Overall Survival (OS)		
Number of OS Events, n (%)	61 (53)	60 (53)
Hazard ratio[c] (95% CI)	0.90 (0.63, 1.30)	
p-value[d] (stratified log-rank)	NS[e]	

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

Diagnostic Study Conclusions

The diagnostic study met the prespecified acceptance criterion associated with its primary objective. Drug efficacy was established by demonstrating that the PFS HR (0.55, 95% CI 0.39 – 0.77) was statistically significant at p=0.0005 for subjects from the RAD1901-308 clinical study positive for *ESR1* missense mutations by Guardant360 CDx (*ESR1*-mut) treated with elacestrant relative to SOC.

Thus, elacestrant demonstrated clinically meaningful efficacy in the Guardant360 CDx-positive intended use population. This supports the clinical validity of Guardant360 CDx to aid in the selection of breast cancer subjects with *ESR1* mutations detected in plasma for treatment with elacestrant.

7.7. Guardant360 CDx Clinical Study for *ESR1* Mutations for the INLURIYO (Imlunestrant) Therapy

EMBER-3 (NCT04975308) Clinical Study Design

The EMBER-3 clinical study is a Phase 3, randomized, open-label, active-controlled, multicenter trial of INLURIYO (imlunestrant) as continuous monotherapy (Arm A), investigator's choice endocrine therapy of either fulvestrant or exemestane (Arm B), and an additional investigational combination regimen in patients with ER+/ HER2- locally advanced (not amenable to curative treatment by surgery) or metastatic breast cancer. The study enrolled patients who have been treated with an aromatase inhibitor (AI), alone or in combination with a CDK4/6 inhibitor. Eligible patients were randomized 1:1:1 between the 3 treatment arms stratified by (1) previous treatment with any CDK4/6 inhibitor, (2) presence of visceral metastases and (3) region.

Guardant360 CDx Clinical Study Design for *ESR1* Mutations

Subjects from the primary EMBER-3 registration population (Arm A and Arm B) with *ESR1* mutations detected by Guardant360 CDx were included in the diagnostic study primary clinical efficacy cohort to assess the clinical validity of Guardant360 CDx to aid in the selection of breast cancer patients with *ESR1* mutations for INLURIYO (imlunestrant) therapy.

a. Guardant360 CDx Diagnostic Study Efficacy Cohort Patient Inclusion Criteria

- Patient randomized into either Arm A or Arm B of the EMBER-3 clinical study.
- Patient is part of the EMBER-3 INLURIYO NDA registration population.
- Adequate pre-treatment plasma sample available for testing by Guardant360 CDx.

b. Follow-up Schedule

The Guardant360 CDx diagnostic study involved only testing and analysis of plasma samples; as such, no additional patient follow-up was conducted regarding the diagnostic study.

c. Clinical Endpoints

The clinical endpoint used to assess efficacy in the EMBER-3 clinical study primary objective was progression free survival (PFS) per RECIST version 1.1 as assessed by the investigator at the local site or death due to any cause in the absence of disease progression.

d. Diagnostic Objective and Endpoints

The primary objective of this diagnostic clinical study was to demonstrate the clinical validity of Guardant360 CDx for the selection of ER+/HER2- breast cancer patients with an *ESR1* mutation detected for treatment with INLURIYO therapy.

This objective was assessed by comparing INLURIYO therapy efficacy relative to fulvestrant or exemestane in the EMBER-3 registration population of patients with an *ESR1* mutation detected by Guardant360 CDx (*ESR1*-mut). The primary endpoint is the same as that used for the clinical study, PFS per RECIST v1.1 as assessed by the investigator.

Accountability of the Guardant360 CDx Clinical Study Cohort for *ESR1* Mutations

Pre-treatment plasma samples from 630 subjects of the primary EMBER-3 registration population (Arms A and B, n=661) were tested with Guardant360 CDx, of which 254 subjects (40.3% of the Guardant360 CDx tested population) have one or more *ESR1* mutation(s) detected and 365 (57.9% of the Guardant360 CDx tested population) have none. 31 subjects did not have samples available for

testing, and 11 samples failed sample QC and did not generate valid Guardant360 CDx results (**Figure 13**).

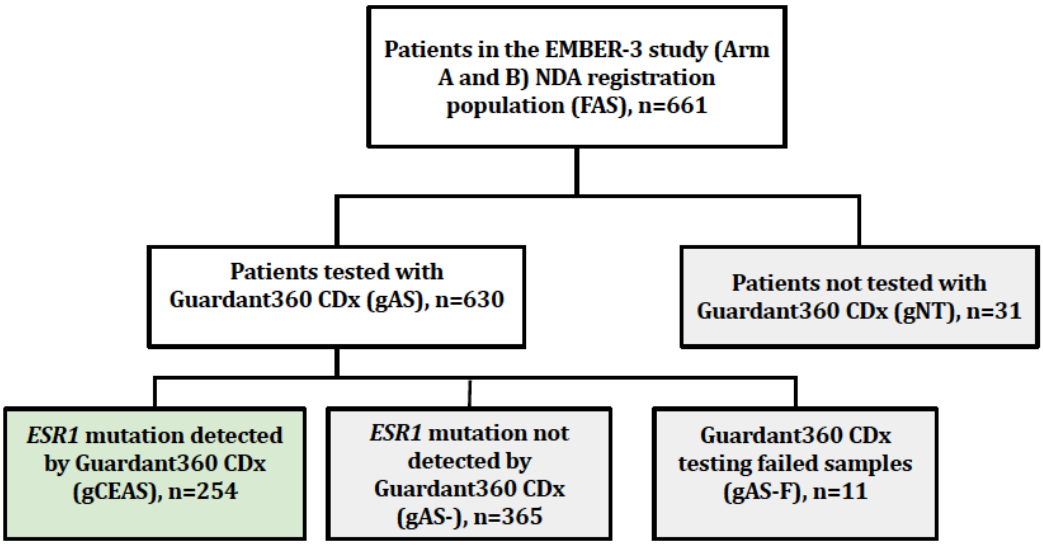


Figure 13. Guardant360 CDx *ESR1* Mutations Efficacy Analysis Patient Accountability and Analysis Set Definitions

Study Population Demographics and Baseline Parameters

Demographics and baseline clinical characteristics of subjects enrolled in Arm A and Arm B of the EMBER-3 clinical study were categorized relative to the diagnostic study populations as defined by Guardant360 CDx results. As shown in **Table 68** and **Table 69**, the *ESR1*-mut and the *ESR1*-mut-nd populations are well balanced. The INLURIYO therapy and fulvestrant or exemestane treatment arms are also well balanced.

Table 68. Demographic and Baseline Characteristics of Full Analysis Set Population

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		Fulvestrant or Exemestane (Arm B)		Total (Arm A and Arm B)		gAS-Unk (gAS-F + gNT)	FAS	p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A
	ESR1-mut	*ESR1*-mut-nd	*ESR1*-mut	*ESR1*-mut-nd	*ESR1*-mut (gCEAS)	*ESR1*-mut-nd (gAS-)			
Analysis set:	136*	172	118	193	254	365	42	661	
Gender n (%)									
n	136	172	118	193	254	365	42	661	-
Female	136 (100)	169 (98.3)	118 (100)	192 (99.5)	254 (100)	361 (98.9)	41 (97.6)	656 (99.2)	
Male	0	3 (1.7)	0	1 (0.5)	0	4 (1.1)	1 (2.4)	5 (0.8)	

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**				
	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**	**gAS-Unk (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
Age									
n	136	172	118	193	254	365	42	661	0.432
Mean	60.7	59.9	59.6	62	60.2	61	59.9	60.6	
Std. Dev.	11.1	12.2	11.2	12.3	11.2	12.3	11.3	11.8	
Median	61	60.5	59.5	63	61	62	61	61	
Min	28	28	33	27	28	27	32	27	
Max	85	87	85	89	85	89	80	89	
Age Category (years) n (%)									
n	136	172	118	193	254	365	42	661	>.999
<65	90 (66.2)	106 (61.6)	78 (66.1)	107 (55.4)	168 (66.1)	213 (58.4)	27 (64.3)	408 (61.7)	
>=65	46 (33.8)	66 (38.4)	40 (33.9)	86 (44.6)	86 (33.9)	152 (41.6)	15 (35.7)	253 (38.3)	
Age Category 2 (years) n (%)									
n	136	172	118	193	254	365	42	661	0.910
<65	90 (66.2)	106 (61.6)	78 (66.1)	107 (55.4)	168 (66.1)	213 (58.4)	27 (64.3)	408 (61.7)	
>=65 - <75	30 (22.1)	46 (26.7)	24 (20.3)	53 (27.5)	54 (21.3)	99 (27.1)	12 (28.6)	165 (25.0)	
>=75 - <85	14 (10.3)	16 (9.3)	15 (12.7)	28 (14.5)	29 (11.4)	44 (12.1)	3 (7.1)	76 (11.5)	
>=85	2 (1.5)	4 (2.3)	1 (0.8)	5 (2.6)	3 (1.2)	9 (2.5)	0	12 (1.8)	

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**				
	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**	**gAS-Unk (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
Race n (%)									
n	136	172	118	193	254	365	42	661	0.646
American Indian or Alaska Native	7 (5.1)	16 (9.3)	3 (2.5)	13 (6.7)	10 (3.9)	29 (7.9)	0	39 (5.9)	
Asian	33 (24.3)	49 (28.5)	31 (26.3)	50 (25.9)	64 (25.2)	99 (27.1)	25 (59.5)	188 (28.4)	
Black or African American	7 (5.1)	4 (2.3)	3 (2.5)	4 (2.1)	10 (3.9)	8 (2.2)	0	18 (2.7)	
White	80 (58.8)	94 (54.7)	76 (64.4)	111 (57.5)	156 (61.4)	205 (56.2)	16 (38.1)	377 (57.0)	
Multiple	1 (0.7)	1 (0.6)	1 (0.8)	6 (3.1)	2 (0.8)	7 (1.9)	0	9 (1.4)	
Missing	8 (5.9)	8 (4.7)	4 (3.4)	9 (4.7)	12 (4.7)	17 (4.7)	1 (2.4)	30 (4.5)	
Ethnicity n (%)									
n	136	172	118	193	254	365	42	661	0.989
Hispanic or Latino	25 (18.4)	51 (29.7)	23 (19.5)	55 (28.5)	48 (18.9)	106 (29.0)	5 (11.9)	159 (24.1)	
Not Hispanic or Latino	97 (71.3)	104 (60.5)	83 (70.3)	119 (61.7)	180 (70.9)	223 (61.1)	31 (73.8)	434 (65.7)	
Not Reported	13 (9.6)	17 (9.9)	12 (10.2)	19 (9.8)	25 (9.8)	36 (9.9)	6 (14.3)	67 (10.1)	
Missing	1 (0.7)	0	0	0	1 (0.4)	0	0	1 (0.2)	
Weight (kg)									
n	135	169	117	188	252	357	41	650	0.977
Mean	70.97	68.86	71.03	68.54	71	68.69	67.1	69.49	
Std. Dev.	15.2	17.44	16.23	16.11	15.65	16.73	12.7	16.12	
Median	69.5	66	69.8	66	69.6	66	64.5	67.1	
Min	42.5	37	38	39.9	38	37	47	37	
Max	111	142.7	127.3	129.1	127.3	142.7	105	142.7	
Missing	1	3	1	5	2	8	1	11	

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**				
	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**	**gAS-Unk (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
Region									
n	136	172	118	193	254	365	42	661	0.972
East Asia	28 (20.6)	45 (26.2)	26 (22.0)	43 (22.3)	54 (21.3)	88 (24.1)	25 (59.5)	167 (25.3)	
North America/ Western Europe	63 (46.3)	57 (33.1)	54 (45.8)	72 (37.3)	117 (46.1)	129 (35.3)	8 (19.0)	254 (38.4)	
Other	45 (33.1)	70 (40.7)	38 (32.2)	78 (40.4)	83 (32.7)	148 (40.5)	9 (21.4)	240 (36.3)	

Std. Dev.= Standard Deviation, Min = Minimum, Max = Maximum, *ESR1*-mut = *ESR1* mutation detected, *ESR1*-mut-nd = *ESR1* mutation not detected

*: Two subjects included in the drug efficacy analysis were not tested by Guardant360 CDx.

Table 64. Baseline Disease Characteristics of Full Analysis Set Population

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**				
	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**	**gAS-Unk (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
Analysis set:	136*	172	118	193	254	365	42	661	
Duration of Disease (Months)									
n[a]	136	172	117	193	253	365	42	660	0.198
Mean	72.2	72.8	85.3	70.4	78.2	71.5	68.3	73.9	
Std. Dev.	76.1	70.9	85.6	71.4	80.8	71.1	66.5	74.6	
Median	39.6	49.6	45.7	45.2	41.6	46.3	48.6	45.5	
Min	8	1	5	2	5	1	3	1	
Max	408	355	377	324	408	355	283	408	
Missing	0	0	1	0	1	0	0	1	

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**				
	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**	**gAS-Unk (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
Initial Pathological Diagnosis Type n(%)									
n[a]	136	168	118	189	254	357	41	652	0.567
Breast cancer	21 (15.4)	28 (16.7)	16 (13.6)	38 (20.1)	37 (14.6)	66 (18.5)	9 (22.0)	112 (17.2)	
Breast cancer metastatic	0	0	2 (1.7)	0	2 (0.8)	0	0	2 (0.3)	
Invasive ductal breast carcinoma	101 (74.3)	118 (70.2)	87 (73.7)	130 (68.8)	188 (74.0)	248 (69.5)	28 (68.3)	464 (71.2)	
Invasive lobular breast carcinoma	11 (8.1)	18 (10.7)	12 (10.2)	21 (11.1)	23 (9.1)	39 (10.9)	4 (9.8)	66 (10.1)	
Mucinous breast carcinoma	3 (2.2)	4 (2.4)	1 (0.8)	0	4 (1.6)	4 (1.1)	0	8 (1.2)	
Initial Histopathological Diagnosis Grade n (%)									
n[a]	133	165	116	185	249	350	41	640	0.462
G1 - Low combined histologic grade (favorable)	11 (8.3)	18 (10.9)	5 (4.3)	20 (10.8)	16 (6.4)	38 (10.9)	2 (4.9)	56 (8.8)	
G2 - Intermediate combined histologic grade (mod favorable)	68 (51.1)	86 (52.1)	69 (59.5)	100 (54.1)	137 (55.0)	186 (53.1)	16 (39.0)	339 (53.0)	
G3 - High combined histologic grade (unfavorable)	34 (25.6)	37 (22.4)	26 (22.4)	40 (21.6)	60 (24.1)	77 (22.0)	8 (19.5)	145 (22.7)	
GX - Grade cannot be assessed	20 (15.0)	24 (14.5)	16 (13.8)	25 (13.5)	36 (14.5)	49 (14.0)	15 (36.6)	100 (15.6)	

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		**Fulvestrant or Exemestane (Arm B)**		**Total (Arm A and Arm B)**				
	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut**	***ESR1*-mut-nd**	***ESR1*-mut (gCEAS)**	***ESR1*-mut-nd (gAS-)**	**gAS-Unk (gAS-F + gNT)**	**FAS**	**p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A**
Study Entry Pathological Diagnosis Disease Stage n (%)									
n	136	172	118	193	254	365	42	661	0.626
Stage IIIB	1 (0.7)	6 (3.5)	0	4 (2.1)	1 (0.4)	10 (2.7)	1 (2.4)	12 (1.8)	
Stage IIIC	3 (2.2)	3 (1.7)	1 (0.8)	4 (2.1)	4 (1.6)	7 (1.9)	0	11 (1.7)	
Stage IV	132 (97.1)	163 (94.8)	117 (99.2)	185 (95.9)	249 (98.0)	348 (95.3)	41 (97.6)	638 (96.5)	
Baseline ECOG Performance Status n (%)									
n	136	172	118	193	254	365	42	661	0.603
0	84 (61.8)	122 (70.9)	77 (65.3)	117 (60.6)	161 (63.4)	239 (65.5)	27 (64.3)	427 (64.6)	
1	52 (38.2)	50 (29.1)	41 (34.7)	76 (39.4)	93 (36.6)	126 (34.5)	15 (35.7)	234 (35.4)	
Menopausal Status n (%)									
n[a]	136	169	118	192	254	361	41	656	>.999
Post Menopausal	120 (88.2)	137 (81.1)	105 (89.0)	165 (85.9)	225 (88.6)	302 (83.7)	35 (85.4)	562 (85.7)	
Premenopausal	16 (11.8)	32 (18.9)	13 (11.0)	27 (14.1)	29 (11.4)	59 (16.3)	6 (14.6)	94 (14.3)	
Missing	0	3	0	1	0	4	1	5	
Prior CDK4/6i in any setting (CRF) n (%)									
n	136	172	118	193	254	365	42	661	0.495
No	44 (32.4)	78 (45.3)	33 (28.0)	94 (48.7)	77 (30.3)	172 (47.1)	28 (66.7)	277 (41.9)	
Yes	92 (67.6)	94 (54.7)	85 (72.0)	99 (51.3)	177 (69.7)	193 (52.9)	14 (33.3)	384 (58.1)	
Measurable Disease n (%)									
n	136	172	118	193	254	365	42	661	0.536
No	26 (19.1)	35 (20.3)	27 (22.9)	48 (24.9)	53 (20.9)	83 (22.7)	12 (28.6)	148 (22.4)	
Yes	110 (80.9)	137 (79.7)	91 (77.1)	145 (75.1)	201 (79.1)	282 (77.3)	30 (71.4)	513 (77.6)	

EMBER-3 (J2J-OX-JZLC, NCT04975308)									
	INLURIYO Therapy (Arm A)		Fulvestrant or Exemestane (Arm B)		Total (Arm A and Arm B)		gAS-Unk (gAS-F + gNT)	FAS	p-value (*ESR1*-mut in Arm B vs. *ESR1*-mut in Arm A
	ESR1-mut	*ESR1*-mut-nd	*ESR1*-mut	*ESR1*-mut-nd	*ESR1*-mut (gCEAS)	*ESR1*-mut-nd (gAS-)			
Number of Organs Involved n (%)									
n	136	172	118	193	254	365	42	661	0.7
1	35 (25.7)	64 (37.2)	35 (29.7)	74 (38.3)	70 (27.6)	138 (37.8)	16 (38.1)	224 (33.9)	
2	44 (32.4)	52 (30.2)	39 (33.1)	54 (28.0)	83 (32.7)	106 (29.0)	14 (33.3)	203 (30.7)	
>=3	57 (41.9)	56 (32.6)	44 (37.3)	65 (33.7)	101 (39.8)	121 (33.2)	12 (28.6)	234 (35.4)	
Visceral Metastasis (CRF) n (%)									
n	136	172	118	193	254	365	42	661	0.610
No	54 (39.7)	76 (44.2)	51 (43.2)	93 (48.2)	105 (41.3)	169 (46.3)	21 (50.0)	295 (44.6)	
Yes	82 (60.3)	96 (55.8)	67 (56.8)	100 (51.8)	149 (58.7)	196 (53.7)	21 (50.0)	366 (55.4)	

Std. Dev.= Standard Deviation, Min = Minimum, Max = Maximum, *ESR1*-mut = *ESR1* mutation detected, *ESR1*-mut-nd = *ESR1* mutation not detected, n= number of subjects, n^a= number of subjects with non-missing data, used as denominator for percentage calculation, CNS= central nervous system, CRF=case report form
*: Two subjects included in the drug efficacy analysis were not tested by Guardant360 CDx.

Safety and Effectiveness Results for the Guardant360 CDx Clinical Study for *ESR1* Mutations

a. Safety Results

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

b. Effectiveness Results

i. PFS in Patients with *ESR1* Mutations Detected by Guardant360 CDx

To demonstrate the clinical validity of Guardant360 CDx for the selection of ER+/HER2- locally advanced/metastatic breast cancer patients with *ESR1* mutations detected for treatment with imlunestrant, the primary diagnostic study objective was assessed by comparing the efficacy of INLURIYO (imlunestrant) therapy relative to fulvestrant or exemestane in *ESR1*-mut patients.

The PFS hazard ratio (HR) observed in the *ESR1*-mut population (gCEAS) treated with INLURIYO therapy vs. fulvestrant or exemestane was 0.635 (95% CI 0.478 – 0.844, p=0.0017, **Table 70**). The clinical efficacy of INLURIYO therapy in the *ESR1*-mut population is further supported by the clear separation of the treatment arms in the Kaplan-Meier plot of PFS (**Figure 14**).

The median PFS in the *ESR1*-mut population treated with INLURIYO therapy was 5.49 months (95% CI 3.78 – 7.16) vs. fulvestrant or exemestane 3.84 months (95% CI 3.68 – 5.52) (**Table 70**).

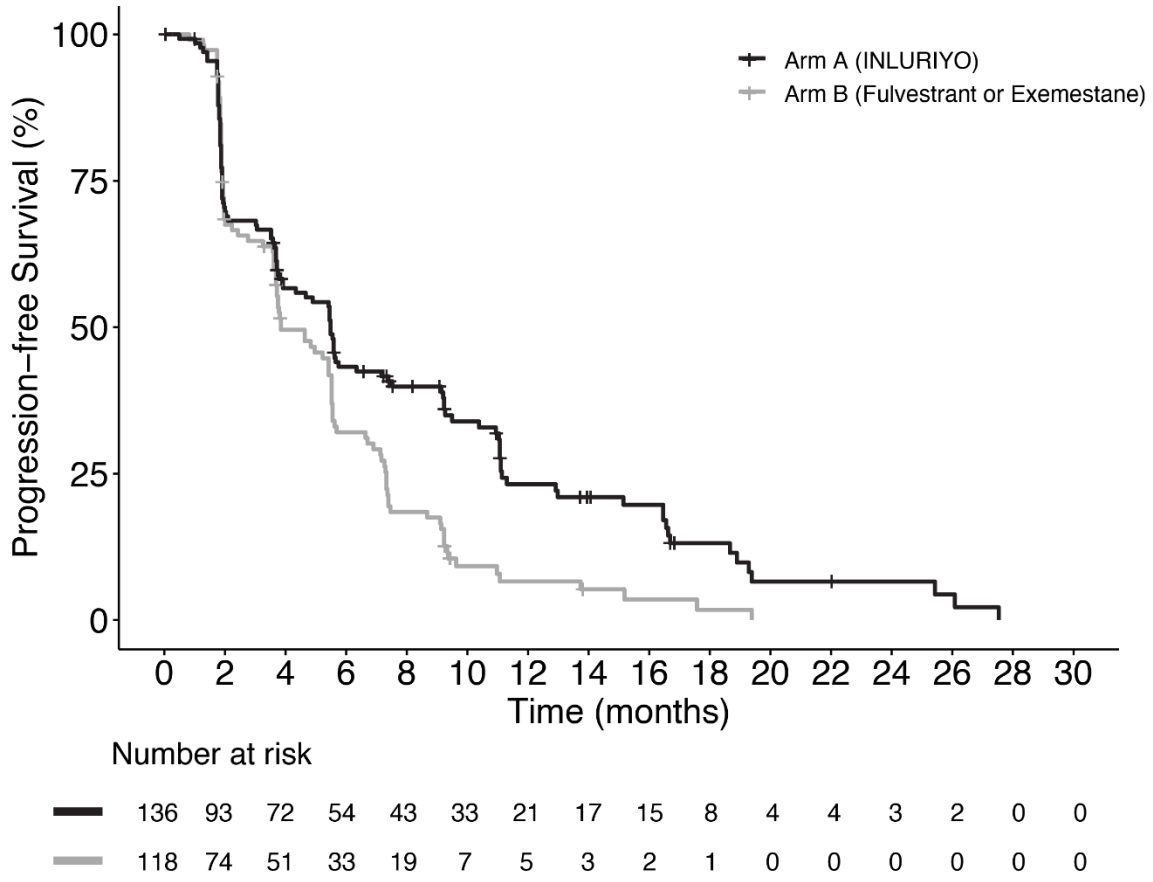


Figure 14. Kaplan-Meier Plot of Investigator-Assessed PFS for INLURIYO (Imlunestrant) Therapy vs. Fulvestrant or Exemestane in *ESR1*-Mut Subjects

Date Cutoff Date = 24-JUN-2024
Note: Two subjects included in the drug efficacy analysis were not tested by Guardant360 CDx.

Table 65. Summary of Investigator-Assessed PFS for INLURIYO (Imlunestrant) Therapy vs. Fulvestrant or Exemestane in *ESR1*-Mut Subjects

	INLURIYO Therapy (N=136*)	Fulvestrant or Exemestane (N=118)
Number of Events, n (%)	109 (80.1)	102 (86.4)
Median PFS Months (95% CI)	5.49 (3.78, 7.16)	3.84 (3.68, 5.52)
Hazard Ratio (95% CI)	0.635 (0.478, 0.844)	
p-value (stratified log-rank)	0.0017	

* Two subjects included in the drug efficacy analysis were not tested by Guardant360 CDx.

Diagnostic Study Conclusions

INLURIYO (imlunestrant) therapy demonstrated clinically meaningful and statistically significant improvement in PFS as compared to fulvestrant or exemestane in BC patients with *ESR1* mutations as determined by Guardant360 CDx.

This supports the clinical validity of Guardant360 CDx to aid in the selection of patients with ER+/HER2- locally advanced/metastatic breast cancer harboring *ESR1* mutations detected in plasma for treatment with the INLURIYO therapy.

7.8. Guardant360 CDx Clinical Study for *BRAF* V600E Mutation

BREAKWATER Clinical Study (NCT04607421) Design

The BREAKWATER clinical study is a multi-center, randomized, open-label, randomized Phase 3 study comparing encorafenib and cetuximab (EC) therapy with or without chemotherapy to SOC chemotherapy in patients with *BRAF* V600E mutation and previously untreated for mCRC. Patients were randomized 1:1:1 to receive EC (Arm A), EC + chemotherapy (Arm B), or SOC chemotherapy (Arm C).

Guardant360 CDx Clinical Study Design for *BRAF* V600E Mutation

Pre-treatment plasma samples from patients enrolled in the phase 3 portion of BREAKWATER clinical study were retrospectively tested with Guardant360 CDx to determine *BRAF* V600E mutation status. The results of this testing were used to support the clinical validity of Guardant360 CDx, as demonstrated by its ability to identify mCRC patients with *BRAF* V600E mutation who may benefit from treatment with EC and chemotherapy.

a. Clinical Study Inclusion and Exclusion Criteria

All patients randomized in Arms B and C in the phase 3 BREAKWATER clinical study were included in the diagnostic study if the criteria below were met.

Guardant360 CDx Diagnostic Study Cohort Patient Inclusion Criteria

- Patients enrolled in the BREAKWATER clinical study with informed consent for blood sample use for diagnostic development.
- The patient is part of the BREAKWATER sNDA registration population.
- Adequate pre-treatment plasma sample available for testing by Guardant360 CDx.

b. Diagnostic Objective and Endpoints

The primary objective of this diagnostic study was to demonstrate the clinical validity of Guardant360 CDx for the selection of mCRC patients with *BRAF* V600E mutation who may benefit from treatment with EC with mFOLFOX6. This objective was assessed by comparing the clinical efficacy of EC with mFOLFOX6 (EC + mFOLFOX6) to SOC chemotherapy, as measured by objective response rate (ORR) by blinded independent central review (BICR) and by progression-free survival (PFS) by BICR in patients with *BRAF* V600E mutation as detected by Guardant360 CDx.

Subject Accountability

The diagnostic study included 343 (71.6%) of the 479 patients randomized in the FAS (**Figure 15**). Of these, *BRAF* V600E mutation was detected in 278 patients (81.0% of the gAS) by Guardant360 CDx and were included in the primary objective analysis set (gCEAS); *BRAF* V600E mutation was not detected (gAS-) in 50 patients (14.6% of the gAS); and 15 patient (4.4% of the gAS) samples failed Guardant360 CDx testing. Plasma samples were not available for 136 patients (28.4%) in the FAS.

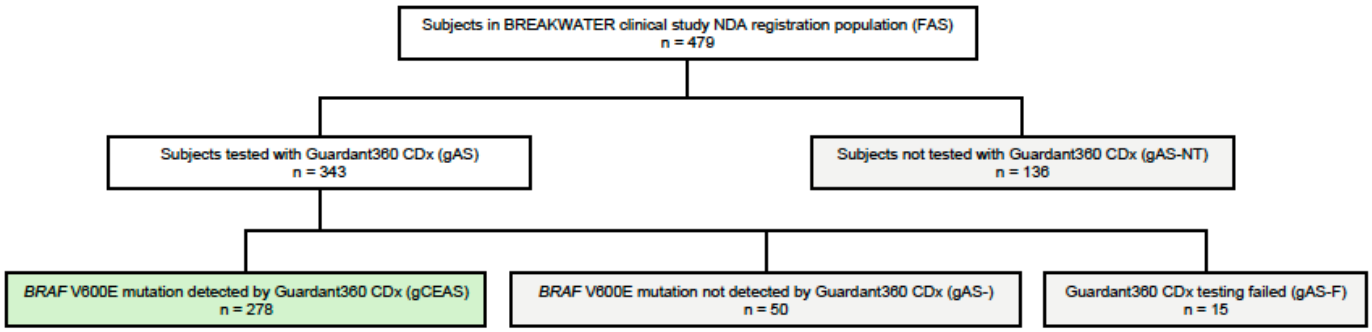


Figure 15. Diagnostic Study Patient Disposition

The sensitivity analysis prevalence sub-study set included 132 patients with matched plasma and tissue samples (**Figure 16**). Samples from 10 (7.6%) patients failed Guardant360 CDx testing and a sample from 1 patient failed CTA testing. No samples failed testing with both Guardant360 CDx and CTA. This resulted in 121 patient samples with valid Guardant360 CDx and tissue CTA results.

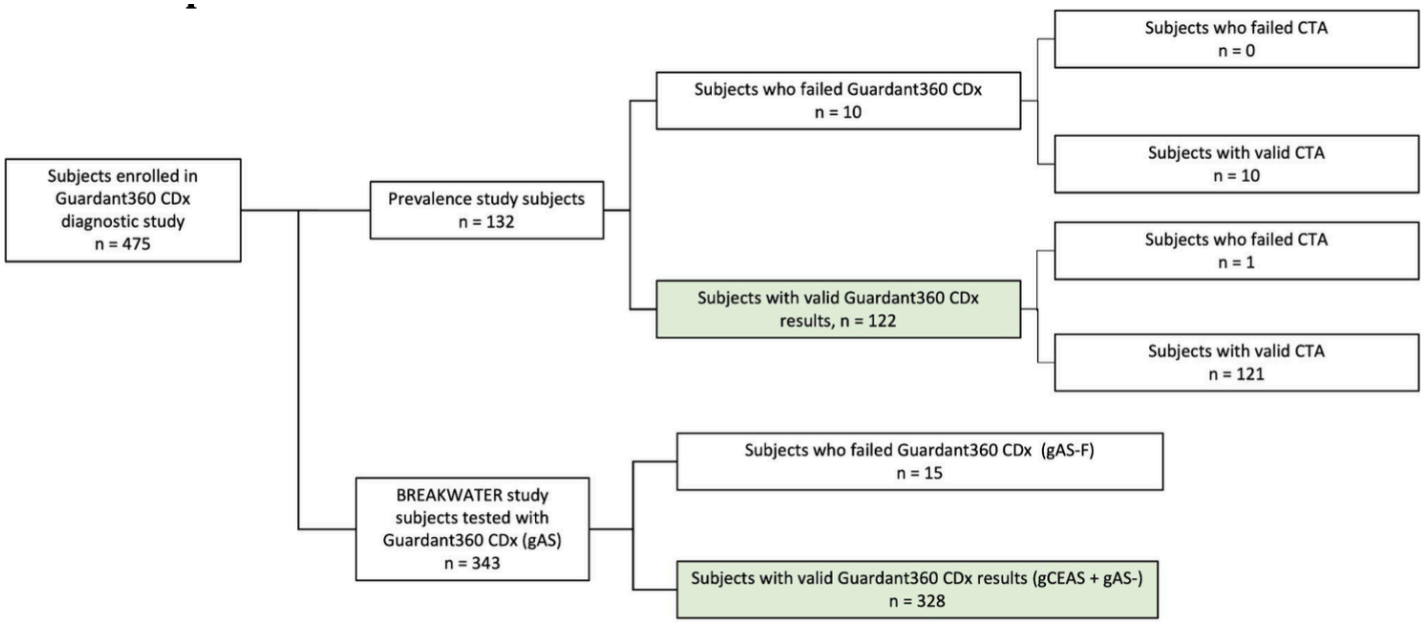


Figure 16. Diagnostic Study Sensitivity Analysis and Prevalence Sub-Study Patient Disposition

Study Population Demographics and Baseline Clinical Characteristics Parameters for the Guardant360 CDx Clinical Study for *BRAF* V600E Mutations [BREAKWATER clinical study]

Demographics and baseline clinical characteristics of patients enrolled in EC + mFOLFOX6 arm and SOC chemotherapy arm of the BREAKWATER clinical study were categorized relative to the diagnostic study populations as defined by Guardant360 CDx results and treatment arm. Demographic and baseline clinical characteristics of the FAS and all subgroups are summarized and reported in **Table 71** and **Table 72**. Demographic and baseline clinical characteristics of the gCEAS subgroup were similar to those of the FAS. The EC + mFOLFOX6 and SOC arms are also similar.

Table 71. Baseline Demographics of the Full Analysis Set Population

	gCEAS		gAS		gAS-		gAS-Unk		FAS	
	EC+ mFOLFOX6 (N = 143)	SOC (N = 135)	EC+ mFOLFOX6 (N = 176)	SOC (N = 167)	EC+ mFOLFOX6 (N = 26)	SOC (N = 24)	EC+ mFOLFOX6 (N = 67)	SOC (N = 84)	EC+ mFOLFOX6 (N = 236)	SOC (N = 243)
Age (years) [a]										
n	143	135	176	167	26	24	67	84	236	243
Mean (SD)	57.95 (12.67)	60.47 (12.62)	58.30 (12.55)	60.40 (13.05)	61.50 (11.93)	59.21 (15.86)	56.57 (12.99)	57.68 (12.36)	57.95 (12.71)	59.38 (12.89)
Median	61.00	63.00	61.00	63.00	62.50	62.00	60.00	61.00	60.00	62.00
Min, Max	24, 80	30, 84	24, 81	29, 84	32, 81	29, 79	29, 81	28, 81	24, 81	28, 84
Age Category (years), n (%) [a]										
< 18	0	0	0	0	0	0	0	0	0	0
18 to < 65	90 (62.9)	72 (53.3)	110 (62.5)	89 (53.3)	14 (53.8)	13 (54.2)	46 (68.7)	54 (64.3)	150 (63.6)	139 (57.2)
65 to < 75	45 (31.5)	48 (35.6)	55 (31.3)	56 (33.5)	9 (34.6)	5 (20.8)	16 (23.9)	27 (32.1)	70 (29.7)	80 (32.9)
≥ 75	8 (5.6)	15 (11.1)	11 (6.3)	22 (13.2)	3 (11.5)	6 (25.0)	5 (7.5)	3 (3.6)	16 (6.8)	24 (9.9)
Sex, n (%)										
Female	75 (52.4)	73 (54.1)	88 (50.0)	91 (54.5)	10 (38.5)	13 (54.2)	28 (41.8)	38 (45.2)	113 (47.9)	124 (51.0)
Male	68 (47.6)	62 (45.9)	88 (50.0)	76 (45.5)	16 (61.5)	11 (45.8)	39 (58.2)	46 (54.8)	123 (52.1)	119 (49.0)
Race, n (%)										
American Indian or Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	42 (29.4)	35 (25.9)	52 (29.5)	46 (27.5)	9 (34.6)	9 (37.5)	37 (55.2)	47 (56.0)	88 (37.3)	91 (37.4)
Black or African American	0	1 (0.7)	0	1 (0.6)	0	0	0	0	0	1 (0.4)
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
White	98 (68.5)	93 (68.9)	119 (67.6)	113 (67.7)	17 (65.4)	15 (62.5)	26 (38.8)	36 (42.9)	141 (59.7)	144 (59.3)
Multiple	0	2 (1.5)	0	2 (1.2)	0	0	0	0	0	2 (0.8)
Not Reported	3 (2.1)	4 (3.0)	5 (2.8)	5 (3.0)	0	0	4 (6.0)	1 (1.2)	7 (3.0)	5 (2.1)
Ethnicity, n (%)										
Hispanic or Latino	14 (9.8)	16 (11.9)	21 (11.9)	17 (10.2)	5 (19.2)	1 (4.2)	9 (13.4)	13 (15.5)	28 (11.9)	30 (12.3)
Not Hispanic or Latino	117 (81.8)	112 (83.0)	141 (80.1)	142 (85.0)	21 (80.8)	23 (95.8)	49 (73.1)	66 (78.6)	187 (79.2)	201 (82.7)
Not Reported	12 (8.4)	7 (5.2)	14 (8.0)	8 (4.8)	0	0	9 (13.4)	5 (6.0)	21 (8.9)	12 (4.9)

Table 72. Baseline Disease Characteristics of the Full Analysis Set Population

	gCEAS		gAS		gAS-		gAS-Unk		FAS	
	EC+ mFOLFOX6 (N = 143)	SOC (N = 135)	EC+ mFOLFOX6 (N = 176)	SOC (N = 167)	EC+ mFOLFOX6 (N = 26)	SOC (N = 24)	EC+ mFOLFOX6 (N = 67)	SOC (N = 84)	EC+ mFOLFOX6 (N = 236)	SOC (N = 243)
Body Site, n (%)										
Colon, Ascending	50 (35.0)	39 (28.9)	61 (34.7)	52 (31.1)	7 (26.9)	10 (41.7)	21 (31.3)	23 (27.4)	78 (33.1)	72 (29.6)
Colon, Descending	11 (7.7)	4 (3.0)	15 (8.5)	7 (4.2)	3 (11.5)	2 (8.3)	4 (6.0)	10 (11.9)	18 (7.6)	16 (6.6)
Colon, Rectosigmoid	9 (6.3)	10 (7.4)	12 (6.8)	12 (7.2)	3 (11.5)	1 (4.2)	5 (7.5)	5 (6.0)	17 (7.2)	16 (6.6)
Colon, Sigmoid	16 (11.2)	14 (10.4)	20 (11.4)	18 (10.8)	4 (15.4)	3 (12.5)	10 (14.9)	15 (17.9)	30 (12.7)	32 (13.2)
Colon, Transverse	25 (17.5)	26 (19.3)	28 (15.9)	28 (16.8)	2 (7.7)	2 (8.3)	12 (17.9)	7 (8.3)	39 (16.5)	35 (14.4)
Colon, Hepatic Flexure	6 (4.2)	6 (4.4)	6 (3.4)	7 (4.2)	0	1 (4.2)	3 (4.5)	7 (8.3)	9 (3.8)	14 (5.8)
Colon, Splenic Flexure	1 (0.7)	5 (3.7)	1 (0.6)	6 (3.6)	0	1 (4.2)	0	1 (1.2)	1 (0.4)	7 (2.9)
Rectum	13 (9.1)	15 (11.1)	17 (9.7)	17 (10.2)	4 (15.4)	2 (8.3)	7 (10.4)	10 (11.9)	24 (10.2)	27 (11.1)
Cecum	12 (8.4)	16 (11.9)	16 (9.1)	20 (12.0)	3 (11.5)	2 (8.3)	5 (7.5)	6 (7.1)	20 (8.5)	24 (9.9)
Side of Tumor, n (%)										
Left	50 (35.0)	48 (35.6)	65 (36.9)	60 (35.9)	14 (53.8)	9 (37.5)	26 (38.8)	41 (48.8)	90 (38.1)	98 (40.3)
Right	93 (65.0)	87 (64.4)	111 (63.1)	107 (64.1)	12 (46.2)	15 (62.5)	41 (61.2)	43 (51.2)	146 (61.9)	145 (59.7)
Stage at Initial Diagnosis, n (%)										
Stage I	1 (0.7)	2 (1.5)	2 (1.1)	2 (1.2)	1 (3.8)	0	1 (1.5)	0	3 (1.3)	2 (0.8)
Stage II	9 (6.3)	8 (5.9)	11 (6.3)	9 (5.4)	2 (7.7)	1 (4.2)	2 (3.0)	1 (1.2)	13 (5.5)	10 (4.1)
Stage III	18 (12.6)	12 (8.9)	28 (15.9)	27 (16.2)	9 (34.6)	11 (45.8)	11 (16.4)	22 (26.2)	38 (16.1)	45 (18.5)
Stage IV	115 (80.4)	113 (83.7)	135 (76.7)	129 (77.2)	14 (53.8)	12 (50.0)	53 (79.1)	61 (72.6)	182 (77.1)	186 (76.5)
Presence of Liver Metastases, n (%)										
Yes	94 (65.7)	98 (72.6)	104 (59.1)	110 (65.9)	8 (30.8)	9 (37.5)	45 (67.2)	53 (63.1)	147 (62.3)	160 (65.8)
No	49 (34.3)	37 (27.4)	72 (40.9)	57 (34.1)	18 (69.2)	15 (62.5)	22 (32.8)	31 (36.9)	89 (37.7)	83 (34.2)
Time Since Initial Diagnosis (months)										
n	143	135	176	167	26	24	67	84	236	243
Mean	7.45	6.34	7.64	7.31	8.82	11.64	4.65	7.49	6.81	7.26
SD	15.62	14.59	14.50	14.41	8.56	13.45	7.66	17.33	13.18	15.51
Median	1.87	1.64	2.14	1.77	4.98	3.19	1.84	2.07	1.94	1.84
Min, Max	0, 112	0, 124	0, 112	0, 124	1, 27	1, 47	0, 37	1, 109	0, 112	0, 124
Time Since Recurrence/Metastatic (months)										
n	142	134	175	166	26	24	67	83	235	241
Mean	2.45	2.05	2.44	2.25	2.24	3.39	1.89	1.61	2.26	2.03
SD	4.44	2.71	4.10	3.50	2.13	6.58	2.13	1.04	3.70	2.97
Median	1.54	1.48	1.58	1.51	1.63	1.86	1.48	1.45	1.54	1.51
Min, Max	0, 40	0, 21	0, 40	0, 34	1, 11	1, 34	0, 16	0, 7	0, 40	0, 34
ECOG Performance Status at Baseline, n (%)										
0	78 (54.5)	81 (60.0)	98 (55.7)	95 (56.9)	17 (65.4)	7 (29.2)	33 (49.3)	43 (51.2)	128 (54.2)	131 (53.9)
1	63 (44.1)	51 (37.8)	75 (42.6)	65 (38.9)	8 (30.8)	13 (54.2)	33 (49.3)	34 (40.5)	104 (44.1)	98 (40.3)
Missing	2 (1.4)	3 (2.2)	3 (1.7)	7 (4.2)	1 (3.8)	4 (16.7)	1 (1.5)	7 (8.3)	4 (1.7)	14 (5.8)

Safety and Effectiveness Results for the Guardant360 CDx Clinical Study for *BRAF* V600E Mutation

a. Safety Results

Data regarding the safety of encorafenib therapy are presented in the drug approval. Refer to the encorafenib label for more information. No adverse events were reported in the conduct of the diagnostic studies.

b. Effectiveness Results

i. ORR in Patients with *BRAF* V600E Mutation Detected by Guardant360 CDx

At the ORR primary completion date (PCD) data cutoff date of 2023-12-23, the observed BICR-assessed ORR among the first 110 randomized patients positive for *BRAF* V600E detected by Guardant360 CDx was 66.7% (95% CI, 54.9%, 76.6%) in the EC + mFOLFOX6 arm, compared to 34.5% (95% CI 23.6%, 47.3%) in the SOC chemotherapy arm, and is similar to the ORR observed in the first 110 randomized patients in the BREAKWATER clinical study [61% (95% CI, 52%, 70%) in the EC + mFOLFOX Arm, as compared to 40% (95% CI, 31%, 49%) in the SOC chemotherapy Arm. This corresponds to an odds ratio of 4.700 (95% CI, 1.973, 11.046; p<.0001; **Table 73**), indicating a significantly higher likelihood of response with EC + mFOLFOX6. These results are compared with that observed in the full analysis set (FAS) for BREAKWATER, where the ORR odds ratio was 2.443 (95% CI, 1.348, 4.380; p=0.0015.

Table 73. Summary of ORR Based on BICR Assessment

Objective Response Rate	EC + mFOLFOX6 (N = 69)	SOC (N = 58)
Objective Response events, n (%)	46 (66.7)	20 (34.5)
95% CI [a]	(54.9, 76.6)	(23.6, 47.3)
Odds Ratio for Objective Response [b]	4.700	
95% CI	(1.973, 11.046)	
2-sided p-value	<0.0001	

[a] Confidence intervals are calculated using the Wilson method.
[b] The odds ratio is estimated using a logistic regression with odds of objective response as the outcome and treatment group as the predictor, adjusted for ECOG status (0, 1) and region (US/Canada, Europe, Rest of World). Odds ratio > 1 indicates better outcome for EC + mFOLFOX6 compared to Control. Confidence intervals use the Wald method.

ii. PFS in Patients with *BRAF* V600E Mutation Detected by Guardant360 CDx

At the PFS PCD data cutoff date of 2025-01-06, the median observed PFS by BICR in the gCEAS population treated with EC + mFOLFOX6 was 12.5 months (95% CI 9.9, 16.4) vs. SOC 7.0 months (95% CI 5.5, 8.3; **Table 74**), which is similar to the median PFS observed in the BREAKWATER clinical trial [12.8 months (95% CI, 11.2, 15.9) in the EC + mFOLFOX6 Arm compared with 7.1 months (95% CI, 6.8, 8.5) in the SOC Arm. The resulting PFS HR was 0.45 (95% CI 0.327, 0.628; p<0.0001; **Table 74**), indicating a substantial reduction in the risk of progression or death with EC + mFOLFOX6, which is similar to the PFS HR of 0.53 reported in the full analysis set (FAS) for the BREAKWATER clinical study (95% CI, 0.41, 0.68), p<0.001 for the EC +mFOLFOX6 Arm vs. SOC chemotherapy. Demonstration of clinical efficacy in the *BRAF V600E*-mutant population is further supported by clear separation of the treatment arms in the Kaplan-Meier plot of PFS (**Figure 17**).

Table 74. Summary of PFS Based on BICR Assessment

	EC + mFOLFOX6 (N = 143)	SOC (N = 135)
Kaplan-Meier estimates of time to event (months)		
Median	12.452	6.965
95% CI [a]	(9.9, 16.4)	(5.5, 8.3)
Hazard ratio	0.45	
95% CI [b]	0.327, 0.628	
2-sided p-value	<0.0001	

[a] Confidence intervals are based on the Brookmeyer and Crowley method.
[b] Confidence intervals are derived using the log-log transformation with back transformation to original scale.

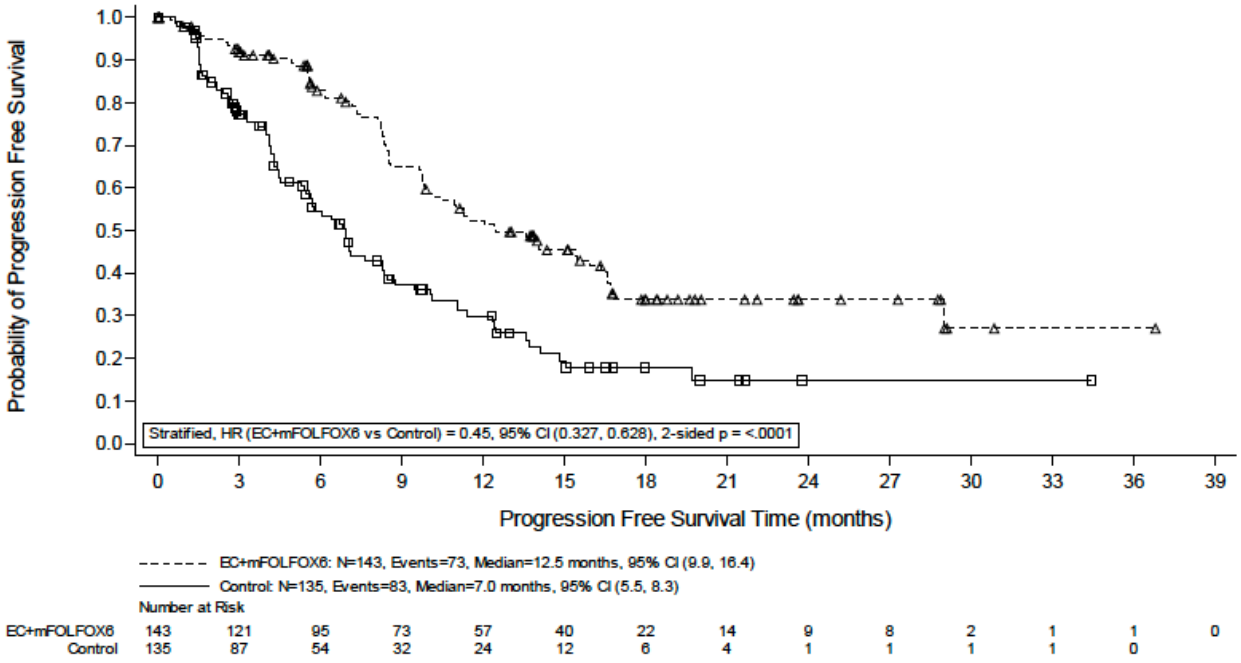


Figure 17. Kaplan-Meier Plot of BICR-Assessed PFS for EC + mFOLFOX6 vs. SOC in gCEAS Population

Concordance between Guardant360 CDx and CTA Testing

The concordance analysis includes all samples from the gCEAS clinical study population, and the sensitivity analysis prevalence sub-study set with both Guardant360 CDx and tissue-based CTA results. Concordance between Guardant360 CDx and tissue-based CTA testing is shown in **Table 75** below. Guardant360 CDx demonstrates high NPA (100%, 95% CI 96.9% - 100.0%) and PPA (85.0%, 95% CI 80.7% - 88.5%) relative to tissue-based CTA results.

Table 75. Concordance Results between Guardant360 CDx and CTA

	CTA Positive [a]	CTA Negative	Total
Guardant360 CDx Positive, n	278	0	278
Guardant360 CDx Negative, n	49	122	171
Total, n	327	122	449
Positive Percent Agreement [b] (95% CI)	85.0 (80.7, 88.5)		
Negative Percent Agreement [b] (95% CI)	100 (96.9, 100.0)		

[a] All subjects enrolled in the BREAKWATER sNDA registration population are considered to be CTA positive.
[b] Subjects with Failed response on Guardant360 CDx are excluded from calculations.

Diagnostic Study Conclusions

Encorafenib in combination with cetuximab and mFOLFOX6 demonstrated clinically meaningful and statistically significant improvement in ORR and PFS as compared to SOC chemotherapy in mCRC patients with *BRAF* V600E mutation as determined by Guardant360 CDx.

In conclusion, the study demonstrated the clinical validity of Guardant360 CDx to aid in the selection of patients with mCRC whose tumors have *BRAF* V600E mutation for encorafenib therapy in combination with cetuximab and mFOLFOX6 chemotherapy.

8. Additional Guardant360 CDx Variant Details

Table 76. 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

Gene (Transcript ID)	Reportable cDNA and Amino Acid Changes
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 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
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

Gene (Transcript ID)	Reportable cDNA and Amino Acid Changes
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
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; G776C; G776D; G776S; G776V; V777A; V777L; V777M; P780L; V794M; T798I; T798M; D808N; D821N; N827S; V842I; N857S; T862A; T862I; L866M; L869R; H878Y; E884K; R896C; R896H

Gene (Transcript ID)	Reportable cDNA and Amino Acid Changes
ESR1 (NM_001122742)	K303R; E380Q; V392I; V422del; 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
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

Gene (Transcript ID)	Reportable cDNA and Amino Acid Changes
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
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 77. 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	-
ESR1 (NM_001122742)	SNV (missense)	-	310-547
KIT (NM_000222)	Indel	All in-frame, excluding splice site	-
MET (NM_000245)	SNV, Indel	14	-
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

Gene (Transcript ID)	Alteration Type	Exon	Codon
PDGFRA (NM_006206)	Indel	All in-frame, excluding splice site	-
PIK3CA (NM_006218)	Indel	2; 8	-
ROS1 (NM_002944)	Indel	37	-

Table 78. 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.

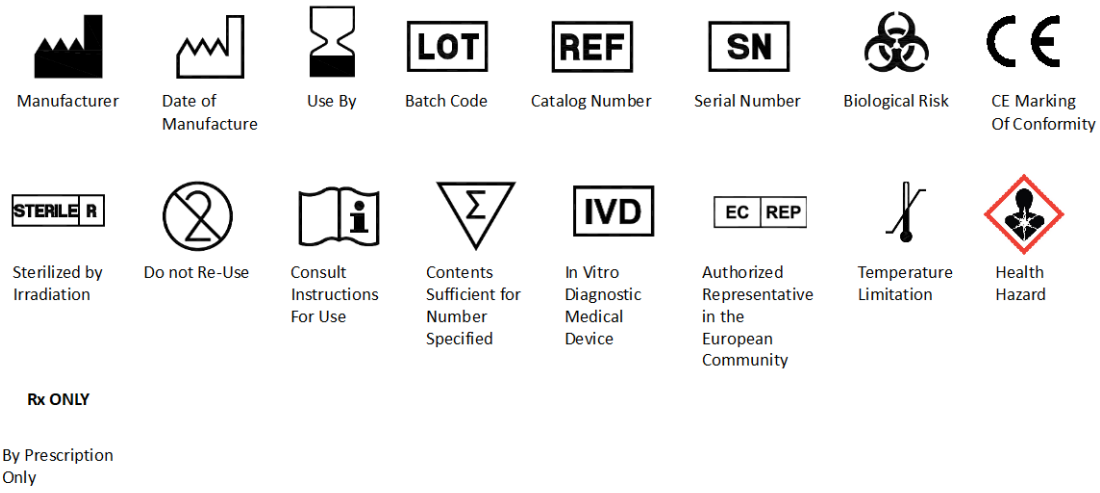
Table 79. Biomarker Rules for Companion Diagnostic Claims Reported by Guardant360 CDx

Indication	Biomarker	Reportable Mutations
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions, L858R, and T790M	Exon 19 deletions, L858R, and T790M
	EGFR exon 20 insertions	Exon 20 insertions
	KRAS G12C	G12C
	ERBB2/*HER2* activating mutations (SNVs and exon 20 insertions)	S310F; S310Y; R678Q; T733I; L755A; L755M; L755P; L755S; L755W; I767F; I767M; D769H; D769N; D769Y; Y772_A775dup; A775_G776insTVMA; A775_G776insV; A775_G776insYVMA; G776C; G776S; G776V; G776_V777delinsCVCG; G776_V777insL; G776_V777insVC; G776_V777insVGC; G776delinsLC; G776delinsVC; V777L; V777M; V777_G778insCG; V777_G778insG; V777_S779dup; G778_P780dup; G778_S779insCPG; G778_S779insLPS; G778dup; S779_P780insVGS; P780_Y781insGSP; T798I; V842I; T862I; L869R; R896C; R896H
Breast cancer	*ESR1* missense mutations between codons 310 and 547	Missense mutations between codons 310 and 547, inclusive
	ESR1 E380, V422del, S463, L469, L536, Y537, and D538 mutations	E380A; E380D; E380K; E380Q; E380V; V422del; S463F; S463P; L469V; L536F; L536G; L536H; L536I; L536K; L536N; L536P; L536Q; L536R; L536V; Y537C; Y537D; Y537G; Y537H; Y537N; Y537P; Y537Q; Y537S; D538E; D538G; D538H; D538N; D538V
Colorectal cancer	*BRAF* V600E	V600E

Mutations found in patients with the corresponding indication will be reported in Category 1 as a companion diagnostic (CDx) for associated therapies as indicated in **Table 1**.

9. Additional Information

9.1. Symbols



10. References

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