SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next Generation Sequencing Oncology Panel, Somatic or Germline Variant Detection System
Device Trade Name:	Guardant360® Liquid CDx
Device Procode:	PQP
Applicant's Name and Address:	Guardant Health, Inc. 505 Penobscot Drive Redwood City, CA 94063 USA
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P250027
Date of FDA Notice of Approval:	May 19, 2026

II. INDICATIONS FOR USE

Guardant360® Liquid 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) and insertions and deletions (indels) in 741 genes, copy number amplifications (CNAs) in two genes, copy number loss (CNL) in one gene, and rearrangements in nine genes. Guardant360 Liquid 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 targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1. Companion Diagnostic Indications

Indication	Biomarker	Therapy
Breast cancer	*ESR1* missense mutations between codons 310 and 547	ORSERDU™ (elacestrant)
	ESR1 E380, V422del, S463, L469, L536, Y537, and D538 mutations	INLURIYO™ (imlunestrant)

Indication	Biomarker	Therapy
Colorectal cancer	*BRAF* V600E	BRAFTOVI® (encorafenib) in combination with ERBITUX® (cetuximab)
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions, L858R, and T790M*	TAGRISSO® (osimertinib)
	EGFR exon 20 insertions	RYBREVANT® (amivantamab-vmjw)
	KRAS G12C	LUMAKRAS™ (sotorasib)
	ERBB2/HER2 activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)

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 solid malignant neoplasms. The test is for use with patients previously diagnosed with cancer and in conjunction with other laboratory and clinical findings.

Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

Warnings and precautions are listed below:

- Alterations reported may include somatic (not inherited) or germline (inherited) alterations. The assay filters germline variants from reporting except for pathogenic alterations in 38 genes. However, if a reported alteration is suspected to be germline, confirmatory testing should be performed using a validated germline test, as this

assay is not intended to replace comprehensive germline testing or to provide definitive information about cancer predisposition.

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

V. DEVICE DESCRIPTION

Guardant360 Liquid CDx is a single-site test performed at Guardant Health, Inc. The test includes reagents, software, and procedures for testing cfDNA from whole blood samples. Plasma is isolated from whole blood collected in Streck Cell-Free DNA BCTs. Using automated methods, cfDNA is extracted from plasma, quantitated, and amplified by PCR to create libraries, which are then enriched for specific regions of interest and sequenced. The test evaluates target genes to identify genomic alterations present in cfDNA. The sequencing data is analyzed using proprietary algorithms to identify single nucleotide variants (SNVs), insertions and deletions (indels), rearrangements, copy number amplifications (CNAs) and copy number loss (CNL) based on the genomic and epigenomic characteristics of molecules identified, including methylation status. A final result of an alteration "Detected" or "Not Detected" is generated.The device is designed to detect alterations in the genes listed in Table 2.

Table 2. Guardant360 Liquid CDx Reportable Gene List

Alteration Type	Genes
Single Nucleotide Variants (SNVs) and Indels	*ABCB1, ABL1, ABL2^, ABRAXAS1, ACVR1, ACVR1B^, ACVR2A^, ADARB2, ADGRA2^, ADGRG4^, AFDN^, AGGF1^, AIP, AKT1, AKT1S1, AKT2, AKT3, ALB, ALK, ALOX12B, ALOX15B, ALOX5, AMER1, APC**, APEX1, APLNR, AR, ARAF, ARFRP1^, ARHGAP35, ARID1A, ARID1B, ARID2, ASXL1, ATM**, ATMIN, ATR, ATRX, AURKA, AURKB, AURKC, AXIN1, AXIN2, AXL, B2M^, BABAM1, BABAM2, BAP1, BARD1**, BCL2, BCL2L1, BCL2L2^, BCL6^, BCOR, BCORL1^, BCR^, BIRC5^, BLM, BMPR1A**, BRAF, BRCA1**, BRCA2**, BRCC3, BRD2^, BRD3^, BRD4^, BRIP1**, BSG, BTG1^, BTG2^, BTK, BUB1B^, C9orf78^, CALR, CARD11, CASP8^, CASR, CAV1, CBFB^, CBL, CBLB^, CCAR1^, CCN6^, CCNA2, CCNB1, CCND1, CCND2, CCND3, CCNE1, CCNE2, CD274, CD276, CD74^, CD79A, CD79B, CDC27, CDC5L^, CDC7, CDC73, CDH1**, CDH6, CDK11A^, CDK12, CDK4, CDK6, CDK7, CDK8^, CDKN1A, CDKN1B, CDKN1C, CDKN2A**, CDKN2B, CDKN2C, CEBPA, CELF4^, CEP295^, CFAP20^, CHD4, CHEK1, CHEK2^**, CIC,*

Alteration Type	Genes
	*CLDN18, CMTM4, CMTM6, CNOT3^, CREBBP^, CRKL^, CRTC1^, CSF1R, CSF3R, CTC1, CTCF, CTLA4, CTNNA1, CTNNB1, CUL3, CUL4A, CUX1^, CWC22^, CXCR4, CYLD^, CYP17A1, CYP19A1, CYP2C19, CYP3A4^, DAXX, DCUN1D1, DDIT3^, DDR1^, DDR2, DDX17^, DDX18^, DDX27^, DDX3X^, DDX41^, DEPDC5, DEPTOR, DHX15, DHX16^, DHX36^, DHX9^, DICER1, DIS3L2, DLL4, DNAJB1, DNMT1^, DNMT3A, DNMT3B, DOT1L^, DPYD, DUSP4, DYNLL1^, DYRK2, E2F3^, ECT2L^, EFTUD2^, EGFR**, EIF1AX^, EIF4A1, EIF4A2, EIF4A3, EIF4B, EIF4E, EIF4E2, ELAVL1^, ELAVL2^, ELF3^, ELOC^, EML4, EMSY, EP300, EPAS1^, EPCAM, EPHA3, EPHA5, EPHA7^, EPHB1^, ERBB2, ERBB3, ERBB4, ERCC1^, ERCC2, ERCC3^, ERCC4^, ERCC5, ERCC6, ERCC6L2, ERCC8, EREG, ERF, ERG, ERRFI1^, ESR1, ETS1, ETV1, ETV4, ETV5^, ETV6, EWSR1^, EXO1^, EZH1, EZH2, FAAP100, FAAP20, FAAP24^, FANCA**, FANCB^, FANCC^, FANCD2^, FANCE^, FANCF, FANCG^, FANCI, FANCL, FANCM^, FAS^, FAT1^, FBXW7, FCGR2A, FCGR3A, FEN1, FGF1, FGF10^, FGF12, FGF14^, FGF19, FGF2, FGF23^, FGF3, FGF4^, FGF5, FGF6^, FGF7^, FGF8, FGF9, FGFR1, FGFR2, FGFR3, FGFR4, FH, FLCN, FLT1, FLT3, FLT4^, FOXA1^, FOXL2, FOXO1^, FOXP1^, FRS2, FUBP1^, FUBP3^, FUS^, FYN, FZD1, FZD10, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, GAS6, GATA1, GATA2, GATA3, GATA4, GATA6, GEN1, GID4^, GLI1, GNA11, GNA13^, GNAQ, GNAS, GPATCH8^, GPC3^, GREM1, GRIN2A^, GSK3B, GSTM1, GSTP1, H3-4, H3F3A^, HACD4^, HDAC2, HDAC6, HELQ, HES1, HEY1, HEYL, HGF, HNF1A, HNRNPDL^, HOXB13, HRAS, HSD3B1^, HSP90AA1^, ICOSLG, ID3, IDH1, IDH2, IDO1, IFNG, IFNGR1, IFNGR2, IFNW1, IGF1, IGF1R, IGF2, IGF2BP3^, IGF2R, IKBKE^, IKZF1^, IL1R1, IL2RA, IL2RB, IL2RG, IL7R, INHBA, INPP4B, INTS6L^, IRF1, IRF2, IRF4^, IRS2^, JAK1, JAK2, JAK3, JUN, KAT6A^, KAT6B, KDM4A, KDM5A^, KDM5B^, KDM5C, KDM6A, KDR, KEAP1, KIN^, KIT, KLF4, KLHL6^, KLHL9, KMT2A, KMT2B, KMT2C, KMT2D, KNSTRN^, KRAS, LATS1, LGR4, LGR5, LGR6, LIG1^, LIG4, LMO1^, LRP1B, LRP2^, LRP5, LRP6, LTK, LYN, LZTR1, MAD2L2, MALT1, MAP2K1, MAP2K2, MAP2K4, MAP3K1, MAP3K13, MAP4K3^, MAPK1, MAPK3, MAPKAP1, MARK2, MAX**, MCL1, MDC1^, MDM2, MDM4, MED12, MEF2B^, MEN1**, MERTK, MET, MGA, MITF**, MKNK1^, MLH1**, MLH3^, MLST8, MPL, MRAS^, MRE11, MSH2**, MSH3^, MSH6**, MTAP, MTHFR, MTOR, MUTYH**, MYB^, MYC, MYCL, MYCN, MYD88, MYOD1, NAB2, NBN, NCOR1^, NCR1,NCR3, NEGR1, NELFE^, NF1, NF2**, NFE2L2, NFKBIA^, NHEJ1^, NKX2-1, NOTCH1, NOTCH2, NOTCH3, NOTCH4^, NOVA1^, NPM1^, NPRL2,*

Alteration Type	Genes
	*NPRL3, NRAS, NRG1, NSD1^, NSD2, NSD3, NSRP1^, NTHL1, NTRK1, NTRK2^, NTRK3^, NUMA1, NUMB, NUP93^, NUTM1^, P2RY8^, PABPC1^, PAK1, PAK3^, PALB2**, PARG, PARP1^, PARP2^, PAX3, PAX5, PAX7, PAX8, PAXIP1, PBRM1^, PCBP1, PCBP2^, PCDH15^, PDCD1, PDCD1LG2, PDE7A, PDGFRA, PDGFRB, PDK1^, PDPK1, PHF6^, PHLPP1, PHLPP2, PHOX2B, PIAS4, PIK3C2B^, PIK3CA, PIK3CB, PIK3CD^, PIK3CG, PIK3R1, PIK3R2^, PIK3R3, PIM1, PIN1, PKM^, PLCG2, PLEKHS1, PLRG1^, PMS1^, PMS2^**, POLA1, POLD1, POLE, POLH, POLQ^, POT1, POU2F2, PPARG, PPIG^, PPM1D, PPP2CA^, PPP2R1A, PPP2R2A, PPP3CA, PPP6C^, PRDM1, PREX1, PREX2, PRKAR1A, PRKCI^, PRKDC^, PRKN, PRMT5, PRPF40B^, PRPF4B^, PSENEN, PSMB10, PSMB8, PSMB9, PTCH1, PTDSS1, PTEN**, PTPN11, PTPN2, PTPRD^, PTPRS, PTPRT, QKI^, RAB35, RAC1^, RAD18^, RAD21^, RAD50, RAD51, RAD51B, RAD51C**, RAD51D**, RAD52^, RAD54L, RAET1E, RAF1, RARA, RASA1^, RB1^**, RBBP6^, RBM10, RBMX^, RECQL, RECQL4, RET**, REV3L, RGS1, RHEB, RHOA, RHOB, RICTOR, RIF1, RILPL1^, RIT1, RNASEH2B, RNF43, ROBO1, ROBO2, ROS1, RPA1^, RPS27A^, RPS6KA3, RPS6KB1, RPS6KB2, RPTOR, RRAGC, RSPO1, RSPO2, RSPO4, RUNX1, RUNX1T1^, RXRA, RYBP, SAMHD1, SDC4, SDHA*, SDHAF2, SDHB*, SDHC*, SDHD*, SEM1^, SERPINB3, SERPINB4, SESN2, SETD2, SF3B1, SF3B3^, SH2D1A, SHLD1, SHLD2^, SLC34A2, SLFN11, SLIT2^, SMAD2, SMAD3^, SMAD4**, SMARCA2, SMARCA4, SMARCAL1, SMARCB1, SMARCD1, SMARCE1^, SMC1A, SMC3, SMO, SNCAIP, SOCS1, SOCS3, SOS1, SOX10^, SOX17, SOX2, SOX9, SPEN, SPOP, SRC, SRSF2, SRY, SS18, STAG2, STAT1, STAT3^, STAT4^, STK11**, STK19^, STK40, STN1, SUFU, SYK^, SYNCRIP^, TACSTD2, TAF1L, TAP1, TAP2, TAPBP, TBC1D7, TBX3^, TCERG1^, TCF7L2, TEK, TEN1, TENT5C, TERT, TET1, TET2, TFE3, TFRC, TGFBR1, TGFBR2, THRAP3^, TIA1^, TIPARP, TMEM127, TMPRSS2, TNFAIP3, TNFRSF14^, TNFRSF1A, TNK2^, TNPO1^, TOP1, TOP2A, TOPAZ1^, TP53**, TP53BP1, TP63^, TP73^, TPMT, TRAF2, TRAF3, TRAF7, TRIM24^, TRIP13, TSC1**, TSC2**, TSHR^, TSHZ2^, TYMP, TYMS, TYRO3^, U2AF1, UBE2T^, UGT1A1^, UIMC1, ULBP1, ULBP3, USP28, USP7, USP9X^, VEGFA^, VEGFB, VHL**, VIRMA^, WBP11^, WEE1, WRN, WT1**, WWP1, XBP1^, XPA, XPC^, XPO1, XRCC1^, XRCC2, XRCC3, XRCC4^, XRCC5^, XRCC6^, YAP1^, YES1, ZC3H13^, ZC3H18^, ZC3H4^, ZMYM3^, ZNF217, ZNF703^, ZNRF3, ZRSR2^*
Copy Number Amplifications (CNAs)	*ERBB2*, *MET*

Alteration Type	Genes
Copy Number Loss (CNL)	*BRCA1*
Rearrangements	*ALK, FGFR2, FGFR3, NRG1, NTRK1, NTRK2, NTRK3, RET, ROS1*

^Regions of interest covered by the panel, some exons are not included.
**Reporting is enabled for both pathogenic germline and somatic alterations.
*Reporting is enabled for pathogenic germline alterations only. Somatic alterations are not reported.

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

Table 3. Category Definitions

FDA Levels of Evidence	Definition	Guardant360 Liquid CDx Reporting Level
Level 1	• ctDNA variants linked to the safe and effective use of the corresponding therapeutic product, for which Guardant360 Liquid CDx has demonstrated clinical performance shown to support therapeutic efficacy and strong analytical performance for the biomarker.	Companion Diagnostic (CDx) Variants
Level 2	• Clinical evidence from FDA-approved liquid biopsy companion diagnostic biomarkers for the specific tumor type at the biomarker or variant level. • Analytical validity supported for each biomarker from accuracy, limit of blank (LoB), limit of detection (LoD), and precision/reproducibility, at the biomarker or variant level.	ctDNA Variants with Evidence of Clinical Significance in Plasma
Level 3	• Clinical evidence from FDA-approved tissue-based companion diagnostic biomarkers, and/or professional guidelines for liquid or tissue. • Analytical validity supported by a representative approach for SNVs and indels from accuracy, LoB, LoD, and precision/reproducibility studies. • Analytical validity supported for each rearrangement, or copy number alteration from accuracy, LoB, LoD, and precision/reproducibility studies, at the gene level.	ctDNA Variants with Evidence of Clinical Significance in Tissue

FDA Levels of Evidence	Definition	Guardant360 Liquid CDx Reporting Level
Level 4	• Biomarkers not categorized into Levels 2 or 3 can be included under Level 4 for informational purposes or to be used to direct patients toward clinical trials for which they may be eligible. Such claims can be supported by clinical rationale for inclusion in the panel. Such rationale could also include peer-reviewed publications for genes/variants in tissue, variant information from well curated public databases, or in vitro pre-clinical models. • Analytical validity supported by a representative approach for SNVs and indels from accuracy, LoB, LoD, and precision/reproducibility studies. • Analytical validity supported for each rearrangement or copy number alteration from accuracy, LoB, LoD, and precision/reproducibility studies, at the gene level.	ctDNA Variants with Potential Clinical Significance

Test Kit Contents

The test includes the Guardant Health Blood Collection Kit (BCK), which is shipped to ordering laboratories. Each BCK contains the following components:

- Streck Cell-Free DNA Blood Collection Tubes (BCTs): 2 per kit
- Absorbent pouch
- Biohazard Bag
- Cushioning material
- Instructions for Use (IFU)
- Labels- Specimen labels, BCK labels and Return shipping label

The test also includes sample preparation reagents and software that are exclusively used in the Guardant Health Clinical Laboratory.

Instruments

Guardant360 Liquid CDx is intended to be performed with serial number-controlled instruments listed below. All instruments are qualified by Guardant Health, Inc. under the Guardant Health Quality System.

- QIAsymphony SP Instrument with QIAsymphony Sample Preparation Module software (Qiagen)
- Microlab STAR with Venus software (Hamilton Robotics)
- NovaSeq X plus Sequencing System (Illumina)

Test Process

Whole Blood Collection and Shipping
BCK is used by the ordering physicians to collect whole blood specimens and ship them to the Guardant Health Clinical Laboratory. A minimum of 5 mL whole blood must be received in order to achieve optimal performance for the Guardant360 CDx assay. Underfilling of tubes with less than 5 mL of blood may lead to incorrect analytical results or poor product performance.

Plasma Isolation
Upon receipt of the BCK, whole blood specimens are processed by laboratory personnel at the Guardant Health Clinical Laboratory within seven days of blood collection. Plasma is isolated from both tubes via centrifugation. One tube of plasma is stored, while the second tube is used for cfDNA extraction.

cfDNA Extraction, Buffer Exchange and cfDNA Quantitation
Extraction occurs using the QIAsymphony SP Instrument and reagent system, which includes lysis, proteinase K digestion, bead binding, washing of the beads, and elution. After extraction, the cfDNA undergoes an automated buffer exchange, and its concentration is determined by quantitation using the 4200 TapeStation system.

Methylation Partitioning
Up to 30 ng of extracted cfDNA is separated into methylated and unmethylated partitions based on the overall methylation state of each molecule. The cfDNA is partitioned based on the differential binding affinity of the methylated nucleic acid molecules to a binding agent (i.e., a binding agent that binds to methylated nucleotides).

Library Preparation and Enrichment
The DNA in each partition is tagged with a distinct set of barcodes, which uniquely identifies the partition associated with every molecule. All partitions are then PCR amplified and enriched via hybridization to oligonucleotides representing genomic or epigenomic regions of interest, enabling targeted enrichment.

DNA Sequencing
Paired-end sequencing by synthesis is performed with the Illumina NovaSeq X Plus Sequencing system. The amplified cfDNA is analyzed by parallel sequencing of amplified target genes.

Sequencing Data Analysis
Guardant360 Liquid CDx Software uses a custom-developed analysis software module, Bioinformatics Pipeline (BIP). The BIP software module uses the raw data (output) from the targeted sequencing, partitions the data based on the sample index sequence (barcode) of each read to separate reads originating from individual samples, and executes a proprietary algorithmic reconstruction of the sequencing signals for high-fidelity molecule-based alteration calling downstream. The sequence data then undergoes an

alignment process where it is mapped to the human genome (hg19), and an analysis of post-sequencing QC metrics and sequence alteration data is performed. Alteration detection is conducted according to pre-defined alteration calling metrics. All alterations must pass alteration calling metrics as described in Table 4.

Table 4. Alteration Analytical Calling Threshold/Cut-Off Metrics

SNV Calling Property	**Threshold**
DNA Molecule Support	≥ 2
Mutant Allele Frequency (MAF) Estimate*	≥ 0.001%
Variant quality filters	PASS
Indel Calling Property	**Threshold**
DNA molecule support	≥ 2
Double stranded molecule support	≥ 1
MAF Estimate*	≥ 0.001%
Variant quality filters	PASS
CNA Calling Property	**Threshold**
ERBB2 copy number	≥ 2.18
ERBB2 amplification quality filter	PASS
ERBB2 amplification is not associated with chromosome-arm aneuploidy	TRUE
MET copy number	≥ 2.20
MET amplification quality filter	PASS
MET amplification is not associated with chromosome arm aneuploidy	TRUE
CNL Calling Property	**Threshold**
BRCA1 CNL quality filter	PASS
Estimated total tumor copy number of overlapping segment	< tumor ploidy
Copy number estimated tumor fraction	≥ 20%
Epigenomic estimated tumor fraction	≥ 1%
Rearrangement Calling Property	**Threshold**
Number of unique fusion molecules	≥ 2

Rearrangement quality filters	PASS

*Estimated MAF and SNV threshold limits may vary based on specific genomic regions or sample coverage.

Result Reporting

For samples passing QC metrics, an IVD results report containing CDx alteration relevant information (Level 1) and all other biomarkers (Levels 2-4) is generated. This information is merged with patient-specific information.

Quality Control Measures

Guardant360 Liquid CDx uses an external control consisting of pre-specified positive and negative results for run validation. Additionally, a no template negative control (NTC) is run in parallel with patient samples and is used to verify assay background is clear of contamination. The controls are run in each batch and are included in the workflow steps; the external control is included from methylation partitioning through sequencing while the NTC is included from cfDNA extraction through sequencing. In addition to assessing performance of the external control within a batch, multiple in-process and post-sequencing QC metrics are assessed for each sample tested.

VI. PREDETERMINED CHANGE CONTROL PLAN

Guardant360 Liquid CDx is approved with a Predetermined Change Control Plan (PCCP). The PCCP describes the specific test methods for (1) implementing software updates to the sequencing instrument and (2) expanding the indications for use as a companion diagnostic (CDx) for additional drugs following FDA approval of the drug and CDx indication for Guardant360 CDx (P200010) comparator device. Each modification is evaluated using pre-specified statistical analyses and performance benchmarks appropriate to the type of change.

Pre-specified software updates to the NovaSeq X Plus (NVX) Sequencing System include a staggered start feature, a sequencing recipe adjustment, and implementation of a software update manager. These software updates are evaluated through comparative studies against the approved device. The equivalency study will utilize samples representing the intended use population. The sample set is designed to ensure adequate representation of the variant types expected in the intended use population, including single nucleotide variants (SNVs), insertions/deletions (indels), rearrangements, copy number amplifications, and copy number losses across Level 1-3 reporting levels. Analytical performance is assessed using measures of agreement for variant detection, including positive percent agreement (PPA) and negative percent agreement (NPA), with confidence interval-based analyses to ensure that performance remains consistent with the approved device.

Additional CDx indications may be added to Guardant360 Liquid CDx under the PCCP provided that the following conditions are met:

- The new CDx indication must fall within the scope of previously authorized indications for Guardant360 Liquid CDx. Specifically, the cancer type for the new follow-on CDx claim must be the same as a previously approved cancer type, and the new CDx biomarker rule/definition must be identical to, or a subset of, the previously approved CDx biomarker rule/definition. If the new biomarker rule includes variants not previously covered, those variants must be of the same variant types already approved (e.g., SNVs and insertions/deletions), and the comparator assay's (Guardant360 CDx) approval for that indication must have been based solely on clinical validation, without requiring additional analytical validation studies.
- The existing analytical validation data authorized for Guardant360 Liquid CDx must be sufficient to support the new CDx indication, such that no additional AV studies are required beyond those already established and authorized for the device.
- Guardant Health must conduct and successfully complete the NI study protocol specified in this PCCP, demonstrating that Guardant360 Liquid CDx is non-inferior to the FDA-approved comparator assay, Guardant360 CDx, for the detection of the relevant biomarker in the applicable cancer type. The NI study must meet the predefined acceptance criteria prior to implementation of the new CDx claim.
- The corresponding therapy must have received FDA approval, and the FDA-approved drug labeling must be consistent with the intended use of Guardant360 Liquid CDx for the new indication. Additionally, Guardant360 CDx must have received FDA approval as a companion diagnostic for the specific biomarker and cancer type indication prior to implementation of the follow-on CDx claim under this PCCP.

The NI study for each new CDx indication will use remnant plasma samples from patients previously tested with Guardant360 CDx, drawn from the intended use patient population for the drug, and selected to represent the range of biomarker-positive and biomarker-negative cases relevant to the new indication. Agreement metrics, including PPA and NPA, are analyzed to demonstrate that clinical performance of the Guardant360 Liquid CDx remains non-inferior to the Guardant360 CDx within predefined statistical margins.

Prior to implementation of NVX Software update, the PCCP require Guardant Health to perform new threat modeling, conduct a comprehensive cybersecurity risk analysis, and carry out new security testing, including penetration testing, to ensure that no new threats or vulnerabilities are introduced by the respective software update. In addition, an assessment of cybersecurity documentation will be conducted for both modifications. These cybersecurity activities will be completed and documented before implementation of the respective modification. The outcomes of these activities will be documented and reflected in updates to the relevant cybersecurity documentation.

Guardant Health will perform testing according to the specified protocols. If the validation data meets the specified acceptance criteria and the updated device remains

cybersecure, then the changes outlined in the PCCP will be implemented without the submission of additional marketing submission(s). The labeling for the device, including the Guardant360 Liquid CDx Technical Information Document, will be updated to reflect any new sequencer software version and any additional CDx indications pursuant to the PCCP. All labeling changes will be reported to FDA in a PMA Annual Report.

VII. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA approved companion diagnostic (CDx) alternatives for the detection of some of the genetic alterations using cfDNA isolated from plasma samples to those that are listed in Table 1 of the Guardant360 Liquid CDx intended use statement. These approved alternative CDx tests are listed in Table 5 below. Each alternative has its own advantages and disadvantages. A patient should fully discuss any alternative with their physician to select the most appropriate method. For additional details, see the list of FDA Cleared or Approved Companion Diagnostic Devices at https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approvedcompanion-diagnostic-devices-in-vitro-and-imaging-tools

Table 5. List of FDA-Approved CDx Assays for genes targeted by Guardant360 Liquid CDx

Biomarker	Indication (Sample Type)	Therapy	Device	Company	Technology
ESR1 mutations	Breast Cancer (Plasma)	ORSERDU™ (elacestrant)	Guardant360® CDx (P200010/S010)	Guardant Health Inc.	NGS
ESR1 mutations	Breast Cancer (Plasma)	INLURIYO™ (imlunestrant)	Guardant360® CDx (P200010/S021)	Guardant Health Inc.	NGS
BRAF V600E	Colorectal Cancer (Plasma)	BRAFTOVI® (encorafenib) in combination with ERBITUX® (cetuximab)	Guardant360® CDx (P200010/S026)	Guardant Health Inc.	NGS
			FoundationOne® Liquid CDx (P190032/S010)	Foundation Medicine, Inc.	NGS
EGFR exon 19 deletions, L858R and T790M	NSCLC (Plasma)	TAGRISSO® (osimertinib)	Guardant360® CDx (P200010)	Guardant Health Inc.	NGS
			Cobas® EGFR Mutation Test v2 (P120019/S016)	Roche Molecular Systems, Inc.	PCR

Biomarker	Indication (Sample Type)	Therapy	Device	Company	Technology
			FoundationOne® Liquid CDx (P190032)	Foundation Medicine, Inc.	NGS
EGFR exon 20 insertions	NSCLC (Plasma)	RYBREVANT® (amivantamab-vmjw)	Guardant360® CDx (P200010/S001)	Guardant Health Inc.	NGS
KRAS G12C	NSCLC (Plasma)	LUMAKRAS™ (sotorasib)	Guardant360® CDx (P200010/S002)	Guardant Health Inc.	NGS
ERBB2/HER2 activating mutations	NSCLC (Plasma)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)	Guardant360® CDx (P200010/S008)	Guardant Health Inc.	NGS

NGS= Next Generation Sequencing; PCR= Polymerase Chain Reaction

VIII. MARKETING HISTORY

Guardant360 Liquid CDx has not been marketed in the United States or any other countries.

IX. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected may lead to incorrect test results, and incorrect interpretation of test results may lead to erroneous conclusions; either scenario may subsequently result in inappropriate patient management decisions. Patients with false positive results may undergo treatment with a therapy listed in the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with an indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment.

X. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Guardant360 Liquid CDx performance characteristics were established using clinical samples from patients with a wide range of cancer types or contrived samples. Studies included CDx variants, as well as a broad range of representative alteration types (SNVs, indels, CNAs, CNL, and rearrangements) in various genomic contexts across a large number of genes in the assay's reportable range. Due to limitations in clinical sample availability, contrived samples were utilized for some analytical validation

studies. A contrived sample functional characterization (CSFC) study was conducted to demonstrate functional comparability between contrived samples and clinical samples.

1. Analytical Accuracy/Concordance with Orthogonal Methods

Analytical accuracy of Guardant360 Liquid CDx test for CDx biomarkers and tumor profiling variants (SNV, indels, rearrangements, CNAs, and CNLs) was assessed by comparing detection by Guardant360 Liquid CDx to validated NGS comparator methods. A total of 1,511 cancer patient samples across 22 cancer types (Table 6) from the intended use population and 2 contrived samples were evaluated. In total, 1,494 samples had passing QC results for both Guardant360 Liquid CDx and the comparator assays.

Table 6. Number of Samples per Cancer Type

Cancer Type	Number of Samples
Breast	294
Cholangiocarcinoma	16
CRC	268
Melanoma	20
NSCLC	787
Ovarian	16
Prostate	38
Urothelial	7
All other (includes Pancreatic, cancer of unknown Primary (CUP), Esophageal, Hepatocellular, astric, Endometrial, Gallbladder, Gastric, Neuroendocrine, Parotid, Renal, SCLC, Squamous cell, and Testicular)	65
TOTAL	1511

Concordance analysis between Guardant360 Liquid CDx (GCDx) and the NGS comparator methods (cNGS) is provided in Table 7 for all variant types tested in the study. In addition, a summary of accuracy results for SNVs in predefined challenging genomic regions (e.g., simple sequence repeats [SSRs], homopolymers, GC content), and for insertions and deletions stratified by predefined size bins (e.g., 1–5 bp, 6–10 bp, 11–15 bp, 16–20 bp, 21–30 bp, and >30 bp) and by challenging genomic regions, excluding unknown results, is presented in Table 8. Concordance results for each reporting level stratified by LoD are shown in Table 9. Concordance results for Level 1 CDx biomarkers demonstrated a PPA of approximately 95% or above, and an NPA of above 98.75% for each biomarker. The analysis demonstrated a PPA of 94.93% and 96.24% for clinically significant SNVs and indels, respectively, and an NPA of 99.86% and 99.98% for clinically significant SNVs and indels, respectively. The analysis also demonstrated a PPA of 76.81% and 87.32% for panel-wide SNVs and indels, respectively, and an NPA of >99.95% for panel-wide SNVs and indels, respectively. Low concordance (4/6, 66.67%) observed for indels >30 bp (≥ LoD), is partially attributed to an alignment error. The analysis for panel-wide rearrangements, CNAs, and CNLs showed a PPA of 94.64% and NPA of 99.76% for rearrangements, a PPA of

91.67% and NPA of 99.97% for CNAs, and a PPA of 100% and NPA of 97.56% for CNLs (Table 7). This study has demonstrated the analytical accuracy of the device.

Table 7. Accuracy Results for All Variant Types at Each Reporting Level

Variant Type	Variant Level	GCDx+ cNGS+	GCDx+ cNGS−	GCDx− cNGS+	GCDx− cNGS−	PPA [95% CI]	NPA [95% CI]
SNV	1	590	68	25	28440	95.93% [94.06%, 97.35%]	99.76% [99.70%, 99.81%]
	2	163	12	9	10677	94.77% [90.30%, 97.58%]	99.89% [99.80%, 99.94%]
	3	277	38	21	44640	92.95% [89.43%, 95.59%]	99.91% [99.88%, 99.94%]
	4	2756	577	1088	1305627	71.70% [70.24%, 73.12%]	99.96% [99.95%, 99.96%]
	All	3786	695	1143	1389384	76.81% [75.61%, 77.98%]	99.95% [99.95%, 99.95%]
Indel	1	268	7	6	46515	97.81% [95.29%, 99.19%]	99.98% [99.97%, 99.99%]
	2	10	0	3	4624	76.92% [46.19%, 94.96%]	100% [99.92%, 100%]
	3	29	8	3	23103	90.62% [74.98%, 98.02%]	99.97% [99.93%, 99.99%]
	4	464	207	100	421315	82.27% [78.86%, 85.33%]	99.95% [99.94%, 99.96%]
	All	771	222	112	495557	87.32% [84.94%, 89.44%]	99.96% [99.95%, 99.96%]
Rearran-gement	2	66	10	4	3203	94.29% [86.01%, 98.42%]	99.69% [99.43%, 99.85%]
	3	37	4	1	1491	97.37% [86.19%, 99.93%]	99.73% [99.32%, 99.93%]
	4	3	2	1	1822	75% [19.41%, 99.37%]	99.89% [99.60%, 99.99%]
	All	106	16	6	6516	94.64% [88.70%, 98.01%]	99.76% [99.60%, 99.86%]

Variant Type	Variant Level	GCDx+ cNGS+	GCDx+ cNGS−	GCDx− cNGS+	GCDx− cNGS−	PPA [95% CI]	NPA [95% CI]
CNA	3	76	1	4	2175	95.00% [87.69%, 98.62%]	99.95% [99.74%, 100%]
	4	12	0	4	686	75.00% [47.62%, 92.73%]	100% [99.46%, 100%]
	All	88	1	8	2861	91.67% [84.24%, 96.33%]	99.97% [99.81%, 100%]
CNL	2	3	0	0	9	100% [29.24%, 100%]	100% [66.37%, 100%]
	3	3	5	0	36	100% [29.24%, 100%]	87.80% [73.80%, 95.92%]
	4	3	0	0	155	100% [29.24%, 100%]	100% [97.65%, 100%]
	All	9	5	0	200	100% [66.37%, 100%]	97.56% [94.40%, 99.20%]

GCDx: Guardant360 Liquid CDx; cNGS: comparator NGS

Table 8. Accuracy Results for SNVs, Insertions, and Deletions in Clinically and Analytically Meaningful Bins

Variant Type	Bin	MAF Level	GCDx+ cNGS+	GCDx+ cNGS−	GCDx− cNGS+	GCDx− cNGS−	PPA [95% CI]	NPA [95% CI]
SNV	GC (0-30%]	All	65	12	10	34,379	86.67% [76.84%, 93.42%]	99.97% [99.94%, 99.98%]
		≥ LoD	49	5	0	34,412	100% [92.75%, 100%]	99.99% [99.97%, 100%]
	GC (30-40%]	All	1,206	201	252	368,593	82.72% [80.68%, 84.62%]	99.95% [99.94%, 99.95%]
		≥ LoD	956	29	9	369,258	99.07% [98.24%, 99.57%]	99.99% [99.99%, 99.99%]
	GC (40-50%]	All	855	175	366	420,040	70.02% [67.37%, 72.58%]	99.96% [99.95%, 99.96%]
		≥ LoD	571	27	5	420,833	99.13% [97.99%, 99.72%]	99.99% [99.99%, 100%]

Variant Type	Bin	MAF Level	GCDx+ cNGS+	GCDx+ cNGS−	GCDx− cNGS+	GCDx− cNGS−	PPA [95% CI]	NPA [95% CI]
	GC (50-60%]	All	1,017	148	313	326,282	76.47% [74.09%, 78.72%]	99.95% [99.95%, 99.96%]
		≥ LoD	763	21	2	326,974	99.74% [99.06%, 99.97%]	99.99% [99.99%, 100%]
	GC (60-100%]	All	643	159	202	240,090	76.09% [73.07%, 78.93%]	99.93% [99.92%, 99.94%]
		≥ LoD	485	47	17	240,545	96.61% [94.63%, 98.02%]	99.98% [99.97%, 99.99%]
Indel	GC (0-30%]	All	11	11	1	15,563	91.67% [61.52%, 99.79%]	99.93% [99.87%, 99.96%]
		≥ LoD	10	8	1	15,567	90.91% [58.72%, 99.77%]	99.95% [99.90%, 99.98%]
	GC (30-40%]	All	182	68	33	171,803	84.65% [79.13%, 89.19%]	99.96% [99.95%, 99.97%]
		≥ LoD	160	17	9	171,900	94.67% [90.13%, 97.54%]	99.99% [99.98%, 99.99%]
	GC (40-50%]	All	221	49	34	108,406	86.67% [81.87%, 90.59%]	99.95% [99.94%, 99.97%]
		≥ LoD	195	8	7	108,500	96.53% [92.99%, 98.60%]	99.99% [99.99%, 100%]
	GC (50-60%]	All	172	34	21	80,739	89.12% [83.85%, 93.14%]	99.96% [99.94%, 99.97%]
		≥ LoD	146	5	3	80,812	97.99% [94.23%, 99.58%]	99.99% [99.99%, 100%]
	GC (60-100%]	All	185	60	23	119,046	88.94% [83.87%, 92.86%]	99.95% [99.94%, 99.96%]
		≥ LoD	170	26	14	119,104	92.39% [87.56%, 95.78%]	99.98% [99.97%, 99.99%]
	1–5 bp	All	441	158	88	339413	83.36% [79.91%, 86.44%]	99.95% [99.95%, 99.96%]

Variant Type	Bin	MAF Level	GCDx+ cNGS+	GCDx+ cNGS−	GCDx− cNGS+	GCDx− cNGS−	PPA [95% CI]	NPA [95% CI]
		≥ LoD	377	46	27	339650	93.32% [90.43%, 95.55%]	99.99% [99.98%, 99.99%]
	6–10 bp	All	124	24	6	71560	95.38% [90.22%, 98.29%]	99.97% [99.95%, 99.98%]
		≥ LoD	112	3	0	71599	100% [96.76%, 100%]	99.996% [99.99%, 100%]
	11–15 bp	All	159	17	10	46226	94.08% [89.39%, 97.13%]	99.96% [99.94%, 99.98%]
		≥ LoD	151	3	3	46255	98.05% [94.41%, 99.60%]	99.994% [99.98%, 100%]
	16–20 bp	All	26	5	2	16885	92.86% [76.50%, 99.12%]	99.97% [99.93%, 99.99%]
		≥ LoD	21	1	1	16895	95.45% [77.16%, 99.88%]	99.994% [99.97%, 100%]
	21–30 bp	All	17	12	3	15456	85.00% [62.11%, 96.79%]	99.92% [99.86%, 99.96%]
		≥ LoD	16	5	1	15466	94.12% [71.31%, 99.85%]	99.97% [99.92%, 99.99%]
	>30 bp	All	4	6	3	6017	57.14% [18.41%, 90.10%]	99.90% [99.78%, 99.96%]
		≥ LoD	4	6	2	6018	66.67% [22.28%, 95.67%]	99.90% [99.78%, 99.96%]
SNV	SSR Regions	All	37	47	0	6,168	100% [90.51%, 100.00%]	99.24% [99.00%, 99.44%]
		≥ LoD	36	23	0	6,193	100% [90.26%, 100%]	99.63% [99.45%, 99.77%]
Indel	SSR Regions	All	0	2	0	442	NA	99.55% [98.38%, 99.95%]
		≥ LoD	0	2	0	442	NA	99.55% [98.38%, 99.95%]

Variant Type	Bin	MAF Level	GCDx+ cNGS+	GCDx+ cNGS−	GCDx− cNGS+	GCDx− cNGS−	PPA [95% CI]	NPA [95% CI]
SNV	Homopolymer Regions	All	14	0	2	5,410	87.50% [61.65%, 98.45%]	100% [99.93%, 100%]
		≥ LoD	11	0	0	5,415	100% [71.51%, 100%]	100% [99.93%, 100%]
Indel	Homopolymer Regions	All	3	4	0	4,469	100% [29.24%, 100%]	99.91% [99.77%, 99.98%]
		≥ LoD	3	2	0	4,471	100% [29.24%, 100%]	99.96% [99.84%, 99.99%]

GCDx: Guardant360 Liquid CDx; cNGS: comparator NGS

Table 9. Accuracy Results Stratified by Variant Level

Variant Category	Variant Level	LoD Level	GCDx+ cNGS+	GCDx+ cNGS-	GCDx- cNGS+	GCDx- cNGS-	PPA [95% CI]	NPA [95% CI]
BRAF V600E	1	All	97	5	5	396	95.10% [89.03%, 97.89%]	98.75% [97.11%, 99.47%]
BRAF V600E	1	≥ 1x LoD	93	1	0	409	100.00% [96.03%, 100.00%]	99.76% [98.63%, 99.96%]
EGFR exon 19 deletions, L858R, and T790M	1	All	252	11	12	12975	95.45% [92.22%, 97.38%]	99.92% [99.85%, 99.95%]
EGFR exon 19 deletions, L858R, and T790M	1	≥ 1x LoD	237	2	1	13010	99.58% [97.66%, 99.93%]	99.98% [99.94%, 100.00%]
EGFR exon 20 insertions	1	All	88	3	1	26659	98.88% [93.91%, 99.80%]	99.99% [99.97%, 100.00%]
EGFR exon 20 insertions	1	≥ 1x LoD	85	0	0	26666	100.00% [95.68%, 100.00%]	100.00% [99.99%, 100.00%]
ERBB2/HER2 activating mutations	1	All	109	4	2	18196	98.20% [93.67%, 99.50%]	99.98% [99.94%, 99.99%]
ERBB2/HER2 activating mutations	1	≥ 1x LoD	101	0	0	18210	100.00% [96.34%, 100.00%]	100.00% [99.98%, 100.00%]

Variant Category	Variant Level	LoD Level	GCDx+ cNGS+	GCDx+ cNGS-	GCDx- cNGS+	GCDx- cNGS-	PPA [95% CI]	NPA [95% CI]
ESR1 mutations	1	All	202	59	11	15327	94.84% [90.99%, 97.09%]	99.62% [99.51%, 99.70%]
ESR1 mutations	1	≥ 1x LoD	179	20	2	15398	98.90% [96.06%, 99.70%]	99.87% [99.80%, 99.92%]
KRAS G12C	1	All	99	4	0	1402	100.00% [96.26%, 100.00%]	99.72% [99.27%, 99.89%]
KRAS G12C	1	≥ 1x LoD	92	2	0	1411	100.00% [95.99%, 100.00%]	99.86% [99.49%, 99.96%]
All Level 1 (CDx) Biomarkers (Aggregate)	1	All	847	86	31	74955	96.47% [95.03%, 97.50%]	99.89% [99.86%, 99.91%]
All Level 1 (CDx) Biomarkers (Aggregate)	1	≥ 1x LoD	787	25	3	75104	99.62% [98.89%, 99.87%]	99.97% [99.95%, 99.98%]
All Level 2 Biomarkers (Aggregate)	2	All	236	24	20	18513	92.19% [88.24%, 94.89%]	99.87% [99.81%, 99.91%]
All Level 2 Biomarkers (Aggregate)	2	≥ 1x LoD	197	9	3	18584	98.50% [95.68%, 99.49%]	99.95% [99.91%, 99.97%]
All Level 3 Biomarkers (Aggregate)	3	All	391	63	53	71445	88.06% [84.72%, 90.76%]	99.91% [99.89%, 99.93%]
All Level 3 Biomarkers (Aggregate)	3	≥ 1x LoD	323	23	21	71585	93.90% [90.85%, 95.97%]	99.97% [99.95%, 99.98%]
All Level 4 Biomarkers (Aggregate)	4	All	2453	1206	1571	1733526	60.96% [59.44%, 62.46%]	99.93% [99.93%, 99.93%]
All Level 4 Biomarkers (Aggregate)	4	≥ 1x LoD	1764	561	241	1736190	87.98% [86.48%, 89.33%]	99.97% [99.96%, 99.97%]

GCDx: Guardant360 Liquid CDx; cNGS: comparator NGS

2. Contrived Sample Functional Characterization (CSFC) Study

Comparable performance between clinical plasma specimens and contrived samples for SNVs, indels, rearrangements, and copy number amplifications was established by testing a dilution series consisting of seven dilution levels for contrived samples and five to seven levels for clinical samples, with at least 24 replicates per level for contrived samples across multiple reagent kit lots (4-7 lots depending on reagent type). Each dilution series was designed to target non-singleton coverage (NSC) 650, a challenging input level, to assess performance between the sample types across a MAF range.

Comparability of performance between clinical plasma specimens and contrived samples was assessed for SNVs (*EGFR* L858R, *EGFR* T790M, *KRAS* G12C, *BRAF* V600E, *PIK3CA* H1047R, *PIK3CA* E545K, *ESR1* D538G, and *KIT* Y823D/D816V), Rearrangements [*ALK* (*ALK-EML4*), *RET* (*KIF5B-RET*/*NCOA4-RET*), *ROS1* (*CD74-ROS1*), *NTRK1* (*TPM3-NTRK1*), *NTRK2* (*PAG1-NTRK2*), *FGFR2* (*FGFR2-BICC1*), *FGFR3* (*FGFR3-TACC3*), and *NRG1* (*HOOK3-NRG1*/*NRG1-CD74*)], Copy Number Amplifications (*MET* and *ERBB2* CNAs), Indels (*EGFR* E746_A750del, *ERBB2* Y772_A775dup, and *BRCA2* K1517Nfs*25/R2645Nfs*3), which demonstrated similar hit rates between clinical and contrived sample.

The results of the evaluation demonstrated that use of contrived samples to assess the analytical performance of the Guardant360 Liquid CDx test does not lead to overestimation of assay performance.

3. Analytical Sensitivity

a. Limit of Blank (LoB)

The LoB for Guardant360 Liquid CDx was assessed using cfDNA derived from cancer-free donor samples that were prescreened by an orthogonal NGS test. A total of 120 sample replicates from 30 unique cancer-free donor samples were assessed at maximum cfDNA input (30 ng cfDNA). Four replicates per sample across one instrument line, one operator group and two reagent lots were tested. LoB data demonstrated false positive rate, predominantly with Level 4 variants. Root-cause analysis indicated that these false positives were due to a substantial contribution from non-tumor hematopoietic somatic variation (CHIP-like signal) and/or similar low-level/background somatic events in presumed-negative samples. Re-analysis of LoB data after implementation of a CHIP caller update showed 0% false positive rate across all the reportable variant classes (SNVs, indels, rearrangements, CNAs and CNL).

b. Limit of Detection (LoD)

i. LoD for Low Input Samples

The LoD was established for Guardant360 Liquid CDx variants with CDx claims, representative SNVs and indels, and all reportable CNAs, CNL, and rearrangements. A total of five (5) sample pools containing targeted variants were created. Four (4) pools containing SNVs, indels, CNAs and rearrangements were created from patient

samples from the intended use population (NSCLC n= 24, Breast Cancer n= 9, CRC n= 3, Prostate cancer n= 5, Cholangiocarcinoma n= 1, and GIST n= 1). One (1) pool containing *BRCA1* CNL was created using cell line-derived cfDNA. Clinical pools were titrated into cfDNA derived from clinical plasma specimens without this study's variants of interest (termed as "WT cfDNA") and the contrived cfDNA sample was titrated into cfDNA from a matching WT cell line.

Detection rates were compared across 5-7 titration levels to establish the LoD at which there is at least 95% probability of detection for each variant class. Six hundred and forty-nine (649) replicates were tested at an analytically challenging level of NSC 650 across three critical reagent lots, three operator groups, and three instrument lines. Table 10 summarizes established LoD for CDx biomarkers. The LoDs for clinically relevant variants were established as 1.0% MAF for panel-wide SNVs and 0.9% MAF for panel-wide indels for low input samples (Table 11).

Table 10. Established LoD of CDx Biomarkers for Low Input Samples

Biomarker	Cancer Type	LoD (MAF%/CN/TF%)
EGFR T790M	NSCLC	0.90%
EGFR L858R	NSCLC	1.00%
EGFR E746_A750del	NSCLC	1.00%
EGFR L747_A755delinsSKG	NSCLC	0.70%
EGFR A767_V769dup	NSCLC	0.40%
ERBB2 S310F	NSCLC	1.00%
ERBB2 Y772_A775dup	NSCLC	0.90%
KRAS G12C	NSCLC	1.40%
ESR1 D538G	Breast	1.00%
ESR1 V422del	Breast	1.10%
BRAF V600E	CRC	0.80%

Table 11. Established LoD of Clinically Relevant Variant for Low Input Samples

Variant Type	Biomarker	Cancer Type	LoD (MAF%/CN/ TF%)
SNV	*ATM* R2227C	Prostate	1.50%
SNV	*KIT* Y823D	GIST	0.90%
SNV	*MET* exon14 splice variant	NSCLC	1.20%
SNV	*PIK3CA* E545K	Breast	0.70%
SNV	*PIK3CA* H1047R	NSCLC	1.00%
Indel	*BRCA1* M1827fs	Prostate	0.90%
Indel	*BRCA2* K1517fs	Prostate	2.20%
Indel	*KIT* W557_V559del	GIST	1.60%

Variant Type	Biomarker	Cancer Type	LoD (MAF%/CN/ TF%)
CNA	*ERBB2* amplification	Breast	2.4 copies
CNA	*MET* amplification	NSCLC	2.3 copies
CNL	*BRCA1* loss	Breast	22.70% TF
Rearrangement	*ALK* rearrangement	NSCLC	1.60%
Rearrangement	*FGFR2* rearrangement	Cholangiocarcinoma	0.90%
Rearrangement	*FGFR3* rearrangement	Breast	1.20%
Rearrangement	*NRG1* rearrangement	Breast	0.70%
Rearrangement	*NTRK1* rearrangement	NSCLC	0.60%
Rearrangement	*NTRK2* rearrangement	Prostate	1.20%
Rearrangement	*NTRK3* rearrangement	Breast	0.70%
Rearrangement	*RET* rearrangement	NSCLC	0.90%
Rearrangement	*ROS1* rearrangement	NSCLC	0.90%

To confirm low input LoD for additional CDx variants, tumor mutation profiling variants, and *BRCA1* CNL, a combined LoD Confirmation and Precision study was performed using one breast cancer patient sample with *BRCA1* deletion and four (4) clinical sample pools (NSCLC (Pool 1), Breast (Pool 2), CRC (Pool 3), and Prostate (Pool 4)). Positive sample pools, containing targeted SNVs, indels, and CNAs, were titrated into wild-type cfDNA (cfDNA derived from clinical specimens without targeted variants), at a low input target of NSC 1000 with variant MAF levels either targeting 1-1.5x LoD for LoD confirmation or targeting 1-2x LoD for precision assessment. All samples were tested with 27 replicates across three (3) batches with three (3) unique lots of critical reagents, three (3) unique instrument lines, three (3) operator groups, one (1) testing site and over three (3) different start days.

Of the samples tested, 135/135 (100%) sample replicates passed all QC metrics and were eligible for data analysis. A PPA ranging from 96.3% to 100% was observed for all targeted variants within 1-1.5x LoD as shown in Table 12, thus indicating LoD confirmation for the targeted variants.

Table 12. Combined LoD Confirmation and Precision Study Summary Results for CDx Variants and Representative Variants

Variant	Variant Type	Cancer Type	Number Positive / Number Expected	PPA Point Estimate [95% CI]	Average Observed MAF/CN/TF
BRCA1 S405	SNV	Breast	26 / 27	96.3% [81.0%, 99.9%]	1.30%
BRCA2 E187	SNV	Breast	27 / 27	100% [87.2%, 100.0%]	1.13%
ERBB2 L755P	SNV	NSCLC	27 / 27	100% [87.2%, 100.0%]	1.30%
ESR1 Y537S	SNV	Breast	27 / 27	100% [87.2%, 100.0%]	1.39%

Variant	Variant Type	Cancer Type	Number Positive / Number Expected	PPA Point Estimate [95% CI]	Average Observed MAF/CN/TF
NRAS G13V	SNV	CRC	27 / 27	100% [87.2%, 100.0%]	1.42%
PIK3CA H1047R	SNV	Breast	27 / 27	100% [87.2%, 100.0%]	1.19%
ATM L585fs	Indel	Prostate	26 / 27	96.3% [81.0%, 99.9%]	1.25%
BRCA2 C1200fs	Indel	Prostate	27 / 27	100% [87.2%,100.0%]	2.58%
EGFR E746_T751delinsVA	Indel	NSCLC	27 / 27	100% [87.2%, 100.0%]	1.52%
MET exon 14 skipping	Indel	NSCLC	26 / 27	96.3% [81.0%, 99.9%]	1.40%
ERBB2	CNA	CRC	27 / 27	100% [87.2%,100.0%]	2.47 copies
BRCA1	CNL	Breast	27 / 27	100% [87.2%, 100.0%]	29% TF

ii. LoD for High Input Samples

The LoDs for high input samples for clinically relevant variants (SNVs, indels, rearrangements) was established at 30ng cfDNA input (the highest input accepted by Guardant360 Liquid CDx) using 58 unique clinical samples across multiple tumor types that are representative of the intended use population *in silico*.

The established *in silico* LoD was confirmed using clinical samples from 46 cancer patients from the intended use population comprising of NSCLC (n=24), Breast Cancer (n=7), CRC (n=5), Prostate cancer (n=6), Cholangiocarcinoma (n=1), Ovarian (n=2) and other cancer types (n=1) at the maximum input level of 30 ng. Five (5) cfDNA clinical sample pools containing target variants (13 SNVs, 9 indels, and 9 rearrangements) were prepared using cfDNA from mutation-positive late-stage cancer patient samples in the background of mutation-negative cfDNA, targeting each variant at 1-1.5x of the *in silico* established LoD at maximum sample input of 30 ng. A total of 118 clinical samples were tested, with at least 18-20 replicates per pool across three (3) critical reagent lots, two (2) instrument combinations, four (4) operator groups, and spanned four (4) independent testing start days at one testing site. The median MAFs for biomarkers tested for LoD confirmation for CDx biomarkers are summarized in Table 13 and for clinically relevant variants are summarized in Table 14. The LoDs for clinically relevant SNVs (0.2% MAF) and indels (0.2% MAF) for high-input samples were calculated from the median LoDs for Level 1-3 variants.

Table 13. LoD for CDx Biomarkers for High Input Samples

Biomarker	Cancer Type	Median MAF in LoD Confirmation	Established LoD
EGFR T790M	NSCLC	0.2%	0.2%
EGFR L858R	NSCLC	0.3%	0.2%
EGFR E746_A750del	NSCLC	0.2%	0.2%

Biomarker	Cancer Type	Median MAF in LoD Confirmation	Established LoD
EGFR H773_V774dup	NSCLC	0.2%	0.2%
ERBB2 S310F	NSCLC	0.3%	0.2%
ERBB2 Y772_A775dup	NSCLC	0.2%	0.2%
KRAS G12C	NSCLC	0.1%	0.1%
ESR1 D538G	Breast	0.2%	0.2%
ESR1 V422del	Breast	0.3%	0.3%
BRAF V600E	CRC	0.2%	0.1%

Table 14. Clinically Relevant Variant LoD for High Input Samples

Variant Type	Biomarker	Cancer Type	Median MAF in LoD Confirmation	Established LoD
SNV	*ATM* mutations	Prostate	0.5%	0.3%
SNV	*BRCA1* inactivating mutations	Ovarian	0.4%	0.3%
SNV	*BRCA2* inactivating mutations	Prostate	0.5%	0.3%
SNV	*MET* exon 14 mutations	NSCLC	0.3%	0.2%
SNV	*NRAS* mutations	CRC	0.4%	0.3%
SNV	*PIK3CA* mutations	Breast	0.3%	0.2%
Indel	*KIT* mutations	Other	0.3%	0.2%
Indel	*ATM* mutations	Prostate	0.2%	0.2%
Indel	BRCA1 inactivating mutations	Prostate	0.2%	0.3%
Indel	*BRCA2* inactivating mutations	Prostate	0.3%	0.2%
Indel	*KIT* mutations	Other	0.3%	0.3%
Indel	*MET* exon 14 mutations	NSCLC	0.2%	0.2%
Rearrangement	*ALK* rearrangements	NSCLC	0.2%	0.2%
Rearrangement	*FGFR3* rearrangements	NSCLC	0.3%	0.2%
Rearrangement	*FGFR2* rearrangements	Cholangica-cinoma	0.2%	0.2%
Rearrangement	*NRG1* rearrangements	Breast	0.3%	0.2%
Rearrangement	*NTRK1* rearrangements	NSCLC	0.3%	0.2%
Rearrangement	*NTRK2* rearrangements	Prostate	0.6%	0.4%
Rearrangement	*NTRK3* rearrangements	Breast	0.2%	0.2%
Rearrangement	*RET* rearrangements	NSCLC	0.2%	0.2%
Rearrangement	*ROS1* rearrangements	NSCLC	0.2%	0.2%

4. Analytical Specificity

a. Endogenous Interfering Substances

To evaluate the potential impact of endogenous interfering substances on the performance of Guardant360 Liquid CDx, a total of 90 samples (49 mutation-positive and 41 mutation-negative) were evaluated with five (5) interferents (Unconjugated bilirubin - 0.4 g/L, Conjugated bilirubin - 0.4 g/L, Triglycerides - 15 g/L, Albumin - 60 g/L and Hemoglobin - 10 g/L). For mutation-positive samples, 14 control replicates (7 replicates per control substance) and 35 treatment replicates (7 replicates per interferent material) were tested. For mutation-negative samples, 11 control replicates and 30 treatment replicates (6 replicates per interferent material) were tested. All samples were tested at a challenging input (targeting NSC 1000), across one reagent lot, one operator group and one instrument line.

All samples (90/90, 100%) passed QC and were eligible for analysis. 100% PPA was observed for variants ≥1x LoD from mutation-positive samples between individual interferent conditions and their respective controls. 100% NPA was observed between individual interferent conditions and their respective negative controls. These results indicate that the endogenous interferents tested had no impact on Guardant360 Liquid CDx performance.

b. *In silico* Primer and Probe Specificity

Primer and probe specificity were assessed by mapping panel probes and primers to the human genome and common microbial genomes. When mapped to the human genome (hg19) with decoy sequences, unplaced contigs, and representative microbial contaminant genomes, 97.2% of probes uniquely map to the human genome with ideal specificity (MAPQ ≥ 60). None of the primers or probes mapped to the representative microbial contaminant genomes.

5. Precision

This study was performed to demonstrate the positive and negative precision and sequencer-to-sequencer precision of the Guardant360 Liquid CDx for SNVs, indels, rearrangements, and CNAs. Additionally, within batch (run on the same batch under the same conditions) and between batch (run across different batches, reagent lots, operators, and instruments) precision was assessed.

a. Precision from cfDNA Clinical Sample Pools

Two precision studies have been performed to demonstrate the repeatability and within-site reproducibility of Guardant360 Liquid CDx.

In the first precision study, four (4) clinical sample pools of different cancer types representing the intended use population, comprising 27 targeted variants (10 SNVs, 6 indels, 2 CNAs, and 9 rearrangements), were created by diluting cfDNA from clinical cancer patients with mutation-negative cfDNA. 27 replicates with MAF/copy number targeting 1-2x LoD were tested at a challenging input target of NSC 650. A total of three (3) MAF levels were tested per clinical sample pool, and 23-27 replicates were eligible for analysis per pool per dilution level.

To assess primary precision, a total of 308 sample replicates were run in seven (7) batches and tested across three (3) unique lots of critical reagents, three (3) unique instrument line combinations encompassing assay workflow from methyl partitioning to sequencing (Hamilton STARs, thermal cyclers, TECAN SPARK microplate readers, and NovaSeq X Plus sequencers), three (3) operator groups, one (1) testing site and six (6) different start days.

To assess for sequencer-to-sequencer precision, a total of 192 sample replicate pairs were sequenced on three (3) unique sequencing instruments for this study. Each sample replicate from four (4) primary precision batches was re-sequenced on a different sequencer than the original run. A total of 516 sample replicates were tested, combining both primary precision and sequencer-to-sequencer precision. Of these, 491 sample replicates passed QC (with inclusion of samples ≥ NSC 400) and were eligible for analysis.

Both primary precision and sequencer-to-sequencer precision were assessed on variants in sample replicates targeted to be in the 1-2x LoD range. The primary precision analysis demonstrated a PPA of 99.3% (95% CI: 98.3% - 99.8%) and NPA of 99.3% (95% CI: 99.0% - 99.5%) across all replicates for 27 targeted variants (Table 15). The sequencer-to-sequencer precision analysis showed an Average Positive Agreement (APA) of 99.7% (95% CI: 98.6% - 100.0%) and Average Negative Agreement (ANA) of 100.0% (95% CI: 99.8% - 100.0%) between paired replicates sequenced on two different sequencers (Table 15).

Table 15. Summary of Precision Results

Cohort	Measurand	N (Concordant Variants / Total)	Point Estimate [95% CI]
Primary Precision	PPA	696 / 701	99.3% [98.3%,99.8%]
	NPA	6650 / 6699	99.3% [99.0%, 99.5%]
Sequencer-to-Sequencer Precision	APA	443 / 444	99.7% [98.6%, 100.0%]
	ANA	3949 / 3950	100.0% [99.8%, 100.0%]

PPA/NPA by each variant type (SNV, indel, CNA, rearrangement) were calculated for targeted pool variants in the 1- 2X LoD range. Agreement levels for each of the variant types exceeded 95% (Table 16).

Table 16. Agreement Rate of Targeted Variants within 1-2x LoD

Pool (Cancer Type)	Variant Type	Variant	Category	Concordant Variants/Total	Point Estimate [95% CI]	Observed Mean MAF%/CN /% TF	Fold LoD
1 (NSCLC)	SNV	*EGFR* L858R	1	26/26	100.0% [86.8%, 100.0%]	2%	2x
		EGFR T790M	1	25/26	96.2% [80.4%, 99.9%]	1.9%	2.1x
		ERBB2 S310F	1	26/26	100.0%	1.7%	1.7x

Pool (Cancer Type)	Variant Type	Variant	Category	Concordant Variants/Total	Point Estimate [95% CI]	Observed Mean MAF%/CN /% TF	Fold LoD
					[86.8%, 100.0%]		
		KRAS G12C	1	26/26	100.0% [86.8%, 100.0%]	3.1%	2.2x
	Indel (15bp)	*EGFR* E746 A750del	1	26/26	100.0% [86.8%, 100.0%]	1.4%	1.4x
	Rearrange -ment	*ALK*	2	26/26	100.0% [86.8%, 100.0%]	1.5%	0.9x
		RET	3	26/26	100.0% [86.8%, 100.0%]	1.5%	1.7x
	CNA	*MET*	3	26/26	100.0% [86.8%, 100.0%]	2.5%	1.1x
2 (NSCLC)	SNV	*MET* exon 14 splice variant	2	25/25	100.0% [86.3%, 100.0%]	2.1%	1.8x
	Indel (9bp)	*EGFR* A767 V769dup	1	25/25	100.0% [86.3%, 100.0%]	0.9%	2.2x
	Indel (12bp)	*ERBB2* Y772 A775dup	1	24/25	96.0% [79.6%, 99.9%]	1.2%	1.3x
	Rearrange ment	*ROS1*	2	25/25	100.0% [86.3%, 100.0%]	1.6%	1.8x
	Rearrange ment	*NTRK1*	2	25/25	100.0% [86.3%, 100.0%]	1.2%	2x
3 (Breast)	SNV	*ESR1* D538G	1	26/26	100.0% [86.8%, 100.0%]	1.6%	1.6x
		PIK3CA E545K	2	26/26	100.0% [86.8%, 100.0%]	1.1%	1.6x
	Indel (3bp)	*ESR1* V422del	1	25/26	96.2% [80.4%, 99.9%]	1.3%	1.2x
	Rearrange ment	*NRG1*	4	27/27	100.0% [87.2%, 100.0%]	1.3%	1.9x
	Rearrange ment	*NTRK3*	2	27/27	100.0% [87.2%, 100.0%]	1.3%	1.9x
	CNA	*ERBB2*	3	26/26	100.0% [86.8%, 100.0%]	2.6%	1.1x
4 (Mixed)	SNV	*ATM* R2227C	2	27/27	100.0% [87.2%, 100.0%]	2.8%	1.9x
		BRAF V600E	1	26/26	100.0% [86.8%, 100.0%]	1.1%	1.4x
		KIT Y823D	4	26/26	100.0% [86.8%, 100.0%]	1.7%	1.9x
	Indel (2bp)	*BRCA1* M1827Rfs*8	2	26/26	100.0% [86.8%, 100.0%]	1.5%	1.7x
	Indel (4bp)	*BRCA2* K1517Nfs*25	2	27/27	100.0% [87.2%, 100.0%]	4.3%	2x
	Rearran-gement	*FGFR2*	3	26/26	100.0% [86.8%, 100.0%]	1.8%	2x

Pool (Cancer Type)	Variant Type	Variant	Category	Concordant Variants/Total	Point Estimate [95% CI]	Observed Mean MAF%/CN /% TF	Fold LoD
	Rearran-gement	*FGFR3*	3	25/26	96.2% [80.4%, 99.9%]	1.3%	1.1x
	Rearran-gement	*NTRK2*	2	25/26	96.2% [80.4%, 99.9%]	1.3%	1.1x

An assessment of the precision for panel-wide SNV and indel variants demonstrated high concordance (PPA: 92.3% - 95.7%; NPA: 98.1% - 98.4%) for within-batch and an APA of 93.8% and ANA of 99.2% across batches. Variant component analysis showed that batch-to-batch variation as well as variability caused by instrument, operator, and reagent lot was minimal. The largest contributor to variance was within-batch technical replicate- variability.

The second precision study is a combined LoD confirmation and precision study. The purpose is to confirm low input LoD for additional CDx variants, tumor mutation profiling variants, and *BRCA1* CNL and verify the positive precision and negative precision for CDx variants, tumor mutation profiling variants, and *BRCA1* CNL (Positive Precision only).

Positive precision for additional CDx variants, tumor mutation profiling variants, and *BRCA1* CNL confirmed in the combined LoD confirmation and precision study described in Section X.A.3.b above.

For negative precision, three (3) negative clinical sample pools representing NSCLC (Pool 1), Breast (Pool 2), and CRC (Pool 3) indications were used to assess the precision of the negative sites. All samples were tested with 27 replicates across three (3) batches with three (3) unique lots of critical reagents, three (3) unique instrument lines, three (3) operator groups, one (1) testing site and over three (3) different start days.

Of the samples tested, 216/216 (100%) samples passed all QC metrics and were eligible for data analysis. The PPA for variant-positive samples was 99.1% (321/324; 95% CI: 97.3% - 99.8%). The NPA for variant-positive samples was 100% (891/891; 95% CI: 99.6% - 100%), and the NPA for variant negative samples was 99.9% (3076/3078; 95% CI: 99.8% - 100%).

b. <u>Plasma Extraction Precision and Precision of Downstream Steps</u>

The purpose of this study was to show the precision of variant calling for the entire sample workflow (from cfDNA extraction through sequencing) and to evaluate within-conditions (run on the same batch under the same conditions) and between-conditions (run across different batches, reagent lots, operators, and instruments) extraction variability.

A total of 26 unique clinical samples carrying targeted variants (SNVs, indels, CNAs, CNL and rearrangements) across 10 different cancer types representing the intended use population were used in the study. Eighteen (18) of 26 clinical samples were pooled and diluted with mutation-negative cancer-type matched pooled plasma to create six (6) unique sample pools targeting MAF level between 1-3x LoD. Remaining eight (8) of 26 clinical samples were selected with variants at challenging MAF near LoD. Table 17 lists the cancer type and targeted variant information per sample/sample pool.

Table 17. Cancer Type and Targeted Variant Information per Sample/Sample Pool

Cancer Type	Targeted Variant	Variant Type	Variant Level	Number of Mutation Positive Clinical Samples	Average Observed MAF Across Replicates	Observed nX LoD
Non-small Cell Lung Carcinoma	*ALK;EML4*	Rearrange-ment	2	5	3.06	1.91x
	EGFR L858R	SNV	1		2.01	2.01x
	EGFR E746_S753delinsV	Indel	1		2.72	3.90x
	ERBB2 S310F	SNV	1		2.05	2.05x
	KRAS G12C	SNV	1		2.49	1.78x
Non-small Cell Lung Carcinoma	*EGFR* T790M	SNV	1	4	1.55	1.73x
	ERBB2 G778_P780	Indel	3		1.48	1.65x
	MET exon14 skipping Indels	Indel	2		1.61	1.15x
	ROS1;CD74	Rearrange-ment	2		1.57	1.75x
Breast Carcinoma	*ESR1 Y537S*	SNV	1	3	1.39	1.40x
	ESR1 V422del	Indel	1		3.35	3.05x
	PIK3CA H1047R	SNV	2		1.87	1.87x
Prostate	*ATM V2424G*	SNV	2	3	2.66	1.78x

Cancer Type	Targeted Variant	Variant Type	Variant Level	Number of Mutation Positive Clinical Samples	Average Observed MAF Across Replicates	Observed nX LoD
Adenocarcinoma	*BRCA2 p.T3085fs*	Indel	2		4.26	1.94x
	NTRK1;MYOC	Rearrange-ment	2		1.25	2.10x
Colorectal Adenocarcinoma	*BRAF V600E*	SNV	1	2	1.53	1.92x
	KRAS G12D	SNV	3		2.87	2.05x
Lung Adenocarcinoma	*EGFR D770_N771insG*	Indel	1	1	0.97	2.43x
Prostate Adenocarcinoma	*BRCA1 Deletion*	CNL	2	1	0.74	3.27x
Breast Carcinoma	*ERBB2* CNA	CNA	3	1	3.29	3.23x
Ovarian Carcinoma	*ERBB2 CNA*	CNA	3	1	3.27	3.19x
Melanoma	*PIK3CA.E545K*	SNV	4	1	1.44	2.06x
Cholangio carcinoma	*PIK3CA.D1029Y*	SNV	4	1	0.94	1.1x
Bladder Carcinoma	*ERBB2 CNA*	CNA	3	1	3.69	4.23x
Pancreatic ductal adenocarcinoma	*KRAS.G12R*	SNV	4	1	2.83	2.02x
Esophageal /gastroesophageal Junction adenocarcinoma	*ERBB2 CNA*	CNA	3	1	5.21	8.05x

All sample/sample pools were tested with six (6) replicates across three (3) extraction batches at a challenging cfDNA input target of NSC 1000. Samples were tested using three (3) unique lots of extraction reagents, three (3) operator groups, one (1) unique critical reagent lot, over three (3) different start days and at one (1) testing site. Three (3) unique instrument groups from cfDNA extraction to automated buffer exchange and quantitation (BEQ) were used in this study. Each instrument group comprises a QIAsymphony, Hamilton STAR, and TapeStation, collectively supporting the assay workflow from cfDNA extraction through BEQ. One (1) instrument line from Methylation Partitioning to sequencing was used in this study. The three precision conditions are described in Table 18.

Table 18. Exaction Precision Study Design

Precision Condition	Day	Extraction Batch	Extraction Reagent Lot	Qiasymphony	Hamilton STAR	Tape Station	Operator Group
PC1	1	1	A	1	2	1	OP1
PC2	2	2	B	2	2	2	OP2
PC3	3	3	C	2	1	1	OP1/OP3

Of the samples tested, 83/84 (98.81%) sample replicates passed all QC metrics and were eligible for data analysis. The within-precision condition analysis demonstrated APA of 100% and ANA of 100% for targeted variants ≥ 1x LoD. The between-precision condition analysis showed APA of 100% and ANA of 100%. Additional analyses were performed on all reportable variants including those not prespecified in the study (Table 19). Except for indels, all the other variant types demonstrated APA of >95%. The slightly lower APA observed for indels (ranging from 89.1% - 92.7%) is primarily attributable to analytical variability for variants present near the LoD. Across the 75 indel replicate comparisons contributing to APA, 12 comparisons (16%) involved replicate measurements spanning below and above the 1xLoD threshold, such that a variant is stochastically detected across replicates while the average value of the MAF is above the LoD, resulting in reduced concordance.

Table 19. Variant-Type Stratified APA and ANA for Reportable Variants Within and Between Precision Conditions

Comparison Pair	Comparison Type	Variant Type	APA [95% CI]	APA Number of Variants	ANA [95% CI]	ANA Number of Variants
PC1	Within PC	SNV	97.6% [96.1%, 98.5%]	662	99.9949% [99.9946%, 99.9952%]	19583202
		Indel	91.8% [80.8%, 96.8%]	49	99.9995% [99.9994%, 99.9996%]	19610326
		CNA	100.0% [51.0%, 100.0%]	4	95.8333% [79.7582%, 99.2607%]	24
		CNL	100.0% [20.7%, 100.0%]	1	100.0000% [77.1905%, 100.0000%]	13
		Rearrange-ment	100.0% [43.9%, 100.0%]	3	100.0000% [96.9714%, 100.0000%]	123
PC2	Within PC	SNV	97.9% [96.6%, 98.7%]	723	99.9948% [99.9945%, 99.9951%]	19583202

Comparison Pair	Comparison Type	Variant Type	APA [95% CI]	APA Number of Variants	ANA [95% CI]	ANA Number of Variants
		Indel	90.4% [79.4%, 95.8%]	52	99.9994% [99.9993%, 99.9995%]	19610326
		CNA	100.0% [51.0%, 100.0%]	4	95.8333% [79.7582%, 99.2607%]	24
		CNL	100.0% [20.7%, 100.0%]	1	100.0000% [77.1905%, 100.0000%]	13
		Rearrange-ment	100.0% [43.9%, 100.0%]	3	100.0000% [96.9714%, 100.0000%]	123
PC3	Within PC	SNV	97.7% [96.3%, 98.5%]	730	99.9948% [99.9944%, 99.9951%]	19583202
		Indel	89.1% [78.2%, 94.9%]	55	99.9995% [99.9993%, 99.9996%]	19610326
		CNA	100.0% [51.0%, 100.0%]	4	95.8333% [79.7582%, 99.2607%]	24
		CNL	100.0% [20.7%, 100.0%]	1	100.0000% [77.1905%, 100.0000%]	13
		Rearrange-ment	100.0% [43.9%, 100.0%]	3	100.0000% [96.9714%, 100.0000%]	123
PC1 vs PC2	Between PC	SNV	96.6% [95.9%, 97.2%]	2891	99.9948% [99.9947%, 99.9950%]	78332808
		Indel	92.7% [88.3%, 95.5%]	206	99.9995% [99.9994%, 99.9995%]	78441304
		CNA	100.0% [80.6%, 100.0%]	16	95.8333% [89.7718%, 98.3679%]	96
		CNL	100.0% [51.0%, 100.0%]	4	100.0000% [93.1208%, 100.0000%]	52
		Rearrange-ment	100.0% [75.8%, 100.0%]	12	100.0000% [99.2253%, 100.0000%]	492
PC1 vs PC3	Between PC	SNV	96.4% [95.6%, 97.0%]	2906	99.9948% [99.9947%, 99.9950%]	78332808

Comparison Pair	Comparison Type	Variant Type	APA [95% CI]	APA Number of Variants	ANA [95% CI]	ANA Number of Variants
		Indel	90.6% [85.9%, 93.8%]	213	99.9995% [99.9994%, 99.9995%]	78441304
		CNA	100.0% [80.6%, 100.0%]	16	95.8333% [89.7718%, 98.3679%]	96
		CNL	100.0% [51.0%, 100.0%]	4	100.0000% [93.1208%, 100.0000%]	52
		Rearrange-ment	100.0% [75.8%, 100.0%]	12	100.0000% [99.2253%, 100.0000%]	492
PC2 vs PC3	Between PC	SNV	97.8% [97.2%, 98.3%]	2906	99.9948% [99.9946%, 99.9950%]	78332808
		Indel	89.3% [84.4%, 92.7%]	214	99.9995% [99.9994%, 99.9995%]	78441304
		CNA	100.0% [80.6%, 100.0%]	16	95.8333% [89.7718%, 98.3679%]	96
		CNL	100.0% [51.0%, 100.0%]	4	100.0000% [93.1208%, 100.0000%]	52
		Rearrange-ment	100.0% [75.8%, 100.0%]	12	100.0000% [99.2253%, 100.0000%]	492

These results confirm the repeatability and reproducibility of the Guardant360 Liquid CDx extraction process in detecting both positive and negative variant calls across technical replicates.

6. Carryover/Cross-Contamination

The study evaluated potential cross-contamination and carry-over contamination in the Guardant360 Liquid CDx workflow using 180 pre-characterized cancer patient plasma samples (87 male, 93 female) across two consecutive batches from the analytical accuracy study. These 180 samples from the intended used population were from different cancer types (NSCLC n=88, Breast Cancer n=22, CRC n=22, Prostate cancer n=19, Melanoma Cancer n=17, Ovarian cancer n=6, Other (Carcinoma of unknown primary (CUP) Bladder Carcinoma, Esophageal/Gastroesophageal junction Adenocarcinoma, Endometrial carcinoma) n=6).

Two full batches of 90 samples each were arranged in a male-female checkerboard pattern with respect to the detection of contamination in chromosome Y signal. The

checkerboard layout was also designed to represent a pattern of high MAF positive and presumed negative variants, based on prior Guardant360 CDx (P200010) variant call data. Only SNVs, indels, and rearrangements were considered, as these variant types could be reliably tracked for contamination. The two batches were consecutively processed on the same instrument line to assess both cross-contamination and carry-over contamination events. The second plate had a flipped checkerboard pattern compared to the first plate to allow for the assessment of carry-over contamination. A total of 180 pre-characterized cancer patient plasma samples were run across two batches and tested using one (1) instrument line, one (1) critical reagent lot, two (2) operator groups and at one (1) testing site. In the primary analysis, a low cross-contamination rate of 0.6% (1 out of 180) was observed, with no evidence of carryover-contamination.

7. Guardbanding/Robustness

This study evaluated the robustness of Guardant360 Liquid CDx across various cfDNA input levels and established the assay's tolerance to input variability.

8. Stability

a. Reagent Stability

The purpose of this study was to establish the shelf-life stability of reagents used in Guardant360 Liquid CDx. Contrived samples containing various clinically relevant variants (SNVs, indels, rearrangements, CNAs and *BRCA1* CNL) are used to assess reagent stability across three (3) lots of reagents over two (2) timepoints (T0 = 0 month, and T1 = 4 months) with T0 defined as the first testing time point. The MAF or tumor fraction level were targeted at 1-3x LoD, and each replicate was diluted to target an input at approximately 1000 NSC. A total of 144 contrived samples were tested at each time point across three (3) instrument line combinations, one (1) operator group, three (3) critical reagent lots and at one (1) testing site.

A total of 143/144 (99.3%) samples passed QC for the T0 timepoint, while 144/144 (100%) passed QC for the T1 timepoint and were eligible for analysis for their respective timepoints. 100% PPA and 100% NPA was observed for targeted positive variants within reagent lots and between baseline (T0) and reagents aged for 4 months (T1). The study showed no significant decline in detection rates over the course of the study, demonstrating that there was no significant difference between the 4-month time point compared to T0 for all three lots. The current data demonstrate that Guardant360 Liquid CDx reagents are stable for up to 3 months.

b. Whole Blood Stability

The objective of this study was to demonstrate the stability of whole blood specimens used for Guardant360 Liquid 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 study evaluated plasma samples from previously collected whole blood into four (4) Streck Cell-Free BCTs from each of 24 donors (10 cancer patients and 14 self-

declared cancer-free healthy donors). The 10 cancer patient study subjects included the following four (4) cancer types from the intended use population (NSCLC, breast cancer, CRC, and pancreatic cancer). One (1) out of the four (4) BCT per patient was processed to plasma immediately upon receipt to serve as the reference condition. The other three (3) BCTs were subjected to summer temperature profile, winter temperature profile, and room temperature (RT) storage conditions, respectively, as summarized in Table 20. Plasma was isolated from these tubes on Day 8 after blood collection. Procedures for whole blood handling and plasma isolation used in processing previously collected whole blood are the same as those used for Guardant360 Liquid CDx.

Table 20. Description of Whole Blood Storage Conditions

Condition	BCT # from Each Patient	Storage Condition / Processing
Reference	1	Reference condition: Plasma processing on Day 0 for healthy donors by Discovery Life Sciences (GH approved vendor) and Day 1 for Cancer donors by GH personnel
C1	2	Summer Profile Storage: 4h at 22°C, 6h at 37°C, 56h at 22°C, 6h at 37°C plus remaining time at room temperature.
C2	3	Winter Profile Storage: 4h at 18°C, 6h at 0°C, 56h at 10°C, 6h at 0°C plus remaining time at room temperature.
C3	4	Room Temperature Storage: Storage at room temperature 18-25°C.

A total of 95 sample replicates from four BCT lots were processed across two operator groups, one reagent lot and one instrument group. One sample was excluded from analysis due to a processing issue, and 95.79% (91/95) were eligible for analysis. All storage conditions demonstrated acceptable performance (Table 21). 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 0.8397 to 0.9229.

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 * 0.0768) was 87.4-88.6%, which demonstrated that there was no preferential drop-out of relative exon-level coverage exceeding expected levels due to random variation, and the entire panel was covered consistently between reference and interfering substance conditions. The lower bound of the 95% exact binomial CI for the fraction of genomic targeted exonic regions with relative exon-level non-singleton coverage is shown in Table 21.

PPAs were also calculated for all the clinically relevant variants (Level 1-3) and panel-wide variants (Level 1-4). PPA for clinically relevant variants above LoD was 100% for all conditions, and for panel-wide variants above LoD, PPA was ranging from 86.9% to 89.2% (Table 21). The data indicate acceptable sensitivity and specificity when using samples across the storage conditions.

The panel-wide NPAs were also calculated between the test storage and reference conditions for reportable loci that are negative in the reference sample. It calculates the NPA based on the number of concordant negative calls out of the total reference negative calls across the set of reportable loci in the Guardant360 Liquid CDx panel. The results indicate >99.99% agreement of negative calls between the test storage and reference conditions (Table 21).

Table 21. Whole Blood Stability Summary Results

Study Endpoint	Metric	Room Temperature	Summer Profile	Winter Profile
		Study Result	Study Result	Study Result
Sample-level Molecule Recovery	Median of NSC fold (condition / reference)	0.8397	0.8650	0.9229
Relative Exon-Level Coverage	Lower bound of 95% CI for fraction of exons outside expected coverage	87.4%	87.9%	88.6%
Variant Call Concordance	PPA (all variant types, clinically relevant)	83.3% (5/6)	66.7% (4/6)	83.3% (5/6)
	PPA (≥ 1× LoD, all variant types, clinically relevant)	100% (4/4)	100% (4/4)	100% (4/4)
	PPA (panel-wide)	76.3% (103/135)	74.1% (100/135)	76.7% (99/129)
	PPA (≥ 1× LoD, all variant types, panel-wide)	89.2% (91/102)	88.2% (90/102)	86.9% (86/99)
	NPA (combined)	>99.99% (27,995,228 / 27,995,275)	>99.99% (27,995,229 / 27,995,275)	>99.99% (25,195,716 / 25,195,740)

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

c. <u>Plasma Stability</u>

The purpose of this analytical verification study is to verify stability of plasma samples stored at -80°C ± 10°C for 45 days and 1 year to support Guardant360 Liquid

CDx test workflow. A total of 112 samples (56 samples per timepoint) from patients representing various cancer types from the intended use population (NSCLC, Breast, Colorectal, Prostate, Melanoma, Urothelial, and Other) and representing all variant classes (SNV, indel, rearrangement, CNA, and CNL) were analyzed in this study. Testing was conducted at a single site, using two (2) instrument groups, one (1) reagent lot, and one (1) operator group per time point. This study is designed to evaluate the impact of plasma storage for 45 days of storage plus 2 freeze/thaws (T1) and long-term storage >1 year (T2) on the performance of Guardant360 Liquid CDx. Currently, the data is available for T1 timepoint of 45 days (Table 22).

Table 22. Description of Plasma Storage Conditions

Condition	BCT # from Each Patient	Storage Condition / Processing
Reference (T0)	1	Enriched library plates (ENT plates), retained as intermediate backup material from the assay workflow, were processed from autopooling through sequencing using the Guardant360 Liquid CDx workflow. The plasma samples used in the T0 batch had minimal freezer storage time (approximately one day).
T1	2	Clinical plasma retains for the same samples were stored at -80°C ± 10°C for 48 days with 2 freeze/thaw cycles and then processed from cfDNA extraction through sequencing on Guardant360 Liquid CDx (for a 45-day stability claim at -80°C
T2	3	Storage of plasma at -80°C ± 10°C plus 2 freeze/thaw cycles for one year (Study is ongoing)

All 112/112 (100.0%) samples evaluated passed all QC metrics and were eligible for analysis. The primary analysis evaluated sample-level and exon-level molecule recovery between the reference time point (T0) and the post-storage time point (T1, 48 days). Sample level molecule recovery showed fold change of 0.8895, and exon-level relative coverage demonstrated 94.94% fraction of exons within 2σ (2 * 0.0768) of expected relative coverage. The lower bound of the 95% exact binomial CI for the fraction of genomic targeted exonic regions with relative exon-level non-singleton coverage is shown in Table 23.

Table 23. Plasma Stability Summary Results

Study Endpoint	Metric	T0 vs T1
Sample-level Molecule Recovery	Median of NSC fold (T0 / T1)	0.8895

Study Endpoint	Metric	T0 vs T1
Relative Exon-Level Coverage	95% CI Lower Bound of fraction of exons outside expected coverage	94.94%
Variant Call Concordance	PPA (all variant types, clinically relevant)	91.7% (33/36)
	PPA (≥ 1× LoD, all variant types, clinically relevant)	100% (27/27)
	PPA (panel-wide)	72.0% (378/525)
	PPA (≥ 1× LoD, all variant types, panel-wide)	91.1% (277 / 304)
	NPA (combined)	>99.99% (156,774,216 / 156,774,331)

PPAs were also calculated for all the clinically relevant variants (Level 1-3) and panel-wide variants (Level 1-4). The PPA for clinically relevant variants above LoD was 100%, and the PPA for panel-wide variants above LoD was 91.1% (Table 23). The panel-wide NPA was >99.99% (Table 23).

This study demonstrates that plasma samples are stable when stored at -80°C±10°C for at least 45 days, with up to two freeze-thaw cycles.

d. cfDNA Stability

The purpose of this study is to establish the stability of cfDNA samples after buffer exchange (BE), where samples are stored at +2°C to +8°C for 24 hours, then at -20°C ± 5°C for 7 days, with two freeze-thaw (F/T) cycles, to support cfDNA sample stability claims for the Guardant360 Liquid CDx.

A total of 114 sample replicates from 57 clinical samples representing the intended use population (NSCLC, CRC, breast, prostate, ovarian, pancreatic, gastric, gastroesophageal junction, melanoma, carcinoma of unknown primary) were evaluated in this study. These samples included all variant types, i.e., SNV, indel, rearrangement, CNA, and CNL. These samples were a subset of those processed as part of the Analytical Accuracy study, and the original batch processed for accuracy served as the "Reference" (T0) condition. For evaluating cfDNA stability, the residual cfDNA from the same batch were subjected to different storage conditions before processing through the remaining workflow. The BE output plate (containing ≥30 ng of cfDNA) was stored at +2°C to +8°C for over 25 hours, then transferred to -20°C for over 8 days. The plate underwent two freeze-thaw cycles before being processed through the remainder of the Guardant360 Liquid CDx workflow (Table 24). This batch served as "Storage or Test" (T8) condition. Both Reference (T0) and

Storage (T8) conditions were tested using two (2) instrument lines, one (1) operator group and one (1) critical reagent lot at one (1) testing site.

Table 24. Description of cfDNA Storage Conditions

Condition	Storage Condition / Processing
Reference (T0)	Reference condition: cfDNA extraction on Day 0
Storage (T8)	The same batch of cfDNA from reference condition was stored at +2°C to +8°C for over 25 hours, then transferred to -20°C for over 8 days with two freeze-thaw cycles (for a 7-day stability claim at -20°C± 5°C).

A total of 112/114 (98.25%) samples passed QC and were eligible for analysis. The primary analysis evaluated sample-level and exon-level molecule recovery between the reference time point (T0) and the post-storage time point (T8, 8 days). Sample level molecule recovery showed fold change of 0.9829, and exon-level relative coverage demonstrated 96.5% fraction of exons within 2σ (2 * 0.0768) of expected relative coverage. The lower bound of the 95% exact binomial CI for the fraction of genomic targeted exonic regions with relative exon-level non-singleton coverage is shown in Table 25.

PPAs were also calculated for all the clinically relevant variants (Level 1-3) and panel-wide variants (Level 1-4). The PPA for clinically relevant variants above LoD was 100%, and the PPA for panel-wide variants above LoD was 96.0% (Table 25). The panel-wide NPA was >99.99% (Table 25).

Table 25. cfDNA Stability Summary Results

Study Endpoint	Metric	Condition 1 (T8)
Sample-level Molecule Recovery	Median of NSC fold (condition / reference)	0.9829
Relative Exon-Level Coverage	95% CI Lower Bound of fraction of exons outside expected coverage	96.5%
Variant Call Concordance	PPA (all variant types, clinically relevant)	96.7% (89/92)
	PPA (≥ 1× LoD, all variant types, clinically relevant)	100% (77/77)
	PPA (all variant types, panel-wide)	83.66% (1024/1224)

Study Endpoint	Metric	Condition 1 (T8)
	PPA (≥ 1× LoD, all variant types, panel-wide)	96.0% (863/899)
	NPA (combined)	>99.99% (159,572,972 / 159,573,173)

This study demonstrates that cfDNA samples are stable when stored at +2°C to +8°C for 24 hours, -20°C ± 5°C for 7 days, and up to two freeze-thaw cycles.

e. Intermediate Sample Stability

The purpose of this study is to define the storage conditions and evaluate the stability of intermediate products used for repeat testing in the Guardant360 Liquid CDx workflow. Three (3) intermediate products are generated during the Guardant360 Liquid CDx workflow and retained as backup material: libraries - Library Preparation Cleanup (LPC) plate retains generated at LPC step, enriched libraries - Enrichment Transfer (ENT) plate retain generated at ENT step, and normalized enriched retain library pool(s) - Normalized Enriched Retain (NRE) tube retain generated during Autopooling Method for NVX Sequencer (APX) step. The stability of these intermediate products at defined temperatures and durations was assessed (Table 26).

A total of 60 cancer patient samples from 10 cancer types representing the intended use population (NSCLC, CRC, breast, prostate, cholangiocarcinoma, ovarian, pancreatic, carcinoma of unknown primary (CUP), testis germ cell tumor (seminoma), and gastroesophageal junction carcinoma) previously tested as part of Analytical Accuracy study were used as the "Reference" condition. Intermediate products were stored under specific conditions before processing in the test condition.

Table 26. Description of Intermediate Product Storage Conditions

Intermediate Product	Target Storage Claim	Executed Test Conditions
Library plates (LPC plate retains)	14 days (up to 2 freeze/thaw cycles) at -20°C±5°C	Condition 1: 15 days (including 2 freeze/thaw cycles) at -20°C±5°C
Enriched library plates (ENT plate retain)	Up to 4 hours at 2-8°C and 14 days (up to 3 freeze/thaw cycles) at -20°C±5°C	Condition 2: 4.5 hours at 2-8°C followed by 19 days (including 3 freeze/thaw cycles)
Normalized Enriched Retain library pool(s)	14 days (up to 2 freeze/thaw cycles) at -20°C±5°C	Condition 3: 16 days (including 2 freeze/thaw cycles) at -20°C±5°C

A total of 240 samples (comprising 60 each from the LPC plates, ENT plate, NRE tube retains of the reference batch, as well as 60 from the reference condition) were processed through the Guardant360 Liquid CDx assay workflow, with 100% of the samples passing all assay QC metric criteria.

The study evaluated the sample-level and exon-level molecule recovery between the reference condition and each of the three test storage conditions to demonstrate that the stability of intermediate products is maintained under the tested storage conditions. Sample level molecule recovery showed fold change of 1.0456, 1.0306 and 1.0160 for the three intermediate products stored at the designated storage conditions, respectively, and exon-level relative coverage demonstrated 99.1%, 99.9% and 99.9% fraction of exons within 2σ (2 * 0.0768) of expected relative coverage for the three intermediate products stored at the designated storage conditions, respectively. The lower bound of the 95% exact binomial CI for the fraction of genomic targeted exonic regions with relative exon-level non-singleton coverage is shown in Table 27.

PPAs were also calculated for all the clinically relevant variants (Level 1-3) and panel-wide variants (Level 1-4). The PPAs for clinically relevant variants above LoD were 100% for all three intermediate products stored at the specific storage conditions, and the PPAs for panel-wide variants above LoD were >95% (Table 27). The panel-wide NPA was >99.99% (Table 27).

Table 27. Intermediate Product Stability Summary Results

Study Endpoint	Metric	Condition 1: LPC plate retains	Condition 2: ENT plate retain	Condition 3: NRE tube retain
Sample-level Molecule Recovery	Median of NSC fold (condition / reference)	1.0456	1.0306	1.0160
Relative Exon-Level Coverage	95% CI Lower Bound of fraction of exons outside expected coverage	99.1%	99.9%	99.9%
Variant Call Concordance	PPA (all variant types, clinically relevant)	97.3% (36/37)	91.9% (34/37)	94.6% (35/37)
	PPA (≥ 1× LoD, all variant types, clinically relevant)	100% (29/29)	100% (29/29)	100% (29/29)
	PPA (all variant types, panel-wide)	85.9% (799/930)	85.3% (793/930)	85.8% (798/930)
	PPA (≥ 1× LoD, all variant types, panel-wide)	96.7% (533/551)	96.9% (534/551)	95.6% (527/551)

Study Endpoint	Metric	Condition 1: LPC plate retains	Condition 2: ENT plate retain	Condition 3: NRE tube retain
	NPA (combined)	>99.99% (167,972,718 / 167,972,867)	>99.99% (167,972,716 / 167,972,860)	>99.99% (167,972,719 / 167,972,865)

These results demonstrate that the stability of the intermediate products in the Guardant360 Liquid CDx workflow; the stability of intermediate products: LPC plate retains are stable when stored at-20°C±5°C for up to 14 days with up to 2 freeze-thaw cycles, ENT retain plates are stable when stored at -20°C±5°C for up to 14 days with up to 3 freeze-thaw cycles, and NRE tube retains are stable when stored at -20°C±5°C for up to 14 days with up to 2 freeze-thaw cycles.

9. Blood Collection Tube Precision

This study assessed the precision (repeatability and reproducibility) of the Guardant360 Liquid CDx assay across different BCT lots and time points from whole blood collection to plasma isolation. It also assesses the equivalency between samples processed to plasma on Day 0 versus Day 1 post-collection based on sample-level and/or exon-level molecule recovery metrics.

To assess the BCT precision, minimally manipulated samples and age-matched healthy donor samples were used. Whole blood from 12 unique age-matched healthy donors (16 tubes per donor) were collected with Guardant Health BCK. Three (3) different BCT lots (Lots A, B and C) were used for sample collection from each donor. Eight (8) of the 16 BCTs per donor were spiked with plasma or plasma pools using 17 cancer patient samples (8 with NSCLC, 4 with breast cancer, 4 with CRC, and 1 with unknown origin) on Day 1 post collection. The variants spiked included 18 clinically relevant variants from the intended use population; 10 SNVs, 4 indels, 3 rearrangements, and 1 CNA. The remaining eight (8) tubes from each donor remained un-spiked. All whole blood samples were inspected for volumes and abnormalities upon arrival (Day 1 post whole-blood collection) and recorded.

Plasma was isolated on Day 1 for 10 out of the 16 tubes per donor (5 spike-in samples +5 non-spike-in samples) while the remaining tubes were held at room temperature and subjected to plasma isolation on Day 8. A total of 120 samples from Day 1 and 72 samples from Day 8 were run across four (4) batches and tested using one (1) critical reagent lot, three (3) instrument lines, one (1) operator group, three (3) BCT lots and at one (1) testing site.

Of the samples tested, 237/238 (99.6%) passed QC and were eligible for data analysis. BCT precision was assessed by measuring agreement in detected variants between pairwise combinations of sample replicates corresponding to Day 1 plasma isolation conditions. Within lot APA was calculated using replicates generated from BCT Lot A, and the within replicate comparisons have an APA of 100% for each of

the comparisons, resulting 100% APA for overall within lot comparisons (Table 28). Between lot APA was calculated across BCT Lots A, B, and C using samples corresponding to Day 1 plasma isolation conditions. Between lot comparisons have an APA of 100% (Table 29).

Table 28. Within lot APA using samples from replicates extracted on Day 1 using BCT Lot A.

Comparison*	APA% [95% CI]	Observed Number of variants	Number of samples
LotA Rep 1 - LotA Rep 2	100.0% [93.6%, 100.0%]	28	12
LotA Rep 1 - LotA Rep 3	100.0% [93.6%, 100.0%]	28	12
LotA Rep 2 - LotA Rep 3	100.0% [93.6%, 100.0%]	28	12
All within lot comparisons	100.0% [95.7%, 100.0%]	84	36

*Variants greater than 1X LoD were considered for the evaluation.

Table 29. Between-lot APA across BCT lots A, B and C.

Comparison*	APA% [95% CI]	Observed Number of variants	Number of samples
LotA Day 1 - LotB Day 1	100.0% [93.4%, 100.0%]	27	12
LotA Day 1 - LotC Day 1	100.0% [93.4%, 100.0%]	27	12
LotB Day 1 - LotC Day 1	100.0% [93.4%, 100.0%]	27	12
All between lot comparisons	100.0% [95.5%, 100.0%]	81	36

*Variants greater than 1X LoD were considered for the evaluation.

Negative precision was estimated using the set of healthy donor samples without spike-ins. Sites contained in the reportable panel were used for SNV and indel negative agreement calculation and variants reported by the device at these sites were considered as positive for the purposes of ANA analysis. Both Within lot ANA and Between-lot ANA were 99.99%.

For the Day0/Day1 equivalency study, samples were collected from 20 healthy donors (2 BCTs per donor) with informed consent, and plasma was isolated by the vendor from one BCT on Day 0 (the day of collection) and from the second BCT on Day 1. The isolated plasma was shipped to Guardant Health on dry ice. Upon receipt, the samples were stored at –80°C ± 10°C until further processing. Plasma isolated on Day 0 and Day 1 was processed together.

A total of 40 samples (20 from Day 0 and 20 from Day 1) were run across one (1) batch and tested using one (1) critical reagent lot, three (3) instrument lines, one (1) operator group, one (1) BCT lot and at one (1) testing site. Of the samples tested, 40/40 (100%) passed QC and were eligible for data analysis. Sample-level molecule recovery analysis showed an overall mean difference in molecule coverage of 14.1%

for the Day 1 storage condition and the exon-level molecule recovery showed that the lower bound of the 95% confidence interval for the fraction of reportable exonic regions with relative coverage within 2σ (2 * 0.0768) of Day 0 is 95.7% (Table 30).

Table 30. Sample-level Molecule Recovery and Exon Coverage between Plasma Isolated on Day 0 and Day 1

Study Endpoint	Metric	Day 1 Storage
Sample-level Molecule Recovery	Median of NSC fold (Day 1 / Day 0)	0.859
Relative Exon-Level Coverage	95% CI Lower Bound of fraction of exons outside expected coverage	95.7%

In summary, the study results demonstrated that Guardant360 Liquid CDx test exhibits high precision and reliability when using whole blood collected in BCTs. This study also confirms that plasma isolated on the day of blood collection (Day 0) is equivalent in performance to plasma isolated one day later (Day 1).

10. <u>NovaseqX Plus Software Equivalency</u>

This study evaluated the equivalence of Guardant360 Liquid CDx results obtained using the NovaseqX Plus (NVX) software version v1.3.1 compared to version v1.2.2, which was previously used to generate part of the analytical validation (AV) data for Guardant360 Liquid CDx. The evaluation involved sequencing enriched library (ENT) retains from four instrument-to-instrument precision batches using NVX v1.3.1 and comparing these results against data from the original sequencing runs conducted with NVX v1.2.2.

A total of 384 samples were tested (48 samples x 4 sequencing library pools x 2 sequencers software versions). 332 out of 384 samples passed QC and were included in data analysis for this study. For each sample, variants that fall in the 1-2x LoD range were used for data analysis. The study used 4 clinical sample pools (Cancer Patient Sample Pools 1-4). 27 clinically relevant variants which meet criteria for categories 1-3 - 10 SNVs, 6 indels, 2 CNAs, and 9 rearrangements were covered by these 4 clinical sample pools. Two unique sequencing events (sequencing on one sequencer with NVX v1.2.2 and another sequencer with NVX v1.3.1) of the same sequencing library pooled from the same ENT retain plates were generated in support of the sequencer software precision study data.

The primary analysis evaluated the PPA and NPA of variant calls generated on the NVX instruments using software versions v1.2.2 and v1.3.1. PPA was assessed for targeted variants with expected MAF is within the 1-2X LoD range. Point estimates for PPA and NPA are both at 100%, demonstrating equivalency between these two software versions.

11. Pan Cancer Analysis

Guardant360 Liquid CDx performance characteristics were established using cfDNA derived from a wide range of cancer types. In total, 4,215 sample replicates from 1,814 unique patient samples representing 29 different cancer types were included across the analytical validation (AV) studies, as summarized in Table 31.

Table 31. Numbers of Clinical Specimens Represented in Analytical Studies by Cancer Type

Cancer Type	Total Number of Unique Samples	Analytical Accuracy	Limit of Detection – High Input	Limit of Detection – Low Input	Precision	Precision II	Blood Collection Tube Precision	Extraction Precision	Cross Contamination	Input Guard Banding	Whole Blood Stability	Plasma Stability	cfDNA Stability	Intermediate Product Stability	NovaSeq X Equivalency
NSCLC	897	787	50	24	24	19	8	10	88	31	4	11	31	18	24
Breast Carcinoma	350	294	15	9	9	10	4	4	22		2	16	7	15	9
CRC	332	268	9	3	3	40	4	2	22		3	6	5	10	3
Prostate Cancer	64	38	14	5	5	2		4	19			2	6	2	5
Pancreatic Cancer	28	22						1			1	4	1	8	
Melanoma	24	20	1					1	17			2	1		
Ovarian Carcinoma	22	16	5					1	6				2	1	
Cholangiocarcinoma	21	16	3	1	1			1						1	1
Carcinoma of Unknown Primary (CUP)	13	10					1		1			2	1	3	
Urothelial Carcinoma	13	7						1	1			5			
Esophageal Adenocarcinoma	11	9	1					1	2				1	1	
Other	11	5	1									5		1	
Gastric Adenocarcinoma	7	5	2										2		

Cancer Type	Total Number of Unique Samples	Analytical Accuracy	Limit of Detection – High Input	Limit of Detection – Low Input	Precision	Precision II	Blood Collection Tube Precision	Extraction Precision	Cross Contamination	Input Guard Banding	Whole Blood Stability	Plasma Stability	cfDNA Stability	Intermediate Product Stability	NovaSeq X Equivalency
Hepatocellular Carcinoma	7	6										1			
GIST	4		2	1	1							1			1
Neuroendocrine Carcinoma	4	3	1												
Endometrial Carcinoma	3	3							2						
Head and Neck Squamous Cell Carcinoma (HNSCC)	1											1			
Renal Cell Carcinoma	1	1													
Small Cell Lung Carcinoma	1	1													

The number of unique molecules with support from multiple reads (non-singleton molecule coverage, or NSC) was examined across cancer types to show the Guardant360 Liquid CDx assay maintains the efficiency of molecule recovery per cfDNA mass added to the assay across cancer types. The NSC normalized to the input cfDNA mass was examined for samples where a single cancer type was present (Figure 1). The median NSC per ng was approximately 140 molecules/ng, and similar across cancer types. This indicates the Guardant360 Liquid CDx has similar recovery of molecules across cancer types.

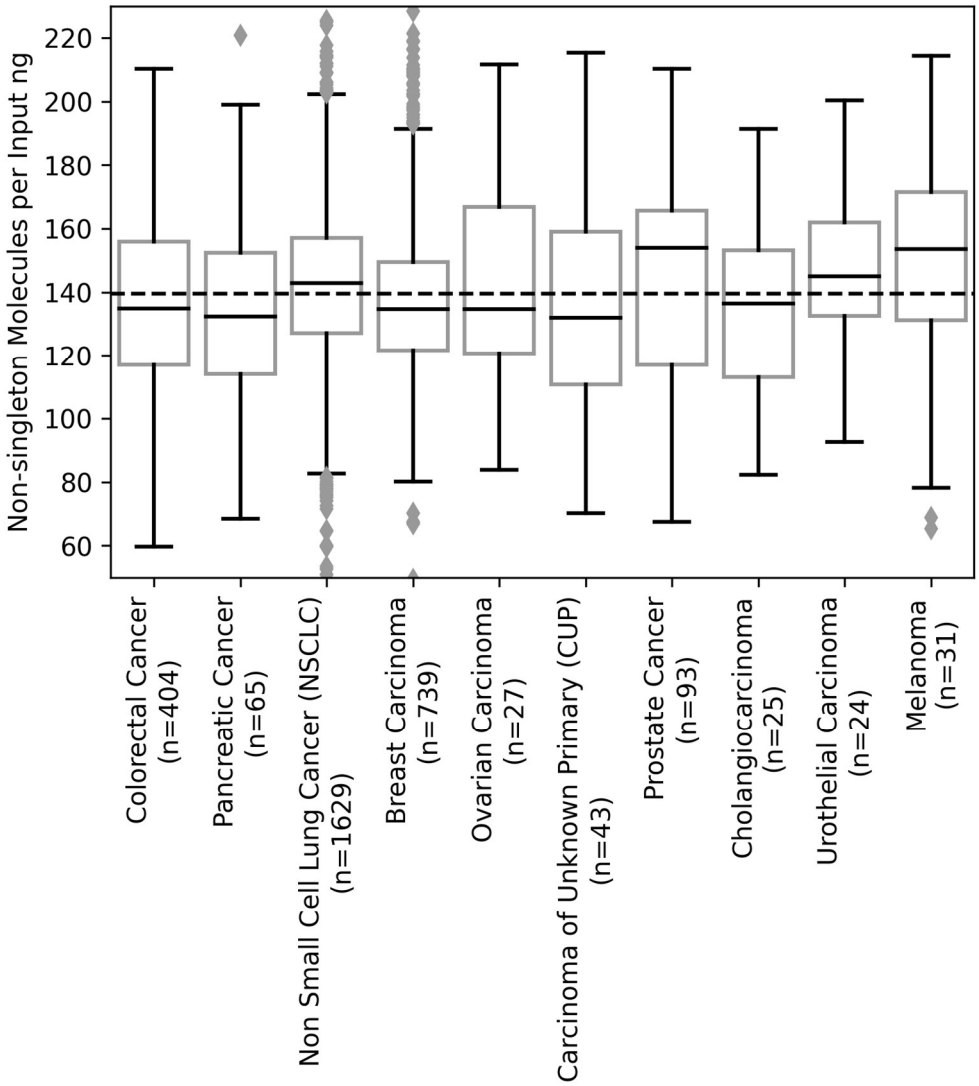

Figure 1. Non-singleton molecules per ng input by cancer type. Only samples with NSC per ng between 50 and 230 are shown, excludes outliers (0.3% [n=9] with <50 NSC per ng and 0.6% [n=18] with >230 NSC per ng).

The total QC pass rate and QC failure rate by cancer type for all the individual clinical samples used in AV studies were analyzed. When a clinical sample was processed across several conditions, only the data for the reference condition was examined and if replicates of the same sample were processed, replicate 1 based on the arbitrary assigned sample IDs was used.

The QC pass rate across cancer types showed similar rates for all cancer types studied (Table 32). The overall final sample QC pass rate was 99.1% (1572/1586) across all patient samples. Final QC pass rate for each individual cancer type was greater than 94% with the lowest rate coming from cholangiocarcinoma samples, which had a single sample QC failure leading to a 94.1% QC pass rate.

Table 32. Sample Success Rate across Different Cancer Types

Cancer type	Total Patient Samples	In-Process QC	Initial* Sample QC Pass Rate	Final Sample QC Pass Rate
Non Small Cell Lung Cancer (NSCLC)	802	99.9% (801/802)	99.4% (797/802)	99.4% (797/802)
Breast Carcinoma	314	100.0% (314/314)	98.4% (309/314)	98.4% (309/314)
Colorectal Cancer	277	100.0% (277/277)	99.3% (275/277)	99.3% (275/277)
Prostate Cancer	41	100.0% (41/41)	97.6% (40/41)	97.6% (40/41)
Pancreatic Cancer	28	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)
Melanoma	23	100.0% (23/23)	100.0% (23/23)	100.0% (23/23)
Ovarian Carcinoma	17	100.0% (17/17)	100.0% (17/17)	100.0% (17/17)
Cholangiocarcinoma	17	100.0% (17/17)	94.1% (16/17)	94.1% (16/17)
Urothelial Carcinoma	13	100.0% (13/13)	92.3% (12/13)	100.0% (13/13)
Carcinoma of Unknown Primary (CUP)	12	100.0% (12/12)	91.7% (11/12)	100.0% (12/12)
Other	10	100.0% (10/10)	100.0% (10/10)	100.0% (10/10)
Esophageal Adenocarcinoma	10	100.0% (10/10)	100.0% (10/10)	100.0% (10/10)
Hepatocellular Carcinoma	7	100.0% (7/7)	100.0% (7/7)	100.0% (7/7)
Gastric Adenocarcinoma	5	100.0% (5/5)	100.0% (5/5)	100.0% (5/5)
Endometrial Carcinoma	3	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Neuroendocrine Carcinoma	3	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Head and Neck Squamous Cell Carcinoma (HNSCC)	1	100.0% (1/1)	100.0% (1/1)	100.0% (1/1)
GIST (Gastrointestinal Stromal Tumor)	1	100.0% (1/1)	100.0% (1/1)	100.0% (1/1)
Renal Cell Carcinoma	1	100.0% (1/1)	100.0% (1/1)	100.0% (1/1)
Small Cell Lung Carcinoma	1	100.0% (1/1)	100.0% (1/1)	100.0% (1/1)

* Initial QC pass rate is the pass rate before repeat testing

Overall, the data presented across cancer types show similar assay performance and QC passing rates. This indicates the performance demonstrated in these AV studies is representative of the expected performance of the Guardant360 Liquid CDx assay across all cancer types.

B. Animal Studies

Not Applicable.

C. Additional Studies

Not Applicable.

XI. SUMMARY OF PRIMARY CLINICAL STUDIES

Guardant360 Liquid CDx performance was established through non-inferiority studies comparing to Guardant360 CDx (P200010), an FDA-approved companion diagnostic that was previously validated against tissue-based testing for NSCLC and CRC indications in the clinical trials supporting the therapeutic approvals. The tissue-relative performance of the comparator assay Guardant360 CDx varied by biomarker, with PPA to tissue ranging from 67% to 91% depending on the specific genomic alteration evaluated; the respective performance for this comparator assay is included in each non-inferiority study section to provide context to the results.

Multiple follow-on non-inferiority (NI) studies were conducted to establish a reasonable assurance of safety and effectiveness of Guardant360 Liquid CDx for CDx indications listed in Table 1. CDx claims were based on a NI statistical testing approach for follow-on CDx devices published by Li (2016). Plasma samples from patients representing the intended use population were used in these studies. A sample from each patient was split equally into three aliquots and tested twice on FDA-approved Guardant360 CDx (comparator CDx denoted as CCD1 or C1 and CCD2 or C2) and once on Guardant360 Liquid CDx (denoted as follow-on CDx denoted as FCD or F). The patient samples included in the diagnostic study were compared against the corresponding intended use population from the applicable primary clinical trial with respect to age and gender to ensure the screening population was representative of the intended use population. Since samples were not obtained from a clinical trial, limited demographic data was available. Both mutation positive and negative patients were used in these studies. Only patients that passed QC on both devices were eligible for the NI analysis.

A. Guardant360 Liquid CDx Concordance Study for the Selection of NSCLC Patients with *EGFR* Exon 19 Deletions, L858R or T790M Mutations for Osimertinib Therapy

1. Non-Inferiority Study Design

The clinical validity of Guardant360 Liquid CDx was established for identifying NSCLC patients with *EGFR* exon 19 deletions, L858R and T790M mutations who may be eligible for treatment with osimertinib. Plasma samples were selected for this study which included two sample sets that were analyzed separately. One sample set included *EGFR* L858R and/or exon 19 deletion positive and negative samples, and the second sample set included *EGFR* T790M mutation positive and negative samples. Some samples were common to both sets. In the primary clinical trial (FLAURA, NCT02296125), the comparator assay Guardant360 CDx showed a PPA of 75.1% (95% CI: 70.4–79.4) and an NPA of 98.7% (95% CI: 92.7–100.0) compared to tissue testing for *EGFR* Exon 19 del/L858R. For *EGFR* T790M, which was tested in the primary clinical trial (AURA3, NCT02151981), the comparator assay Guardant360 CDx

demonstrated a notably lower concordance, with both PPA of 67.4% (95% CI: 61.6–72.8) and NPA of 67.1% (95% CI: 58.9–74.7), indicating reduced agreement with tissue testing for this biomarker.

For the first set, plasma samples from 285 patients (179 *EGFR* L858R and/or exon 19 deletions positive and 106 *EGFR* L858R and/or exon 19 deletions negative samples) were tested. 14 samples failed sample QC metrics and did not generate valid results with either Guardant360 CDx and/or Guardant360 Liquid CDx. As a result, a total of 271 samples were eligible for analysis. For the second set, plasma samples from 221 patients (103 *EGFR* T790M positive and 118 *EGFR* T790M negative) were tested. 11 samples failed sample QC metrics and did not generate valid results with either Guardant360 CDx and/or Guardant360 Liquid CDx. As a result, a total of 210 samples were included in the analysis. Age and gender data available for the subjects enrolled in the non-inferiority study was comparable to that from the primary clinical trial FLAURA (*EGFR* exon 19 deletions and L858R mutations) and AURA3 (*EGFR* T790M mutations), respectively.

2. Study Results

The results of the NI study for the detection of *EGFR* mutations indicated for treatment of osimertinib in NSCLC patients are summarized in Tables 33 – 35 for *EGFR* Exon 19 deletions and/or L858R mutations and in Tables 36 -38 for *EGFR* T790M mutations.

Table 33. Concordance for *EGFR* Exon 19 Deletions and/or L858R Mutations

	CCD0+					CCD0-				
CCD1/2	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**
FCD+	157	2	1	1	161	0	0	0	0	0
FCD-	4	3	2	1	10	0	100	0	0	100
Total	161	5	3	2	171	0	100	0	0	100

*CCD1+/CCD2-; ^CCD1-/CCD2+

Table 34. Individual Agreement Estimates for *EGFR* Exon 19 Deletions and/or L858R Mutations

	% Agreement	
	Unadjusted (%)	**Adjusted (11.1% Prevalence)**
PPA_{C1C2}	98.17	98.17
PPA_{C1F}	96.34	96.34
PPA_{C2C1}	98.77	98.77
PPA_{C2F}	96.93	96.93

	% Agreement	
	Unadjusted (%)	**Adjusted (11.1% Prevalence)**
NPA_{C1C2}	98.13	99.85
NPA_{C1F}	97.20	99.78
NPA_{C2C1}	97.22	99.78
NPA_{C2F}	97.22	99.78

Agreement difference estimates comparing CCD to CCD concordance with CCD to FCD concordance are summarized in Table 35, along with the corresponding two-sided 95% CIs.

Table 35. Agreement Difference Estimates for Non-Inferiority Evaluation for *EGFR* Exon 19 Deletions and/or L858R Mutations

	Unadjusted		Adjusted (11.1% Prevalence)	
	Point Estimate (%)	**95% two-sided CI (%)**	**Point Estimate (%)**	**95% two-sided CI (%)**
ζPPA1	1.83	(-0.61, 4.40)	1.83	(-0.61, 4.38)
ζPPA2	1.84	(-0.61, 4.32)	1.84	(-0.61, 4.35)
ζNPA1	0.93	(-1.90, 3.79)	0.07	(-0.15, 0.29)
ζNPA2	0.00	(-3.64, 3.62)	0.00	(-0.29, 0.29)

ζPPA1 = PPAC1C2 - PPAC1F, ζPPA2 = PPAC2C1 - PPAC2F and ζNPA1 = NPAC1C2 - NPAC1F, ζNPA2 = NPAC2C1 - NPAC2F

Table 36. Concordance for *EGFR* T790M Mutations

	CCD0+					CCD0-				
CCD1/2	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**
FCD+	69	3	1	2	75	0	0	0	0	0
FCD-	2	16	1	4	23	0	112	0	0	112
Total	71	19	2	6	98	0	112	0	0	112

*CCD1+/CCD2-; ^CCD1-/CCD2+

Table 37. Individual Agreement Estimates for *EGFR* T790M Mutations

	% Agreement	
	Unadjusted (%)	**Adjusted (60% Prevalence)**
PPA_{C1C2}	97.26	97.26
PPA_{C1F}	95.89	95.89
PPA_{C2C1}	92.21	92.21
PPA_{C2F}	92.21	92.21
NPA_{C1C2}	95.62	93.36
NPA_{C1F}	96.35	94.46
NPA_{C2C1}	98.50	97.68
NPA_{C2F}	96.99	95.37

Agreement difference estimates comparing CCD to CCD concordance with CCD to FCD concordance are summarized in Table 38, along with the corresponding two-sided 95% CIs.

Table 38. Agreement Difference Estimates for Non-Inferiority Evaluation for *EGFR* T790M Mutations

	Unadjusted		Adjusted (60% Prevalence)	
	Point Estimate (%)	**95% two-sided CI (%)**	**Point Estimate (%)**	**95% two-sided CI (%)**
ζPPA1	1.37	(-2.86, 5.80)	1.37	(-2.90, 6.10)
ζPPA2	0.00	(-4.97, 5.19)	0.00	(-5.00, 5.19)
ζNPA1	-0.73	(-4.35, 2.95)	-1.11	(-6.79, 4.41)
ζNPA2	1.50	(-1.46, 4.44)	2.32	(-2.24, 6.91)

ζPPA1 = PPAC1C2 - PPAC1F, ζPPA2 = PPAC2C1 - PPAC2F and ζNPA1 = NPAC1C2 - NPAC1F, ζNPA2 = NPAC2C1 - NPAC2F

Based on these results, Guardant360 Liquid CDx is non-inferior to Guardant360 CDx for the detection of *EGFR* mutations. Therefore, this study establishes the clinical validity of

Guardant360 Liquid CDx for identifying NSCLC patients with *EGFR* exon 19 deletions, L858R and T790M mutations who may be eligible for treatment with osimertinib.

Because the clinical validity of Guardant360 Liquid CDx was established using a non-inferiority framework with Guardant360 CDx (P200010) as the reference device, clinical limitations applicable to Guardant360 CDx are also applicable to Guardant360 Liquid CDx. Specifically, for *EGFR* T790M, concordance between Guardant360 CDx plasma testing and tissue testing in the AURA3 clinical trial was notably low, with a PPA of 67.4% (95% CI: 61.6%, 72.8%) and an NPA of 67.1% (95% CI: 58.9%, 74.7%), indicating substantial discordance between plasma and tissue results for this biomarker. Because the AURA3 trial enrolled patients based exclusively on tissue confirmation of T790M positivity, clinical efficacy data for osimertinib in the T790M plasma-positive, tissue-negative or unknown population are limited and have not been established. Accordingly, as reflected in the labeling for both Guardant360 CDx and Guardant360 Liquid CDx, plasma-based testing for *EGFR* T790M is most appropriate for consideration in patients from whom a tumor biopsy cannot be obtained.

B. Guardant360 Liquid CDx Concordance Study for the Selection of NSCLC Patients with *EGFR* Exon 20 Insertion Mutations for Amivantamab-vmjw Therapy

1. Non-Inferiority Study Design

The clinical validity of Guardant360 Liquid CDx was established for identifying NSCLC patients with *EGFR* exon 20 insertions who may be eligible for treatment with amivantamab-vmjw. In the primary clinical trial (CHRYSALIS, NCT02609776), the comparator assay Guardant360 CDx demonstrated a PPA of 80.4% (95% CI: 71.4–87.1) and an NPA of 100% (95% CI: 97.7–100) compared to tissue testing. Plasma samples from 222 patients (104 *EGFR* exon 20 insertion positive and 118 *EGFR* exon 20 insertion negative) were tested. 14 samples failed sample QC metrics and did not generate valid results with either Guardant360 CDx and/or Guardant360 Liquid CDx. As a result, a total of 208 samples were included in the analysis. Age and gender data available for all the subjects enrolled in the non-inferiority study was comparable to that from the primary clinical trial CHRYSALIS.

2. Study Results

The results of the NI study for the detection of *EGFR* exon 20 insertion mutations indicated for treatment of amivantamab-vmjw therapy in NSCLC patients are summarized in Tables 39-41.

Table 39. Concordance for *EGFR* Exon 20 Insertion Mutations

	CCD0+					CCD0-				
CCD1/2	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**
FCD+	87	2	3	0	92	0	0	0	0	0
FCD-	1	2	1	0	4	0	112	0	0	112

	CCD0+					CCD0-				
CCD1/2	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**
Total	88	4	4	0	96	0	112	0	0	112

*CCD1+/CCD2-; ^CCD1-/CCD2+

Table 40. Individual Agreement Estimates for *EGFR* Exon 20 Insertion Mutations

	% Agreement	
	Unadjusted (%)	**Adjusted (0.9% Prevalence)**
PPA_{C1C2}	95.65	95.65
PPA_{C1F}	97.83	97.83
PPA_{C2C1}	100.00	100.00
PPA_{C2F}	98.86	98.86
NPA_{C1C2}	100.00	100.00
NPA_{C1F}	98.28	99.98
NPA_{C2C1}	96.67	99.96
NPA_{C2F}	95.83	99.95

Agreement difference estimates comparing CCD to CCD concordance with CCD to FCD concordance are summarized in Table 41, along with the corresponding two-sided 95% CIs.

Table 41. Agreement Difference Estimates for Non-Inferiority Evaluation for *EGFR* Exon 20 Insertion Mutations

	Unadjusted		Adjusted (0.9% Prevalence)	
	Point Estimate (%)	**95% two-sided CI (%)**	**Point Estimate (%)**	**95% two-sided CI (%)**
ζPPA1	-2.17	(-6.59, 2.15)	-2.17	(-6.59, 2.13)
ζPPA2	1.14	(0.00, 3.57)	1.14	(0.00, 3.57)
ζNPA1	1.72	(0.00, 4.24)	0.02	(0.00, 0.05)

	Unadjusted		Adjusted (0.9% Prevalence)	
	Point Estimate (%)	95% two-sided CI (%)	Point Estimate (%)	95% two-sided CI (%)
ζNPA2	0.83	(-1.69, 4.07)	0.01	(-0.02, 0.04)

ζPPA1 = PPAC1C2 - PPAC1F, ζPPA2 = PPAC2C1 - PPAC2F and ζNPA1 = NPAC1C2 - NPAC1F, ζNPA2 = NPAC2C1 - NPAC2F

Based on these results, Guardant360 Liquid CDx is non-inferior to Guardant360 CDx for the detection of *EGFR* exon 20 insertion mutations. Therefore, this study establishes the clinical validity of Guardant360 Liquid CDx for identifying NSCLC patients with *EGFR* exon 20 insertion mutations who may be eligible for treatment with amivantamab-vmjw.

C. Guardant360 Liquid CDx Concordance Study for the Selection of NSCLC Patients with *KRAS* G12C Mutations for Sotorasib Therapy

1. Non-Inferiority Study Design

The clinical validity of Guardant360 Liquid CDx was established for identifying NSCLC patients with *KRAS* G12C mutations who may be eligible for treatment with sotorasib. In the primary clinical trial (CodeBreak 100, NCT03600883), the comparator assay Guardant360 CDx showed a PPA of 71.6% (95% CI: 62.1–79.8) and an NPA of 100% (95% CI: 95.0–100) compared to tissue testing. Plasma samples from 214 patients (106 *KRAS* G12C positive and 108 *KRAS* G12C negative) were tested. 12 samples failed sample QC metrics and did not generate valid results with either Guardant360 CDx and/or Guardant360 Liquid CDx. As a result, a total of 202 samples were included in the analysis. Age and gender data available for the subjects enrolled in the non-inferiority study was comparable to that from the primary clinical trial CodeBreak 100 (NCT03600883).

2. Study Results

The results of the NI study for the detection of *KRAS* G12C mutations indicated for treatment of sotorasib in NSCLC patients are summarized in Tables 42-44.

Table 42. Concordance for *KRAS* G12C Mutations

	CCD0+					CCD0-				
CCD1/2	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**
FCD+	89	2	2	1	94	0	0	0	0	0
FCD-	0	5	1	0	6	0	102	0	0	102
Total	89	7	3	1	100	0	102	0	0	102

*CCD1+/CCD2-; ^CCD1-/CCD2+

Table 43. Individual Agreement Estimates for *KRAS* G12C Mutations

	% Agreement	
	Unadjusted (%)	**Adjusted (6.7% Prevalence)**
PPA_{C1C2}	96.74	96.74
PPA_{C1F}	98.91	98.91
PPA_{C2C1}	98.89	98.89
PPA_{C2F}	100.00	100.00
NPA_{C1C2}	99.09	99.93
NPA_{C1F}	97.27	99.79
NPA_{C2C1}	97.32	99.79
NPA_{C2F}	96.43	99.72

Agreement difference estimates comparing CCD to CCD concordance with CCD to FCD concordance are summarized in Table 44, along with the corresponding two-sided 95% CIs.

Table 44. Agreement Difference Estimates for Non-Inferiority Evaluation for *KRAS* G12C

	Unadjusted		Adjusted (6.7% Prevalence)	
	Point Estimate (%)	**95% two-sided CI (%)**	**Point Estimate (%)**	**95% two-sided CI (%)**
ζPPA1	-2.17	(-5.43, 0.00)	-2.17	(-5.49, 0.00)
ζPPA2	-1.11	(-3.45, 0.00)	-1.11	(-3.37, 0.00)
ζNPA1	1.82	(0.00, 4.42)	0.14	(0.00, 0.35)
ζNPA2	0.89	(-1.80, 3.60)	0.07	(-0.14, 0.28)

ζPPA1 = PPAC1C2 - PPAC1F, ζPPA2 = PPAC2C1 - PPAC2F and ζNPA1 = NPAC1C2 - NPAC1F, ζNPA2 = NPAC2C1 - NPAC2F

Based on these results, Guardant360 Liquid CDx is non-inferior to Guardant360 CDx for the detection of *KRAS* G12C mutations. Therefore, this study establishes the clinical

validity of Guardant360 Liquid CDx for identifying NSCLC patients with *KRAS* G12C mutations who may be eligible for LUMAKRAS (sotorasib) therapy.

D. Guardant360 Liquid CDx Concordance Study for the Selection of NSCLC Patients with *ERBB2/HER2* Activating Mutations for Fam-trastuzumab deruxtecan-nxki Therapy

1. Non-Inferiority Study Design

The clinical validity of Guardant360 Liquid CDx was established for identifying NSCLC patients with *ERBB2/HER2* activating mutations who may be eligible for treatment with fam-trastuzumab deruxtecan-nxki. In the primary clinical trial (DESTINY Lung-01, NCT03505710), the comparator assay Guardant360 CDx achieved a PPA of 91.1% (95% CI: 83.2–96.1) and an NPA of 100% (95% CI: 96.7–100) compared to tissue testing. Plasma samples from 220 patients (104 *ERBB2/HER2* positive and 116 *ERBB2/HER2* negative) were tested. 11 samples failed sample QC metrics and did not generate valid results with either Guardant360 CDx and/or Guardant360 Liquid CDx. As a result, a total of 209 samples were included in the analysis. Age and gender data available for the subjects enrolled in the non-inferiority study was comparable to that from the primary clinical trial DESTINY Lung-01.

2. Study Results

The results of the NI study for the detection of *ERBB2/HER2* activating mutations indicated for treatment of fam-trastuzumab deruxtecan-nxki in NSCLC patients are summarized in Tables 45-47.

Table 45. Concordance for *ERBB2/HER2* Activating Mutations

	CCD0+					CCD0-				
CCD1/2	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**
FCD+	90	1	0	0	91	0	0	0	0	0
FCD-	0	6	1	1	8	0	110	0	0	110
Total	90	7	1	1	99	0	110	0	0	110

*CCD1+/CCD2-; ^CCD1-/CCD2+

Table 46. Individual Agreement Estimates for *ERBB2/HER2* Activating Mutations

	% Agreement	
	Unadjusted (%)	**Adjusted (1.3% Prevalence)**
PPA_{C1C2}	98.90	98.90
PPA_{C1F}	98.90	98.90

	% Agreement	
	Unadjusted (%)	Adjusted (1.3% Prevalence)
PPA_{C2C1}	98.90	98.90
PPA_{C2F}	98.90	98.90
NPA_{C1C2}	99.15	99.99
NPA_{C1F}	99.15	99.99
NPA_{C2C1}	99.15	99.99
NPA_{C2F}	99.15	99.99

Agreement difference estimates comparing CCD to CCD concordance with CCD to FCD concordance are summarized in Table 47, along with the corresponding two-sided 95% CIs.

Table 47. Agreement Difference Estimates for Non-Inferiority Evaluation for *ERBB2/HER2* Activating Mutations

	Unadjusted		Adjusted (1.3% Prevalence)	
	Point Estimate (%)	95% two-sided CI (%)	Point Estimate (%)	95% two-sided CI (%)
ζPPA1	0.00	(-4.49, 4.49)	0.00	(-4.49, 4.49)
ζPPA2	0.00	(-4.49, 4.49)	0.00	(-4.49, 4.49)
ζNPA1	0.00	(-2.49, 2.48)	0.00	(-0.04, 0.04)
ζNPA2	0.00	(-2.49, 2.49)	0.00	(-0.04, 0.04)

ζPPA1 = PPAC1C2 - PPAC1F, ζPPA2 = PPAC2C1 - PPAC2F and ζNPA1 = NPAC1C2 - NPAC1F, ζNPA2 = NPAC2C1 - NPAC2F

Based on these results, Guardant360 Liquid CDx is non-inferior to Guardant360 CDx for the detection of *ERBB2/HER2* activating mutations. Therefore, this study establishes the clinical validity of Guardant360 Liquid CDx for identifying NSCLC patients with *ERBB2/HER2* activating mutations who may be eligible for treatment with fam-trastuzumab deruxtecan-nxki therapy.

E. Guardant360 Liquid CDx Concordance Study for the Selection of Breast Cancer Patients with *ESR1* Mutations for Elacestrant Therapy

1. Non-Inferiority Study Design

The clinical validity of Guardant360 Liquid CDx was established for identifying breast cancer patients with *ESR1* mutations who may be eligible for treatment with elacestrant. Plasma samples from 212 patients (109 *ESR1* positive and 103 *ESR1* negative) were tested. 10 samples failed sample QC metrics and did not generate valid results with either Guardant360 CDx and/or Guardant360 Liquid CDx. As a result, a total of 202 samples were included in the analysis. Age and gender data available for the subjects enrolled in the non-inferiority study was comparable to that from the primary clinical trial EMERALD.

2. Study Results

The results of the NI study for the detection of *ESR1* missense mutations indicated for treatment of elacestrant in breast cancer patients are summarized in Tables 48-50.

Table 48. Concordance for *ESR1* Mutations for Elacestrant Therapy

	CCD0+					CCD0-				
CCD1/2	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**
FCD+	89	3	3	2	97	0	2	0	0	2
FCD-	0	4	0	0	4	1	97	1	0	99
Total	89	7	3	2	101	1	99	1	0	101

*CCD1+/CCD2-; ^CCD1-/CCD2+

Table 49. Individual Agreement Estimates for *ESR1* Mutations for Elacestrant Therapy

	% Agreement	
	Unadjusted (%)	**Adjusted (21.6% Prevalence)**
PPA_{C1C2}	95.74	93.32
PPA_{C1F}	97.87	92.68
PPA_{C2C1}	97.83	97.89
PPA_{C2F}	98.91	96.16
NPA_{C1C2}	98.15	99.46
NPA_{C1F}	93.52	96.67

	% Agreement	
	Unadjusted (%)	**Adjusted (21.6% Prevalence)**
NPA_{C2C1}	96.36	98.22
NPA_{C2F}	92.73	96.45

Agreement difference estimates comparing CCD to CCD concordance with CCD to FCD concordance are summarized in Table 50, along with the corresponding two-sided 95% CIs.

Table 50. Agreement Difference Estimates for Non-Inferiority Evaluation for *ESR1* Mutations for Elacestrant Therapy

	Unadjusted		Adjusted (21.6% Prevalence)	
	Point Estimate (%)	**95% two-sided CI (%)**	**Point Estimate (%)**	**95% two-sided CI (%)**
ζPPA1	-2.13	(-6.45, 2.03)	0.64	(-6.12, 8.46)
ζPPA2	-1.09	(-4.65, 2.17)	1.72	(-4.49, 9.71)
ζNPA1	4.63	(0.94, 9.09)	2.78	(0.27, 5.91)
ζNPA2	3.64	(-0.89, 8.22)	1.78	(-1.68, 5.36)

ζPPA1 = PPAC1C2 - PPAC1F, ζPPA2 = PPAC2C1 - PPAC2F and ζNPA1 = NPAC1C2 - NPAC1F, ζNPA2 = NPAC2C1 - NPAC2F

Based on these results, Guardant360 Liquid CDx is non-inferior to Guardant360 CDx for the detection of *ESR1* mutations. Therefore, this study establishes the clinical validity of Guardant360 Liquid CDx for identifying breast cancer patients with *ESR1* mutations who may be eligible for treatment with elacestrant.

F. Guardant360 Liquid CDx Concordance Study for the Selection of Breast Cancer Patients with *ESR1* Mutations for Imlunestrant Therapy

1. Non-Inferiority Study Design

The clinical validity of Guardant360 Liquid CDx was established for identifying breast cancer patients with *ESR1* mutations who may be eligible for treatment with imlunestrant therapy. Plasma samples from 210 patients (104 *ESR1* positive and 106 *ESR1* negative) were tested. 10 samples failed sample QC metrics and did not generate valid results with either Guardant360 CDx and/or Guardant360 Liquid CDx. As a result, a total of 200 samples were included in the analysis. Age and gender data available for the subjects

enrolled in the non-inferiority study was comparable to that from the primary clinical trial EMBER-3.

2. **Study Results**

The results of the NI study for the detection of *ESR1* mutations indicated for treatment of imlunestrant in breast cancer patients are summarized in Tables 51-53.

Table 51. Concordance for *ESR1* Mutations for Imlunestrant Therapy

	CCD0+					CCD0-				
CCD1/2	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**
FCD+	86	3	2	2	93	0	1	0	0	1
FCD-	0	3	0	0	3	1	101	1	0	103
Total	86	6	2	2	96	1	102	1	0	104

*CCD1+/CCD2-; ^CCD1-/CCD2+

Table 52. Individual Agreement Estimates for *ESR1* Mutations for Imlunestrant Therapy

	% Agreement	
	Unadjusted (%)	**Adjusted (20.9% Prevalence)**
PPA_{C1C2}	96.67	94.22
PPA_{C1F}	97.78	92.64
PPA_{C2C1}	97.75	97.81
PPA_{C2F}	98.88	96.18
NPA_{C1C2}	98.18	99.45
NPA_{C1F}	94.55	97.67
NPA_{C2C1}	97.30	98.51
NPA_{C2F}	94.59	97.69

Agreement difference estimates comparing CCD to CCD concordance with CCD to FCD concordance are summarized in Table 53, along with the corresponding two-sided 95% CIs.

Table 53. Agreement Difference Estimates for Non-Inferiority Evaluation for *ESR1* Mutations for Imlunestrant Therapy

	Unadjusted		Adjusted (20.9% Prevalence)	
	Point Estimate (%)	95% two-sided CI (%)	Point Estimate (%)	95% two-sided CI (%)
ζPPA1	-1.11	(-5.32, 2.25)	1.57	(-4.52, 9.49)
ζPPA2	-1.12	(-5.38, 2.26)	1.63	(-4.60, 9.78)
ζNPA1	3.64	(0.89, 7.21)	1.78	(0.27, 4.14)
ζNPA2	2.70	(-0.93, 6.55)	0.82	(-2.00, 3.45)

ζPPA1 = PPAC1C2 - PPAC1F, ζPPA2 = PPAC2C1 - PPAC2F and ζNPA1 = NPAC1C2 - NPAC1F, ζNPA2 = NPAC2C1 - NPAC2F

Based on these results, Guardant360 Liquid CDx is non-inferior to Guardant360 CDx for the detection of *ESR1* mutations. Therefore, this study establishes the clinical validity of Guardant360 Liquid CDx for identifying breast cancer patients with *ESR1* mutations who may be eligible for treatment with imlunestrant.

G. Guardant360 Liquid CDx Concordance Study for the Selection of CRC Patients with *BRAF* Mutations for Encorafenib plus Cetuximab Therapy

1. Non-Inferiority Study Design

The clinical validity of Guardant360 Liquid CDx was established for identifying CRC patients with *BRAF* V600E mutations who may be eligible for treatment with encorafenib plus cetuximab. In the primary clinical trial (BREAKWATER, NCT02928224), the comparator assay Guardant360 CDx showed a PPA of 85.0% (95% CI: 80.7–88.5) and an NPA of 100% (95% CI: 96.9–100.0) compared to tissue testing. Plasma samples from 208 patients (105 *BRAF* V600E positive and 103 *BRAF* V600E negative) were tested. 19 samples failed sample QC metrics and did not generate valid results with either Guardant360 CDx and/or Guardant360 Liquid CDx. As a result, a total of 189 samples were included in the analysis. Age and gender data available for the subjects enrolled in the non-inferiority study was comparable to that from the primary clinical trial BREAKWATER.

2. Study Results

The results of the NI study for the detection of *BRAF* V600E mutations indicated for treatment of encorafenib in combination with cetuximab in CRC patients are summarized in Tables 54-56.

Table 54. Concordance for *BRAF* V600E Mutations

	CCD0+					CCD0-				
CCD1/2	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**	**(+,+)**	**(-,-)**	**(+,-)***	**(-,+)^**	**Total**
FCD+	88	2	2	2	94	0	0	0	1	1
FCD-	2	2	0	0	4	0	90	0	0	90
Total	90	4	2	2	98	0	90	0	1	91

*CCD1+/CCD2-; ^CCD1-/CCD2+

Table 55. Individual Agreement Estimates for *BRAF* V600E Mutations

	% Agreement	
	Unadjusted (%)	**Adjusted (5.3% Prevalence)**
PPA_{C1C2}	97.83	97.83
PPA_{C1F}	97.83	97.83
PPA_{C2C1}	96.77	80.99
PPA_{C2F}	97.85	98.20
NPA_{C1C2}	96.91	98.79
NPA_{C1F}	94.85	98.68
NPA_{C2C1}	97.92	99.88
NPA_{C2F}	95.83	99.77

Agreement difference estimates comparing CCD to CCD concordance with CCD to FCD concordance are summarized in Table 56, along with the corresponding two-sided 95% CIs.

Table 56. Agreement Difference Estimates for Non-Inferiority Evaluation for *BRAF* V600E Mutations

	Unadjusted		Adjusted (5.3% Prevalence)	
	Point Estimate (%)	**95% two-sided CI (%)**	**Point Estimate (%)**	**95% two-sided CI (%)**
ζPPA1	0.00	(-4.26, 4.30)	0.00	(-4.26, 4.35)

	Unadjusted		Adjusted (5.3% Prevalence)	
	Point Estimate (%)	**95% two-sided CI (%)**	**Point Estimate (%)**	**95% two-sided CI (%)**
ζPPA2	-1.08	(-5.38, 3.37)	-17.21	(-40.02, 3.30)
ζNPA1	2.06	(0.00, 5.05)	0.11	(0.00, 0.29)
ζNPA2	2.08	(0.00, 5.10)	0.12	(0.00, 0.29)

ζPPA1 = PPAC1C2 - PPAC1F, ζPPA2 = PPAC2C1 - PPAC2F and ζNPA1 = NPAC1C2 - NPAC1F, ζNPA2 = NPAC2C1 - NPAC2F

Based on these results, Guardant360 Liquid CDx is non-inferior to Guardant360 CDx for the detection of *BRAF* V600E mutations. Therefore, this study establishes the clinical validity of Guardant360 Liquid CDx for identifying CRC patients with *BRAF* V600E mutations who may be eligible for treatment with encorafenib in combination with cetuximab.

XII. Financial Disclosure

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

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

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

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

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

XIV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The analytical performance of Guardant360 Liquid CDx for the detection of SNVs, indels, CNAs, CNL and rearrangements in plasma-derived cfDNA across solid tumors was established in the analytical studies reported above. Analytical accuracy, sensitivity, specificity, and precision are reported in Section X.

The clinical benefit of Guardant360 Liquid CDx in the detection of alterations listed in Table 1 of the intended use statement was demonstrated in seven clinical concordance studies using previously approved CDx tests as the comparator methods. All studies based on the NI statistical testing approach passed the acceptance criteria. The concordance observed between Guardant360 Liquid CDx, and the approved companion diagnostics test supports the effectiveness of Guardant360 Liquid CDx to identify patients who are positive for the alterations listed in Table 1 of the intended use.

B. Safety Conclusions

The risks of the device are based on nonclinical laboratory studies as well as data collected in clinical studies conducted to support PMA approval as described above. Failure of Guardant360 Liquid CDx to perform as expected or failure to correctly interpret test results may lead to inappropriate patient management decisions in cancer treatment. Patients with false positive results may undergo treatment with one of the therapies listed in Table 1 of the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with an indicated therapy. There is also a risk of delayed results which may lead to delay of treatment. For the specific adverse events related to the approved therapeutics referenced in Table 1, please see the approved drug product labels available at Drugs@FDA.

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

C. Benefit-Risk Determination

The probable benefits of the device are based on data collected in the analytical validation and clinical concordance studies conducted to support PMA approval.

Treatment with the therapies listed in Table 1 of the intended use provides meaningful clinical benefit to patients selected for those therapies based on the finding of the associated genomic alterations. Based on data provided in the clinical concordance studies, which compared the ability of Guardant360 Liquid CDx to detect the relevant mutations to the Guardant360 CDx companion diagnostic assay, Guardant360 Liquid CDx is non-inferior to Guardant360 CDx for all biomarker-indication pairs evaluated. The performance of Guardant360 Liquid CDx with respect to the Guardant360 CDx companion diagnostic test was also considered to be clinically acceptable beyond passing the statistical acceptance criteria; therefore, there is probable benefit for the use of Guardant360 Liquid CDx for selection of patients with the alterations listed in Table 1, in the specific tumor types identified, for administration of the corresponding approved therapeutics. For reference to the individual clinical concordance studies, see Section XI above, which provides detailed performance for each biomarker.

Concordance between Guardant360 Liquid CDx and the comparator companion diagnostic was highest for variants at MAF levels at or above the assay LoD. The concordance of clinically relevant variants at or above 1x LoD across all Level 1 CDx biomarkers was 99.62% PPA and 99.97% NPA in the analytical accuracy study.

In addition, Guardant360 Liquid CDx provides significant probable clinical benefit through tumor mutation profiling of 741 genes across solid tumor types for use in accordance with professional oncology guidelines. Guardant360 Liquid CDx enables detection of a broad range of SNVs, indels, CNAs, CNL, and rearrangements, providing qualified healthcare professionals with comprehensive genomic information to inform clinical decision-making beyond the companion diagnostic indications. The blood-based nature of the test reduces morbidity associated with invasive tissue biopsy acquisition, improves access to genotyping for patients in whom tissue is insufficient or inaccessible, and may facilitate faster return of results relative to tissue-based testing.

The risks associated with the use of Guardant360 Liquid CDx are principally those of: (1) false positive results, (2) false negative results, (3) failure to provide a result, and (4) incorrect interpretation of test results by the user. Patients with false positive results may undergo treatment without clinical benefit and may experience adverse reactions associated with an inappropriate therapy or may have delayed access to more beneficial treatments. Patients with false negative results may not be considered for treatment with an indicated targeted therapy; however, this risk is mitigated by the recommendation to reflex negative plasma results to tissue biopsy when feasible, as stated in the device labeling and in accordance with professional guidelines. The false negative risk is further mitigated by the device's broad genomic coverage, which can provide contextual information to assess sample adequacy. In addition, there is a risk of potential false positivity, particularly with the *EGFR* T790M alteration, based on the current data; thus, the Intended Use stipulates that plasma-based testing for *EGFR* T790M is most appropriate for consideration in patients from whom a tumor biopsy cannot be obtained. The risks associated with incorrect interpretation are mitigated by

the clinical reporting structure, labeling language, and the limitation statements in the device labeling, including the explicit statement that a negative plasma result does not assure tumor negativity and that reflex tissue testing is recommended.

In addition, risks are mitigated by the analytical and clinical performance of the device, as provided above. Guardant360 Liquid CDx has demonstrated non-inferiority to Guardant360 CDx for all evaluated companion diagnostic indications, and therefore the risk of the device is considered to be clinically acceptable for the indications listed.

Additional factors considered in determining probable benefits and probable risks include the the representativeness of variants across analytical and clinical studies.

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

In conclusion, given the above information, the data support that the probable benefits of Guardant360 Liquid CDx outweigh the probable risks, for the tumor profiling and companion diagnostic indications delineated above.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the clinical studies support the use of Guardant360 Liquid CDx in the identification of patients for whom treatment with the therapies listed in the Intended Use Statement may be indicated.

E. Pediatric Extrapolation

Patients aged 18 years older were eligible for enrollment into the non-inferiority clinical validation studies if they fulfilled other inclusion criteria. The youngest patient enrolled in the study was 21 years of age. Notwithstanding the adult composition of the clinical validation study datasets, the assay workflow for Guardant360 Liquid CDx, including sample processing, library preparation, sequencing on the NovaSeq X Plus platform, and bioinformatics analysis, is identical regardless of patient age. Therefore, clinical data from adult patients aged 21 years and older are considered generalizable to the pediatric population aged 18-21 years and can be relied on to establish safety and effectiveness of the device within the 18-21 years age group.

XV. CDRH DECISION

CDRH issued an approval order on May 19, 2026. The final condition of approval cited in the approval order is described below.

Guardant Health, Inc. must provide data from a blood collection tube (BCT) incomplete mixing study using healthy donor and intended use biomarker-positive samples to evaluate the tolerance of Guardant360 Liquid CDx to variations in mixing after blood collection. Study data must be sufficient to assess the impact of under-mixing and over-mixing on the performance of Guardant360 Liquid CDx. The final study data, study conclusions, and labeling revisions should be submitted within one (1) year of the PMA approval date.

The applicant's manufacturing facility was inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820), which was in effect at the time of the inspection. As of February 2, 2026, the revised part 820, referred to as the Quality Management System Regulation (QMSR), is effective.

XVI. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

XVII. REFERENCES

Li M. Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study. Statistics in Biopharmaceutical Research 8(3), 355-363 (2016).