GUARDANT360®
Liquid CDx

Technical Information

Guardant Health, Inc.

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1. Intended 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 1-1 in accordance with the approved therapeutic product labeling.

Table 1-1. 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)
	ESR1 K303R, V422del, L549P, and missense mutations between codons 310 and 547	VEPPANU™ (vepdegestrant)
	ESR1 E380, V422del, S463, L536, Y537, and D538 mutations	ETCAMAH™ (camizestrant)+
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)
	ERBB2/HER2 activating mutations (SNVs in exons 18-21 within the tyrosine kinase domain and exon 20 insertions)	HERNEXEOS® (zongertinib)

+ See the therapeutic product label or Device Description section for specific clinical circumstances guiding *ESR1* testing.

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 1-1 should be reflexed to tissue biopsy testing for Table 1-1 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 1-1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

2. Contraindications

There are no known contraindications.

3. Warnings and Precautions

- Alterations reported may include somatic (not inherited) or germline (inherited) alterations. The assay filters germline variants from reporting except for pathogenic 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.

4. Limitations

- For *in vitro* diagnostic use.
- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- The test is not intended to be used for standalone diagnostic purposes.
- The test is intended to be performed on specific serial number-controlled instruments by Guardant Health, Inc.
- A negative result for any given variant does not preclude the presence of this variant in tumor tissue.
- 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.
- ORSERDU efficacy has not been established in patients with *ESR1* missense mutations < 0.03% MAF.
- INLURIYO efficacy has not been established in patients with *ESR1* V422 deletion < 0.19% MAF and in patients with *ESR1* SNVs < 0.03% MAF.
- VEPPANU efficacy has not been established in patients with *ESR1* V422 deletion < 0.11% MAF and with ESR1 SNVs <0.03% MAF.
- ETCAMAH efficacy has not been established in patients with *ESR1* V422 deletion < 0.11% MAF and in patients with *ESR1* SNVs <0.03% MAF
- BRAFTOVI + ERBITUX efficacy has not been established in patients with *BRAF* V600E mutation < 0.06% MAF.
- TAGRISSO efficacy has not been established in patients with *EGFR* exon 19 deletions < 0.07% MAF, in patients with *EGFR* L858R < 0.12% MAF, and in patients with *EGFR* T790M < 0.05% MAF.
- RYBREVANT efficacy has not been established in patients with *EGFR* exon 20 insertions < 0.05% MAF.
- LUMAKRAS efficacy has not been established in patients with *KRAS* G12C biomarkers < 0.05% MAF.
- ENHERTU efficacy has not been established in patients with *ERBB2* exon 20 insertions < 0.03% MAF and in patients with *ERBB2* SNVs < 0.18% MAF.
- HERNEXEOS efficacy has not been established in patients with ERBB2 kinase domain activating insertions/deletions <0.11% MAF and in patients with ERBB2 kinase domain activating SNVs <0.05% MAF.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care.
- ctDNA shedding rate may be lower in patients with primary central nervous system (CNS) tumors.

5. Guardant360 Liquid CDx Overview and Device Description

5.1. Test Summary and Explanation

Guardant360 Liquid CDx is a next-generation, sequencing-based test for the detection of genetic alterations in 741 genes frequently mutated in cancer. It is a companion diagnostic to identify patients who may benefit from treatment with the targeted therapy listed in Table 1-1 of the Intended Use. For patients with HR-positive, HER2-negative locally advanced or metastatic breast cancer who have received at least 6 months of aromatase inhibitor and CDK4/6 inhibitor therapy, testing with Guardant360 Liquid CDx should be performed every 3 months during aromatase inhibitor and CDK4/6 inhibitor therapy until an *ESR1* mutation (refer to Table 1-1) has been detected, or disease progression occurs. Detection of an *ESR1* mutation may be used to support treatment with ETCAMAH. Additionally, the test is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with any malignant neoplasm.

The test report includes variants reported in the following categories (Table 5-1).

Table 5-1. Level 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
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

5.2. Sample Collection and Test Ordering

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

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

5.3. Principles of the Procedure

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

- Whole Blood Collection and Shipping
- Plasma Isolation
- cfDNA Extraction, Buffer Exchange and cfDNA Quantitation
- Methylation Partitioning
- Library Preparation and Enrichment
- DNA Sequencing
- Data Analysis and Reporting

The Guardant Health BCK will be used by the ordering laboratories/physicians to collect whole blood specimens and ship them to the Guardant Health Clinical Laboratory. The BCK contains Streck Cell-Free DNA BCTs that contain a reagent that stabilizes cfDNA in plasma and nucleated blood cells. A minimum amount of whole blood (one tube with at least 3 mL whole blood) must be received in order to process the sample for Guardant360 Liquid CDx.

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 via centrifugation, and cfDNA is extracted from plasma. Up to 30 ng of extracted cfDNA undergoes methylation partitioning. Partitioned cfDNA is then used to prepare sequencing libraries which are enriched by hybridization capture. The enriched libraries are then sequenced using next generation sequencing on the Illumina NovaSeq X Plus Sequencing system.

Sequencing data are analyzed using a custom-developed bioinformatics pipeline designed to detect SNVs, indels, CNAs, CNLs, and rearrangements from cfDNA. Results (detected or not detected) are presented in a results report for all samples that pass QC, including minimum unique molecule coverage that ensures sufficient input material was successfully processed through the assay. This QC is applied to all samples with cfDNA inputs spanning the input range up to 30 ng. A "not detected" result from a plasma specimen for any given variant does not preclude the presence of this variant in tumor tissue.

The device is designed to detect variants in the genes outlined in Table 5-2.

Table 5-2. Genes Containing Alterations Reported by Guardant360 Liquid CDx

Alteration Type	Genes
SNVs and Indels	*ABCB1*, *ABL1*, *ABL2**, *ABRAXAS1*, *ACVR1*, *ACVR1B**, *ACVR2A**, *ADARB2*, *ADGRA2**, *ADGRG4**, *AFDN**, *AGGF1**, *AIP*, *AKT1*, *AKT1S1*, *AKT2*, *AKT3*, *ALB*, *ALK*, *ALOX5*, *ALOX12B*, *ALOX15B*, *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*, *CD74**, *CD79A*, *CD79B*, *CD274*, *CD276*, *CDC5L**, *CDC7*, *CDC27*, *CDC73*, *CDH1*‡, *CDH6*, *CDK4*, *CDK6*, *CDK7*, *CDK8**, *CDK11A**, *CDK12*, *CDKN1A*, *CDKN1B*, *CDKN1C*, *CDKN2A*‡, *CDKN2B*, *CDKN2C*, *CEBPA*, *CELF4**, *CEP295**, *CFAP20**, *CHD4*, *CHEK1*, *CHEK2**‡, *CIC*, *CLDN18*, *CMTM4*, *CMTM6*, *CNOT3**, *CREBBP**, *CRKL**, *CRTC1**, *CSF1R*, *CSF3R*, *CTC1*, *CTCF*, *CTLA4*, *CTNNA1*, *CTNNB1*, *CUL3*, *CUL4A*, *CUX1**, *CWC22**, *CXCR4*, *CYLD**, *CYP2C19*, *CYP3A4**, *CYP17A1*,

Alteration Type	Genes
	CYP19A1, *DAXX*, *DCUN1D1*, *DDIT3**, *DDR1**, *DDR2*, *DDX3X**, *DDX17**, *DDX18**, *DDX27**, *DDX41**, *DEPDC5*, *DEPTOR*, *DHX9**, *DHX15*, *DHX16**, *DHX36**, *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*, *FAAP20*, *FAAP24**, *FAAP100*, *FANCA*‡, *FANCB**, *FANCC**, *FANCD2**, *FANCE**, *FANCF*, *FANCG**, *FANCI*, *FANCL*, *FANCM**, *FAS**, *FAT1**, *FBXW7*, *FCGR2A*, *FCGR3A*, *FEN1*, *FGF1*, *FGF2*, *FGF3*, *FGF4**, *FGF5*, *FGF6**, *FGF7**, *FGF8*, *FGF9*, *FGF10**, *FGF12*, *FGF14**, *FGF19*, *FGF23**, *FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, *FH*, *FLCN*, *FLT1*, *FLT3*, *FLT4**, *FOXA1**, *FOXL2*, *FOXO1**, *FOXP1**, *FRS2*, *FUBP1**, *FUBP3**, *FUS**, *FYN*, *FZD1*, *FZD2*, *FZD3*, *FZD4*, *FZD5*, *FZD6*, *FZD7*, *FZD8*, *FZD9*, *FZD10*, *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*, *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*, *PRPF4B**, *PRPF40B**, *PSENEN*, *PSMB8*, *PSMB9*, *PSMB10*, *PTCH1*, *PTDSS1*, *PTEN*‡, *PTPN2*, *PTPN11*, *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**, *RPS6KA3*, *RPS6KB1*, *RPS6KB2*, *RPS27A**, *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*,

Alteration Type	Genes
	SMC3, *SMO*, *SNCAIP*, *SOCS1*, *SOCS3*, *SOS1*, *SOX2*, *SOX9*, *SOX10*[*], *SOX17*, *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*, *TNFRSF1A*, *TNFRSF14*[*], *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*, *USP7*, *USP9X*[*], *USP28*, *VEGFA*[*], *VEGFB*, *VHL*[‡], *VIRMA*[*], *WBP11*[*], *WEE1*, *WRN*, *WT1*[‡], *WWP1*, *XBP1*[*], *XPA*, *XPC*[*], *XPO1*, *XRCC1*[*], *XRCC2*, *XRCC3*, *XRCC4*[*], *XRCC5*[*], *XRCC6*[*], *YAP1*[*], *YES1*, *ZC3H4*[*], *ZC3H13*[*], *ZC3H18*[*], *ZMYM3*[*], *ZNF217*, *ZNF703*[*], *ZNRF3*, *ZRSR2*[*]
CNAs	*ERBB2*, *MET*
CNLs	*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 pathogenic germline alterations only. Somatic alterations are not reported.
[‡] Reporting is enabled for both pathogenic germline and somatic alterations.

5.4. Reagent, Material, and Equipment

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

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

6. Summary of Performance Characteristics

6.1. Analytical Accuracy/Concordance

6.1.1. Concordance – Comparison to NGS Comparators

Analytical accuracy of Guardant360 Liquid CDx for CDx biomarkers and tumor profiling variants was assessed by comparing detection by Guardant360 Liquid CDx to validated NGS comparator methods.

Analytical accuracy for Guardant360 Liquid CDx for SNVs, indels, rearrangements, CNAs, and CNLs was evaluated using a total of 1511 cancer patient samples across different cancer types in the intended use population (787 NSCLC, 294 breast cancer, 268 colorectal cancer (CRC), 38 prostate cancer, 16 cholangiocarcinoma, 20 melanoma, 16 ovarian, 7 urothelial, 65 other cancers), and 2 contrived samples.

The analysis demonstrated a PPA of 94.93% (95% CI: 93.45%-96.16%) and 96.24% (95% CI: 93.52%-98.04%) and NPA of 99.86% (95% CI: 99.83% - 99.88%) and 99.98% (95%CI: 99.97%- 99.99%) for clinical significant SNVs and indels, respectively, and PPA of 76.81% (95% CI: 75.61% - 77.98%) and 87.32% (95% CI: 84.94%-89.44%) and NPA of 99.99973% (95% CI: 99.99971% - 99.99975%) and 99.99992% (95% CI: 99.99990% - 99.99993%) for panel-wide SNVs and indels, respectively (Table 6-1).

The analysis for panel-wide rearrangements, CNAs, and CNLs showed a PPA of 94.64% (95% CI: 88.70% - 98.01%) and NPA of 99.76% (95% CI: 99.60% - 99.86%) for rearrangements, a PPA of 91.67% (95% CI: 84.24% - 96.33%) and NPA of 99.97% (95% CI: 99.81%- 99.99%) for CNAs, and a PPA of 100% (95% CI: 66.37% - 100.0%) and NPA of 97.56% (95% CI: 94.40% - 99.20%) for CNL (Table 6-1).

Table 6-1. Concordance between Guardant360 Liquid CDx and Comparator NGS

Variant Type		G360LCDx+ cNGS+	G360LCDx+ cNGS-	G360LCDx- cNGS+	G360LCDx- cNGS-	PPA (%)	NPA (%)
SNV	Clinically Significant	1030	118	55	83757	94.93	99.86
Indel	Clinically Significant	307	15	12	74242	96.24	99.98
Rearrangement	Panel-Wide	106	16	6	6516	94.64	99.76
CNA	Panel-Wide	88	1	8	2861	91.67	99.97
CNL	Panel-Wide	9	5	0	200	100	97.56

G360LCDx: Guardant360 Liquid CDx; cNGS: comparator NGS

Concordance results for each reporting level stratified by LoD are shown in Table 6-2.

Table 6-2. CDx Biomarker Concordance between Guardant360 Liquid CDx and Comparator NGS

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
BRAF V600E	1	All	97	5	5	396	95.10% (97/102) [89.03%, 97.89%]	98.75% (396/401) [97.11%, 99.47%]
BRAF V600E	1	≥ 1x LoD	93	1	0	409	100.00% (93/93) [96.03%, 100.00%]	99.76% (409/410) [98.63%, 99.96%]
BRAF V600E	1	< 1x LoD	4	4	5	490	44.44% (4/9) [18.88%, 73.34%]	99.19% (490/494) [97.94%, 99.68%]
EGFR exon 19 deletions, L858R, and T790M	1	All	252	11	12	12975	95.45% (252/264) [92.22%, 97.38%]	99.92% (12975/12986) [99.85%, 99.95%]

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
EGFR exon 19 deletions, L858R, and T790M	1	≥ 1x LoD	237	2	1	13010	99.58% (237/238) [97.66%, 99.93%]	99.98% (13010/13012) [99.94%, 100.00%]
EGFR exon 19 deletions, L858R, and T790M	1	< 1x LoD	15	9	11	13215	57.69% (15/26) [38.95%, 74.46%]	99.93% (13215/13224) [99.87%, 99.96%]
EGFR exon 20 insertions	1	All	88	3	1	26659	98.88% (88/89) [93.91%, 99.80%]	99.99% (26659/26662) [99.97%, 100.00%]
EGFR exon 20 insertions	1	≥ 1x LoD	85	0	0	26666	100.00% (85/85) [95.68%, 100.00%]	100.00% (26666/26666) [99.99%, 100.00%]
EGFR exon 20 insertions	1	< 1x LoD	3	3	1	26744	75.00% (3/4) [30.06%, 95.44%]	99.99% (26744/26747) [99.97%, 100.00%]
ERBB2/HER2 activating mutations	1	All	109	4	2	18196	98.20% (109/111) [93.67%, 99.50%]	99.98% (18196/18200) [99.94%, 99.99%]
ERBB2/HER2 activating mutations	1	≥ 1x LoD	101	0	0	18210	100.00% (101/101) [96.34%, 100.00%]	100.00% (18210/18210) [99.98%, 100.00%]
ERBB2/HER2 activating mutations	1	< 1x LoD	8	4	2	18297	80.00% (8/10) [49.02%, 94.33%]	99.98% (18297/18301) [99.94%, 99.99%]

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
ESR1 mutations	1	All	202	59	11	15327	94.84% (202/213) [90.99%, 97.09%]	99.62% (15327/15386) [99.51%, 99.70%]
ESR1 mutations	1	≥ 1x LoD	179	20	2	15398	98.90% (179/181) [96.06%, 99.70%]	99.87% (15398/15418) [99.80%, 99.92%]
ESR1 mutations	1	< 1x LoD	23	39	9	15528	71.88% (23/32) [54.63%, 84.44%]	99.75% (15528/15567) [99.66%, 99.82%]
KRAS G12C	1	All	99	4	0	1402	100.00% (99/99) [96.26%, 100.00%]	99.72% (1402/1406) [99.27%, 99.89%]
KRAS G12C	1	≥ 1x LoD	92	2	0	1411	100.00% (92/92) [95.99%, 100.00%]	99.86% (1411/1413) [99.49%, 99.96%]
KRAS G12C	1	< 1x LoD	7	2	0	1496	100.00% (7/7) [64.57%, 100.00%]	99.87% (1496/1498) [99.51%, 99.96%]
All Level 1 (CDx) Biomarkers (Aggregate)	1	All	847	86	31	74955	96.47% (847/878) [95.03%, 97.50%]	99.89% (74955/75041) [99.86%, 99.91%]
All Level 1 (CDx) Biomarkers (Aggregate)	1	≥ 1x LoD	787	25	3	75104	99.62% (787/790) [98.89%, 99.87%]	99.97% (75104/75129) [99.95%, 99.98%]
All Level 1 (CDx) Biomarkers (Aggregate)	1	< 1x LoD	60	61	28	75770	68.18% (60/88) [57.87%, 76.98%]	99.92% (75770/75831) [99.90%, 99.94%]

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
ALK rearrangements	2	All	25	6	2	750	92.59% (25/27) [76.63%, 97.94%]	99.21% (750/756) [98.28%, 99.64%]
ALK rearrangements	2	≥ 1x LoD	19	4	0	760	100.00% (19/19) [83.18%, 100.00%]	99.48% (760/764) [98.66%, 99.80%]
ALK rearrangements	2	< 1x LoD	6	2	2	772	75.00% (6/8) [40.93%, 92.85%]	99.74% (772/774) [99.06%, 99.93%]
ATM mutations	2	All	5	0	1	210	83.33% (5/6) [43.65%, 96.99%]	100.00% (210/210) [98.20%, 100.00%]
ATM mutations	2	≥ 1x LoD	5	0	1	210	83.33% (5/6) [43.65%, 96.99%]	100.00% (210/210) [98.20%, 100.00%]
BRAF V600E	2	All	23	0	0	1482	100.00% (23/23) [85.69%, 100.00%]	100.00% (1482/1482) [99.74%, 100.00%]
BRAF V600E	2	≥ 1x LoD	18	0	0	1487	100.00% (18/18) [82.41%, 100.00%]	100.00% (1487/1487) [99.74%, 100.00%]
BRAF V600E	2	< 1x LoD	5	0	0	1500	100.00% (5/5) [56.55%, 100.00%]	100.00% (1500/1500) [99.74%, 100.00%]
BRCA1 deletion	2	All	2	0	1	9	66.67% (2/3) [20.77%, 93.85%]	100.00% (9/9) [70.08%, 100.00%]

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
BRCA1 deletion	2	≥ 1x LoD	2	0	1	9	66.67% (2/3) [20.77%, 93.85%]	100.00% (9/9) [70.08%, 100.00%]
BRCA1/2 inactivating mutations	2	All	13	1	5	3048	72.22% (13/18) [49.13%, 87.50%]	99.97% (3048/3049) [99.81%, 99.99%]
BRCA1/2 inactivating mutations	2	≥ 1x LoD	11	0	0	3056	100.00% (11/11) [74.12%, 100.00%]	100.00% (3056/3056) [99.87%, 100.00%]
BRCA1/2 inactivating mutations	2	< 1x LoD	2	1	5	3059	28.57% (2/7) [8.22%, 64.11%]	99.97% (3059/3060) [99.82%, 99.99%]
MET exon 14 mutations	2	All	12	0	1	7940	92.31% (12/13) [66.69%, 98.63%]	100.00% (7940/7940) [99.95%, 100.00%]
MET exon 14 mutations	2	≥ 1x LoD	12	0	0	7941	100.00% (12/12) [75.75%, 100.00%]	100.00% (7941/7941) [99.95%, 100.00%]
MET exon 14 mutations	2	< 1x LoD	0	0	1	7952	0.00% (0/1) [0.00%, 79.35%]	100.00% (7952/7952) [99.95%, 100.00%]
PIK3CA activating SNVs	2	All	115	13	8	2621	93.50% (115/123) [87.69%, 96.67%]	99.51% (2621/2634) [99.16%, 99.71%]
PIK3CA activating SNVs	2	≥ 1x LoD	102	3	0	2652	100.00% (102/102) [96.37%, 100.00%]	99.89% (2652/2655) [99.67%, 99.96%]

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
PIK3CA activating SNVs	2	< 1x LoD	13	10	8	2726	61.90% (13/21) [40.88%, 79.25%]	99.63% (2726/2736) [99.33%, 99.80%]
ROS1 rearrangements	2	All	19	1	1	762	95.00% (19/20) [76.39%, 99.11%]	99.87% (762/763) [99.26%, 99.98%]
ROS1 rearrangements	2	≥ 1x LoD	12	1	0	770	100.00% (12/12) [75.75%, 100.00%]	99.87% (770/771) [99.27%, 99.98%]
ROS1 rearrangements	2	< 1x LoD	7	0	1	774	87.50% (7/8) [52.91%, 97.76%]	100.00% (774/774) [99.51%, 100.00%]
NTRK1/2/3 Rearrangements	2	All	22	3	1	1691	95.65% (22/23) [79.01%, 99.23%]	99.82% (1691/1694) [99.48%, 99.94%]
NTRK1/2/3 Rearrangements	2	≥ 1x LoD	16	1	1	1699	94.12% (16/17) [73.02%, 98.95%]	99.94% (1699/1700) [99.67%, 99.99%]
NTRK1/2/3 Rearrangements	2	< 1x LoD	6	2	0	1706	100.00% (6/6) [60.97%, 100.00%]	99.88% (1706/1708) [99.57%, 99.97%]
All Level 2 Biomarkers (Aggregate)	2	All	236	24	20	18513	92.19% (236/256) [88.24%, 94.89%]	99.87% (18513/18537) [99.81%, 99.91%]
All Level 2 Biomarkers (Aggregate)	2	≥ 1x LoD	197	9	3	18584	98.50% (197/200) [95.68%, 99.49%]	99.95% (18584/18593) [99.91%, 99.97%]

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
All Level 2 Biomarkers (Aggregate)	2	< 1x LoD	39	15	17	18717	69.64% (39/56) [56.66%, 80.10%]	99.92% (18717/18732) [99.87%, 99.95%]
ATM mutations	3	All	5	1	0	2217	100.00% (5/5) [56.55%, 100.00%]	99.95% (2217/2218) [99.75%, 99.99%]
ATM mutations	3	≥ 1x LoD	5	1	0	2217	100.00% (5/5) [56.55%, 100.00%]	99.95% (2217/2218) [99.75%, 99.99%]
BRAF V600E	3	All	5	0	0	33	100.00% (5/5) [56.55%, 100.00%]	100.00% (33/33) [89.57%, 100.00%]
BRAF V600E	3	≥ 1x LoD	5	0	0	33	100.00% (5/5) [56.55%, 100.00%]	100.00% (33/33) [89.57%, 100.00%]
BRAF V600K	3	All	7	1	1	12	87.50% (7/8) [52.91%, 97.76%]	92.31% (12/13) [66.69%, 98.63%]
BRAF V600K	3	≥ 1x LoD	6	1	0	14	100.00% (6/6) [60.97%, 100.00%]	93.33% (14/15) [70.18%, 98.81%]
BRAF V600K	3	< 1x LoD	1	0	1	19	50.00% (1/2) [9.45%, 90.55%]	100.00% (19/19) [83.18%, 100.00%]
BRCA1 deletion	3	All	3	5	0	36	100.00% (3/3) [43.85%, 100.00%]	87.80% (36/41) [74.46%, 94.68%]
BRCA1 deletion	3	≥ 1x LoD	3	5	0	36	100.00% (3/3) [43.85%, 100.00%]	87.80% (36/41) [74.46%, 94.68%]

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
BRCA1/2 inactivating mutations	3	All	37	10	6	31903	86.05% (37/43) [72.74%, 93.44%]	99.97% (31903/31913) [99.94%, 99.98%]
BRCA1/2 inactivating mutations	3	≥ 1x LoD	30	2	2	31922	93.75% (30/32) [79.85%, 98.27%]	99.99% (31922/31924) [99.98%, 100.00%]
BRCA1/2 inactivating mutations	3	< 1x LoD	7	8	4	31937	63.64% (7/11) [35.38%, 84.83%]	99.97% (31937/31945) [99.95%, 99.99%]
EGFR activating SNVs	3	All	28	0	1	3586	96.55% (28/29) [82.82%, 99.39%]	100.00% (3586/3586) [99.89%, 100.00%]
EGFR activating SNVs	3	≥ 1x LoD	22	0	0	3593	100.00% (22/22) [85.13%, 100.00%]	100.00% (3593/3593) [99.89%, 100.00%]
EGFR activating SNVs	3	< 1x LoD	6	0	1	3608	85.71% (6/7) [48.69%, 97.43%]	100.00% (3608/3608) [99.89%, 100.00%]
ERBB2 activating indels	3	All	7	0	0	5054	100.00% (7/7) [64.57%, 100.00%]	100.00% (5054/5054) [99.92%, 100.00%]
ERBB2 activating indels	3	≥ 1x LoD	6	0	0	5055	100.00% (6/6) [60.97%, 100.00%]	100.00% (5055/5055) [99.92%, 100.00%]
ERBB2 activating indels	3	< 1x LoD	1	0	0	5060	100.00% (1/1) [20.65%, 100.00%]	100.00% (5060/5060) [99.92%, 100.00%]

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
ERBB2 amplification	3	All	30	1	16	1432	65.22% (30/46) [50.77%, 77.32%]	99.93% (1432/1433) [99.61%, 99.99%]
ERBB2 amplification	3	≥ 1x LoD	24	1	8	1446	75.00% (24/32) [57.89%, 86.75%]	99.93% (1446/1447) [99.61%, 99.99%]
ERBB2 amplification	3	< 1x LoD	6	0	8	1465	42.86% (6/14) [21.38%, 67.41%]	100.00% (1465/1465) [99.74%, 100.00%]
FGFR2 rearrangements	3	All	12	2	0	3	100.00% (12/12) [75.75%, 100.00%]	60.00% (3/5) [23.07%, 88.24%]
FGFR2 rearrangements	3	≥ 1x LoD	11	2	0	4	100.00% (11/11) [74.12%, 100.00%]	66.67% (4/6) [30.00%, 90.32%]
FGFR2 rearrangements	3	< 1x LoD	1	0	0	15	100.00% (1/1) [20.65%, 100.00%]	100.00% (15/15) [79.61%, 100.00%]
FGFR3 rearrangements	3	All	3	0	0	20	100.00% (3/3) [43.85%, 100.00%]	100.00% (20/20) [83.89%, 100.00%]
FGFR3 rearrangements	3	≥ 1x LoD	2	0	0	21	100.00% (2/2) [34.24%, 100.00%]	100.00% (21/21) [84.54%, 100.00%]
FGFR3 rearrangements	3	< 1x LoD	1	0	0	21	100.00% (1/1) [20.65%, 100.00%]	100.00% (21/21) [84.54%, 100.00%]
KRAS G12C	3	All	20	1	1	481	95.24% (20/21)	99.79% (481/482)

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
							[77.33%, 99.15%]	[98.83%, 99.96%]
KRAS G12C	3	≥ 1x LoD	17	0	0	486	100.00% (17/17) [81.57%, 100.00%]	100.00% (486/486) [99.22%, 100.00%]
KRAS G12C	3	< 1x LoD	3	1	1	498	75.00% (3/4) [30.06%, 95.44%]	99.80% (498/499) [98.87%, 99.96%]
KRAS activating SNVs	3	All	140	25	10	20163	93.33% (140/150) [88.16%, 96.34%]	99.88% (20163/20188) [99.82%, 99.92%]
KRAS activating SNVs	3	≥ 1x LoD	124	8	3	20203	97.64% (124/127) [93.28%, 99.19%]	99.96% (20203/20211) [99.92%, 99.98%]
KRAS activating SNVs	3	< 1x LoD	16	17	7	20298	69.57% (16/23) [49.13%, 84.40%]	99.92% (20298/20315) [99.87%, 99.95%]
MET amplification	3	All	20	3	11	743	64.52% (20/31) [46.95%, 78.88%]	99.60% (743/746) [98.82%, 99.86%]
MET amplification	3	≥ 1x LoD	19	2	8	748	70.37% (19/27) [51.52%, 84.15%]	99.73% (748/750) [99.03%, 99.93%]
MET amplification	3	< 1x LoD	1	1	3	772	25.00% (1/4) [4.56%, 69.94%]	99.87% (772/773) [99.27%, 99.98%]

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
NRAS activating SNVs	3	All	52	12	6	4294	89.66% (52/58) [79.21%, 95.17%]	99.72% (4294/4306) [99.51%, 99.84%]
NRAS activating SNVs	3	≥ 1x LoD	33	1	0	4330	100.00% (33/33) [89.57%, 100.00%]	99.98% (4330/4331) [99.87%, 100.00%]
NRAS activating SNVs	3	< 1x LoD	19	11	6	4328	76.00% (19/25) [56.57%, 88.50%]	99.75% (4328/4339) [99.55%, 99.86%]
RET rearrangements	3	All	22	2	1	1468	95.65% (22/23) [79.01%, 99.23%]	99.86% (1468/1470) [99.51%, 99.96%]
RET rearrangements	3	≥ 1x LoD	16	0	0	1477	100.00% (16/16) [80.64%, 100.00%]	100.00% (1477/1477) [99.74%, 100.00%]
RET rearrangements	3	< 1x LoD	6	2	1	1483	85.71% (6/7) [48.69%, 97.43%]	99.87% (1483/1485) [99.51%, 99.96%]
All Level 3 Biomarkers (Aggregate)	3	All	391	63	53	71445	88.06% (391/444) [84.72%, 90.76%]	99.91% (71445/71508) [99.89%, 99.93%]
All Level 3 Biomarkers (Aggregate)	3	≥ 1x LoD	323	23	21	71585	93.90% (323/344) [90.85%, 95.97%]	99.97% (71585/71608) [99.95%, 99.98%]
All Level 3 Biomarkers (Aggregate)	3	< 1x LoD	68	40	32	71809	68.00% (68/100) [58.34%, 76.33%]	99.94% (71809/71849) [99.92%, 99.96%]

Variant Category	Variant Level	LoD Level	CDx+ cNGS+	CDx+ cNGS-	CDx- cNGS+	CDx- cNGS-	PPA	NPA
All Level 4 Biomarkers (Aggregate)	4	All	2453	1206	1571	1733526	60.96% (2453/4024) [59.44%, 62.46%]	99.93% (1733526/1734732) [99.93%, 99.93%]
All Level 4 Biomarkers (Aggregate)	4	≥ 1x LoD	1764	561	241	1736190	87.98% (1764/2005) [86.48%, 89.33%]	99.97% (1736190/1736751) [99.96%, 99.97%]
All Level 4 Biomarkers (Aggregate)	4	< 1x LoD	689	645	1330	1736090	34.13% (689/2019) [32.09%, 36.22%]	99.96% (1736090/1736735) [99.96%, 99.97%]

CDx: Guardant360 Liquid CDx; cNGS: comparator NGS

6.2. Contrived Sample Functional Characterization

A contrived sample functional characterization (CSFC) study was performed to demonstrate commutability between contrived and clinical samples to support their use for establishing analytical performance characteristics for Guardant360 Liquid CDx. A total of 212 contrived samples and 539 clinical samples were tested to evaluate the functional performance of contrived samples and compare it to that of clinical samples. Of 212 contrived samples, 189 samples were deemed eligible for analysis. Comparable hit rates were observed between contrived and clinical samples across all variant types tested-including SNVs, indels, CNAs, and rearrangements-across a range of mutant allele frequency (MAF) percentages. Based on the results of this study, commutability between clinical and contrived material has been established.

6.3. Limit of Detection (LoD)

6.3.1. LoD Establishment and LoD Confirmation for High Input Samples

The LoDs for high input samples for clinically relevant variants (SNVs, indels, rearrangements) were established *in silico* at the maximum input level of the assay.

The established LoDs were confirmed using clinical samples from 46 cancer patients from the intended use population at the maximum input level of the assay. Five cfDNA clinical sample pools containing 31 targeted variants at 1-1.5x LoD were tested, with at least 18-20 replicates per pool across three reagent lots, two instrument combinations, and four operator groups. The median MAFs for biomarkers tested for LoD confirmation for CDx biomarkers are summarized in Table 6-3 and for clinically relevant variants are summarized in Table 6-4.

Table 6-3. CDx Biomarker LoD Confirmation for High Input Samples

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

Biomarker	Cancer Type	Median MAF in LoD Confirmation	Established LoD
EGFR L858R	NSCLC	0.3%	0.2%
EGFR E746_A750del	NSCLC	0.2%	0.2%
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 6-4. Clinically Relevant Variant LoD Confirmation 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	0.3%	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	*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%	0.2%
Indel	*MET* exon 14 mutations	NSCLC	0.2%	0.2%
Rearrangement	*ALK* rearrangements	NSCLC	0.2%	0.2%
Rearrangement	*FGFR2* rearrangements	Cholangiocarcinoma	0.2%	0.2%
Rearrangement	*FGFR3* rearrangements	NSCLC	0.3%	0.2%
Rearrangement	*NRG1* rearrangements	Breast	0.3%	0.3%
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%

The panel-wide LoDs for 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 6-5).

Table 6-5. Established LoDs for High Input Samples with Clinically Relevant Small Variants

Variant Type	LoD
Clinically relevant SNVs	0.2%
Clinically relevant Indels	0.2%

6.3.2. LoD Establishment for Low Input Samples

The LoDs for SNVs, indels, rearrangements, CNAs, and CNL detected by Guardant360 Liquid CDx were established at the most challenging low input level for the assay. The LoDs for SNVs, indels, CNAs, and rearrangements were established using sample pools created from clinical specimens from the intended use population, and for *BRCA1* CNL, contrived cfDNA was used.

A total of five sample pools containing targeted variants were created, and detection rates were compared across 5-7 titration levels to establish the LoD. A total of 629 replicates were assessed across three reagent lots, three operator groups and three instrument lines. The LoDs were established using a combination of probit and empirical approaches. Table 6-6 summarizes established LoDs for CDx biomarkers, and Table 6-7 summarizes the established LoDs for other clinically relevant biomarkers.

Table 6-6. Established LoD for Companion Diagnostic Biomarkers for Low Input Samples

Biomarker	Cancer Type	LoD (MAF)
EGFR T790M	NSCLC	0.9%
EGFR L858R	NSCLC	1.0%
EGFR E746_A750del	NSCLC	1.0%
EGFR L747_A755delinsSKG	NSCLC	0.7%
EGFR A767_V769dup	NSCLC	0.4%
ERBB2 S310F	NSCLC	1.0%
ERBB2 Y772_A775dup	NSCLC	0.9%
KRAS G12C	NSCLC	1.4%
ESR1 D538G	Breast	1.0%
ESR1 V422del	Breast	1.1%
BRAF V600E	CRC	0.8%

Table 6-7 Established LoD for Clinically Relevant Variants for Low Input Samples

Biomarker Type	Biomarker	Cancer Type	LoD (MAF/CN/TF)
SNV	*ATM* R2227C	Prostate	1.5%
SNV	*KIT* Y823D	GIST	0.9%
SNV	*MET* exon14 splice variant	NSCLC	1.2%
SNV	*PIK3CA* E545K	Breast	0.7%
SNV	*PIK3CA* H1047R	NSCLC	1.0%
Indel	*BRCA1* M1827fs	Prostate	0.9%
Indel	*BRCA2* K1517fs	Prostate	2.2%
Indel	*KIT* W557_V559del	GIST	1.6%
CNA	*ERBB2* amplification	Breast	2.4 copies
CNA	*MET* amplification	NSCLC	2.3 copies
CNL	*BRCA1* loss	Breast	22.7%
Rearrangement	*ALK* rearrangement	NSCLC	1.6%
Rearrangement	*FGFR2* rearrangement	Cholangiocarcinoma	0.9%
Rearrangement	*FGFR3* rearrangement	Breast	1.2%
Rearrangement	*NRG1* rearrangement	Breast	0.7%
Rearrangement	*NTRK1* rearrangement	NSCLC	0.6%
Rearrangement	*NTRK2* rearrangement	Prostate	1.2%
Rearrangement	*NTRK3* rearrangement	Breast	0.7%

Biomarker Type	Biomarker	Cancer Type	LoD (MAF/CN/TF)
Rearrangement	*RET* rearrangement	NSCLC	0.9%
Rearrangement	*ROS1* rearrangement	NSCLC	0.9%

The LoDs for clinically relevant variants were established as 1.0% MAF for SNVs and 0.9% MAF for indels for low input samples (Table 6-8).

Table 6-8. Established LoDs for Low Input Samples with Clinically Relevant Small Variants

Variant Type	LoD
Clinically relevant SNVs	1.0%
Clinically relevant Indels	0.9%

6.4. Limit of Blank (LoB)

The LoB for Guardant360 Liquid CDx was assessed using cfDNA derived from cancer-free donor samples to represent analytically blank samples. A total of 120 sample replicates from 30 unique healthy donor samples were assessed at maximum cfDNA input. The false positive rate did not exceed 5% for any of the identified reportable variants (Table 6-9).

Table 6-9. Limit of Blank by Variant Type

Variant Type	Number of variants with >5% False Positive Rate
SNVs	0
Indels	0
Rearrangements	0
CNAs	0
CNLs	0

6.5. Endogenous Interfering Substances

To evaluate the potential impact of endogenous interfering substances on the performance of Guardant360 Liquid CDx, these studies evaluated a total of 90 samples subjected to unconjugated bilirubin, conjugated bilirubin, triglycerides, albumin, or hemoglobin.

The studies resulted in 100% PPA and 100% NPA in calling positive and negative variants for all conditions, demonstrating minimal impact of endogenous interfering substances on the performance of Guardant360 Liquid CDx.

6.6. *In-silico* Primer and Probe Specificity

Primer and probe specificity were addressed 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 contaminants genomes, 97.2% of probes uniquely mapped to the human genome with ideal specificity (MAPQ ≥ 60). None of the primers or probes mapped to the representative microbial contaminant genomes.

6.7. Precision and LoD Confirmation

Guardant360 Liquid CDx precision for CDx variants and tumor mutation profiling variants were evaluated using cancer patient sample pools at challenging cfDNA input and challenging MAFs for SNVs, indels, rearrangements, and CNAs ("Primary Precision" and "Sequencer-to-Sequencer Precision" cohorts; Table 6-10).

A total of 516 samples were tested, and 491 samples were used for data analysis. 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 27/27 targeted variants (Table 6-10). The sequencer-to-sequencer precision analysis showed an APA of 99.7% (95% CI: 98.6% - 100.0%) and ANA of 100.0% (95% CI: 99.8% - 100.0%) between paired replicates sequenced on two different sequencers (Table 6-10).

An additional precision study evaluated the positive precision and LoD confirmation for additional CDx variants, tumor profiling variants, and CNLs ("Variant Positive" cohort; Table 6-10). The negative precision performance was determined using variant-negative cancer patient samples ("Variant Negative" cohort; Table 6-10).

A total of 216 samples were tested, all samples passed QC, and all 216 samples were used 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% - 100.0%). The NPA for variant negative samples was 99.9% (3076/3078; 95% CI: 99.8% - 100.0%).

Table 6-10. Summary of Precision Results

Cohort	Measurand	N (Concordant Variants / Total)	Point Estimate (95% Confidence Interval)
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%)
Variant Positive	PPA	321 / 324	99.1% (97.3% - 99.8%)
	NPA	891 / 891	100% (99.6% - 100.0%)
Variant Negative	NPA	3076 / 3078	99.9% (99.8% - 100.0%)

Table 6-11 summarizes the precision results for the CDx biomarkers tested at a MAF target of 1-2x LoD.

Table 6-11. Summary of Precision Results for Companion Diagnostic Biomarkers

Biomarker	Variant Type	N	Point Estimate	Average MAF
BRAF V600E	SNV	26/26	100% (86.8% - 100.0%)	0.9%
EGFR T790M	SNV	25/26	96.2% (80.4% - 99.9%)	1.8%
EGFR L858R	SNV	26/26	100% (86.8% - 100.0%)	1.8%
ERBB2 S310F	SNV	26/26	100% (86.8% - 100.0%)	1.8%
ESR1 D538G	SNV	26/26	100% (86.8% - 100.0%)	1.6%
KRAS G12C	SNV	26/26	100% (86.8% - 100.0%)	2.7%
EGFR E746_A750del	Indel	26/26	100% (86.8% - 100.0%)	1.7%
EGFR A767_V769dup	Indel	25/25	100% (86.3% - 100.0%)	0.7%
ERBB2 Y772_A775dup	Indel	24/25	96.0% (79.6% - 99.9%)	1.1%
ESR1 V422del	Indel	25/26	96.2% (80.4% - 99.9%)	1.2%

LoD was confirmed for SNVs, indels, CNAs, and CNLs (Table 6-12) in clinical samples prepared at a MAF target of 1-1.5x LoD.

Table 6-12. Summary of Precision and LoD Confirmation Results

Variant	Variant Type	Cancer Type	N	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%
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% tumor fraction

6.8. Blood Collection Tube Precision

BCT precision for Guardant360 Liquid CDx was evaluated by assessing repeatability within a single BCT lot and reproducibility across three different lots. Whole blood from healthy donors was 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). The variants spiked in included 18 clinically relevant variants: 10 SNVs, 4 indels, 3 rearrangements, and 1 copy number alteration. A total of 239 samples were tested, and 237 passed QC and were included in the final analysis. All targeted variants ≥1x LoD showed 100% APA and 99.99% ANA within and between BCT lots. These results demonstrate high precision and reliability of Guardant360 Liquid CDx using whole blood collected in BCTs.

Day 0 / Day 1 equivalency was assessed using 40 samples from 20 healthy donors, with plasma isolated from each donor on both Day 0 and Day 1. The analysis evaluated sample-level molecule recovery and exon-level molecule recovery between Day 0 and Day 1. Both acceptance criteria were met, demonstrating that specimens processed into plasma on the same day as blood collection (Day 0) are equivalent to specimens processed into plasma the day after blood collection (Day 1).

6.9. Extraction Precision

Within- and between-batch extraction precision, or preanalytical workflow precision, starting from cfDNA extraction, was assessed using a total of 14 individual patient samples and pooled patient samples with 6 replicates across 3 extraction batches. Samples tested included 26 unique clinical specimens from 10 cancer types (10 NSCLC, 4 breast carcinoma, 4 prostate adenocarcinoma, 2 CRC, 1 ovarian carcinoma, 1 melanoma, 1 cholangiocarcinoma, 1 bladder carcinoma, 1 pancreatic ductal adenocarcinoma, 1 esophageal/gastroesophageal junction adenocarcinoma). The samples represented all variant types (SNVs, indels, CNAs, CNLs, and rearrangements) and were tested at challenging MAF levels (1-3x LoD) and input levels.

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%. These results have met

acceptance criteria and confirm the repeatability and reproducibility of the Guardant360 Liquid CDx extraction process in detecting both positive and negative variants across technical replicates.

6.10. Cross-Contamination / Carry-Over

The cross-contamination / carryover study evaluated the prevalence of cross-contamination when material is transferred between samples in the same batch and carry-over contamination when material is transferred between samples across batches processed sequentially on the same instrument using Guardant360 Liquid CDx.

A total of 180 plasma samples across 2 batches were run in a consecutive order across instruments within the analytical accuracy study and sequenced on 4 flow cells. There was no evidence of high positive variants from near-by wells detected in negative samples. In conclusion, no carryover contamination and low cross-contamination rate (1 out of 180) were observed in 180 samples processed across 2 consecutive batches.

6.11. Guard Banding / Robustness

The robustness of Guardant360 Liquid CDx across its analytical input range was evaluated by testing samples at low and high cfDNA input levels in the cfDNA guard banding study.

A total of 183 samples (120 low-input samples, 63 high-input samples) that included clinical and contrived samples were tested in the cfDNA guard banding study. Of 183 samples, 127 samples processed passed sample QC and were further evaluated. The study demonstrated 98.5% PPA and 100% NPA for low input guard banding conditions and 100% PPA and 100% NPA for high input guard banding conditions.

6.12. Whole Blood Stability

To demonstrate the stability of whole blood specimen collected in Streck Cell-Free DNA BCTs, whole blood specimens were subjected to storage under room temperature, summer, and winter profiles. The analysis evaluated the sample-level and exon-level molecule recovery between the reference condition and each of the three test storage temperature profiles. These metrics remained equivalent under the tested conditions.

These results demonstrate the stability of stored whole blood specimens up to 7 days.

6.13. Plasma Stability

To demonstrate the stability of plasma specimens isolated from whole blood, isolated plasma specimens were subjected to storage at -80°C±10°C. The analysis evaluated the sample-level and exon-level molecule recovery between the reference condition (plasma isolation on Day 0) and the test storage condition. These metrics remained equivalent under the tested conditions.

These results demonstrate the stability of stored plasma specimens at -80°C±10°C up to 45 days.

6.14. Cell-Free DNA Stability

To demonstrate the stability of cfDNA isolated from plasma, extracted cfDNA was subjected to storage at +2°C to +8°C for 24 hours, -20°C ± 5°C for 7 days. The analysis evaluated the sample-level and exon-level molecule recovery between the reference condition (cfDNA extraction on Day 0) and the test storage condition. These metrics remained equivalent under the tested conditions.

These results demonstrate the stability of stored cfDNA at +2°C to +8°C for 24 hours and -20°C±5°C up to 7 days with up to two freeze-thaw cycles.

6.15. Reagent Stability

The stability of reagents used in sample processing for Guardant360 Liquid CDx were evaluated using three lots of reagents stored under the specified storage conditions and then tested at the 4-month time point. Under the tested conditions, results from 4-month time point were compared against samples tested at day 0 (time point T0).

The study showed no significant difference between the 4-month time point compared to T0 for all three lots, demonstrating that there was no significant decline in detection rates over the course of the study. All of the expected positive and negative variants were observed in agreement across all replicates, indicating 100% PPA and 100% NPA.

These results demonstrate the stability of Guardant360 Liquid CDx reagents for a minimum of 3 months.

6.16. Intermediate Product Stability

The stability of the intermediate products (libraries, enriched libraries, and normalized enriched library pools) was established for the following:

- Library: -20°C±5°C for 15 days (including 2 freeze-thaw cycles)
- Enriched library: 2-8°C for up to 4.5 hours, then stored at -20°C±5°C for 19 days (including 3 freeze-thaw cycles)
- Normalized enriched library pool: -20°C±5°C for 16 days (including 2 freeze-thaw cycles)

The analysis 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. These metrics remained equivalent under the tested conditions.

6.17. Pan-Cancer Analysis

Guardant360 Liquid CDx analytical performance characteristics were established using clinical samples from patients with a wide range of cancer types. In total, 1814 unique patient samples representing 29 cancer types were included across the analytical studies as summarized in Table 6-13. Overall, the data presented across cancer types show similar assay performance and QC passing rates. This indicates the performance demonstrated in these studies is representative of the expected performance of Guardant360 Liquid CDx across all cancer types.

Table 6-13. 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 I	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		
Hepatocellular Carcinoma	7	6										1			
GIST	4		2	1	1							1			1
Neuroendocrine Carcinoma	4	3	1												

Cancer Type	Total Number of Unique Samples	Analytical Accuracy	Limit of Detection - High Input	Limit of Detection - Low Input	Precision I	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
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													

7. Clinical Validation Studies

7.1. Concordance Study for Companion Diagnostic Biomarkers

To demonstrate the clinical validity of Guardant360 Liquid CDx for the companion diagnostic claims listed in the Intended Use Statement, non-inferiority studies comparing Guardant360 Liquid CDx to the FDA-approved Guardant360 CDx (P200010) were conducted using samples from the representative intended use population for the respective biomarker (Li, 2016). Guardant360 CDx 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 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 CCD comparator assay is included in each non-inferiority study section to provide context to the results.

Each study compared Guardant360 Liquid CDx (FCD) to the FDA-approved Guardant360 CDx (with the two CCD replicates denoted CCD1 and CCD2), using a non-inferiority statistical approach (Li, 2016). Concordance counts, individual agreement estimates (positive percent agreement, PPA; negative percent agreement, NPA), and agreement difference estimates (with two-sided 95% confidence intervals) are presented for each biomarker. Both unadjusted and prevalence-adjusted estimates are reported.

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

7.1.1.1. 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. In the primary clinical trial (FLAURA, NCT02296125, for *EGFR* exon 19 deletions and L858R mutations), Guardant360 CDx demonstrated 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. In the primary clinical trial (AURA3, NCT02151981 for *EGFR* T90M mutations), Guardant360 CDx demonstrated a PPA of 67.4% (95% CI: 61.6–72.8) and an NPA of 67.1% (95% CI: 58.9–74.7) compared to tissue testing. 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.

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.

7.1.1.2. 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 7-1–7-3 for *EGFR* Exon 19 deletions and/or L858R mutations and in Tables 7-4 -7-6 for *EGFR* T790M mutations.

Table 7-1. 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 7-2. 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 the table below, along with the corresponding two-sided 95% CIs.

Table 7-3. 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, ζNPA1 = NPAC1C2 − NPAC1F, ζNPA2 = NPAC2C1 − NPAC2F.

Table 7-4. 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 7-5. 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 the table below, along with the corresponding two-sided 95% CIs.

Table 7-6. 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, 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 eligible for treatment with osimertinib.

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

7.1.2.1. 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), 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.

7.1.2.2. 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 7-7-7-9.

Table 7-7. 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
Total	88	4	4	0	96	0	112	0	0	112

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

Table 7-8. 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

	% Agreement	
	Unadjusted (%)	Adjusted (0.9% Prevalence)
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 the table below, along with the corresponding two-sided 95% CIs.

Table 7-9. 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)
ζNPA2	0.83	(-1.69, 4.07)	0.01	(-0.02, 0.04)

ζPPA1 = PPAC1C2 − PPAC1F, ζPPA2 = PPAC2C1 − PPAC2F, 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 for treatment with amivantamab-vmjw.

7.1.3. Concordance Study for the Selection of NSCLC Patients with *KRAS* G12C Mutations for Sotorasib Therapy

7.1.3.1. 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 (CodeBreak100 (NCT03600883), Guardant360 CDx demonstrated 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).

7.1.3.2. 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 7-10 – 7-12.

Table 7-10. 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 7-11. 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 the table below, along with the corresponding two-sided 95% CIs.

Table 7-12. Agreement Difference Estimates for Non-Inferiority Evaluation for *KRAS* G12C Mutations

	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, 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 for LUMAKRAS (sotorasib) therapy.

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

7.1.4.1. 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), Guardant360 CDx demonstrated 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.

7.1.4.2. 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 7-13-7-15.

Table 7-13. 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 7-14. 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
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 the table below, along with the corresponding two-sided 95% CIs.

Table 7-15. 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, 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 for treatment with fam-trastuzumab deruxtecan-nxki therapy.

7.1.5. Concordance Study for the Selection of Breast Cancer Patients with *ESR1* Mutations for Elacestrant Therapy

7.1.5.1. 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.

7.1.5.2. 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 7-16 - 7-18.

Table 7-16. Concordance for *ESR1* Mutations (Elacestrant)

	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 7-17. Individual Agreement Estimates for *ESR1* Mutations (Elacestrant)

	% 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
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 the table below, along with the corresponding two-sided 95% CIs.

Table 7-18. Agreement Difference Estimates for Non-Inferiority Evaluation for *ESR1* Mutations (Elacestrant)

	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)

	Unadjusted		Adjusted (21.6% Prevalence)	
	Point Estimate (%)	95% two-sided CI (%)	Point Estimate (%)	95% two-sided CI (%)
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, 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 eligible for treatment with elacestrant.

7.1.6. Concordance Study for the Selection of Breast Cancer Patients with *ESR1* Mutations for Imlunestrant Therapy

7.1.6.1. 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.

7.1.6.2. 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 7-19-7-21.

Table 7-19. Concordance for *ESR1* Mutations (Imlunestrant)

	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 7-20. Individual Agreement Estimates for *ESR1* Mutations (Imlunestrant)

	% 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

	% Agreement	
	Unadjusted (%)	Adjusted (20.9% Prevalence)
NPA_{C2F}	94.59	97.69

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

Table 7-21. Agreement Difference Estimates for Non-Inferiority Evaluation for *ESR1* Mutations (Imlunestrant)

	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, 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 eligible for treatment with imlunestrant.

7.1.7. Concordance Study for the Selection of CRC Patients with *BRAF* V600E Mutations for Encorafenib plus Cetuximab Therapy

7.1.7.1. 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), Guardant360 CDx demonstrated a PPA of 85.0% (95% CI: 80.7–88.5) and an NPA of 100% (95% CI: 96.9–100) 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.

7.1.7.2. 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 7-22-7-24.

Table 7-22. 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 7-23. 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 the table below, along with the corresponding two-sided 95% CIs.

Table 7-24. 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)
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, 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 eligible for treatment with encorafenib in combination with cetuximab.

7.1.8. Concordance Study for the Selection of Breast Cancer Patients with *ESR1* Mutations for Vepdegestrant Therapy

7.1.8.1. 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 vepdegestrant therapy. 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 VERITAC-2 (NCT05654623).

7.1.8.2. Study Results

The results of the NI study for the detection of *ESR1* mutations indicated for treatment of vepdegestrant in breast cancer patients are summarized in Tables 7-19-7-21.

Table 7-19. Concordance for *ESR1* Mutations (Vepdegestrant)

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

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

Table 7-20. Individual Agreement Estimates for *ESR1* Mutations (Vepdegestrant)

	% Agreement	
	Unadjusted (%)	Adjusted (21.6% Prevalence)
PPAC1C2	96.77	94.27
PPAC1F	97.85	92.61
PPAC2C1	96.77	96.86
PPAC2F	98.92	96.20
NPAC1C2	97.25	99.19
NPAC1F	92.66	96.41
NPAC2C1	97.25	98.49
NPAC2F	93.58	96.71

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

Table 7-21. Agreement Difference Estimates for Non-Inferiority Evaluation for *ESR1* Mutations (Vepdegestrant)

	Unadjusted		Adjusted (21.6% Prevalence)	
	Point Estimate (%)	95% two-sided CI (%)	Point Estimate (%)	95% two-sided CI (%)
ζPPA1	-1.08	(-4.55, 2.16)	1.66	(-4.48, 9.41)
ζPPA2	-2.15	(-6.52, 2.04)	0.66	(-6.35, 8.82)
ζNPA1	4.59	(0.94, 8.98)	2.78	(0.27, 5.89)
ζNPA2	3.67	(-0.90, 8.26)	1.78	(-1.69, 5.39)

ζPPA1 = PPAC1C2 − PPAC1F, ζPPA2 = PPAC2C1 − PPAC2F, 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 eligible for treatment with vepdegestrant.

7.1.9. Concordance Study for the Selection of NSCLC Patients with *ERBB2/HER2* Activating Mutations for Zongertinib Therapy

7.1.9.1. Non-Inferiority Study Design

The clinical validity of Guardant360 Liquid CDx was established for identifying NSCLC patients with *ERBB2/HER2* activating mutations (SNVs in exons 18–21 within the tyrosine kinase domain and exon 20 insertions) who may be eligible for treatment with zongertinib. Plasma samples from 250 patients (108 *ERBB2/HER2* positive and 142 *ERBB2/HER2* 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 236 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 (Beamion LUNG-1, NCT04886804).

7.1.9.2. Study Results

The results of the NI study for the detection of *ERBB2/HER2* activating mutations indicated for treatment of zongertinib in NSCLC patients are summarized in Tables 7-22-7-24.

Table 7-22. Concordance for *ERBB2/HER2* Activating Mutations

	CCD0+					CCD0-				
CCD1/2	(+,+)	(-,-)	(+,-)*	(-,+)^	Total	(+,+)	(-,-)	(+,-)*	(-,+)^	Total
FCD+	95	1	0	0	96	0	1	0	0	1
FCD-	0	4	1	0	5	0	134	0	0	134
Total	95	5	1	0	101	0	135	0	0	135

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

Table 7-23. Individual Agreement Estimates for *ERBB2/HER2* Activating Mutations

	% Agreement	
	Unadjusted (%)	Adjusted (1.3% Prevalence)
PPAC1C2	98.96	98.96
PPAC1F	98.96	98.96
PPAC2C1	100.00	100.00
PPAC2F	100.00	100.00
NPAC1C2	100.00	100.00
NPAC1F	98.57	99.25
NPAC2C1	99.29	99.99
NPAC2F	98.58	99.25

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

Table 7-24. 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	(-4.27, 4.27)	0.00	(-4.27, 4.27)
ζPPA2	0.00	(-3.89, 3.89)	0.00	(-3.89, 3.89)
ζNPA1	1.43	(0.00, 3.57)	0.75	(0.00, 2.25)
ζNPA2	0.71	(-1.43, 2.90)	0.74	(-0.03, 2.23)

ζPPA1 = PPAC1C2 − PPAC1F, ζPPA2 = PPAC2C1 − PPAC2F, 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 for treatment with zongertinib therapy.

7.1.10. Concordance Study for the Selection of Breast Cancer Patients with *ESR1* Mutations for Camizestrant Therapy

7.1.10.1 Non-Inferiority Study Design

The clinical validity of Guardant360 Liquid CDx was established to identify breast cancer patients with *ESR1* mutations who may be eligible for treatment with camizestrant. Plasma samples from 224 patients (118 *ESR1* positive and 106 *ESR1* negative) were tested. 17 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 207 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 (SERENA-6, NCT04964934).

7.1.10.2 Study Results

The results of the NI study for the detection of *ESR1* mutations indicated for treatment of camizestrant in breast cancer patients are summarized in **Tables 7-31- 7-33**.

Table 7-31. Concordance for *ESR1* Mutations (Camizestrant)

	CCD0+					CCD0-				
CCD1/2	(+,+)	(-,-)	(+,-)*	(-,+)^	Total	(+,+)	(-,-)	(+,-)*	(-,+)^	Total
FCD+	92	3	2	2	99	0	1	0	0	1
FCD-	0	3	1	0	4	1	101	1	0	103
Total	92	6	3	2	103	1	102	1	0	104

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

Table 7-32. Individual Agreement Estimates for *ESR1* Mutations (Camizestrant)

% Agreement		
	Unadjusted (%)	Adjusted (20.8% Prevalence)
PPA_{C1C2}	95.88	93.41
PPA_{C1F}	96.91	91.71
PPA_{C2C1}	97.89	97.95
PPA_{C2F}	98.95	96.16
NPA_{C1C2}	98.18	99.49
NPA_{C1F}	94.55	97.76
NPA_{C2C1}	96.43	98.29
NPA_{C2F}	94.64	97.79

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

Table 7-33. Agreement Difference Estimates for Non-Inferiority Evaluation for *ESR1* Mutations (Camizestrant)

	Unadjusted		Adjusted (20.8% Prevalence)	
	Point Estimate (%)	95% two-sided CI (%)	Point Estimate (%)	95% two-sided CI (%)

ζPPA1	-1.03	(-4.35, 2.08)	1.71	(-4.12, 9.65)
ζPPA2	-1.05	(-4.49, 2.11)	1.79	(-4.35, 9.83)
ζNPA1	3.64	(0.90, 7.21)	1.73	(0.25, 3.96)
ζNPA2	1.79	(-2.55, 6.09)	0.51	(-2.34, 3.20)

ζPPA1 = PPAC1C2 – PPAC1F, ζPPA2 = PPAC2C1 – PPAC2F, 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 for treatment with camizestrant therapy.

7.1.11. Concordance study for the Selection of Breast Cancer Patients with *ESR1* mutations for Imlunestrant plus Abemaciclib Therapy

7.1.11.1 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 plus abemaciclib. Plasma samples from 224 patients (118 *ESR1* positive and 106 *ESR1* negative) were tested. 17 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 207 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, NCT04975308).

7.1.11.2 Study Results

The results of the NI study for the detection of *ESR1* mutations indicated for treatment of imlunestrant plus abemaciclib in breast cancer patients are summarized in **Tables 7-34 -7-36**.

Table 7-34. Concordance for *ESR1* Mutations (Imlunestrant plus Abemaciclib)

	CCD0+					CCD0-				
CCD1/2	(+,+)	(-,-)	(+,-)*	(-,+)^	Total	(+,+)	(-,-)	(+,-)*	(-,+)^	Total
FCD+	92	3	2	2	99	0	1	0	0	1
FCD-	0	3	1	0	4	1	101	1	0	103
Total	92	6	3	2	103	1	102	1	0	104

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

Table 7-35. Individual Agreement Estimates for *ESR1* Mutations (Imlunestrant plus Abemaciclib)

% Agreement		
	Unadjusted (%)	Adjusted (20.8% Prevalence)
PPA_{C1C2}	95.88	93.41

PPA_{C1F}	96.91	91.71
PPA_{C2C1}	97.89	97.95
PPA_{C2F}	98.95	96.16
NPA_{C1C2}	98.18	99.49
NPA_{C1F}	94.55	97.76
NPA_{C2C1}	96.43	98.29
NPA_{C2F}	94.64	97.79

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

Table 7-36. Agreement Difference Estimates for Non-Inferiority Evaluation for *ESR1* Mutations (Imlunestrant plus Abemaciclib)

	Unadjusted		Adjusted (20.8% Prevalence)	
	Point Estimate (%)	95% two-sided CI (%)	Point Estimate (%)	95% two-sided CI (%)
ζPPA1	-1.03	(-4.35, 2.08)	1.71	(-4.12, 9.65)
ζPPA2	-1.05	(-4.49, 2.11)	1.79	(-4.35, 9.83)
ζNPA1	3.64	(0.90, 7.21)	1.73	(0.25, 3.96)
ζNPA2	1.79	(-2.55, 6.09)	0.51	(-2.34, 3.20)

ζPPA1 = PPAC1C2 − PPAC1F, ζPPA2 = PPAC2C1 − PPAC2F, 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 for treatment with imlunestrant plus abemaciclib therapy.

8. Additional Guardant360 Liquid CDx Biomarker Information

Table 8-1. Reported Alterations for Guardant360 Liquid CDx Companion Diagnostic Biomarkers

Indication	Biomarker	Reportable Mutations
Breast cancer	*ESR1* missense mutations between codons 310 and 547	For elacestrant: Missense mutations between codons 310 and 547, inclusive
	ESR1 E380, V422del, S463, L469, L536, Y537, and D538 mutations	For imlunestrant: E380A; E380D; E380K; E380Q; E380V; V422del; S463F; S463P; L469V; L536F; L536G; L536H; L536I; L536K; L536N; L536P; L536Q; L536R; L536V; Y537C; Y537D; Y537G; Y537H; Y537N; Y537P; Y537Q; Y537S; D538E; D538G; D538H; D538N; D538V
	ESR1 K303R, V422del, L549P, and missense mutations between codons 310 and 547	For vepdegestrant: K303R, V422del, L549P, and missense mutations between codons 310 and 547, inclusive
	ESR1 E380, V422del, S463, L536, Y537, and D538 mutations	For camizestrant: E380Q; V422del; S463P; L536H; L536P; L536R; Y537C; Y537D; Y537N; Y537S; D538G
CRC	*BRAF* V600E	V600E
NSCLC	*EGFR* exon 19 deletions, L858R, and T790M	Exon 19 deletions, L858R, and T790M
	EGFR exon 20 insertions	Exon 20 insertions
	KRAS G12C	G12C
	ERBB2/*HER2* activating mutations (SNVs and exon 20 insertions)	S310F; S310Y; R678Q; T733I; L755A; L755M; L755P; L755S; L755W; I767F; I767M; D769H; D769N; D769Y; Y772_A775dup; A775_G776insTVMA; A775_G776insV; A775_G776insYVMA; G776C; G776S; G776V; G776_V777delinsCVCG; G776_V777insL; G776_V777insVC; G776_V777insVGC; G776delinsLC; G776delinsVC; V777L; V777M; V777_G778insCG; V777_G778insG; V777_S779dup; G778_P780dup; G778_S779insCPG; G778_S779insLPS; G778dup; S779_P780insVGS; P780_Y781insGSP; T798I; V842I; T862I; L869R; R896C; R896H

Indication	Biomarker	Reportable Mutations
	ERBB2/HER2 activating mutations (SNVs in exons 18-21 within the tyrosine kinase domain and exon 20 insertions)	L726V; T733I; P749A; L755A; L755M; L755P; L755S; L755W; A760_G761insYVMA; I767F; I767M; D769H; D769N; D769Y; E770_A771insAYVM; Y772_A775dup; Y772_A775dupYVMA; Y772_V773insMMAY; V773_M774insWTYV; A775_G776insL; A775_G776insSVMA; A775_G776insTVMA; A775_G776insV; A775_G776insVVMA; A775_G776insYVMA; A775delinsML; G776C; G776L; G776S; G776V; G776_V777delinsCVCG; G776_V777delinsVCD; G776_V777insL; G776_V777insVC; G776_V777insVGC; G776delinsLC; G776delinsVC; G776delinsVV; V777L; V777M; V777_G778insCG; V777_G778insG; V777_S779dup; G778_P780dup; G778_S779insCPG; G778_S779insLPS; G778dup; S779_P780insRS; S779_P780insVGS; P780_Y781insGSP; T798I; C805S; V842I; T862I; L869R

9. Symbols



Manufacturer



Date of Manufacture



Use By Date



Batch Code



Catalog Number



Serial Number



Biological Risks



CE Marking of Conformity



Country of Manufacture



Do Not Re-Use



Consult Instructions for Use



Contains Sufficient for Number of Tests Specified



In Vitro Diagnostic Medical Device



Authorized Representative in the European Community



Temperature Limitation



By Prescription Only



Fragile, Handle with Care



Do Not Use if Package is Damaged or Broken

10. References

Li, M. (2016). Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study. *Statistics in Biopharmaceutical Research*, 8(3), 355–363. https://doi.org/10.1080/19466315.2016.1202859

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