

## SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

### I. GENERAL INFORMATION

Device Generic Name:	Next generation sequencing oncology panel, somatic or germline variant detection system
Device Trade Name:	MI Cancer Seek
Device Procode:	PQP
Applicant's Name and Address:	Caris Life Sciences 4610 South 44th Place Phoenix, AZ 85040
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P240010
Date of FDA Notice of Approval:	November 5, 2024

### II. INDICATIONS FOR USE

MI Cancer Seek is a next-generation sequencing (NGS) based in vitro diagnostic (IVD) device using total nucleic acid (TNA) isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens for the detection of single nucleotide variants (SNVs) and insertions and deletions (indels) in 228 genes, microsatellite instability (MSI), tumor mutational burden (TMB) in patients with previously diagnosed solid tumors, and copy number amplification (CNA) in one gene in patients with breast cancer.

MI Cancer Seek is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in **Table 1** below, in accordance with the approved therapeutic product labeling.

Additionally, MI Cancer Seek is intended to provide tumor mutational profiling to be used by qualified healthcare professionals in accordance with professional oncology guidelines for cancer patients with previously diagnosed solid malignant neoplasms. Genomic findings other than those listed in **Table 1** are not prescriptive or conclusive for labeled use of any specific therapeutic product.

**Table 1. MI Cancer Seek Companion Diagnostic Indications**

Indication	Biomarker	Therapy
Breast Cancer	<i>PIK3CA</i> (C420R; E542K; E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, H1047Y)	PIQRAY® (alpelisib)
Colorectal Cancer (CRC)	<i>KRAS</i> wild-type (absence of mutations in exons 2, 3, and 4) and <i>NRAS</i> wild type (absence of mutations in exons 2, 3, and 4)	VECTIBIX® (panitumumab)
	<i>BRAF</i> V600E	<i>BRAFTOVI</i> ® (encorafenib) in combination with ERBITUX® (cetuximab)
Melanoma	<i>BRAF</i> V600E	<i>BRAF</i> Inhibitors approved by FDA*
	<i>BRAF</i> V600E or V600K	MEKINIST® (trametinib) or <i>BRAF</i> /MEK Inhibitor Combinations approved by FDA*
Non-small cell lung cancer (NSCLC)	<i>EGFR</i> exon 19 deletions and exon 21 L858R alterations	<i>EGFR</i> Tyrosine Kinase Inhibitors approved by FDA*
Solid Tumors	MSI-H	KEYTRUDA® (pembrolizumab), JEMPERLI (dostarlimab-gxly)
Endometrial Carcinoma	Not MSI-H	KEYTRUDA® (pembrolizumab) in combination with LENVIMA® (lenvatinib)
*For the most current information about the device indications for the therapeutic products in this group, go to: <a href="https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools#Group_Labeling">https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools#Group_Labeling</a>		

MI Cancer Seek is a single-site assay performed at Caris Life Sciences, Phoenix, AZ.

### **III. CONTRAINDICATIONS**

There are no known contraindications.

### **IV. WARNINGS AND PRECAUTIONS**

The warnings and precautions can be found in the MI Cancer Seek labeling.

### **V. DEVICE DESCRIPTION**

MI Cancer Seek is a single-site assay performed at Caris Life Sciences located at 4610 South 44th Place, Phoenix, AZ 85040. The test includes reagents, software, and procedures for

testing of total nucleic acid (TNA) from formalin-fixed paraffin-embedded (FFPE) tumor tissue. The test employs a custom whole exome sequencing panel to detect and report SNVs and indels within 228 genes outlined in **Table 2** across solid tumors and amplifications in *ERBB2* in patients with breast cancer only. The test also detects MSI determined from 3,210 genes and whole exome based TMB in patients with solid tumors.

**Table 2. MI Cancer Seek Reportable Gene List for SNVs and indels**

<i>ABL1</i>	<i>BRCA1</i>	<i>CYLD</i>	<i>FGFR1</i>	<i>JAK2</i>	<i>MPL</i>	<i>PDGFRA</i>	<i>RAF1</i>	<i>STAG2</i>
<i>ACVR1</i>	<i>BRCA2</i>	<i>DDR2</i>	<i>FGFR2</i>	<i>JAK3</i>	<i>MRE11</i>	<i>PDGFRB</i>	<i>RASA1</i>	<i>STAT3</i>
<i>AIP</i>	<i>BRIP1</i>	<i>DICER1</i>	<i>FGFR3</i>	<i>KDM5C</i>	<i>MSH2</i>	<i>PIK3CA</i>	<i>RB1</i>	<i>STK11</i>
<i>AKT1</i>	<i>BTB</i>	<i>DNMT3A</i>	<i>FGFR4</i>	<i>KDM6A</i>	<i>MSH3</i>	<i>PIK3CB</i>	<i>RET</i>	<i>SUFU</i>
<i>AKT2</i>	<i>CALR</i>	<i>EGFR</i>	<i>FH</i>	<i>KDR</i>	<i>MSH6</i>	<i>PIK3R1</i>	<i>RHOA</i>	<i>TCF7L2</i>
<i>AKT3</i>	<i>CARD11</i>	<i>EP300</i>	<i>FLCN</i>	<i>KEAP1</i>	<i>MTOR</i>	<i>PIK3R2</i>	<i>RNF43</i>	<i>TERT</i>
<i>ALK</i>	<i>CBFB</i>	<i>EPHA2</i>	<i>FLT1</i>	<i>KIT</i>	<i>MUTYH</i>	<i>PIM1</i>	<i>ROS1</i>	<i>TET2</i>
<i>AMER1</i>	<i>CCND1</i>	<i>ERBB2</i>	<i>FLT3</i>	<i>KLF4</i>	<i>MYC</i>	<i>PMS2</i>	<i>RUNX1</i>	<i>TMEM127</i>
<i>APC</i>	<i>CCND2</i>	<i>ERBB3</i>	<i>FOXA1</i>	<i>KMT2A</i>	<i>MYCN</i>	<i>POLD1</i>	<i>SDHA</i>	<i>TNFAIP3</i>
<i>AR</i>	<i>CCND3</i>	<i>ERBB4</i>	<i>FOXL2</i>	<i>KMT2C</i>	<i>MYD88</i>	<i>POLE</i>	<i>SDHAF2</i>	<i>TNFRSF14</i>
<i>ARAF</i>	<i>CD79B</i>	<i>ERCC2</i>	<i>FUBP1</i>	<i>KMT2D</i>	<i>NBN</i>	<i>POT1</i>	<i>SDHB</i>	<i>TP53</i>
<i>ARID1A</i>	<i>CDC73</i>	<i>ESR1</i>	<i>GATA3</i>	<i>KRAS</i>	<i>NF1</i>	<i>PPP2R1A</i>	<i>SDHC</i>	<i>TRAF7</i>
<i>ARID2</i>	<i>CDH1</i>	<i>EZH2</i>	<i>GNAI1</i>	<i>LZTR1</i>	<i>NF2</i>	<i>PPP2R2A</i>	<i>SDHD</i>	<i>TSC1</i>
<i>ASXL1</i>	<i>CDK12</i>	<i>FANCA</i>	<i>GNAI3</i>	<i>MAP2K1</i>	<i>NFE2L2</i>	<i>PRDM1</i>	<i>SETD2</i>	<i>TSC2</i>
<i>ATM</i>	<i>CDK4</i>	<i>FANCB</i>	<i>GNAQ</i>	<i>MAP2K2</i>	<i>NFKBIA</i>	<i>PRKACA</i>	<i>SF3B1</i>	<i>U2AF1</i>
<i>ATRX</i>	<i>CDKN1B</i>	<i>FANCC</i>	<i>GNAS</i>	<i>MAP2K4</i>	<i>NOTCH1</i>	<i>PRKARIA</i>	<i>SMAD2</i>	<i>VHL</i>
<i>AXIN2</i>	<i>CDKN2A</i>	<i>FANCD2</i>	<i>H3F3A</i>	<i>MAP3K1</i>	<i>NPM1</i>	<i>PRKDC</i>	<i>SMAD4</i>	<i>WRN</i>
<i>B2M</i>	<i>CHEK1</i>	<i>FANCE</i>	<i>H3F3B</i>	<i>MAPK1</i>	<i>NRAS</i>	<i>PTCH1</i>	<i>SMARCA4</i>	<i>WT1</i>
<i>BAP1</i>	<i>CHEK2</i>	<i>FANCF</i>	<i>HIST1H3B</i>	<i>MAX</i>	<i>NSD1</i>	<i>PTEN</i>	<i>SMARCB1</i>	<i>XPO1</i>
<i>BARD1</i>	<i>CIC</i>	<i>FANCG</i>	<i>HNF1A</i>	<i>MED12</i>	<i>NSD2</i>	<i>PTPN11</i>	<i>SMARCE1</i>	<i>XRCC1</i>
<i>BCL2</i>	<i>CREBBP</i>	<i>FANCI</i>	<i>HOXB13</i>	<i>MEF2B</i>	<i>NTHL1</i>	<i>RAC1</i>	<i>SMO</i>	
<i>BCL9</i>	<i>CSF1R</i>	<i>FANCL</i>	<i>HRAS</i>	<i>MEN1</i>	<i>NTRK1</i>	<i>RAD50</i>	<i>SOCS1</i>	
<i>BCOR</i>	<i>CTCF</i>	<i>FANCM</i>	<i>IDH1</i>	<i>MET</i>	<i>NTRK2</i>	<i>RAD51B</i>	<i>SOS1</i>	
<i>BLM</i>	<i>CTNNA1</i>	<i>FAS</i>	<i>IDH2</i>	<i>MITF</i>	<i>NTRK3</i>	<i>RAD51C</i>	<i>SPEN</i>	
<i>BMPRI1A</i>	<i>CTNNB1</i>	<i>FAT1</i>	<i>IRF4</i>	<i>MLH1</i>	<i>PALB2</i>	<i>RAD51D</i>	<i>SPOP</i>	
<i>BRAF</i>	<i>CXCR4</i>	<i>FBXW7</i>	<i>JAK1</i>	<i>MLH3</i>	<i>PBRM1</i>	<i>RAD54L</i>	<i>SRC</i>	

## A. Test Output

The test report includes variants reported in the following levels:

Level 1: Companion Diagnostic (CDx) Claims noted in **Table 1** of the Intended Use

Level 2: Cancer Mutations with Evidence of Clinical Significance

Level 3: Cancer Mutations with Potential Clinical Significance

Genomic findings other than those listed in **Table 1** of the Companion diagnostic indications table of the intended use statement (i.e., Levels 2 and 3) are not prescriptive or conclusive for labeled use of any specific therapeutic product.

## **B. Test Kit Contents**

The MI Cancer Seek assay consists of five sub-kits that include the critical reagents required to execute the assay workflow:

1. MI Cancer Seek Extraction Kit
2. MI Cancer Seek Quantification Kit
3. MI Cancer Seek Library Prep Kit
4. MI Cancer Seek Sequencing Kit
5. MI Cancer Seek Library Quant Kit

All reagents needed to perform the MI Cancer Seek assay are used exclusively at the Caris facility, as this is a single site assay.

## **C. Instruments**

MI Cancer Seek is intended to be performed with serial numbered controlled instruments (**Table 3**) that are qualified and maintained by Caris' Quality System. All instruments needed to perform the MI Cancer Seek assay are used exclusively at the Caris facility, as this is a single site assay.

**Table 2. Overview of MI Cancer Seek Instruments**

<b>Instrument</b>
Beckman Biomek i7 Automated Workstation
Promega GloMax® Explorer Microplate Reader
BioMicroLab Inc BioMicroLab XL 100
Agilent Bravo NGS workstation Option B
Illumina NovaSeq 6000 Sequencing System
Hamilton LabElite ID Capper
Life Technologies Fleet Control Software Thermocycler Server
Life Technologies Qubit 4.0 Fluorometer
Life Technologies Veriti (Pro) 96 Well Thermal Cycler
Thermo Fisher Scientific VisionMate Sample Barcode Scanner
Thermo Fisher Scientific KingFisher Flex

## **D. Principles of Operation**

The MI Cancer Seek involves the process described below:

### **a. Specimen Requirements, Collection and Preparation for Analysis**

The MI Cancer Seek requires TNA isolated from FFPE tissue specimens. Formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens are collected and prepared following standard pathology practice. FFPE specimens may be received as either unstained slides or FFPE tissue blocks. Prior to MI Cancer Seek testing, a Hematoxylin and Eosin (H&E) stained slide is prepared and reviewed by a board-certified pathologist to confirm that invasive cancer is present and ensure there is adequate tissue and tumor content to proceed with the assay. A minimum tissue area of 25 mm<sup>2</sup> with  $\geq 20\%$  tumor content is required for MI Cancer Seek. When necessary, Caris will perform manual microdissection of specimens to increase cell density for testing and avoid interferences as much as possible. H&E slides will be annotated for manual microdissection and microdissection will be performed to enrich tumor content and/or avoid areas with potential interferences such as necrotic tissue, fat cells, or melanin. 120 mm<sup>2</sup> tissue area is recommended for dissection as an optimal tissue input into TNA extraction.

#### **b. Nucleic Acid Extraction**

Deparaffinization and protease digestion of the dissected tissue is performed, followed by automated TNA extraction using the MI Cancer Seek Extraction Kit with Biomek i7 Automated Liquid Handling Workstation and KingFisher Flex automated extraction instrument. Tissue specimens are scraped into a tube containing lysis buffer and proceed to TNA extraction. The extraction-negative control (EXT NEG), extraction-positive control (HBCTL) and clinical samples are placed onto the Biomek i7 Automated Liquid Handling Workstation for addition of Proteinase K, decrosslinking and lysis incubations. The 96-well plate is placed onto the KingFisher Flex automated extraction instrument to perform the TNA extraction and elution process with the MI Cancer Seek Extraction Kit. After completion of TNA extraction, double-stranded DNA (dsDNA) is quantified using the MI Cancer Seek Quantification Kit and the GloMax Plate Reader. The sample must yield a minimum of 50 ng of DNA to continue workflow (1.43 ng/ $\mu$ L QC Check), while NTC should not exceed 0.5 ng/ $\mu$ L. The optimal or maximum DNA input for the test is 220 ng.

#### **c. Library Preparation and Enrichment**

Libraries are prepared through a series of automated processes using the MI Cancer Library Preparation Kit, Bravo liquid handlers, thermocyclers, and GloMax plate readers. The process begins with enzymatic fragmentation of the extracted TNA, followed by cDNA synthesis. cDNA molecules are chemically labelled to later allow for mutation origin differentiation (RNA or DNA-based) by the pipeline. After 3' adenylation, end-repair and ligation, libraries are amplified in a pre-capture polymerase chain reaction (PCR) followed by quantification using the MI Cancer Seek Quantification Kit and GloMax plate reader. Genomic targets are enriched through hybridization with a custom bait panel, covering the whole exome with boosted regions for clinically relevant genes. Baits are 120 nucleotides long, double-

stranded and biotinylated. The enriched library is amplified by post-capture PCR and purified.

Samples are normalized and pooled through an automated process on the Bravo liquid handlers using the MI Cancer Seek Quantification Kit. The resulting pooled library is quantified using the MI Cancer Seek Library Quantification Kit and is then manually denatured prior to use of the MI Cancer Seek Sequencing Kits for flow cell loading and sequencing on the Illumina NovaSeq 6000 platform.

#### **d. DNA Sequencing**

The pooled libraries are subject to sequencing-by-synthesis using the standard NovaSeq6000 workflow and the MI Cancer Seek Sequencing Kits. NovaSeq6000 systems will automatically load pooled libraries onto the flow cell and proceed for cluster generation and sequencing of the prepared library.

Base calls are detected from the signal intensity measurements during each cycle. Base Call (BCL) files generated from the Illumina NovaSeq systems are stored on a secure High-Performance Computing (HPC) server on rapid solid-state hard drives. The initial sequencing metrics for the flow cells are reviewed by the NGS bioinformatics pipeline which includes the following run validity criteria, quality control (QC) check: Reads Passing Filter (M), Yield (G) and Q30 scores for each read 1 and read 4 (forward and reverse). The pipeline initiates automatic conversion and de-multiplex using the FPGA-adapted version of bcl2fastq via Illumina DRAGEN devices to convert the Illumina-generated BCL files to FASTQ file formats for downstream analysis. Demultiplexing is performed on the TNA reads, creating sample-specific FASTQ files. Custom software is applied to differentiate RNA and DNA molecules using the synthetic labels added during cDNA synthesis. Once separated, RNA data proceeds through the whole transcriptome pipeline and DNA data proceeds through the whole exome pipeline. DNA and RNA Seq data are aligned to the NCBI hg38reference genome (GRCh38). DNA variants detection (SNVs, indels, TMB, MSI and *ERBB2* CNAs) are evaluated separately and the data are stored on secured servers. While the test has the capability to detect alterations in RNA via whole transcriptome sequencing (WTS), the test is only approved to report alterations using genomic DNA consistent with the test's intended use.

#### **e. Data Analysis and Reporting**

Sequencing results are analyzed by the MI Cancer Seek NGS Bioinformatic Pipeline, and reportable results passing run, control, and sample validity criteria are provided through Report Generation software. The pipeline performs an assessment of DNA-based NGS data as described below to generate the reportable calls for variants (SNV and indel), *ERBB2* CNA, MSI, and TMB. The Report Generation software makes applicable CDx therapy associations based on pipeline results.



The pipeline automatically initiates after the sequencer has finished generating the sequencing data in base call (BCL) format. Upon conclusion of copy of BCL data to a secure and designated directory, the pipeline commences data analysis with conversion of BCL files into demultiplexed sample-specific FASTQ files containing both DNA and RNA reads. These FASTQ files are split into DNA and RNA FASTQ file sets using the RNA labels to identify the RNA reads. Each FASTQ pair (one for DNA and RNA each) consists of two FASTQ files: each corresponding to forward and reverse reads due to paired-end sequencing. The DNA and RNA FASTQ files are then processed through WES and WTS workflows, respectively. Results from both WES and WTS workflows are required in order to assess the validity of the control samples and the run. The DNA FASTQ files are aligned with hg38 reference genome, using Sentieon BWA, creating a binary alignment map (BAM) where the duplicates are marked to be excluded from downstream steps.

Next, as part of SNVs and indel identification, the BAM file is processed in a series of steps using several in-house and industry-standard tools. These steps include indel realignment and initial variant calling. Subsequently, high quality SNVs and indels are retained and annotated by removal of false positives, flagging low-quality variants and adding annotation including variant clinical impact.

Next, *ERBB2* CNA assessment is performed using CNV kit. This step uses the DNA BAM and VCF file as inputs and detects any *ERBB2* amplifications. The CNV kit uses both the on-target reads and the nonspecifically captured off-target reads along with a segmentation algorithm to infer discrete copy number segments for an accurate amplification status of *ERBB2*.

Subsequently, MSI status of a given sample is determined by calculating the number of observed frame shift mutations among 5,721 specific candidate loci present in the VCF file. Caris MI Cancer Seek CDx test uses tumor-only sequencing for MSI status determination.

Finally, as the last step in WES workflow, TMB is calculated for a given sample as the number of variants per Mega bases (total number of variants divided by the size of target region in Mega bases) after filtering out non-coding, low-quality, low-variant frequency (VF) synonymous variants along with variants containing 'gnomAD' and 'Benign' tags.

As the last step, the WES and WTS results are combined together and evaluated together to perform the following validity criteria checks:

- Run validity on the sequencer output to classify the run as pass/fail
- Run Controls QC to classify the run as pass or fail
- Analyte Controls QC to determine which capabilities are reportable
- DNA and RNA validity to pass/fail the individual samples
- Variant calling criteria to determine which biomarkers are reportable for successful samples

## f. Assay Validity Criteria

Validity criteria has been established at various levels and stages to evaluate each run, control sample, clinical sample and individual biomarker. Details are provided below for each type of validity criteria. **Table 4** provides an overview of the MI Cancer Seek QC Process.

### i. Run validity criteria

Run validity criteria are evaluated to determine if the sequencing run has passed. If a run fails its validity criteria, all clinical and control samples on that run are marked as fail. As part of this validity check, number of reads passing filter (PF) should be > 16 billion. This is calculated by the Illumina sequencers that perform an internal quality filtering procedure and reads that pass this filter are reported as PF reads. The run yield should be > 2,400 Gb and the percentage (%) of Q30 reads for both R1 and R4 should > 75%. A quality score of 30 (Q30) represents an error rate (for calling the base wrong) of 1 in 1000 (meaning every 1000 bp sequencing read may contain an error), with a corresponding call accuracy of 99.9%. When sequencing quality reaches Q30, virtually all of the reads will be perfect, with no errors or ambiguities. This is why Q30 is considered a benchmark for quality in next-generation sequencing (NGS). Finally, all three run control samples (NTC, HBCTL and POSCTL) need to pass for the run to pass. The validity criteria for these run controls is described in subsection ii. below.

### ii. Control validity criteria

Control validity is used to determine if the control samples have passed or failed. There are three run controls that all have to pass for the run to pass. Validity criteria is established separately for each of them. In order to pass, run controls have to pass both WES and WTS validity criteria which are established separately.

The first run control sample, No Template Control (NTC), does not contain any template DNA and is usually replaced with an equal amount of nuclease-free water and hence should ideally receive very few reads. For NTC to pass, the average read depth in WES should be <1x. Also, ratio of mapped reads to NTC and median of reads mapped to clinical samples within that flowcell should be < 5%.

For POSCTL, the second run control, average depth over the boosted panel should be at least 100x in WES and it should receive at least 20 million DNA reads to ensure that the biomarkers are called confidently. In addition, the variant allele frequency (VAF) of several reference biomarkers specific to this POSCTL should be in their allowable range of the pipeline.

For HBCTL, sample average depth should be at least 100x in WES and it should receive at least 20 million reads. In addition, the variant allele frequency (VAF) of several control biomarkers should be in their intended range. Additionally, for a



given set of reference genes, HBCTL should not report any pathogenic variants in them.

In addition to these run controls, an analyte control sample is also used (HBCNA), failure of which results in an invalid TMB, MSI and amplification results. For HBCNA to pass, the average WES depth should be >100x. Also, MSI, TMB and *ERBB2* copy values for HBCNA should be in a certain range. In addition, it should also not report amplification in any genes in a reference list.

iii. Sample validity criteria

Several WES and WTS validity criteria are also established for classifying a clinical sample as PASS or FAIL. A sample failing any number of these criteria is also referred to as QNS. WES and WTS QC results are recorded separately which are then used to determine which biomarkers are reportable for that sample. On WES side, the average read depth should be at least 100x and the sample should also receive at least 20 million reads. In addition, all cancer-type drug rules genes which are of high clinical significance to different cancer types should have valid results (non-indeterminate results). On WTS side, the clinical sample should have a certain number of total and on-target RNA reads.

**Table 4. MI Cancer Seek QC Process**

QC No.	QC Steps within Assay Workflow	Description	Impact of Failure
1	MI Cancer Seek Extraction Kit: QC Check for potential cross-contamination during TNA extraction with a negative control.	An extraction-negative control (EXT NEG) is added to each plate and does not contain tissue. The control is expected to not have measurable levels of TNA. The control must have DNA below a threshold DNA concentration measured with the MI Cancer Seek Quantification Kit.	If extraction negative control exceeds threshold, the whole plate will be re-extracted.
	MI Cancer Seek Extraction Kit: QC Check for sufficient yield during TNA extraction with an extraction positive control.	An extraction positive control (HBCTL) is a kit component and must meet a minimum threshold DNA concentration measured with the MI Cancer Seek Quantification Kit.	If extraction positive control does not exceed threshold, the whole plate will be re-extracted.
	MI Cancer Seek Extraction Kit: QC Check criteria for sufficient TNA extraction for each specimen.	Each specimen must meet a minimum threshold DNA concentration measured with the MI Cancer Seek Quantification Kit.	If the specimen does not exceed threshold, the sample will be re-extracted, with increased tissue input if possible.
2	Library Pooling QC Check for quantification of final library pool concentration.	Pooled library must be above the lower spec limit and below the upper spec limit DNA concentrations	For pools outside of the concentration range, the Qubit quantification is repeated, or the samples are re-normalized. The re-pool is

QC No.	QC Steps within Assay Workflow	Description	Impact of Failure
		measured with the MI Cancer Seek Library Quant Kit.	retested with the MI Cancer Seek Library Quant Kit.  If the re-pool fails, remake libraries or re-extract TNA.
3	MI Cancer Seek NGS sequencing metrics run validity criteria for DNA and RNA combined.	Reads Passing Filter (B) > 16 Yield (G) > 2,400 % of Q30 Reads (both R1 and R4) ≥ 75%	Run will be re-sequenced if one of the specified metrics fails. Repeat library preparation if necessary.
4	MI Cancer Seek NGS bioinformatics pipeline QC Check for negative control validity criteria for the run.	The EXT-NEG (NTC) DNA criteria will be below specified thresholds: depth of <1x and ratio of mapped reads <5%.	Repeat library preparation for whole plate using extracted TNA.
	MI Cancer Seek NGS bioinformatics pipeline QC Check for HBCTL positive control validity criteria for the run.	The HBCTL DNA target variants detected within lower and upper specification limits and validity criteria.	Repeat library preparation for whole plate using extracted TNA.
	MI Cancer Seek NGS bioinformatics pipeline QC Check for POSCTL positive control DNA validity criteria for the run.	The POSCTL DNA and RNA target variants within lower and upper specification limits and validity criteria.	Repeat library preparation for whole plate using extracted TNA.
	MI Cancer Seek NGS bioinformatics pipeline QC Check for HBCNA positive control DNA validity criteria for capabilities.	The HBCNA DNA validity criteria average depth ≥100x, DNA capabilities MSI value, TMB value and ERBB2 copy value for CNA will each be between a lower and upper specification limit criteria will be met.	The HBCNA specified capabilities will not be reported for the run.
	MI Cancer Seek NGS bioinformatics pipeline QC Check for DNA sample validity criteria (also referred to as QNS).	The sample DNA validity criteria will be met including ≥20 M total reads and average depth of ≥100x	If DNA read criteria is not met, then remake library from TNA.
	MI Cancer Seek NGS bioinformatics pipeline QC Check for Negative drug rules genes.	The sample is Negative for DNA variants if exons associated to the drug decision for the corresponding tumor type and validity criteria.	If validity criteria are not met, then remake the library from TNA.

## E. Variant calling criteria

In addition to the run and clinical sample validity criteria listed above, several variant calling criteria established separately for different biomarkers are used to determine which biomarkers are reportable in a passing sample in a successful run.

### a. SNV and Indels

For SNV and indels to be reported, i.e., receive a “Variant Detected” result, they should achieve  $\geq 20\times$  read depth with at least 5 supporting reads, have a minimum VAF of  $>5\%$  and should not have any poor-quality flags such as strand bias, false positives due to sequence reads containing residue to RNA or indel repeat of more than 8 nucleotides. A “low coverage” classification is assigned to any gene in the Reportable Gene list (**Table 2**) if there is no reportable variant from the gene and any exon in that gene has an average depth  $<100\times$  with the exception of a list of exons in which frequently report depth below threshold and no important variant have been observed. This category, also referred to as “No Call”, does not rule out the presence or absence of a mutation. With low coverage, a call cannot be reliably determined.

b. ERBB2 CNA

For *ERBB2* CNA to be reported, *ERBB2*  $\geq 6.9$  copies should be observed. *ERBB2* CNAs may also be reported if *ERBB2* has  $\geq 4.1$  copies with HER2/CEP17 ratio  $\geq 2.1$ . HER2/CEP17 ratio is a test that measures the number of HER2 gene copies on chromosome 17 (*ERBB2*) in relation to the number of chromosome 17 centromere (CEP17) copies per nucleus. Samples with  $\geq 3.3$  copies and *ERBB2*/CEP17  $\geq 2.1$  are deemed to be “Intermediate” for *ERBB2* CNAs. Samples with *ERBB2* CNA results not covered above are deemed not amplified for *ERBB2*. Patients with breast cancer whose samples receive *ERBB2* CNA “Intermediate” calls should be tested with another FDA approved or cleared test to ascertain *ERBB2* CNA status in their tumor. Patient’s specimens with average read depth of  $< 100\times$  are determined to be “Indeterminate” for *ERBB2* CNAs.

c. MSI

For MSI to be reported as high, MSI-High (MSI-H), the clinical sample should have  $\geq 39$  frameshift mutations. MSI is reported as not MSI-H if there are  $<39$  frameshift mutations and the average read depth of a panel of reference genes ( $N=753$ ) is  $\geq 100\times$ . Patient’s specimens with average read depth of  $< 100\times$  are determined to be “Indeterminate” for MSI.

d. TMB

TMB is reported quantitatively (mutations/megabase) and therefore has no reportable positive/negative result. Samples with a depth of coverage lower than  $100\times$  are considered “Indeterminate” and a result for TMB will not be provided. For samples with sufficient coverage, variants with at least  $5\%$  allele frequency identified across the whole exome are filtered to remove low quality, low depth, non-coding, synonymous, and any presumed germline variants which belong to gnomAD ( $AC>0$ ), dbSNP 151 common. From the filtered list, missense, nonsense, in-frame indel, and frameshift variants in selected coding regions with sufficient depth are counted. The final value is the variant count divided by the length of the assessed genomic regions (25 Mb), presented as mutations/Mb.

## F. Variant Classification for Companion Diagnostic Biomarkers

**Table 5** describes the biomarker rules for CDx claims in **Table 1** of the intended use reported by MI Cancer Seek.

**Table 5. Biomarker Rules for Companion Diagnostic Claims Reported by MI Cancer Seek**

Indication	Biomarker	Reportable Mutations
Breast Cancer	<i>PIK3CA</i> SNVs	C420R E542K; E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R H1047L, H1047R, H1047Y
Colorectal Cancer (CRC)	<i>KRAS</i> wild-type (exons 2, 3, and 4) and <i>NRAS</i> wild-type (exons 2, 3, and 4)	Wild-type as determined by a “Variant Not Detected” result in the following locations:  <i>KRAS</i> Exon 2: G12A, G12C, G12D, G12F, G12N, G12R, G12S, G12V, G12W, G13C, G13D (c.38G>A; c.38_39delinsAT), G13E, G13R, G13V <i>KRAS</i> Exon 3: A59G, A59T, Q61E, Q61H (c.183A>C; c.183A>T), Q61K, Q61L, Q61R <i>KRAS</i> Exon 4: A146P, A146T, A146V, K117N (c.351A>C; c.351A>T) <i>NRAS</i> Exon 2: G12A, G12C, G12D, G12F, G12N, G12R, G12S, G12V, G12W, G13C, G13D, G13E (c.38_39delinsAA; c.38_39delinsAG), G13R, G13V <i>NRAS</i> Exon 3: A59G, A59T, Q61E, Q61H (c.183A>T, c.183A>C), Q61K, Q61L, Q61R <i>NRAS</i> Exon 4: A146V, A146T, A146P, K117N (c.351G>C; c.351G>T)
	<i>BRAF</i> SNV	V600E
Melanoma	<i>BRAF</i> SNV	V600E, V600K
NSCLC	<i>EGFR</i> activating mutations ( <i>EGFR</i> exon 19 deletions and exon 21 SNV)*	Exon 21: L858R Exon 19 deletions: E746_S752delinsA, K745_A750del, E746_R748del, T751_E758del, T751_N756del, K757_I759delinsN, L747_K754del, E746_E749delinsQ, R748_T751del, E746_T751del, R748_K754del, P753_I759del, S752_A755del, E746del, R748_E749del, T751_A755del, L858R, E746_A750del, L747_T751del, S752_I759del, E746_T751delinsA, L747_P753delinsS, E746_T751delinsVP, E746_S752delinsV, L747_K754delinsATSPE, E746_T751delinsI, L747_E749del, L747_T751delinsP, L747_A755delinsSKD, L747_A750delinsP, T751_I759delinsN, E746_T751delinsIP, E746_S752delinsC, E746_A750delinsQP, A750_I759delinsSN, E746_T751delinsVA, L747_P753delinsQQ, L747_K754delinsG, L747_A750delinsS, E746_A750delinsIP, E746_T751delinsAA, L747_S752del, A750_I759delinsSSS, L747_A755delinsAT, E746_E749del,

		E746_K754delinsVSE, K754_D761delinsN, L747_T751delinsN, E746_A750delinsFP, L747_K754delinsPRE, E746_P753delinsVS, L747_S752delinsQ, L747_A755delinsSKG, L747_A755delinsAN, A750_I759delinsGD, L747_K754delinsSPE, E746_A750delinsKP, E746_T751delinsV, I744_E749delinsMKL, L747_K754delinsAISPE, E746_T751delinsL, E746_S752delinsI, L747_A750del, L747_P753delinsQ, E746_L747delinsIP, L747_K754delinsQQ, L747_A755delinsSQQG, L747_A755delinsGN, L747_P753del, E746_A750delinsAP, L747_A755delinsSRD, A750_I759delinsPT, T751_I759delinsS, I744_E749delinsLKR, E746_S752delinsD, E746_A750delinsSP, L747_A755delinsATSPEG, E746_S752delinsL, E746_P753delinsLS, R748_A750del, E746_S752delinsIPVA, A750_I759delinsSQQG, L747_A755delinsNRET, E746_T751delinsKP, E746_T751delinsLP, E746_T751delinsAP, L747_P753delinsT, E749_S752delinsD, L747_A755delinsTKD, L747_S752delinsSRD, E746_A750delinsRP, L747delinsFPSLS, E746_R748delinsIPVAIKE, K754_I759del, R748_A755delinsT, L747_A755delinsGT, L747_K754delinsSPQ, E746_P753delinsIS, E746_A750delinsYP, L747_I759delinsSKANKEL, E746_T751delinsFPS, E746_T751delinsIS, A750_I759delinsGG, E746_P753delinsSS, L747_A755delinsNNNN, E746_A750delinsVP, E746_T751delinsLA, E746_T751delinsQ, L747_S752delinsQH, E746_P753delinsAS, L747_K754delinsNIE, E746_E749delinsK, A750_T751delinsVP, L747_T751delinsS, E749_A755delinsD, L747_P753delinsQT, A750_E758delinsP, L747_K754delinsQE, L747_S752delinsPF
Solid Tumors	MSI	MSI-H
Endometrial Carcinoma	MSI	Not MSI-H
*More than one cDNA change may be associated with the same protein change. Mutations found in patients with the corresponding indication will be reported as a CDx for the associated therapies in the MI Cancer Seek Intended Use.		

## VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA-approved CDx alternatives for the detection of genetic alterations using FFPE tumor specimens, as listed in **Table 1** of the MI Cancer Seek intended use statement. The approved CDx tests are listed in **Table 6** below; for additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at <https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools>. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

**Table 6. List of FDA-Approved CDx Assays for Biomarkers Identified by MI Cancer Seek**

<b>Biomarker</b>	<b>Device</b>	<b>Company</b>	<b>Technology</b>	<b>Therapy</b>	<b>Indication</b>
<b>BRAF V600E</b>	<i>therascreen BRAF V600E RGQ PCR Kit</i>	<i>QIAGEN GmbH</i>	PCR <sup>2</sup>	Braftovi (encorafenib) in combination with Erbitux (cetuximab)	Colorectal Cancer
	<i>cobas 4800 BRAF V600 Mutation Test</i>	<i>Roche Molecular Systems, Inc.</i>	PCR <sup>2</sup>	Zelboraf (vemurafenib)	Melanoma Cancer
	<i>cobas 4800 BRAF V600 Mutation Test</i>	<i>Roche Molecular Systems, Inc.</i>	PCR <sup>2</sup>	Cotellic (cobimetinib) in combination with Zelboraf (vemurafenib)	Melanoma Cancer
	<i>FoundationOne CDx</i>	<i>Foundation Medicine, Inc.</i>	NGS <sup>1</sup>	Mekinist (trametinib)	Melanoma Cancer
	<i>THXID BRAF Kit</i>	<i>bioMérieux Inc.</i>	PCR <sup>2</sup>	Mekinist (trametinib)	Melanoma Cancer
	<i>THXID BRAF Kit</i>	<i>bioMérieux Inc.</i>	PCR <sup>2</sup>	Tafinlar (dabrafenib)	Melanoma Cancer
	<i>THXID BRAF Kit</i>	<i>bioMérieux Inc.</i>	PCR <sup>2</sup>	Braftovi (encorafenib) in combination with Mektovi (binimetinib)	Melanoma Cancer
<b>BRAF V600E/ BRAF V600K</b>	<i>FoundationOne CDx</i>	<i>Foundation Medicine, Inc.</i>	NGS <sup>1</sup>	Mekinist (trametinib) or BRAF/MEK Inhibitor Combinations approved by FDA	Melanoma Cancer
	<i>THXID BRAF Kit</i>	<i>bioMérieux Inc.</i>	PCR <sup>2</sup>	Mekinist (trametinib) or Braftovi (encorafenib) in combination with Mektovi (binimetinib)	Melanoma Cancer



Biomarker	Device	Company	Technology	Therapy	Indication
<b>EGFR – Exon 19 deletions &amp; L858R</b>	<i>cobas EGFR Mutation Test v2</i>	<i>Roche Molecular Systems, Inc.</i>	PCR <sup>2</sup>	EGFR Tyrosine Kinase Inhibitors approved by FDA*	NSCLC
	<i>FoundationOne CDx</i>	<i>Foundation Medicine, Inc.</i>	NGS <sup>1</sup>	EGFR Tyrosine Kinase Inhibitors approved by FDA*	NSCLC
	<i>ONCO/Reveal Dx Lung &amp; Colon Cancer Assay (O/RDx-LCCA)</i>	<i>Pillar Biosciences, Inc.</i>	NGS <sup>1</sup>	EGFR Tyrosine Kinase Inhibitors approved by FDA*	NSCLC
	<i>Oncomine Dx Target Test</i>	<i>Life Technologies Corporation</i>	NGS <sup>1</sup>	Iressa (gefitinib)	NSCLC
	<i>therascreen EGFR RGQ PCR Kit</i>	<i>Qiagen Manchester, Ltd.</i>	PCR <sup>2</sup>	Gilotrif (afatinib)	NSCLC
	<i>therascreen EGFR RGQ PCR Kit</i>	<i>Qiagen Manchester, Ltd.</i>	PCR <sup>2</sup>	Iressa (gefitinib)	NSCLC
	<i>therascreen EGFR RGQ PCR Kit</i>	<i>Qiagen Manchester, Ltd.</i>	PCR <sup>2</sup>	Vizimpro (dacomitinib)	NSCLC
<b>KRAS and NRAS</b>	<i>Praxis Extended RAS Panel</i>	<i>Illumina</i>	NGS <sup>1</sup>	Vectibix (panitumumab)	Colorectal Cancer
<b>MSI-H/dMMR</b>	<i>FoundationOne CDx</i>	<i>Foundation Medicine, Inc.</i>	NGS <sup>1</sup>	Keytruda (pembrolizumab)	Solid Tumor
	<i>Ventana MMR RxDx Panel</i>	<i>Ventana Medical Systems, Inc.</i>	IHC <sup>3</sup>	Keytruda (pembrolizumab)	Solid Tumor
	<i>Ventana MMR RxDx Panel</i>	<i>Ventana Medical Systems, Inc.</i>	IHC <sup>3</sup>	Jemperli (dostarlimab-gxly)	Solid Tumor

Biomarker	Device	Company	Technology	Therapy	Indication
Not MSI-H/ pMMR	<i>Ventana MMR RxDx Panel</i>	<i>Ventana Medical Systems, Inc.</i>	IHC <sup>3</sup>	Keytruda (pembrolizumab) in combination with Lenvima (lenvatinib)	Endometrial Cancer
PIK3CA	<i>FoundationOne CDx</i>	<i>Foundation Medicine, Inc.</i>	NGS <sup>1</sup>	Piqray (alpelisib)	Breast Cancer
	<i>therascreen PIK3CA RGQ PCR Kit</i>	<i>QIAGEN GmbH</i>	PCR <sup>2</sup>	Piqray (alpelisib)	Breast Cancer
<p>*For the most current information about the device indications for the therapeutic products in this group, go to: <a href="https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools#Group_Labeling">https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools#Group_Labeling</a></p> <p>Abbreviations:</p> <p><sup>1</sup> NGS = Next Generation Sequencing</p> <p><sup>2</sup> PCR = Polymerase Chain Reaction</p> <p><sup>3</sup> IHC = Immunohistochemistry</p>					

## VII. MARKETING HISTORY

Caris Life Sciences indicates the MI Cancer Seek assay was offered as a laboratory developed test (LDT) with the name “MTS LDT” since November 2022. As described in the preamble to FDA’s LDT final rule, FDA uses the term "IVDs offered as LDTs" for IVDs that are manufactured and offered as LDTs by laboratories that are certified under CLIA and that meet the regulatory requirements under CLIA to perform high complexity testing, and used within such laboratories, even if those IVDs do not fall within FDA’s traditional understanding of an LDT because they are not designed, manufactured, and used within a single laboratory.

## VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions. Patients with false positive results may be inappropriately treated with one of the therapies listed in the above intended use statement without clinical benefit and may experience adverse reactions associated with inappropriate therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy.

For the specific adverse events related to the approved therapeutics, please see approved drug product labels which are available at [Drugs@FDA](mailto:Drugs@FDA).

## **IX. SUMMARY OF NON-CLINICAL STUDIES**

### **A. Laboratory Studies**

Caris Life Sciences executed 16 analytical validation studies to support the CDx and tumor profiling claims indicated in the intended use statement. Studies were performed using FFPE specimens and TNA extracted from a wide range of FFPE tissue types having various genomic alterations across a number of genes. Studies included CDx variants, *ERBB2* copy number amplification, as well as a broad range of representative SNVs and indels for tumor profiling. Analysis of the genomic signatures for MSI and TMB was also performed. See Section IX-A below for analytical validation study summaries.

#### **1. Analytical Accuracy**

##### **a. Comparison to an Orthogonal Method for Tumor Profiling (SNV and indels) and TMB**

This study demonstrated accuracy of Tumor Profiling variants (SNVs, MNVs and indels) and TMB for the MI Cancer Seek assay by comparing variant calling using two orthogonal validated NGS assays. A total of 500 clinical FFPE samples across 38 tumor types were consecutively enrolled based on tumor type and tissue requirements to support testing with three assays (MI Cancer Seek and two orthogonal NGS assays). To evaluate variant accuracy, samples were tested on an FDA-cleared commercially available Targeted Gene Panel assay and processed per the assay Instructions for Use (IFU) using optimal input (100 ng) where possible.

To evaluate TMB accuracy, samples were tested using the MI Cancer Seek and an externally validated WES assay. Samples for MI Cancer Seek were processed using the complete test workflow starting with extraction and the majority were tested at the minimal allowable input (50 ng) to allow for more challenging comparisons.

##### **Accuracy of SNVs, MNVs, and Indels Concordance for Tumor Profiling**

A total of 500 samples were tested on both MI Cancer Seek and the Targeted Gene Panel assay, of which 454 samples were analyzed to determine variant accuracy across 131 genes and 527 exons having reportable pathogenic or likely pathogenic variants detected by both assays. Forty-six (46) samples were excluded from the concordance analysis due to either sample quality or sample swaps that occurred during orthogonal method testing. The apparent sample swap for 30 cases involved the Targeted Gene Panel assay, therefore these samples were excluded because definitive evidence for the sample swap outside of the data analysis could not be obtained. The accuracy results are summarized by variant type, and further stratified by FDA's Biomarker Class Level for tumor profiling in **Table 7**.

**Table 7: Agreement Summary for SNVs, MNVs, Insertions and Deletions for Tumor Profiling**

Variant Type	Total Unique Variants	MI Cancer Seek (+) Comparator (+)	MI Cancer Seek (+) Comparator (-)	MI Cancer Seek (-) Comparator (+)	MI Cancer Seek (-) Comparator (-)	PPA (n/N) [95% CI]	NPA (n/N) [95% CI]
<b>All Variants</b>	170636	1177	215	54	77467298	95.61% (1177/1231) [94.31-96.69]	100.00% (77467298/77467513) [100.00-100.00]
<b>All SNVs</b>	50425	923	86	46	22891895	95.25% (923/969) [93.72-96.50]	100.00% (22891895/22891981) [100.00-100.00]
Level 1 SNVs	15	67	0	4	6739	94.37% (67/71) [86.20-98.44]	100.00% (6739/6739) [99.95-100.00]
Level 2 SNVs	25018	617	46	31	11357478	95.22% (617/648) [93.28-96.73]	100.00% (11357478/11357524) [100.00-100.00]
Level 3 SNVs	25392	239	40	11	11527678	95.60% (239/250) [92.26-97.78]	100.00% (11527678/11527718) [100.00-100.00]
<b>All MNVs</b>	4563	3	12	0	2071587	100.00% (3/3) [29.24-100.00]	100.00% (2071587/2071599) [100.00-100.00]
Level 1 MNVs	29	0	0	0	13166	NA (0/0) [0-0]	100.00% (13166/13166) [99.97-100.00]
Level 2 MNVs	2879	3	10	0	1307053	100.00% (3/3) [29.24-100.00]	100.00% (1307053/1307063) [100.00-100.00]
Level 3 MNVs	1655	0	2	0	751368	NA (0/0) [0-0]	100.00% (751368/751370) [100.00-100.00]
<b>All Insertions</b>	31090	59	23	0	14114778	100.00% (59/59) [93.94-100.00]	100.00% (14114778/14114801) [100.00-100.00]
Level 1 Insertions	2	0	0	0	908	NA (0/0) [0-0]	100.00% (908/908) [99.59-100.00]
Level 2 Insertions	16851	27	12	0	7650315	100.00% (27/27) [87.23-100.00]	100.00% (7650315/7650327) [100.00-100.00]
Level 3 Insertions	14237	32	11	0	6463555	100.00% (32/32) [89.11-100.00]	100.00% (6463555/6463566) [100.00-100.00]

Insertions 1-5bp	26065	54	21	0	11833435	100.00% (54/54) [93.40-100.00]	100.00% (11833435/11833456) [100.00-100.00]
Insertions 6-10bp	2044	2	0	0	927974	100.00% (2/2) [15.81-100.00]	100.00% (927974/927974) [100.00-100.00]
Insertions 11-15bp	669	2	0	0	303724	100.00% (2/2) [15.81-100.00]	100.00% (303724/303724) [100.00-100.00]
Insertions 16-20bp	643	1	1	0	291920	100.00% (1/1) [2.50-100.00]	100.00% (291920/291921) [100.00-100.00]
Insertions 21-25bp	457	0	0	0	207478	NA (0/0) [0-0]	100.00% (207478/207478) [100.00-100.00]
Insertions >25bp	1212	0	1	0	550247	NA (0/0) [0-0]	100.00% (550247/550248) [100.00-100.00]
<b>All Deletions</b>	84558	192	94	8	38389038	96.00% (192/200) [92.27-98.26]	100.00% (38389038/38389132) [100.00-100.00]
Level 1 Deletions	166	0	0	1	75363	0.00% (0/1) [0.00-97.50]	100.00% (75363/75363) [100.00-100.00]
Level 2 Deletions	48564	100	37	3	22047916	97.09% (100/103) [91.72-99.40]	100.00% (22047916/22047953) [100.00-100.00]
Level 3 Deletions	35828	92	57	4	16265759	95.83% (92/96) [89.67-98.85]	100.00% (16265759/16265816) [100.00-100.00]
Deletions 1-5bp	51675	179	84	8	23460179	95.72% (179/187) [91.74-98.14]	100.00% (23460179/23460263) [100.00-100.00]
Deletions 6-10bp	8958	7	4	0	4066921	100.00% (7/7) [59.04-100.00]	100.00% (4066921/4066925) [100.00-100.00]
Deletions 11-15bp	7013	1	1	0	3183900	100.00% (1/1) [2.50-100.00]	100.00% (3183900/3183901) [100.00-100.00]
Deletions 16-20bp	5553	0	0	0	2521062	NA (0/0) [0-0]	100.00% (2521062/2521062) [100.00-100.00]
Deletions 21-25bp	3198	2	1	0	1451889	100.00% (2/2) [15.81-100.00]	100.00% (1451889/1451890) [100.00-100.00]
Deletions >25bp	8161	3	4	0	3705087	100.00% (3/3) [29.24-100.00]	100.00% (3705087/3705091) [100.00-100.00]

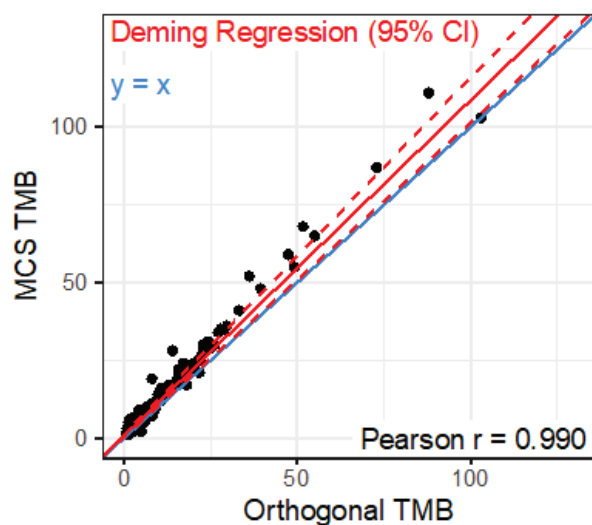
The discordances observed were due to design differences, including variant calling thresholds and variant calling rules, between the orthogonal Targeted Gene Panel Assay and MI Cancer Seek. Twenty-seven (27) false negative variants were observed because the orthogonal Targeted Gene Panel threshold for positive results is lower than the MI Cancer Seek threshold of 5% variant frequency. An additional 19 false

negatives were the result of differences in merging of multiple variant types between the two assays. For the majority of false positives, variant alignments were present for the Targeted Gene Panel assay, but the calls were not reported.

### **TMB Concordance**

A total of 500 samples were tested on both MI Cancer Seek and the externally validated whole exome sequencing assay, of which 497 samples were analyzed to determine TMB accuracy across 38 different tumor types. Three (3) samples were excluded from analysis due to sample swaps (there is an apparent sample swap for 2 cases involved with the whole exome sequencing assay, therefore these samples were excluded because definitive evidence for the sample swap outside of the data analysis could not be obtained) or QC failure during orthogonal method testing. A linear regression analysis was completed using Deming regression to estimate slope and intercept along with 95% confidence intervals (CIs) for the measured TMB values between the two assays, as shown in **Figure 1**. This analysis reflects low-coverage regions (<50x) that underperformed in the orthogonal method and artificially underestimates the orthogonal method TMB value, which contributed to the observed discordances. Variants were not detected in regions of low coverage by the orthogonal method and therefore did not contribute to the TMB value but were detected in these regions by MI Cancer Seek and contributed to the TMB value for a given sample. This was further evaluated by calculating TMB for a subset of exome regions where both assays (MI Cancer Seek and orthogonal assay) had greater or equal to 50x depth of coverage. The slope and intercept of the Deming regression both improved from 0.856 to 0.927 and -0.859 to -0.68, respectively. These data indicate TMB calls between MI Cancer Seek and the orthogonal method are concordant when coverage regions  $\geq 50x$  are evaluated.

**Figure 1: TMB Accuracy Using Deming Regression Analysis**





**b. Comparison to an Orthogonal Fluorescence In Situ Hybridization (FISH) Assay for *ERBB2* CNAs**

The accuracy of MI Cancer Seek was evaluated for the detection of *ERBB2* CNAs in patient samples with breast cancer. A total of 288 unique FFPE tissue samples from patients histologically confirmed as breast cancer were enrolled and tested with FDA approved fluorescence in situ hybridization (FISH), PathVysion HER2 DNA Probe Kit, and MI Cancer Seek. Eight samples did not meet MI Cancer Seek testing requirements; therefore, 280 samples were tested on MI Cancer Seek.

In the 280 samples tested on both MI Cancer Seek and FISH assay, five (5) samples did not have valid FISH assay results. Out of the 275 samples that had valid FISH results, one sample did not have valid MI Cancer Seek result. One (1) sample had intermediate MI Cancer Seek result. Concordance between MI Cancer Seek and the FISH assay is shown in **Table 8**.

**Table 8. Agreements between the MI Cancer Seek and Orthogonal FISH assay for *ERBB2* CNAs in samples from patients with breast cancer**

	FISH+	FISH-	Total*
MI Cancer Seek +	99	1	100
MI Cancer Seek Intermediate	1	0	1
MI Cancer Seek -	18	155	173
MI Cancer Seek Invalid	1	0	1
Total	119	156	275

\*There were another 5 FISH invalid samples.

Concordance results from **Table 8**, treating the MI Cancer Seek “Intermediate” result as positive or negative result were used to determine agreements shown in **Table 9**. Using the FISH assay results as reference and treating the MI Cancer Seek “Intermediate” results as “Positive” results, the PPA is 84.7% with two-sided 95% confidence interval (77.2%, 90.1%), and NPA is 99.4% with two-sided 95% confidence interval (96.5%, 99.9%). When treating MI Cancer Seek “Intermediate” results as “Negative” results, the PPA is 83.9% with two-sided 95% confidence interval (76.2%, 89.4%) and NPA is 99.4% with two-sided 95% confidence interval (96.5%, 99.9%).

**Table 9. Agreement Summary for *ERBB2* CNAs in breast cancer for Tumor Profiling**

	Agreement (95% CI*) Intermediate as Positive	Agreement (95% CI*) Intermediate as Negative
PPA	84.7% (77.2%, 90.1%)	83.9% (76.2%, 89.4%)
NPA	99.4% (96.5%, 99.9%)	99.4% (96.5%, 99.9%)

\*95% confidence intervals were calculated by Wilson score method.

Based on the PPA observed for the detection of *ERBB2* CNAs, this alteration may not be detected in patients with breast cancer. Additional clinical investigation to confirm the presence of *ERBB2* CNAs in the breast cancer patient's tumor with another FDA approved or cleared test is strongly recommended.

For additional concordance data for the CDx-associated variants, refer to the Summary of Primary Clinical Studies in Section X.

## 2. Analytical Sensitivity

### a. Limit of Blank

This study was designed to estimate the Limit of Blank (LoB) or false positive call rate of MI Cancer Seek assay using adjacent non-tumor tissue across multiple tissue types. A total of 168 data points were generated across 28 FFPE non-tumor samples and 15 tissue types using two lots of reagents. Each sample was extracted in triplicate using one lot of extraction reagent and further utilized to prepare libraries at maximum DNA input (220 ng). A total of six library replicates were prepared and sequenced per sample using two lots of reagents. No false positives were detected for Level 1 CDx biomarkers, Level 2 SNV/indels, or Level 3 indels; for Level 3 SNVs, <0.01% false positives were detected, refer to **Table 10**.

**Table 10. LoB Study Summary Results**

Category	Per Position False Positive Rate	Per Sample False Positive Rate*
Level 1: <i>BRAF</i> V600E	0%	0%
Level 1: <i>BRAF</i> V600K	0%	0%
Level 1: <i>EGFR</i> Exon 19 deletions	0%	0%
Level 1: <i>EGFR</i> Exon 21 substitution mutations	0%	0%
Level 1: <i>PIK3CA</i> C420R	0%	0%
Level 1: <i>PIK3CA</i> E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R	0%	0%
Level 1: <i>PIK3CA</i> H1047L, H1047R, H1047Y	0%	0%
Level 1: <i>KRAS</i> SNVs	0%	0%
Level 1: <i>NRAS</i> SNVs	0%	0%
Level 1: MSI-H	N/A	0%
Level 2: <i>ERBB2</i> CNA	N/A	0%
Level 2: Panel-wide SNVs (924)	0%	0%

Level 2: Panel wide Indels (950)	0%	0%
Level 3: Panel-wide SNVs (50710)	0.000035% (3/50710*168)	1.8% (3/168)
Level 3: Panel wide Indels (115407)	0%	0%
*Calculated from 2 reagent lots (n=168 data points)		

The 3 false positives in Level 3: Panel wide SNVs were each from TERT c.-124C>T and had variant frequencies near the threshold of 5% at 5.3%, 5.2% and 5.8%.

An additional study was performed using 10 WT FFPE samples, including 4 breast tissue and 6 lung tissue samples, across two reagent lots. A total of 240 additional data points were generated across two tissue types using two reagent lots at maximum DNA input for the assay. There were no false positive calls for *ERBB2* amplification in both breast tissue and lung tissue. The data also demonstrated 0% false positive rates for each SNV, indel, and MSI in both breast tissue and lung tissue, as shown in Table 11.

**Table 11. Supplemental LoB Study Results Per Position and Sample**

Level & Variant Class	Per Position False Positive Rate	Per Sample False Positive Rate*
Level 1: <i>BRAF</i> V600E	0%	0%
Level 1: <i>BRAF</i> V600K	0%	0%
Level 1: <i>EGFR</i> Exon 19 Dels	0%	0%
Level 1: <i>EGFR</i> L858R	0%	0%
Level 1: <i>PIK3CA</i> C420R	0%	0%
Level 1: <i>PIK3CA</i> E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R	0%	0%
Level 1: <i>PIK3CA</i> H1047L, H1047R, H1047Y	0%	0%
Level 2: Panel-wide SNVs (N=924)	0%	0%
Level 2: Panel-wide Indels (N=950)	0%	0%
Level 3: Panel-wide SNVs (N=50710)	0%	0%
Level 3: Panel-wide Indels (N=115407)	0%	0%
Level 1: MSI	N/A	0%
Level 1: <i>ERBB2</i> Amplifications	N/A	0%
*Calculated from 2 reagent lots (n=240 data points)		

#### **b. Limit of Detection for SNVs and Indels**

Due to the large number of variants detected by the MI Cancer Seek across multiple tissue types and the limitation of testing them all to support tumor profiling claims, Limit of Detection (LoD) of SNVs and indels were determined using a representative approach. A total of 29 samples were tested at minimal DNA input (50 ng), including

12 samples to support CDx biomarker claims and 17 samples to support tumor profiling claims. At least one variant was tested per CDx biomarker for its respective tumor type. A total of 100 data points (ten replicates x five dilution levels x two reagent lots) were generated per panel member for the 12 CDx biomarkers and 25 data points (five replicates x five dilution levels x one reagent lot) were generated per sample for the 17 tumor profiling markers. Additional testing was performed for tumor profiling marker *PIK3CARI* N453 Y463del, and total of 35 data points were generated across seven dilution levels as repeat testing using one lot of reagents. The LoD for each panel member was determined as the lowest VAF level with  $\geq 95\%$  detection (Hit rate =0.95) based on the positivity threshold of 5%. The LoD for SNVs and insertions ranged from 6-11% VAF, and LoD for deletions ranged from 6-8%VAF. The LoD for CDx biomarkers and tumor profiling markers are shown in **Table 12** and **Table 13**, respectively.

**Table 12. LoD for CDx Biomarkers**

Variant Type	Mutation	Tumor Type	LoD (%VAF)
SNV	<i>BRAF</i> V600E	Colorectal Adenocarcinoma	11%
SNV	<i>BRAF</i> V600E	Melanoma	7%
SNV	<i>BRAF</i> V600K	Melanoma	8%
Indel	<i>EGFR</i> L747 T751del	NSCLC)	7%
SNV	<i>EGFR</i> L858R	NSCLC	7%
SNV	<i>KRAS</i> A146T	Colorectal Adenocarcinoma	8%
SNV	<i>KRAS</i> G12C	Colorectal Adenocarcinoma	7%
SNV	<i>KRAS</i> Q61H	Colorectal Adenocarcinoma	10%
SNV	<i>NRAS</i> A146T	Colorectal Adenocarcinoma	11%
SNV	<i>NRAS</i> G12A	Colorectal Adenocarcinoma	7%
SNV	<i>NRAS</i> Q61R	Colorectal Adenocarcinoma	10%
SNV	<i>PIK3CA</i> E542K	Breast Carcinoma	11%

**Table 13. LoD for Tumor Profiling Alterations**

Variant Type	Mutation	Tumor Type	LoD (%VAF)
Insertion (Long homopolymer)	<i>ARID1A</i> A330fs	Uterine Neoplasms - Endometrial carcinoma	8%
SNV	<i>ARID1A</i> Q575*	Uterine Neoplasms - Endometrial carcinoma	6%

Variant Type	Mutation	Tumor Type	LoD (%VAF)
Deletion (Long non-homopolymer)	<i>ASXL1</i> E635fs	Ovarian Surface Epithelial Carcinomas	7%
SNV	<i>BRCA1</i> M1775R	Breast Carcinoma	7%
SNV	<i>BRCA2</i> S1882*	Prostatic Adenocarcinoma	8%
Insertion	<i>BRCA2</i> T2125fs	Prostatic Adenocarcinoma	11%
Insertion	<i>EGFR</i> A767 V769dup	NSCLC	9%
Insertion	<i>ERBB2</i> G776delinsVC	NSCLC	9%
Insertion (Long non-homopolymer)	<i>FANCA</i> D944fs	Colorectal Adenocarcinoma	8%
Insertion	<i>NF1</i> I679fs	Uterine Neoplasms - Endometrial carcinoma	6%
SNV	<i>NOTCH1</i> Y550fs	Low Grade Glioma	7%
Deletion (Long homopolymer)	<i>PIK3R1</i> N453_Y463del	Colorectal Adenocarcinoma	8%
Insertion (Short homopolymer)	<i>SMAD2</i> L175fs	Breast Carcinoma	9%
SNV	<i>SMARCA4</i> R1005*	Colorectal Adenocarcinoma	7%
Deletion (Short homopolymer)	<i>SUFU</i> T13fs	Melanoma	6%
SNV	<i>TERT</i> c.-146C>T	Glioblastoma	11%
SNV	<i>TP53</i> G266V	Ovarian Surface Epithelial Carcinomas	6%

**c. Limit of Detection for *ERBB2* CNA**

The LoD for *ERBB2* CNA was also established by testing two *ERBB2* breast cancer samples at five different dilution levels, using two reagent lots and 10 replicates per lot per dilution level for a total of 100 observations per sample. The samples were run at minimum DNA input (50ng). The LoD for each panel member was determined to be the lowest level of copies (CNA) with  $\geq 95\%$  detection (Hit rate =0.95). and these data established 8.3 copies as the LoD for *ERBB2* CNA.

#### **d. Tumor Purity**

The lowest level of tumor content that supports robust performance of MI Cancer Seek was evaluated. A total of 12 unique samples from nine tumor types were prepared and tested at minimal DNA input (50ng) across different variant classes including CNA (2), MSI (9), and TMB (10). At least five dilution levels were prepared per sample resulting in a total of 100 data points (ten replicates x five dilution levels x two reagent lots) per sample for 14 CDx biomarkers using two reagent lots. The results demonstrated LoD for tumor content was between 5 -20% across the various samples and variant classes, which confirmed performance of MI Cancer Seek at the minimum requirement of 20% tumor content.

#### **e. DNA Input Study**

The study evaluated performance of MI Cancer Seek at six DNA input levels to not only verify performance at the assay minimum (50 ng) and maximum/optimum DNA input (220 ng) but to also guard band around these required levels. The DNA input levels tested included 25, 37.5, 50, 220, 275 and 330 ng, which correspond to -25% and -50% of the minimum requirement and +25% and +50% of the maximum requirement. The study tested 16 samples near LoD (1-3x). A minimum of three replicates per sample were processed through library preparation and sequencing using one lot of reagents for each input level. A total of 18 data points were generated per panel member for SNVs, indels, MSI and TMB, and 36 data points were generated for *ERBB2* CNA. The PPA and NPA for SNVs, indels, *ERBB2* CNA, and MSI were 100% between minimum and maximum DNA input. In addition, for TMB the upper bound of the 95% CI for absolute percent difference in mean TMB values between minimal and optimal inputs ranged 0.0% - 10.5%.

### **3. Analytical Specificity**

#### **a. Interfering Substances – Exogenous**

This study evaluated tolerance to exogenous substances that are introduced during sample processing and are possibly carried over to the next steps in the assay workflow when using MI Cancer Seek assay. Substances tested included paraffin, xylene, Proteinase K, and 80% ethanol, spiked at the appropriate step to mimic carryover of the substance being tested. A total of 17 FFPE clinical samples from eight different tissue types containing genetic alterations at 2-3x LoD were tested across all capabilities including SNVs, indel, *ERBB2* CNA, MSI, and TMB using a single reagent lot. A total of 425 data points (17 FFPE clinical samples x five replicates x five conditions) were generated across all interferent conditions including test and nominal/control condition.

For *ERBB2* CNA, PPA was reported at 100% for all conditions and NPA was reported at 100% for all conditions except Control vs Proteinase K, where NPA was reported at 98.7%. For indels, PPA and NPA were both reported at 100% for all



conditions. For SNVs, PPA was reported at 100% for all conditions except Control vs Proteinase K, where PPA was reported at 97.4% and NPA was reported at 100% for all conditions. For MSI, PPA was reported at 100% for all conditions except Control vs Proteinase K, where PPA was reported at 93.3% and NPA was reported at 100% for all conditions.

There was no significant impact to TMB detection ability in the presence of the tested exogenous substances. TMB included 0 for the 95% 1-sided CI around the mean difference between all TMB test and control condition.

#### **b. Interfering Substances – Endogenous**

To determine the potential impact of endogenous interfering substances on the performance of the MI Cancer Seek assay, in their respective tumor tissue samples for both CDx claims and tumor profiling claims, this study evaluated 23 clinical FFPE samples from ten tumor types (breast carcinoma, CRC, melanoma, NSCLC, small intestine malignancies, pancreatic adenocarcinoma, uterine neoplasms-endometrial carcinoma, prostatic adenocarcinoma, thyroid, cholangiocarcinoma), which were mutation positive samples selected at 2-3x LoD from the tissue types associated with the endogenous interfering substances. For each sample, five replicates each were processed in parallel through the complete assay workflow using one reagent lot at minimum DNA input (50 ng) for both a sample spiked with the potential endogenous interfering substance (refer to **Table 14** for the interferents evaluated) and for the corresponding control condition (without the introduction of potentially interfering substance). A total of 130 data points were obtained for both endogenous interferent positive and endogenous interferent negative (control) replicates yielding a total of 260 data points.

**Table 14. Interferents Evaluated**

<b>Substance</b>	<b>Tumor Type</b>
Control Condition	All tumor types
Hemoglobin	All tumor types
Colloid	Thyroid
Calcium Phosphate	Thyroid
Mucin	CRC/Prostate
Bile acids, conjugated	Cholangiocarcinoma

For *ERBB2* CNA, indel, and SNVs, both NPA and PPA were 100% and no deleterious effect between control and test conditions were observed when tested with endogenous potential interfering substances in intended tumor types.

For TMB, the mean of each sample at each condition was calculated. The mean difference between test and control conditions across all samples was determined and the distribution of TMB differences were evaluated to obtain the one-sided 95% CI for the mean difference. The results demonstrated that comparison of conditions with hemoglobin, calcium, colloid, mucin, and bile acids (Test) to without interfering

substance (Control) included the 0 for the one-sided 95% CI of the mean difference thus passing the study acceptance criteria for all conditions tested.

For MSI, NPA was reported at 97.6% (Control vs Hemoglobin), 93.8% (Control vs Calcium), 100% (Control vs Mucin), 100% (Control vs Bile Acids) and 100% (Control vs Colloid). PPA was reported at 91.3% (Control vs Hemoglobin), 100% (Control vs Calcium), 100% (Control vs Mucin), 100% (Control vs Bile Acids) and 50% (Control vs Colloid). NPA for MSI was impacted by the presence of Colloid. However, the only positive sample selected had replicate values that were near the assay cut-off in the control replicates and test replicates. Thus, a lower PPA is expected for this sample. Further no significant difference between the test and control sample using the same analysis method described above for TMB, this condition passes, showing that it is the sample itself, rather than colloid, that is the root cause of lower NPA.

Performance of MI Cancer Seek in samples with high level presence of necrotic tissue, melanin and fatty acids has not been demonstrated yet. A post market study will be conducted to assess the impact of these potential interferents.

**c. Carryover and Cross-Contamination**

This study was designed to assess the potential of erroneous results due to carryover (run to run) and cross-contamination (within run) throughout the complete MI Cancer Seek assay workflow, at the highest DNA input of 220 ng. A total of 172 data points (85 positive and 87 negative sample replicates) were generated for cross-contamination and 172 data points (86 positive and 86 negative samples replicates) were obtained for carryover using 30 unique FFPE samples (14 high positive and 16 negative) from five tumor types across one lot of reagents and one set of instruments. For the purpose of this study, TMB values were divided into two levels, 1-9 mut/Mb, and  $\geq 10$  mut/Mb. The results demonstrated that the NPA for SNV, indels, MSI, TMB, and *ERBB2* CNA was 100% for cross-contamination and carryover.

**d. Index Cross-Contamination**

The study evaluated index cross-contamination due to potential index hopping and was designed to demonstrate that the rates of index cross contamination do not significantly affect the MI Cancer Seek assay results. The study utilized previously obtained results from 300 randomly selected samples across 20 different flowcells each having a unique index per plate, from previously performed MI Cancer Seek analytical validation studies. Results demonstrated that there was <0.00% cross contamination in all 20 flowcells evaluated. Additionally, all 300 samples had <0.00% cross contamination, which shows that the degree of index hopping does not impact MI Cancer Seek assay performance.

#### e. Hybrid Capture Bait Specificity- *in silico* Analysis

Specificity of the Hybrid Capture Baits (MI Cancer Seek Baits) was evaluated for reportable regions in the MI Cancer Seek assay, including the ability to differentiate between target analyte sequences and sequences generated from other sources (i.e. off-target sequences), using results from random selection of ten flowcells and 300 samples previously tested across MI Cancer Seek analytical validation studies. This study demonstrated that capture of off-targeted sequences does not significantly affect MI Cancer Seek performance. The study confirmed bait specificity, as the mean average depths of coverage were 757x in reportable regions and 1.5x in off-targeted regions. The mean percent off-target coverage was 0.19% of the on-target coverage in reportable regions (ranged from 0.08% to 0.33%). CDx-specific regions represent a small portion of the overall reportable regions, which was confirmed by the percentage of reads mapping to CDx regions (mean of 0.22%), and this study indicates high quality reads are also obtained in the CDx regions given the mean average depth of coverage was 958x.

#### 4. Precision

This study was performed to evaluate MI Cancer Seek repeatability (within-run) and reproducibility (total within-laboratory) under varied conditions including different reagent lots, instruments, operators, and non-consecutive run days. A total of 49 panel members representing 14 different tumor types were selected to evaluate CDx markers, challenging tumor profiling variant types including short/long indels in non-homopolymer/homopolymer regions, and the other biomarkers reported by MI Cancer Seek (MSI, *ERBB2* CNA, and TMB). Of the 49 panel members, 37 were evaluated at low and high dilution level relative to LoD at 1-1.5x and 2-3x for targeted SNVs and indels yielding 74 samples. Two panel members consisted of *ERBB2* CNA positive samples with 1-1.5x and 2-3x LoD levels. The remaining panel members consisted of samples selected to have MSI-H or Not MSI-H status, TMB with various mutations/Mb levels, or no specific mutation (2 panel members). In total there were 86 samples evaluated in the precision study. The study included three operator teams, three instrument sets, three reagent lots and was minimally executed across three non-consecutive days over a 20-day span. This study design resulted in 36 data points per sample. There were no invalid data points for the expected positive results across of replicates per all the samples tested in this study. A call was considered no call if the corresponding exon has a depth equal to or higher than 100x and the target variant was not detected.

Agreements for targeted CDx variants and tumor profiling biomarkers evaluated are presented in **Table 15**. Targeted variants refer to the variants that were the basis for selection of the sample for evaluation in the study. All the CDx biomarkers tested for SNVs and indels had 100% positive agreements for both the levels. *ERBB2* CNA at 2-3x LoD and MSI-H with medium positives (level well above the cut-off) resulted in 100% positive agreement as well, *ERBB2* CNA at 1-1.5x LoD had positive agreement of 97.2% and MSI-H with low positives (close to the cut-off) had agreements of 91.7% and 97.2% in the 2 Panel Members tested. The two samples with not MSI-H had agreements of

100% and 94.4%; lower agreement corresponding to the not MSI-H sample that had a level close to the cut-off.

**Table 15. Agreements for Targeted CDx and Tumor Profiling Variants reported by MI Cancer Seek**

Sample	Lineage	Variant	Bio-marker Type	Fold LoD Level***	Average VAF, MSI Score, or ERBB2 Copies	Number Positive / Number Expected	Agreement (95% CI)
1	Breast Carcinoma	PIK3CA C420R	CDx	1-1.5x	0.139	36/36	100% (90.3%, 100.0%)
				2-3x	0.289	36/36	100% (90.3%, 100.0%)
2	Breast Carcinoma	PIK3CA E542K	CDx	1-1.5x	0.159	36/36	100% (90.3%, 100.0%)
				2-3x	0.187	36/36	100% (90.3%, 100.0%)
3	Breast Carcinoma	PIK3CA H1047R	CDx	1-1.5x	0.135	36/36	100% (90.3%, 100.0%)
				2-3x	0.236	36/36	100% (90.3%, 100.0%)
4	Colorectal Adenocarcinoma	BRAF V600E	CDx	1-1.5x	0.124	36/36	100% (90.3%, 100.0%)
				2-3x	0.21	36/36	100% (90.3%, 100.0%)
5	Colorectal Adenocarcinoma	KRAS A146T	CDx	1-1.5x	0.112	36/36	100% (90.3%, 100.0%)
				2-3x	0.169	36/36	100% (90.3%, 100.0%)
6	Colorectal Adenocarcinoma	KRAS G12C	CDx	1-1.5x	0.302	36/36	100% (90.3%, 100.0%)
				2-3x	0.518	36/36	100% (90.3%, 100.0%)
7	Colorectal Adenocarcinoma	KRAS Q61K	CDx	1-1.5x	0.093	36/36	100% (90.3%, 100.0%)
				2-3x	0.3	36/36	100% (90.3%, 100.0%)
8	Colorectal Adenocarcinoma	NRAS A146V	CDx	1-1.5x	0.143	36/36	100% (90.3%, 100.0%)
				2-3x	0.223	36/36	100% (90.3%, 100.0%)
9	Colorectal Adenocarcinoma	NRAS G12A	CDx	1-1.5x	0.094	36/36	100% (90.3%, 100.0%)
				2-3x	0.189	36/36	100% (90.3%, 100.0%)
10	Colorectal Adenocarcinoma	NRAS Q61R	CDx	1-1.5x	0.153	36/36	100% (90.3%, 100.0%)
				2-3x	0.214	36/36	100% (90.3%, 100.0%)
11	Female Genital Tract Malignancy	MSI-H	CDx	Medium Positive	58.417	36/36	100% (90.3%, 100.0%)
12	Lung Non-small cell lung cancer (NSCLC)	EGFR L747_T751del	CDx	1-1.5x	0.108	36/36	100% (90.3%, 100.0%)
				2-3x	0.195	36/36	100% (90.3%, 100.0%)
13	Lung Non-small cell lung cancer (NSCLC)	EGFR L858R	CDx	1-1.5x	0.089	36/36	100% (90.3%, 100.0%)
				2-3x	0.187	36/36	100% (90.3%, 100.0%)
14**	Melanoma	BRAF V600E	CDx	1-1.5x	0.078	36/36	100% (90.3%, 100.0%)
				2-3x	0.18	36/36	100% (90.3%, 100.0%)
15	Ovarian Surface Epithelial Carcinomas	MSI-H	CDx	Medium Positive	106.722	36/36	100% (90.3%, 100.0%)

Sample	Lineage	Variant	Bio-marker Type	Fold LoD Level***	Average VAF, MSI Score, or ERBB2 Copies	Number Positive / Number Expected	Agreement (95% CI)
16	Uterine Neoplasms - Endometrial carcinoma	MSI-H	CDx	Low positive	47.5	35/36	97.2% (85.5%, 99.9%)
17	Uterine Serous Carcinoma	MSI-H	CDx	Low Positive	45.556	33/36	91.7% (77.5%, 98.2%)
18	Breast Carcinoma	<i>ERBB2</i> CNA	Tumor Profiling	2-3x	11.627	36/36	100% (90.3%, 100.0%)
19	Breast Carcinoma	<i>ERBB2</i> CNA	Tumor Profiling	1-1.5x	7.877	35/36	97.2% (85.5%, 99.9%)
20	Breast Carcinoma	<i>PALB2</i> M723fs	Tumor Profiling	1-1.5x	0.088	36/36	100% (90.3%, 100.0%)
				2-3x	0.183	36/36	100% (90.3%, 100.0%)
21	Breast Carcinoma	<i>SMAD2</i> L175fs	Tumor Profiling	1-1.5x	0.101	35/36	97.2% (85.5%, 99.9%)
				2-3x	0.196	36/36	100% (90.3%, 100.0%)
22	Colorectal Adenocarcinoma	<i>MLH1</i> K618del	Tumor Profiling	1-1.5x	0.088	36/36	100% (90.3%, 100.0%)
				2-3x	0.192	36/36	100% (90.3%, 100.0%)
23	Colorectal Adenocarcinoma	<i>MSH2</i> E12fs	Tumor Profiling	1-1.5x	0.079	35/36	97.2% (85.5%, 99.9%)
				2-3x	0.201	36/36	100% (90.3%, 100.0%)
24	Colorectal Adenocarcinoma	<i>MSH2</i> V705fs	Tumor Profiling	1-1.5x	0.088	34/36	94.4% (81.3%, 99.3%)
				2-3x	0.164	36/36	100% (90.3%, 100.0%)
25	Colorectal Adenocarcinoma	<i>MSH6</i> R1068*	Tumor Profiling	1-1.5x	0.105	36/36	100% (90.3%, 100.0%)
				2-3x	0.189	36/36	100% (90.3%, 100.0%)
26	Colorectal Adenocarcinoma	<i>PIK3R1</i> N453_Y463del	Tumor Profiling	1-1.5x	0.107	36/36	100% (90.3%, 100.0%)
				2-3x	0.198	35/35	100% (90.0%, 100.0%)
27	Colorectal Adenocarcinoma	<i>PMS2</i> R211*	Tumor Profiling	1-1.5x	0.113	36/36	100% (90.3%, 100.0%)
				2-3x	0.177	36/36	100% (90.3%, 100.0%)
28	Gastrointestinal Stromal Tumors (GIST)	<i>KIT</i> K550_K558del	Tumor Profiling	1-1.5x	0.103	36/36	100% (90.3%, 100.0%)
				2-3x	0.158	36/36	100% (90.3%, 100.0%)
29	Gastrointestinal Stromal Tumors (GIST)	<i>NF1</i> I1679_Y1680del	Tumor Profiling	1-1.5x	0.095	36/36	100% (90.3%, 100.0%)
				2-3x	0.162	36/36	100% (90.3%, 100.0%)
30	Glioblastoma	<i>TERT</i> c.-146C>T	Tumor Profiling	1-1.5x	0.1	36/36	100% (90.3%, 100.0%)
				2-3x	0.159	36/36	100% (90.3%, 100.0%)
31	Lung Non-small cell lung cancer (NSCLC)	<i>ARID1A</i> Q1409fs	Tumor Profiling	1-1.5x	0.097	36/36	100% (90.3%, 100.0%)
				2-3x	0.189	36/36	100% (90.3%, 100.0%)
32	Lung Non-small cell lung cancer (NSCLC)	<i>EGFR</i> A767_V769dup	Tumor Profiling	1-1.5x	0.108	36/36	100% (90.3%, 100.0%)
				2-3x	0.211	36/36	100% (90.3%, 100.0%)

Sample	Lineage	Variant	Bio-marker Type	Fold LoD Level***	Average VAF, MSI Score, or ERBB2 Copies	Number Positive / Number Expected	Agreement (95% CI)
33	Lung Non-small cell lung cancer (NSCLC)	<i>EGFR</i> T790M	Tumor Profiling	1-1.5x	0.115	36/36	100% (90.3%, 100.0%)
				2-3x	0.189	36/36	100% (90.3%, 100.0%)
34	Lung Non-small cell lung cancer (NSCLC)	<i>ERBB2</i> G776delinsVC	Tumor Profiling	1-1.5x	0.118	36/36	100% (90.3%, 100.0%)
				2-3x	0.18	36/36	100% (90.3%, 100.0%)
14**	Melanoma	<i>TERT</i> c.-146C>T	Tumor Profiling	1-1.5x	0.113	36/36	100% (90.3%, 100.0%)
				2-3x	0.275	36/36	100% (90.3%, 100.0%)
35	Ovarian Surface Epithelial Carcinomas	<i>BRCA1</i> E23fs	Tumor Profiling	1-1.5x	0.103	36/36	100% (90.3%, 100.0%)
				2-3x	0.25	36/36	100% (90.3%, 100.0%)
36	Prostatic Adenocarcinoma	<i>BRCA2</i> S1882*	Tumor Profiling	1-1.5x	0.113	36/36	100% (90.3%, 100.0%)
				2-3x	0.197	36/36	100% (90.3%, 100.0%)
37**	Prostatic Adenocarcinoma	<i>BRCA2</i> T2125fs	Tumor Profiling	1-1.5x	0.127	36/36	100% (90.3%, 100.0%)
				2-3x	0.177	36/36	100% (90.3%, 100.0%)
37**	Prostatic Adenocarcinoma	<i>CTNNB1</i> T41A	Tumor Profiling	1-1.5x	0.097	36/36	100% (90.3%, 100.0%)
				2-3x	0.146	36/36	100% (90.3%, 100.0%)
38	Small Intestinal Malignancies	<i>APC</i> T1556fs	Tumor Profiling	1-1.5x	0.108	36/36	100% (90.3%, 100.0%)
				2-3x	0.208	36/36	100% (90.3%, 100.0%)
39	Small Intestinal Malignancies	<i>KRAS</i> G13D	Tumor Profiling	1-1.5x	0.259	36/36	100% (90.3%, 100.0%)
				2-3x	0.428	36/36	100% (90.3%, 100.0%)
40	Soft Tissue Tumors	<i>TP53</i> Q167*	Tumor Profiling	1-1.5x	0.117	36/36	100% (90.3%, 100.0%)
				2-3x	0.176	36/36	100% (90.3%, 100.0%)
41	Uterine Neoplasms - Endometrial carcinoma	<i>ARID1A</i> A330fs	Tumor Profiling	1-1.5x	0.163	36/36	100% (90.3%, 100.0%)
				2-3x	0.097	34/36	94.4% (81.3%, 99.3%)
42**	Uterine Serous Carcinoma	<i>TP53</i> F113fs c.332dupT	Tumor Profiling	1-1.5x	0.117	36/36	100% (90.3%, 100.0%)
				2-3x	0.197	36/36	100% (90.3%, 100.0%)
42**	Uterine Serous Carcinoma	<i>TP53</i> F113fs c.336_337delC T	Tumor Profiling	1-1.5x	0.117	36/36	100% (90.3%, 100.0%)
				2-3x	0.198	36/36	100% (90.3%, 100.0%)
43	Uterine Serous Carcinoma	<i>TP53</i> N263fs	Tumor Profiling	1-1.5x	0.123	36/36	100% (90.3%, 100.0%)
				2-3x	0.209	36/36	100% (90.3%, 100.0%)
44	Uterine Neoplasms - Endometrial carcinoma	Not MSI-H	CDx	Low Negative	21.5	36/36	100% (90.3%, 100.0%)



Sample	Lineage	Variant	Bio-marker Type	Fold LoD Level***	Average VAF, MSI Score, or ERBB2 Copies	Number Positive / Number Expected	Agreement (95% CI)
45	Uterine Neoplasms - Endometrial carcinoma	Not-MSI-H	CDx	High negative	30.056	34/36	94.4% (81.3%, 99.3%)
*Stop codon **Samples contained more than 1 tracked variant. *** For MSI, level refers to level in relation to closeness to positivity/negativity threshold.							

Positive and negative call rates were determined for 45 samples with targeted variants or alterations (SNV/indels, *ERBB2* CNA, and MSI) and the results are summarized per variant class in **Table 16** and **Table 17**. No call events, which arise from passing sample-level validity criteria but insufficient exon-level depth to make a positive or negative call for a particular mutation, were treated in two different ways. In the “No Calls Included” columns, the observations are subtracted from the total denominator, representing the concordance when a categorical positive or negative call is made for a given mutation. In the “No Calls Excluded” columns, a no call event is treated as discordant, thus representing the worst-case result. The positive agreement rates were 100% for SNV, MNV, and *ERBB2* CNA at alteration levels 2-3x LoD, 97.2% for MSI-H and *ERBB2* CNA (1-1.5x LoD), and >99% for indels. The negative call rates were 100% for *ERBB2* CNA and >97% for all others.

**Table 16. Positive Call Rates for Tracked Variants by Variant Class**

Variant Class	Level	Expected Positive Calls	Observed Positive Calls	Observed Negative Calls	Observed No calls	Positive Call Rate (No Calls Included)	Positive Call Rate, (No Calls Included 95% CI)	Positive Call Rate, (No Calls Excluded)	Positive Call Rate, (No Calls Excluded 95% CI)
SNV	1-1.5x LoD	720	720	0	0	100%	(99.5%, 100%)	100%	(99.5%, 100.0%)
SNV	2-3x LoD	720	720	0	0	100%	(99.5%, 100%)	100%	(99.5%, 100.0%)
MNV	1-1.5x LoD	36	36	0	0	100%	(90.3%, 100%)	100%	(90.3%, 100.0%)
MNV	2-3x LoD	36	36	0	0	100%	(90.3%, 100%)	100%	(90.3%, 100.0%)
Indel	1-1.5x LoD	684	680	4	0	99.4%	(98.5%, 99.8%)	99.4%	(98.5%, 99.8%)
Indel	2-3x LoD	684	681	2	1	99.7%	(98.9%, 100%)	99.6%	(98.7%, 99.9%)
<i>ERBB2</i> CNA	1-1.5x LoD	36	35	1	0	97.2%	(85.5%, 99.9%)	97.2%	(85.5%, 99.9%)
<i>ERBB2</i> CNA	2-3x LoD	36	36	0	0	100%	(90.3%, 100.0%)	100%	(90.3%, 100.0%)
MSI	H	144	140	4	0	97.2%	(93.0%, 99.2%)	97.2%	(93.0%, 99.2%)

**Table 17. Negative Call Rates for Tracked Variant Sample Set by Variant Class**

Variant Class	Level	Expected Negative Calls	Observed Positive Calls	Observed Negative Calls	Observed No calls	Negative Call Rate (No Calls Included)	Negative Call Rate (No Calls, Included 95% CI)	Negative Call Rate, (No Calls Excluded)	Negative Call Rate, (No Calls Excluded 95% CI)
SNV	2-3x LoD	66390588	68	64780005	1610515	100%	(100.0%, 100.0%)	97.6%	(97.6%, 97.6%)
SNV	1-1.5x LoD	66424140	199	64528287	1895654	100%	(100.0%, 100.0%)	97.1%	(97.1%, 97.1%)
MNV	2-3x LoD	6049332	0	5967205	82127	100%	(100.0%, 100.0%)	98.6%	(98.6%, 98.7%)
MNV	1-1.5x LoD	6049980	0	5951811	98169	100%	(100.0%, 100.0%)	98.4%	(98.4%, 98.4%)
Indel	2-3x LoD	153123084	129	150267525	2855430	100%	(100.0%, 100.0%)	98.1%	(98.1%, 98.1%)

Variant Class	Level	Expected Negative Calls	Observed Positive Calls	Observed Negative Calls	Observed No calls	Negative Call Rate (No Calls Included)	Negative Call Rate (No Calls, Included 95% CI)	Negative Call Rate, (No Calls Excluded)	Negative Call Rate, (No Calls Excluded 95% CI)
Indel	1-1.5x LoD	153091656	167	149770010	3321479	100%	(100.0%, 100.0%)	97.8%	(97.8%, 97.8%)
<i>ERBB2</i> CNA	1-1.5x LoD	72	0	72	0	100%	(95.0%, 100.0%)	100%	(95.0%, 100.0%)
<i>ERBB2</i> CNA	2-3x LoD	72	0	72	0	100%	(95.0%, 100.0%)	100%	(95.0%, 100.0%)
MSI	Not MSI-H	72	2	70	0	97.2%	(90.3%, 99.7%)	97.2%	(90.3%, 99.7%)
Note: The total expected negative calls reflect the sum of all observed calls									

Positive and negative call rates were assessed for the 49 panel member samples. A total of 86 samples were evaluated containing 1-19 variants per sample. The results are summarized in **Table 18**.

**Table 18. Precision Study Positive and Negative Call Rates per Sample**

Sample	Level*	Unique Variants	Replicates	Positive Call Rate (n/N) (95% 2-sided CI)	Negative Call Rate (n/N) (95% 2-sided CI)
1	2-3x LoD	5	36	100% (180/180) 98.0%, 100%)	98.1% (5990116/6107364) 98.1%, 98.1%)
	1-1.5x LoD	5	36	100% (180/180) 98.0%, 100%)	97.7% (5967519/6107364) 97.7%, 97.7%)
2	2-3x LoD	7	36	100% (252/252) 98.5%, 100%)	98.7% (6074567/6155856) 98.7%, 98.7%)
	1-1.5x LoD	7	36	100% (252/252) 98.5%, 100%)	95.5% (5829502/6106608) 95.4%, 95.5%)
3	2-3x LoD	2	36	100% (72/72) 95.0%, 100%)	98.5% (6038700/6128244) 98.5%, 98.5%)
	1-1.5x LoD	4	36	100% (144/144) 97.5%, 100%)	97.5% (5910149/6063552) 97.5%, 97.5%)

Sample	Level*	Unique Variants	Replicates	Positive Call Rate (n/N) (95% 2-sided CI)	Negative Call Rate (n/N) (95% 2-sided CI)
4	2-3x LoD	9	36	100% (324/324) 98.9%, 100%)	99.0% (6073944/6135552) 99.0%, 99.0%)
	1-1.5x LoD	9	36	98.1% (318/324) 96.0%, 99.3%)	97.0% (5923102/6107760) 97.0%, 97.0%)
5	2-3x LoD	10	36	99.2% (357/360) 97.6%, 99.8%)	97.2% (5936756/6106500) 97.2%, 97.2%)
	1-1.5x LoD	10	36	99.7% (359/360) 98.5%, 100%)	96.9% (5930678/6118668) 96.9%, 96.9%)
6	2-3x LoD	12	36	100% (432/432) 99.1%, 100%)	99.3% (6141825/6182424) 99.3%, 99.3%)
	1-1.5x LoD	16	36	100% (576/576) 99.4%, 100%)	99.3% (6141359/6182172) 99.3%, 99.3%)
7	2-3x LoD	9	36	100% (324/324) 98.9%, 100%)	98.0% (5940139/6060600) 98.0%, 98.0%)
	1-1.5x LoD	9	36	94.1% (305/324) 91.0%, 96.4%)	97.7% (5948989/6089616) 97.7%, 97.7%)
8	2-3x LoD	10	36	97.8% (352/360) 95.7%, 99.0%)	99.8% (6106066/6118668) 99.8%, 99.8%)
	1-1.5x LoD	10	36	99.7% (359/360) 98.5%, 100%)	99.7% (6099303/6118668) 99.7%, 99.7%)
9	2-3x LoD	3	36	100% (108/108) 96.6%, 100%)	95.7% (5873273/6135876) 95.7%, 95.7%)
	1-1.5x LoD	3	36	84.3% (91/108) 76.0%, 90.6%)	97.3% (5969814/6135876) 97.3%, 97.3%)
10	2-3x LoD	7	36	99.6% (251/252) 97.8%, 100%)	97.9% (5998738/6126840) 97.9%, 97.9%)
	1-1.5x LoD	7	36	100% (252/252) 98.5%, 100%)	99.3% (6059851/6103548) 99.3%, 99.3%)

Sample	Level*	Unique Variants	Replicates	Positive Call Rate (n/N) (95% 2-sided CI)	Negative Call Rate (n/N) (95% 2-sided CI)
11	2-3x LoD	5	36	100% (180/180) 98.0%, 100%)	97.7% (5964449/6106680) 97.7%, 97.7%)
	1-1.5x LoD	5	36	100% (180/180) 98.0%, 100%)	99.1% (6078601/6135696) 99.1%, 99.1%)
12	2-3x LoD	4	36	100% (144/144) 97.5%, 100%)	98.8% (6060158/6135840) 98.8%, 98.8%)
	1-1.5x LoD	4	36	100% (144/144) 97.5%, 100%)	97.0% (5948803/6135840) 96.9%, 97.0%)
13	2-3x LoD	6	36	95.8% (207/216) 92.2%, 98.1%)	99.1% (6099799/6155892) 99.1%, 99.1%)
	1-1.5x LoD	6	36	100% (216/216) 98.3%, 100%)	98.3% (6050914/6155892) 98.3%, 98.3%)
14	2-3x LoD	4	36	95.8% (138/144) 91.2%, 98.5%)	99.6% (6132661/6155964) 99.6%, 99.6%)
	1-1.5x LoD	2	36	100% (72/72) 95.0%, 100%)	99.2% (6105674/6156036) 99.2%, 99.2%)
15	2-3x LoD	10	36	99.7% (359/360) 98.5%, 100%)	99.2% (6104779/6155064) 99.2%, 99.2%)
	1-1.5x LoD	8	36	94.4% (272/288) 91.1%, 96.8%)	98.3% (6053069/6155820) 98.3%, 98.3%)
16	2-3x LoD	7	36	100% (252/252) 98.5%, 100%)	99.4% (6090355/6128640) 99.4%, 99.4%)
	1-1.5x LoD	7	36	100% (252/252) 98.5%, 100%)	99.0 % (6039609/6100704) 99.0%, 99.0%)
17	2-3x LoD	9	36	100% (324/324) 98.9%, 100%)	98.7% (6027075/6106176) 98.7%, 98.7%)
	1-1.5x LoD	8	36	96.9% (279/288) 94.2%, 98.6%)	96.0% (5860846/6106896) 96.0%, 96.0%)

Sample	Level*	Unique Variants	Replicates	Positive Call Rate (n/N) (95% 2-sided CI)	Negative Call Rate (n/N) (95% 2-sided CI)
18	2-3x LoD	5	36	100% (180/180) 98.0%, 100%)	94.8% (5817473/6135696) 94.8%, 94.8%)
	1-1.5x LoD	6	36	100% (216/216) 98.3%, 100%)	96.9% (5610986/5787972) 96.9%, 97.0%)
19	2-3x LoD	6	36	100% (216/216) 98.3%, 100%)	96.6% (5897983/6107868) 96.5%, 96.6%)
	1-1.5x LoD	6	36	100% (216/216) 98.3%, 100%)	98.2% (6023465/6135660) 98.2%, 98.2%)
20	2-3x LoD	6	36	98.6% (213/216) 96.0%, 99.7%)	97.9% (6003252/6135084) 97.8%, 97.9%)
	1-1.5x LoD	7	36	95.2% (240/252) 91.8%, 97.5%)	97.3% (5967338/6134940) 97.3%, 97.3%)
21	2-3x LoD	5	36	99.4% (179/180) 96.9%, 100%)	96.4% (5888629/6106680) 96.4%, 96.4%)
	1-1.5x LoD	7	36	95.2% (240/252) 91.8%, 97.5%)	99.3% (6075626/6119460) 99.3%, 99.3%)
22	2-3x LoD	4	36	98.6% (142/144) 95.1%, 99.8%)	99.3% (6095067/6135732) 99.3%, 99.3%)
	1-1.5x LoD	6	36	88.9% (192/216) 83.9%, 92.7%)	99.0% (6060389/6119496) 99.0%, 99.0%)
23	2-3x LoD	5	36	96.7% (174/180) 92.9%, 98.8%)	99.2% (6107077/6155820) 99.2%, 99.2%)
	1-1.5x LoD	3	36	100% (108/108) 96.6%, 100%)	98.3% (6007704/6111792) 98.3%, 98.3%)
24	2-3x LoD	15	36	89.1% (481/540) 86.1%, 91.6%)	94.6% (5354299/5659992) 94.6%, 94.6%)
	1-1.5x LoD	10	36	85.8% (309/360) 81.8%, 89.3%)	97.0% (5839547/6019164) 97.0%, 97.0%)

Sample	Level*	Unique Variants	Replicates	Positive Call Rate (n/N) (95% 2-sided CI)	Negative Call Rate (n/N) (95% 2-sided CI)
25	2-3x LoD	12	36	92.8% (401/432) 90.0%, 95.1%)	98.8% (6000139/6075216) 98.8%, 98.8%)
	1-1.5x LoD	5	36	100% (180/180) 98.0%, 100%)	98.3% (5924863/6027624) 98.3%, 98.3%)
26	2-3x LoD	10	36	95.8% (345/360) 93.2%, 97.6%)	98.6% (6033684/6118560) 98.6%, 98.6%)
	1-1.5x LoD	8	36	88.2% (254/288) 83.9%, 91.7%)	97.9% (5946155/6073092) 97.9%, 97.9%)
27	2-3x LoD	21	36	97.2% (735/756) 95.8%, 98.3%)	97.0% (5921927/6105348) 97.0%, 97.0%)
	1-1.5x LoD	12	36	86.1% (372/432) 82.5%, 89.2%)	98.8% (5990290/6060456) 98.8%, 98.9%)
28	2-3x LoD	17	36	91.8% (562/612) 89.4%, 93.9%)	98.6% (5986344/6073884) 98.5%, 98.6%)
	1-1.5x LoD	4	36	100% (144/144) 97.5%, 100%)	98.0% (5994119/6116184) 98.0%, 98.0%)
29	2-3x LoD	6	36	100% (216/216) 98.3%, 100%)	97.5% (5955332/6106968) 97.5%, 97.5%)
	1-1.5x LoD	6	36	100% (216/216) 98.3%, 100%)	94.7% (5762447/6082056) 94.7%, 94.8%)
30	2-3x LoD	2	36	100% (72/72) 95.0%, 100%)	97.4% (5929674/6085476) 97.4%, 97.5%)
	1-1.5x LoD	2	36	100% (72/72) 95.0%, 100%)	97.1% (5936354/6113808) 97.1%, 97.1%)
31	2-3x LoD	4	36	99.3% (143/144) 96.2%, 100%)	99.2% (6085799/6135732) 99.2%, 99.2%)
	1-1.5x LoD	4	36	91.0% (131/144) 85.1%, 95.1%)	97.4% (5959985/6119568) 97.4%, 97.4%)



Sample	Level*	Unique Variants	Replicates	Positive Call Rate (n/N) (95% 2-sided CI)	Negative Call Rate (n/N) (95% 2-sided CI)
32	2-3x LoD	5	36	100% (180/180) 98.0%, 100%)	96.0% (5626124/5859864) 96.0%, 96.0%)
	1-1.5x LoD	5	36	100% (180/180) 98.0%, 100%)	96.6% (5886586/6090840) 96.6%, 96.7%)
33	2-3x LoD	19	36	99.7% (682/684) 98.9%, 100%)	98.2% (6022843/6134508) 98.2%, 98.2%)
	1-1.5x LoD	19	36	98.0% (670/684) 96.6%, 98.9%)	99.2% (6082551/6134508) 99.1%, 99.2%)
34	2-3x LoD	19	36	99.1% (678/684) 98.1%, 99.7%)	96.0% (5774423/6015276) 96.0%, 96.0%)
	1-1.5x LoD	19	36	100% (684/684) 99.5%, 100%)	97.6% (5919148/6062796) 97.6%, 97.6%)
35	2-3x LoD	5	36	98.9% (178/180) 96.0%, 99.9%)	99.3% (6081963/6127596) 99.2%, 99.3%)
	1-1.5x LoD	5	36	98.9% (178/180) 96.0%, 99.9%)	97.8% (5971503/6107472) 97.8%, 97.8%)
36	2-3x LoD	5	36	95.0% (171/180) 90.7%, 97.7%)	96.7% (5857723/6056208) 96.7%, 96.7%)
	1-1.5x LoD	3	36	98.1% (106/108) 93.5%, 99.8%)	93.0% (5593906/6012432) 93.0%, 93.1%)
37	2-3x LoD	4	36	97.2% (140/144) 93.0%, 99.2%)	98.2% (5921580/6029316) 98.2%, 98.2%)
	1-1.5x LoD	4	36	83.3% (120/144) 76.2%, 89.0%)	95.4% (5779364/6055740) 95.4%, 95.5%)
38	N/A	1	36	100% (36/36) 90.3%, 100%)	98.4% (5992219/6090264) 98.4%, 98.4%)
39	2-3x LoD	1	36	100% (36/36) 90.3%, 100%)	98.3% (6026487/6127632) 98.3%, 98.4%)

Sample	Level*	Unique Variants	Replicates	Positive Call Rate (n/N) (95% 2-sided CI)	Negative Call Rate (n/N) (95% 2-sided CI)
40	1-1.5x LoD	3	36	95.4% (103/108) 89.5%, 98.5%)	98.8% (6063639/6135084) 98.8%, 98.8%)
41	N/A	1	36	100% (36/36) 90.3%, 100%)	97.9% (6005476/6135948) 97.9%, 97.9%)
42	MSI-H	8	36	99.0% (285/288) 97.0%, 99.8%)	99.2% (6084167/6135588) 99.2%, 99.2%)
43	Not MSI-H	2	36	100% (72/72) 95.0%, 100%)	99.5% (6122169/6155928) 99.4%, 99.5%)
44	Not MSI-H	4	36	100% (144/144) 97.5%, 100%)	97.3% (5928011/6090552) 97.3%, 97.3%)
45	MSI-H	8	36	100% (288/288) 98.7%, 100%)	97.8% (5917010/6049692) 97.8%, 97.8%)
46	MSI-H	13	36	99.4% (465/468) 98.1%, 99.9%)	96.7% (5855908/6055056) 96.7%, 96.7%)
47	MSI-H	13	36	95.9% (449/468) 93.7%, 97.5%)	97.8% (5982617/6118560) 97.8%, 97.8%)
48	TMB 10 mutations/Mb	4	36	100% (144/144) 97.5%, 100%)	96.4% (5751390/5965956) 96.4%, 96.4%)
49	TMB 15 mutations/Mb	5	36	100% (180/180) 98.0%, 100%)	93.8% (5684972/6063408) 93.7%, 93.8%)

\*Level corresponds to the level originally associated with each sample's targeted variant.

N/A = not applicable.

An analysis of panel-wide precision that includes non-targeted or passenger variants (i.e., variants that may be present in the samples that were not the basis for sample selection in the study) assessing agreement based on variant type stratified by VAF ( $\geq 0\%$ ,  $\geq 5\%$ ,  $\geq 8\%$ ,  $\geq 10\%$ , and  $\geq 15\%$ ) is presented in **Table 19** below. Positive call rates near the reporting threshold of  $\geq 5\%$  VAF are less than the performance at higher VAFs and range from 96.5% (7595/7873) for Short Indel Non-Homopolymer to 100% (72/72) for Long Indel Non-Homopolymer. For  $\text{VAF} \geq 8\%$ , all variant types but Long Indel in Homopolymer Region had  $\geq 99.2\%$  positive call rates. Performance of this challenging variant type at  $\text{VAF} \geq 8\%$  was still high at 98.6% (141/143). As VAFs increase, positive call rates are either at 100% or very close ( $>99\%$ ) for all variant types.

**Table 19. Panel Wide Precision Per Variant Type Stratified by VAF**

Variant Type	VAF	VAF Range	Variant Depth Range	Total Depth Range	N Valid Samples	Positive Calls	Negative Calls	No Calls	Positive Call Rate
All Indel	≥5%	6%-74%	7.48-1706.53	74.48-4054.89	8460	8167	281	12	96.7% (8167/8448)
All MNV	≥5%	8%-30%	46.25-174.42	501.81-975.36	180	176	4	0	97.8% (176/180)
All SNV	≥5%	6%-86%	8.32-1392.97	66.84-4156.83	13608	13318	267	23	98.0% (13318/13585)
Long Indel Homopolymer	≥5%	10%-20%	29.19-152.08	264.11-940.72	144	141	2	1	98.6% (141/143)
Long Indel Non-Homopolymer	≥5%	10%-16%	35.06-53.50	334.78-346.58	72	72	0	0	100% (72/72)
MNV	≥5%	8%-30%	46.25-174.42	501.81-975.36	180	176	4	0	97.8% (176/180)
SNV	≥5%	6%-86%	8.32-1392.97	66.84-4156.83	13608	13318	267	23	98.0% (13318/13585)
Short Indel Homopolymer	≥5%	10%-57%	30.69-693.36	269.86-2282.64	360	359	1	0	99.7% (359/360)
Short Indel Non-Homopolymer	≥5%	6%-74%	7.48-1706.53	74.48-4054.89	7884	7595	278	11	96.5% (7595/7873)
All Indel	≥8%	8%-74%	7.48-1706.53	74.48-4054.89	7380	7313	56	11	99.2% (7313/7369)
All MNV	≥8%	9%-30%	46.25-174.42	501.81-975.36	144	144	0	0	100% (144/144)
All SNV	≥8%	8%-86%	8.32-1392.97	66.84-4156.83	12492	12382	90	20	99.3% (12382/12472)
Long Indel Homopolymer	≥8%	10%-20%	29.19-152.08	264.11-940.72	144	141	2	1	98.6% (141/143)
Long Indel Non-Homopolymer	≥8%	10%-16%	35.06-53.50	334.78-346.58	72	72	0	0	100% (72/72)
MNV	≥8%	9%-30%	46.25-174.42	501.81-975.36	144	144	0	0	100% (144/144)
SNV	≥8%	8%-86%	8.32-1392.97	66.84-4156.83	12492	12382	90	20	99.3% (12382/12472)
Short Indel Homopolymer	≥8%	10%-57%	30.69-693.36	269.86-2282.64	360	359	1	0	99.7% (359/360)
Short Indel Non-Homopolymer	≥8%	8%-74%	7.48-1706.53	74.48-4054.89	6804	6741	53	10	99.2% (6741/6794)

Variant Type	VAF	VAF Range	Variant Depth Range	Total Depth Range	N Valid Samples	Positive Calls	Negative Calls	No Calls	Positive Call Rate
All Indel	≥10%	10%-74%	7.48-1706.53	74.48-3591.64	6444	6409	26	9	99.6% (6409/6435)
All MNV	≥10%	18%-30%	171.08-174.42	580.97-975.36	72	72	0	0	100% (72/72)
All SNV	≥10%	10%-86%	8.32-1392.97	66.84-4156.83	11088	11043	36	9	99.7% (11043/11079)
Long Indel Homopolymer	≥10%	11%-20%	29.19-152.08	264.11-940.72	108	107	0	1	100% (107/107)
Long Indel Non-Homopolymer	≥10%	10%-16%	35.06-53.50	334.78-346.58	72	72	0	0	100% (72/72)
MNV	≥10%	18%-30%	171.08-174.42	580.97-975.36	72	72	0	0	100% (72/72)
SNV	≥10%	10%-86%	8.32-1392.97	66.84-4156.83	11088	11043	36	9	99.7% (11043/11079)
Short Indel Homopolymer	≥10%	10%-57%	30.69-693.36	269.86-2115.36	324	323	1	0	99.7% (323/324)
Short Indel Non-Homopolymer	≥10%	10%-74%	7.48-1706.53	74.48-3591.64	5940	5907	25	8	99.6% (5907/5932)
All Indel	≥15%	15%-74%	39.43-1706.53	202.47-3591.64	4428	4426	0	2	100% (4426/4426)
All MNV	≥15%	18%-30%	171.08-174.42	580.97-975.36	72	72	0	0	100% (72/72)
All SNV	≥15%	15%-86%	24.51-1392.97	111.11-4156.83	8892	8886	5	1	99.9% (8886/8891)
Long Indel Homopolymer	≥15%	16%-20%	61.91-152.08	312.80-940.72	72	71	0	1	100% (71/71)
Long Indel Non-Homopolymer	≥15%	16%-16%	53.50-53.50	346.58-346.58	36	36	0	0	100% (36/36)
MNV	≥15%	18%-30%	171.08-174.42	580.97-975.36	72	72	0	0	100% (72/72)
SNV	≥15%	15%-86%	24.51-1392.97	111.11-4156.83	8892	8886	5	1	99.9% (8886/8891)
Short Indel Homopolymer	≥15%	19%-57%	51.72-693.36	269.86-2115.36	288	288	0	0	100% (288/288)
Short Indel Non-Homopolymer	≥15%	15%-74%	39.43-1706.53	202.47-3591.64	4032	4031	0	1	100% (4031/4031)

A variance component analysis was performed for 45 samples using the underlying quantitative metric for the tracked variant (i.e., VF for SNV and indel, copies for CNA, MSI value for MSI). Mean values were determined using all valid data points per sample (36) and the results are shown in **Table 20**. The results demonstrated <20% CV between operators/instrument and with-in run and <22% CV for reagent lot-to-lot. Between run and within-lab demonstrated <44% CV and <49% CV, respectively with the majority being <25% for both components.

**Table 20. Variance Component Analysis Results by Operator, Instrument, Run and Lot Number**

Sample	Level*	Tracked Variant Per Sample Corresponding to Dilution Levels	Mean Values Per Tracked Variant <sup>s</sup>	Operator/Instrument		Reagent Lot		Within-Run		Between-Run		Within-Lab	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	2-3x LoD	<i>ERBB2</i> Copy	11.6	0	0	0	0	0	0	1.652	14.21	1.652	14.21
2	1-1.5x LoD	<i>ERBB2</i> Copy	7.9	0.401	5.1	0.384	4.87	0	0	0.864	10.97	1.027	13.04
3	MSI-H	MSI	58.4	0	0	1.324	2.27	0	0	5.412	9.26	5.572	9.54
4	Not MSI-H	MSI	21.5	0.826	3.84	1.281	5.96	0.606	2.82	3.064	14.25	3.476	16.17
5	Not MSI-H	MSI	30.1	0.598	1.99	2.121	7.06	0	0	3.53	11.75	4.161	13.85
6	MSI-H	MSI	47.5	0	0	1.955	4.12	0	0	3.772	7.94	4.249	8.94
7	MSI-H	MSI	45.6	0	0	0	0	1.15	2.53	3.883	8.52	4.05	8.89
8	MSI-H	MSI	106.7	0	0	1.724	1.62	0	0	6.154	5.77	6.391	5.99
9	2-3x LoD	<i>PIK3CA</i> H1047R	0.236	0	0	0.003	1.11	0	0	0.02	8.55	0.02	8.62
	1-1.5x LoD	<i>PIK3CA</i> H1047R	0.135	0	0	0.003	2.3	0	0	0.018	13.46	0.018	13.66
10	2-3x LoD	<i>BRAF</i> V600E	0.228	0	0	0	0	0.043	18.74	0.034	14.95	0.055	23.98
	1-1.5x LoD	<i>BRAF</i> V600E	0.095	0	0	0.001	1.33	0.015	15.66	0.016	16.58	0.022	22.85
11	2-3x LoD	<i>EGFR</i> A767_V769dup	0.211	0.006	2.73	0	0	0.008	3.64	0.022	10.28	0.024	11.24
	1-1.5x LoD	<i>EGFR</i> A767_V769dup	0.108	0	0	0.007	6.25	0	0	0.017	16.02	0.019	17.2
12	2-3x LoD	<i>EGFR</i> L747_T751del	0.195	0.006	3.09	0	0	0	0	0.011	5.77	0.013	6.54
	1-1.5x LoD	<i>EGFR</i> L747_T751del	0.108	0	0	0.001	1.06	0	0	0.008	7.17	0.008	7.25
13	2-3x LoD	<i>BRAF</i> V600E	0.21	0	0	0.01	4.74	0	0	0.028	13.1	0.029	13.93
	1-1.5x LoD	<i>BRAF</i> V600E	0.124	0.006	5.2	0.011	8.78	0	0	0.019	15.7	0.023	18.72
14	2-3x LoD	<i>KRAS</i> G12C	0.518	0.003	0.62	0.024	4.57	0	0	0.038	7.31	0.045	8.64
	1-1.5x LoD	<i>KRAS</i> G12C	0.302	0	0	0.02	6.54	0	0	0.034	11.27	0.039	13.04

15	2-3x LoD	<i>MLH1</i> K618del	0.192	0.013	6.75	0.003	1.38	0	0	0.03	15.82	0.033	17.25
	1-1.5x LoD	<i>MLH1</i> K618del	0.088	0.007	8.24	0.002	2.63	0.012	13.23	0.03	34.49	0.034	37.94
16	2-3x LoD	<i>MSH2</i> V705fs	0.164	0.025	14.99	0	0	0.009	5.52	0.029	17.67	0.039	23.82
	1-1.5x LoD	<i>MSH2</i> V705fs	0.088	0.008	9.42	0.005	5.75	0	0	0.027	30.55	0.029	32.48
17	2-3x LoD	<i>PMS2</i> R211*	0.177	0.012	6.81	0	0	0.004	2.49	0.029	16.44	0.032	17.97
	1-1.5x LoD	<i>PMS2</i> R211*	0.113	0.006	5.22	0.011	10.17	0.006	4.87	0.021	18.51	0.025	22.29
18	2-3x LoD	<i>KIT</i> K550 K558del	0.158	0.012	7.79	0.014	8.67	0.009	5.94	0.031	19.62	0.037	23.58
	1-1.5x LoD	<i>KIT</i> K550 K558del	0.103	0.006	6.01	0.012	11.35	0.006	6.23	0.022	21.87	0.027	26.12
19	2-3x LoD	<i>NFI</i> I1679_Y1680de l	0.162	0.012	7.43	0.006	3.7	0.011	6.51	0.028	17.53	0.033	20.46
	1-1.5x LoD	<i>NFI</i> I1679_Y1680de l	0.095	0.018	19.32	0.021	21.78	0.013	14.04	0.026	27.57	0.04	42.49
20	2-3x LoD	<i>PIK3CA</i> C420R	0.289	0	0	0.001	0.5	0	0	0.024	8.33	0.024	8.34
	1-1.5x LoD	<i>PIK3CA</i> C420R	0.139	0.004	3.14	0	0	0	0	0.038	27.13	0.038	27.31
21	2-3x LoD	<i>NRAS</i> Q61R	0.214	0.002	0.94	0	0	0	0	0.022	10.12	0.022	10.17
	1-1.5x LoD	<i>NRAS</i> Q61R	0.153	0	0	0	0	0	0	0.018	12.04	0.018	12.04
22	2-3x LoD	<i>BRCA2</i> S1882*	0.197	0	0	0.005	2.68	0	0	0.021	10.63	0.022	10.96
	1-1.5x LoD	<i>BRCA2</i> S1882*	0.113	0	0	0	0	0	0	0.018	15.53	0.018	15.53
23	2-3x LoD	<i>TERT</i> c.- 146C>T	0.159	0.007	4.13	0	0	0	0	0.017	11.01	0.019	11.76
	1-1.5x LoD	<i>TERT</i> c.- 146C>T	0.1	0	0	0	0	0	0	0.021	20.55	0.021	20.55
24	2-3x LoD	<i>SMAD2</i> L175fs	0.196	0.008	4.17	0	0	0	0	0.046	23.36	0.046	23.73
	1-1.5x LoD	<i>SMAD2</i> L175fs	0.101	0.011	11.32	0.02	19.28	0	0	0.037	36.69	0.043	42.97
25	2-3x LoD	<i>EGFR</i> L858R	0.187	0.002	1.12	0	0	0.002	1.21	0.011	5.9	0.011	6.13
	1-1.5x LoD	<i>EGFR</i> L858R	0.089	0.002	2.29	0	0	0	0	0.007	7.51	0.007	7.86
26	2-3x LoD	<i>ARID1A</i> A330fs	0.097	0.004	4	0.008	7.96	0.001	1.01	0.02	20.44	0.022	22.32
	1-1.5x LoD	<i>ARID1A</i> A330fs	0.163	0.008	4.92	0.011	6.62	0	0	0.024	14.66	0.027	16.82
27	2-3x LoD	<i>NRAS</i> G12A	0.189	0.006	2.95	0.008	4.1	0.005	2.64	0.022	11.53	0.024	12.86
	1-1.5x LoD	<i>NRAS</i> G12A	0.094	0.006	6	0.002	1.84	0.004	4.33	0.013	14.08	0.015	16.02

28	2-3x LoD	<i>KRAS</i> A146T	0.169	0	0	0	0	0	0	0.022	13.03	0.022	13.03
	1-1.5x LoD	<i>KRAS</i> A146T	0.112	0	0	0	0	0	0	0.02	18.07	0.02	18.07
29	2-3x LoD	<i>PIK3R1</i> N453 Y463del	0.198	0.017	8.48	0	0	0	0	0.037	18.83	0.041	20.66
	1-1.5x LoD	<i>PIK3R1</i> N453 Y463del	0.107	0.007	6.93	0.008	7.73	0.004	3.83	0.03	28.33	0.033	30.41
30	2-3x LoD	<i>NRAS</i> A146V	0.223	0	0	0	0	0	0	0.019	8.33	0.019	8.33
	1-1.5x LoD	<i>NRAS</i> A146V	0.142	0	0	0.003	2.01	0.005	3.76	0.011	7.82	0.013	8.91
31	2-3x LoD	<i>BRCA2</i> T2125fs	0.162	0.007	4.45	0.004	2.74	0.016	9.93	0.028	17.15	0.033	20.5
	1-1.5x LoD	<i>BRCA2</i> T2125fs	0.112	0.005	4.34	0.009	8.37	0.015	13.51	0.023	20.91	0.03	26.62
32	2-3x LoD	<i>TP53</i> F113fs c.332dupT	0.197	0.012	6.08	0.022	11.14	0.011	5.73	0.021	10.6	0.035	17.5
	1-1.5x LoD	<i>TP53</i> F113fs c.332dupT	0.117	0.009	7.35	0.008	7.11	0	0	0.028	23.93	0.031	26.03
33	2-3x LoD	<i>TP53</i> N263fs	0.209	0.006	3.04	0.01	4.97	0	0	0.026	12.18	0.028	13.5
	1-1.5x LoD	<i>TP53</i> N263fs	0.123	0.01	8.27	0.007	6.01	0	0	0.024	19.49	0.027	22.01
34	2-3x LoD	<i>ERBB2</i> G776delinsVC	0.18	0	0	0	0.27	0.003	1.78	0.013	7.09	0.013	7.31
	1-1.5x LoD	<i>ERBB2</i> G776delinsVC	0.118	0	0	0.003	2.93	0.004	3.57	0.011	8.93	0.012	10.05
35	2-3x LoD	<i>BRCAl</i> E23fs	0.25	0.006	2.5	0.009	3.75	0	0	0.042	16.8	0.043	17.4
	1-1.5x LoD	<i>BRCAl</i> E23fs	0.103	0.015	14.81	0.014	13.17	0	0	0.037	35.7	0.042	40.83
36	2-3x LoD	<i>PIK3CA</i> E542K	0.187	0	0	0.013	6.76	0.005	2.75	0.025	13.42	0.029	15.28
	1-1.5x LoD	<i>PIK3CA</i> E542K	0.159	0	0	0.01	6.01	0.008	4.99	0.019	11.94	0.023	14.27
37	2-3x LoD	<i>TP53</i> Q167*	0.176	0	0	0.003	1.89	0	0	0.021	12.04	0.021	12.18
	1-1.5x LoD	<i>TP53</i> Q167*	0.117	0.009	7.56	0.002	1.65	0.012	9.96	0.017	14.97	0.023	19.58
38	2-3x LoD	<i>KRAS</i> Q61K	0.3	0.004	1.2	0	0	0.006	2.15	0.015	4.98	0.017	5.55
	1-1.5x LoD	<i>KRAS</i> Q61K	0.093	0	0	0.005	4.83	0	0	0.019	20.24	0.019	20.81
39	2-3x LoD	<i>APC</i> T1556fs	0.208	0	0	0.018	8.55	0	0	0.038	18.33	0.042	20.22
	1-1.5x LoD	<i>APC</i> T1556fs	0.108	0	0	0	0	0.012	11.16	0.037	34.1	0.039	35.88
40	2-3x LoD	<i>KRAS</i> G13D	0.428	0.015	3.59	0.007	1.58	0	0	0.059	13.83	0.061	14.37
	1-1.5x LoD	<i>KRAS</i> G13D	0.259	0.007	2.61	0.031	11.9	0.003	1.03	0.045	17.32	0.055	21.2
41	2-3x LoD	<i>MSH6</i> R1068*	0.189	0.009	4.69	0.005	2.74	0	0	0.03	15.74	0.031	16.65



	1-1.5x LoD	<i>MSH6</i> R1068*	0.105	0.006	5.36	0.009	8.67	0	0	0.035	33.51	0.037	35.03
42	2-3x LoD	<i>MSH2</i> E12fs	0.201	0	0	0	0	0	0	0.042	21.05	0.042	21.05
	1-1.5x LoD	<i>MSH2</i> E12fs	0.079	0.009	12.01	0.014	17.34	0	0	0.034	43.57	0.038	48.41
43	2-3x LoD	<i>EGFR</i> T790M	0.189	0.002	1	0	0	0.001	0.7	0.018	9.62	0.018	9.7
	1-1.5x LoD	<i>EGFR</i> T790M	0.115	0	0	0	0	0	0	0.014	11.86	0.014	11.86
44	2-3x LoD	<i>ARID1A</i> Q1409fs	0.189	0.01	5.29	0.007	3.47	0	0	0.026	13.59	0.028	14.99
	1-1.5x LoD	<i>ARID1A</i> Q1409fs	0.097	0.01	10.3	0.009	9.6	0	0	0.026	26.88	0.029	30.35
45	2-3x LoD	<i>PALB2</i> M723fs	0.183	0	0	0.007	3.57	0.006	3.36	0.032	17.58	0.033	18.25
	1-1.5x LoD	<i>PALB2</i> M723fs	0.088	0.008	9.06	0.005	5.77	0	0	0.024	27.51	0.026	29.54

§Mean results as either VAF for SNV and indel or copies for *ERRB2* CNA or MSI score for MSI.

\*Level corresponds to the level originally associated with each sample's targeted variant.

The entire precision study samples, n=86 were evaluated with regard to TMB. The mean TMB value, standard deviation (SD), and %CV were determined using all valid data points per sample (36) and the results are shown in **Table 21**. Samples had mean TMB scores ranging from 2 to 30 mutations/megabase (mb). The results demonstrated <12% CV between operators/instrument and <13% CV for reagent lot-to-lot across all levels tested. Overall precision and between-run were <37% CV and <30% CV respectively for all TMB levels tested. The combined impact of reagent lots, instruments, operators, and non-consecutive run days had no effect on the performance of the MI Cancer Seek assay to call TMB >3 mutation/Mb.

**Table 21. Precision Analysis for TMB**

Sample	Mean TMB Value *	Operator/Instrument		Reagent Lot		Within-Run (Repeatability)		Between-Run		Total (Within-Lab)	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	3.9	0	0	0.069	1.75	0	0	0.232	5.88	0.242	6.13
2	5.1	0.025	0.49	0.143	2.83	0.118	2.34	0.299	5.92	0.353	6.98
3	4.5	0	0	0	0	0	0	0.522	11.67	0.522	11.67
4	4	0	0	0	0	0	0	0	0	0	0
5	10.9	0	0	0.11	1.01	0.058	0.53	0.435	3.98	0.452	4.14
6	5.8	0	0	0.153	2.66	0	0	0.549	9.55	0.57	9.92
7	5.6	0.115	2.04	0.115	2.04	0.21	3.75	0.451	8.04	0.524	9.33
8	10.8	0.133	1.22	0.133	1.22	0	0	0.35	3.23	0.397	3.66
9	10	0	0	0	0	0	0	0	0	0	0
10	7.3	0	0	0.035	0.48	0.108	1.47	0.485	6.61	0.498	6.79
11	6.9	0	0	0.109	1.57	0.042	0.61	0.359	5.18	0.377	5.45
12	6.1	0.142	2.31	0	0	0	0	0.347	5.65	0.375	6.11
13	2.3	0.135	5.85	0.296	12.84	0.077	3.35	0.378	16.38	0.504	21.88
14	7.6	0.12	1.57	0	0	0	0	0.496	6.5	0.511	6.69

Sample	Mean TMB Value *	Operator/Instrument		Reagent Lot		Within-Run (Repeatability)		Between-Run		Total (Within-Lab)	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
15	7.9	0.076	0.95	0	0	0	0	0.541	6.81	0.546	6.87
16	19.3	2.034	10.52	1.143	5.91	0	0	3.462	17.91	4.175	21.59
17	10.2	0.426	4.18	1.158	11.36	2.064	20.25	2.893	28.37	3.762	36.9
18	11.7	0	0	0.648	5.54	0	0	2.631	22.49	2.709	23.17
19	9.7	0	0	0	0	0	0	2.335	24.15	2.335	24.15
20	17.1	0.862	5.05	0	0	0	0	2.274	13.31	2.432	14.23
21	12.4	0	0	0	0	0	0	2.911	23.39	2.911	23.39
22	4.8	0	0	0.13	2.73	0	0	0.494	10.33	0.511	10.69
23	4.5	0.134	3	0.105	2.35	0	0	0.755	16.89	0.774	17.32
24	4.3	0.21	4.91	0.152	3.56	0	0	0.409	9.57	0.484	11.33
25	3.8	0	0	0.331	8.82	0	0	0.668	17.8	0.745	19.86
26	4.1	0	0	0	0	0	0	0.333	8.22	0.333	8.22
27	4.5	0	0	0.211	4.71	0	0	0.494	11.06	0.537	12.02
28	9.7	0	0	0.175	1.8	0	0	0.571	5.87	0.597	6.14
29	8.4	0.111	1.32	0.441	5.25	0.013	0.16	0.824	9.79	0.942	11.19
30	5	0.079	1.57	0.184	3.67	0	0	0.347	6.9	0.401	7.97
31	26.9	0.311	1.15	0.577	2.14	0	0	0.931	3.46	1.139	4.23
32	7.2	0.105	1.44	0.258	3.56	0	0	0.449	6.2	0.529	7.29
33	7.2	0.107	1.49	0.136	1.89	0.136	1.89	0.336	4.69	0.401	5.6
34	5.9	0	0	0	0	0	0	0.243	4.1	0.243	4.1
35	19.4	0	0	0	0	0.106	0.54	0.62	3.19	0.629	3.23
36	5	0	0	0	0	0	0	0.167	3.35	0.167	3.35
37	4.7	0	0	0.148	3.14	0	0	0.514	10.89	0.535	11.33
38	10.9	0.49	4.5	0.152	1.39	0	0	0.805	7.4	0.955	8.77
39	9.6	0.156	1.62	0	0	0	0	1.348	14.07	1.357	14.16
40	9	0	0	0.107	1.19	0.208	2.31	0.236	2.63	0.332	3.7
41	8.8	0	0	0.038	0.44	0	0	0.421	4.79	0.423	4.81
42	7.8	0	0	0	0	0	0	0.634	8.09	0.634	8.09
43	8.3	0.375	4.52	0.225	2.7	0.123	1.48	0.65	7.82	0.793	9.55
44	8.2	0	0	0.255	3.09	0	0	0.467	5.67	0.533	6.46
45	7.9	0.095	1.2	0.209	2.64	0	0	0.475	6	0.528	6.67
46	8.6	0	0	0.235	2.72	0.054	0.62	0.445	5.15	0.506	5.86
47	9.5	0.105	1.11	0.065	0.68	0	0	0.558	5.89	0.571	6.03
48	6.5	0.125	1.94	0.356	5.5	0	0	1.085	16.77	1.149	17.75
49	10	0.777	7.79	0.74	7.42	0	0	1.139	11.42	1.565	15.69
50	18.9	0	0	0.079	0.42	0.085	0.45	0.347	1.84	0.366	1.94
51	19.6	0	0	0.101	0.51	0	0	0.489	2.49	0.499	2.54
52	4.9	0.123	2.5	0.035	0.71	0	0	0.262	5.32	0.291	5.93
53	4.8	0	0	0	0	0.173	3.57	0.371	7.68	0.41	8.47
54	4.1	0.169	4.14	0.027	0.67	0	0	0.494	12.09	0.522	12.79
55	3.9	0.103	2.66	0.103	2.66	0	0	0.485	12.57	0.507	13.12
56	4	0.101	2.54	0.101	2.54	0	0	0.269	6.77	0.304	7.66
57	3.9	0.105	2.73	0.105	2.73	0	0	0.477	12.36	0.5	12.95
58	3.5	0	0	0.091	2.63	0	0	0.512	14.73	0.52	14.97
59	3.3	0.072	2.21	0.331	10.09	0	0	0.363	11.06	0.496	15.14
60	5.9	0.072	1.22	0.072	1.22	0	0	0.219	3.69	0.242	4.07
61	6	0.046	0.77	0	0	0.055	0.91	0.24	3.99	0.25	4.17
62	11.1	0	0	0.209	1.87	0.156	1.4	0.506	4.54	0.569	5.11

Sample	Mean TMB Value *	Operator/Instrument		Reagent Lot		Within-Run (Repeatability)		Between-Run		Total (Within-Lab)	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
63	10.3	0.11	1.06	0	0	0	0	0.466	4.52	0.478	4.64
64	5.5	0.184	3.36	0.164	2.99	0	0	0.675	12.33	0.718	13.13
65	4.9	0	0	0	0	0	0	0.613	12.39	0.613	12.39
66	4.6	0	0	0.269	5.88	0	0	0.461	10.07	0.534	11.66
67	6	0	0	0	0	0	0	0.167	2.79	0.167	2.79
68	22.9	0.925	4.03	0	0	0	0	3.393	14.79	3.516	15.33
69	25.8	1.081	4.19	0.708	2.74	0	0	2.027	7.85	2.404	9.32
70	27.3	0.668	2.45	0	0	0	0	2.084	7.64	2.189	8.02
71	28.6	0.722	2.53	0.222	0.78	0	0	1.507	5.28	1.686	5.9
72	14.9	0.286	1.92	0	0	0	0	0.891	5.96	0.936	6.26
73	30.2	3.728	12.36	0.692	2.29	0	0	4.302	14.26	5.734	19.01
74	17.6	0	0	0.21	1.19	0	0	5.334	30.24	5.338	30.26
75	30.1	3.034	10.09	1.485	4.94	0	0	5.122	17.04	6.135	20.41
76	17.4	0	0	1.082	6.21	0	0	5.052	29.01	5.167	29.67
77	10.1	0.071	0.71	0.138	1.36	0.131	1.3	0.365	3.61	0.418	4.13
78	17.9	0	0	0.075	0.42	0	0	0.357	2	0.365	2.04
79	18.1	0	0	0.012	0.07	0	0	0.331	1.83	0.331	1.83
80	17.7	0.039	0.22	0	0	0	0	0.585	3.31	0.587	3.32
81	18.9	0	0	0.069	0.37	0	0	1.178	6.24	1.18	6.25
82	18.9	0.184	0.97	0	0	0	0	1.645	8.72	1.655	8.78
83	7.8	0.253	3.23	0.253	3.23	0	0	0.479	6.12	0.598	7.64
84	6	0	0	0.133	2.2	0	0	0.563	9.34	0.578	9.59
85	3	0	0	0	0	0	0	0	0	0	0
86	4	0	0	0	0	0	0	0.167	4.14	0.167	4.14
*Level corresponds to the level originally associated with each sample's targeted variant. N/A not applicable.											

## 5. Stability

### a. Closed Kit Reagent Stability

The closed-kit stability of MI Cancer Seek reagents was evaluated using three kit-level reagent lots and 22 FFPE clinical samples representing each variant class tested by the assay (SNV/indel, MSI, *ERBB2* CNA, and TMB). Two replicates of each sample at 1-3x LoD were tested minimum DNA input (50 ng) for all timepoints starting from extraction. All five MI Cancer Seek single use kitted reagent sets were stored at their specified storage temperatures, and an unopened kit was tested at each timepoint.

The baseline (T0), three-and-a-half (3.5) month (T1), and four (4) month (T2) timepoints were completed by calculating concordance between T0 and T1 timepoints, and T0 and T2 timepoints. The PPA was 100% for SNV, indel, *ERBB2* CNA, and MSI across each time point. For TMB, the slope was between [0.8, 1.2] with Pearson Correlation  $r > 0.9$ . The results demonstrated that the reagents were

stable for a minimum of three-and-a-half (3.5) months when stored at the correct storage temperature.

**b. Open Kit Reagent Stability**

This study was performed to evaluate in-use (open kit) stability for the MI Cancer Seek Quantification Kit and MI Cancer Seek Library Quantification Kit up to 30 days when stored in refrigerator under the recommended temperature (2 to 8 °C) following first use (T0). A total of 11 unique clinical FFPE specimens were tested following the MI Cancer Seek workflow and concordance was calculated between baseline (T0) and each time point [T1 (30 days) and T2 (35 days)]. Both, PPA and NPA values were found to be 100% for SNVs, indels and MSI while no deleterious effects were noted in both T1 and T2 with respect to T0 for Fusions and CNA. For TMB, the 95% CI for the Deming regression slope and y-intercept spanned within acceptance criteria of 1 and 0 respectively. Thus, in-use (open kit) stability for MI Cancer Seek Quantification Kit and MI Cancer Seek Library Quantification Kit was established for up to 30 days when stored at 2 to 8 °C.

**c. Extracted TNA Stability**

This study evaluated stability of extracted TNA when stored frozen (-65°C to -85°C) for up to 6 months or more for 451 FFPE clinical samples (including 43 panel members) across 34 tissue types, stored at 10-20 ng/uL concentration. The study utilized previously extracted and stored TNA that was tested using the earlier version of MI Cancer Seek (concordance between the earlier version and MI Cancer Seek is provided under [Section IX.C.b](#)) as baseline (T0) and TNA stored frozen (-65°C to -85°C) for 90-180 days, 181-300 days or >300 were tested on the MI Cancer Seek assay for 202, 210 and 39 samples, respectively for T1. Concordance was calculated between T0 and T1 for the targeted markers near LoD in a given sample to establish the stability of extracted TNA.

Results showed 100% PPA for SNV, indel, and CNA. PPA was 92.5% for MSI, and for TMB, slope of the regression line was 1.00 and the Pearson Correlation of the T0 vs T1 TMB values was calculated at 0.99. For MSI, the three observed discordances were due to samples that were edge cases, with one of the false negatives being at the positivity threshold for MSI-H at T0 (39) but not MSI-H at T1 (25), one at the edge of the cut-off for not MSI-H at T0 (34) and MSI-H at T1 (48) and the other false negative being higher at T0 (62) but detected as not MSI-H at T1 (27) due to regions of low coverage. The study data collectively suggests MSI discordance is not due to instability of extracted TNA and that extracted TNA was stable for up to 10 months when TNA is stored frozen (-65°C to -85°C).

**d. Extracted TNA Freeze-Thaw stability**

This study established sample stability for extracted TNA for up to four freeze-thaw cycles when stored frozen (-65°C to -85°C). Testing for extracted TNA was

performed at baseline (F0) and after five consecutive freeze-thaw cycles (F5). Extracted TNA from 13 clinical FFPE clinical samples containing a minimum of 20 representative mutations at 1-3x LoD were evaluated using one lot of reagents. Each sample was tested at the minimum input (50 ng) with five replicates, yielding a total of 130 valid sequencing data points. The stability of extracted TNA was established by calculating concordance between F0 and F5 timepoints.

Results showed that after five freeze-thaw cycles, 100% PPA and NPA was achieved for SNVs, indels and, *ERBB2* CNA, with 100% PPA and 98% NPA achieved for MSI. For TMB, the Kolmogorov–Smirnov test was performed for each replicate and tested whether timepoint-F5 and timepoint-F0 data came from different distributions. For 100% of the samples, the Kolmogorov–Smirnov test failed to reject the null hypothesis that the timepoint (Baseline –F0) and F5 are from the same distribution.

This study also evaluated FFPE Slide Stability, and the data supports an initial 30 day claim. However, a post market study will be conducted to confirm and establish FFPE slide stability and FFPE block stability.

## 6. General Lab Equipment and Reagent Evaluation

### a. TNA Extraction

Extraction performance of MI Cancer Seek across multiple tumor types was evaluated by including 123 unique clinical FFPE samples covering 14 tumor types that went through the complete MI Cancer Seek workflow starting from extraction. The set included challenging samples run at the minimum assay input (50 ng) having mutation levels near LoD (1-3x) and low tumor content (20%). The number of replicates per sample varied from two to 24 (average was 8), thus, a total of 1,038 sample replicates were available for analysis, of which there were 27 failures, resulting in a 2.6% failure rate (27/1,038), and of these, 0.7% (7/1038) were due to library prep/sequencing failure and 1.9% (20/1038) were due to failed sample validity criteria. The 1,011 sample replicates passing validity criteria were evaluated for average DNA yield and concordance, which is shown for variants by type and level, along with MSI and for TMB in **Table 22** and **Table 23**. Overall concordance for all variant classes was high, as most were >99% and the average SD and %CV were low for TMB.

**Table 22. DNA Extraction Performance for Variants and MSI in 14 Tumor Types**

Variant Class	Samples per Variant Class*	Total Replicates	Average DNA yield (ng/ul)	# of concordant positives	# total variants	Overall concordance	95% Confidence Interval
Level 1 SNV	33	226	25.09	228	229	99.6%	97.6%, 100%)
Level 1 Indel	7	65	18.40	65	65	100%	94.5%, 100%)
MSI – high	27	188	25.54	184	188	97.9%	94.6%, 99.4%)

Level 2 SNV	98	773	34.30	1787	1795	99.6%	99.1%, 99.8%)
Level 2 Indel	50	373	29.94	945	954	99.1%	98.2%, 99.6%)
Level 3 SNV	64	533	41.16	1218	1228	99.2%	98.5%, 99.6%)
Level 3 Indel	42	304	27.75	1132	1148	98.6%	97.7%, 99.2%)
ALL SNV	104	826	34.47	3233	3252	99.4%	99.1%, 99.6%)
ALL Indel	69	512	27.84	2142	2167	98.9%	98.3%, 99.3%)
*Some samples had more than one variant detected per variant class.							

**Table 23. DNA Extraction Performance for TMB in 14 Tumor Types**

DNA yield	TMB (mutations/Mb)	SD	%CV
Average	12	0.3	4.2
Minimum	2	0.0	0.0
Maximum	182	2.8	21.1

## 7. Robustness

### a. Guard Banding

This study evaluated robustness of the MI Cancer Seek assay while guard banding critical parameters of the workflow including pooled library concentration and NaOH denaturation time at three levels: nominal (8 min), high (+20% and +1 min, respectively) and low (-20% and -1 min, respectively). Testing was carried out across five variant classes (SNV, indel, ERBB2 CNA, MSI and TMB) representing genetic alterations at 1-3x LoD from both CDx and tumor profiling biomarkers. A total of 420 data points (seven FFPE clinical samples x twelve replicates x five conditions) were generated across all guard banding conditions.

The results demonstrated that the guard banding conditions had no effect on the detection of all the capabilities. **Table 24** shows PPA and NPA were 100% for all conditions tested for the qualitative variant classes, and TMB included 0 for the 95% 1-sided CI around the mean difference between the TMB test and control condition.

**Table 24. Study Results for SNV, Indel, CNA, MSI, and TMB**

Comparison to Nominal Condition		SNV/Indel Results	CNA/MSI Results		TMB Results
		PPA	NPA	PPA	Mean Diff
Pool Loading Concentration	Low (-20%)	100%	100%	100%	0.4
	High (+20%)	100%	100%	100%	-0.4
NaOH Denaturing Time	Low (-1 min)	100%	100%	100%/	0.0
	High (+1 min)	100%	100%	100%	0.3

## **b. Processing Hold Times**

This study was designed to confirm the robustness of MI Cancer Seek when processing hold times are introduced during different steps of the assay workflow. TNA was extracted in replicates of four from seven clinical FFPE samples having mutations at 1-3x LoD. The samples, sourced from six tumor types, were tested under six conditions including without hold times (nominal) and with five different hold times (test condition) during the complete assay workflow using one or more reagent lots. Concordance was calculated between the nominal and test conditions. A total of 84 data points (seven FFPE clinical samples x 12 replicates) were generated for each hold time/test condition and nominal/control condition across different capabilities to evaluate impact of the Processing Hold time during the MI Cancer Seek assay workflow.

For all qualitative variant classes, both PPA and NPA were 100%, and the 95% 1-sided CI around the mean difference between the mean TMB test conditions and control condition included zero. This demonstrated that there was no significant impact to the performance of MI Cancer Seek when processing hold times were introduced at different steps of the assay workflow.

## **8. Reagent Interchangeability**

The study demonstrated that different lots of the MI Cancer Seek assay kit reagents are interchangeable. This study evaluated the performance of the MI Cancer Seek when the sub-kits, including MI Cancer Seek Extraction Kit, Quantification Kit, Library Preparation Kit, Library Quantification Kit, and Sequencing Kit were interchanged across three different lots of each kit tested with varying combinations across 11 sequencing runs, and two technical replicates of 11 FFPE clinical samples containing different genetic alterations across the variant classes.

The results demonstrated 100% PPA and NPA for SNV, indel, CNA, and MSI. For TMB, each sample had  $\leq 20\%$  CV when the average TMB was  $>6$  mut/Mb. When the average TMB was  $\leq 6$  mut/Mb for a given sample, the standard deviation was  $\leq 1$  mut/Mb.

## **B. Animal Studies**

No animal studies were conducted using MI Cancer Seek.

## **C. Additional Studies**

### **a. Comparison to earlier version of MI Cancer Seek**

The earlier version of MI Cancer Seek is used to support MI Cancer Seek performance claims for Extracted TNA Stability Study ([Section IX.A.5.d](#)) and Pan-Tumor Tissue Comparability Study ([Section IX.C.b](#)). MI Tumor Seek Hybrid V2 is an earlier version of MI Cancer Seek which shares the same workflows except HBCNA (amplification



positive control) source material and controls for MSI, TMB, and expression use different source materials between assays. Additionally, the earlier version normalization concentration, sample plate volume and tris plate volume are different, that allows for the earlier version to have a lower minimum DNA input of 25 ng as compared to the MCS minimum of 50 ng.

Concordance between MI Cancer Seek and its earlier version was demonstrated through concordance of variants and MSI in 111 clinical FFPE samples representing 14 different tumor types tested from extraction through sequencing and results are summarized in **Table 25** for SNVs, indels and MSI and **Figure 2** for TMB. These data indicate there is a high degree of concordance between the MI Cancer Seek and its earlier version, thereby justifying the use of data from the earlier version to support MI Cancer Seek performance claims for Extracted TNA Stability Study and Pan-Tumor Comparability Study. The impact of the differences between the earlier version stated above was eliminated by excluding samples that did not meet MCS testing criteria (for TNA input and other requirements such as % tumor content) from inclusion in the Extracted TNA Stability and Pan-Tumor Comparability Study.

**Table 25. Summary of Concordance Between MI Cancer Seek and the earlier version of MI Cancer Seek\***

Alteration Type	MCS <sup>1+</sup> EV <sup>2+</sup> (TP)	MCS <sup>1-</sup> EV <sup>2-</sup> (TN)	MCS <sup>1+</sup> EV <sup>2-</sup> (FP)	MCS <sup>1-</sup> EV <sup>2+</sup> (FN)	PPA% (n/N) [95% CI]	NPA% (n/N) [95% CI]
SNV, Level 1	24	2594	1	0	100% (24/24) [0.833, 1.024]	100% (2594/2595) [0.998, 1]
Indel, Level 1	2	1209	0	0	100% (2/2) [0.289, 1.044]	100% (1209/1209) [0.996, 1.001]
SNV, Level 2	21	88933	0	0	100% (21/21) [0.814, 1.026]	100% (88933/88933) [1, 1]
Indel, Level 2	3	90916	0	0	100% (3/3) [0.38, 1.049]	100% (90916/90916) [1, 1]
SNV, Level 3	306	4578470	1	0	100% (306/306) [0.985, 1.002]	100% (4578470/4578471) [1, 1]
Indel, Level 3	208	10277361	2	0	100% (208/208) [0.978, 1.004]	100% (10277361/10277363) [1, 1]
MSI	19	91	1	0	95% (19/20) [0.743, 1.007]	100% (91/91) [0.95, 1.008]
<i>ERBB2</i> CNAs, Level 2	12	20	0	0	100% (12/12) [0.713, 1.037]	100% (20/20) [0.806, 1.027]

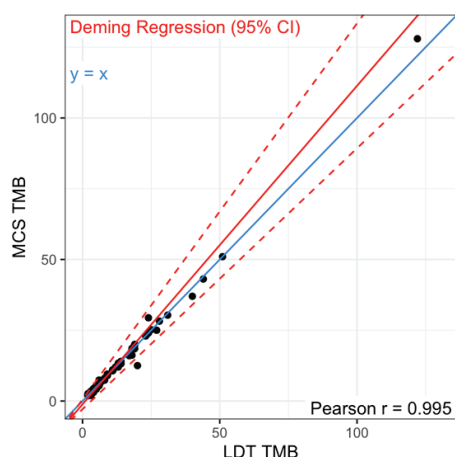
\*The earlier version of the test was considered the reference method.

Abbreviations:

<sup>1</sup>MCS= MI Cancer Seek

Alteration Type	MCS <sup>1</sup> + EV <sup>2</sup> + (TP)	MCS <sup>1</sup> - EV <sup>2</sup> - (TN)	MCS <sup>1</sup> + EV <sup>2</sup> - (FP)	MCS <sup>1</sup> - EV <sup>2</sup> + (FN)	PPA% (n/N) [95% CI]	NPA% (n/N) [95% CI]
<sup>2</sup> EV=Earlier version of MI Cancer Seek						

**Figure 2. Deming Regressions for TMB (n=111 sample cohort)**



## b. Pan Tumor Tissue Comparability

To assess the comparability between tumor types when using the MI Cancer Seek, a retrospective analysis compiled pan tumor QC parameters using data from the earlier version of MI Cancer Seek for 119,952 samples across 46 solid tumor types (**Table 26**). The concordance analysis in [Section IX.C.a](#) provides evidence that these two assays have high concordance and are deemed to be comparable. The concordance analysis between the earlier version and MI Cancer Seek considered the same sample testing criteria (for TNA input and other requirements such as % tumor content) used for the MI Seek Cancer Seek.

**Table 26. Pan Tumor QC Pass Rates by Tumor Type**

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/ $\mu$ L)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
Anaplastic Thyroid Carcinoma	2	Thyroid (63), Head (1)	64	24.2	100.0%, (64/64)	100.0%, (64/64)	100.0%, (64/64)	200.3
Bladder cancer - non-urothelial	8	Bladder (116), Urethra (24), Ureter (5), Renal pelvis (4), Kidney (1), Unknown primary site (1), Other (9)	160	35.4	98.1%, (157/160)	98.1%, (154/157)	97.5%, (153/157)	205.8

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/μL)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
Bladder cancer - urothelial	17	Bladder (2133), Renal pelvis (291), Ureter (182), Urethra (45), Kidney (26), Unknown primary site (4), Prostate (2), Other (88)	2771	31.6	97.7%, (2706/2771)	99.3%, (2686/2706)	99.3%, (2686/2706)	200.8
Breast Carcinoma	8	Breast (11360), Bladder (1), Ileum (1), Stomach (1), Unknown primary site (1), Other (54)	11418	21.6	96.1%, (10977/11418)	98.7%, (10830/10977)	98.6%, (10827/10977)	190.7
Cancer of Unknown Primary	19	Unknown primary site (2094), Connective (2), Liver (2), Lung (2), Skin (2), Appendix (1), Breast (1), Kidney (1), Ovary (1), Pleura (1), Other (15)	2122	19.8	93.1%, (1975/2122)	98.9%, (1954/1975)	98.9%, (1953/1975)	176.4
Cervical Cancer	11	Cervix (874), Endocervix (275), Cervix uteri (262), Overlapping lesion of cervix uteri (135), Exocervix (113), Vagina (3), Endometrium (1), Other (28)	1691	46.7	98.7%, (1669/1691)	99.3%, (1657/1669)	99.2%, (1656/1669)	207.4
Cholangio-carcinoma	14	Intrahepatic bile duct (1245), Bladder (509), Liver (97), Bile duct (61), Common bile duct (22), Extrahepatic bile duct (18), Unknown primary site (7), Ampulla of Vater (2), Other (49)	2010	16.6	95.4%, (1917/2010)	99.7%, (1912/1917)	99.7%, (1912/1917)	184.8
Colorectal Adeno-carcinoma	38	Rectum (3985), Colon (2887), Sigmoid colon (2686), Ascending colon (1478), Cecum (1463), Transverse colon (746), Rectosigmoid colon (623), Descending colon (511), Appendix (440), Hepatic flexure of colon (287), Splenic flexure of colon (235), Overlapping lesion of colon (214), Right colon (201), Rectosigmoid junction (138), Ileocecal valve	16442	36.4	98.3%, (16159/16442)	99.5%, (16075/16159)	99.5%, (16078/16159)	205.3

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/μL)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
		(135), Rectosigmoid (129), Left colon (68), Sigmoid (58), Anorectum (35), Anus (21), Anal canal (14), Duodenum (4), Small intestine (3), Unknown primary site (3), Lung (2), Common bile duct (1), Peritoneum (1), Stomach (1), Other (73)						
Esophageal Carcinoma	5	Esophagus (578), Gastroesophageal junction (14), Esophagogastric junction (4), Cardia (1), Stomach (1)	598	24.5	98.0%, (586/598)	100.0%, (586/586)	100.0%, (586/586)	201.3
Esophago-gastric Junction Carcinoma	10	Esophagus (1823), Gastroesophageal junction (601), Esophagogastric junction (189), Cardia (21), Stomach (17), Gastric cardia (7), Gastric (1), Pyloric antrum (1), Sigmoid colon (1), Other (1)	2662	23.7	97.9%, (2607/2662)	99.7%, (2599/2607)	99.7%, (2599/2607)	199.2
Extra-hepatic Bile Duct Adeno-carcinoma	11	Extrahepatic bile duct (113), Common bile duct (93), Bile duct (36), Intrahepatic bile duct (3), Ampulla of Vater (2), Other (25)	272	17.7	94.5%, (257/272)	100.0%, (257/257)	100.0%, (257/257)	183.5
Female Genital Tract Malignancy	36	Uterus (338), Endometrium (217), Vagina (142), Corpus uteri (129), Uterine (32), Vulva (27), Ovary (11), Cervix (9), Cervix uteri (5), Connective (5), Skin (3), Unknown primary site (3), Ileum (2), Overlapping lesion of cervix uteri (2), Peritoneum (1), Retroperitoneum (1), Other (83)	1010	45.3	98.9%, (999/1010)	99.7%, (996/999)	99.8%, (997/999)	209.6
Gastric Adeno-carcinoma	13	Stomach (1654), Cardia (108), Gastric (105), Gastric cardia (104), Pyloric antrum (77), Gastroesophageal junction (27),	2152	21.5	97.6%, (2101/2152)	99.8%, (2097/2101)	99.8%, (2096/2101)	194.2

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/μL)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
		Esophagus (4), Esophagogastric junction (3), Other (70)						
Gastro-intestinal Stromal Tumors (GIST)	42	Stomach (371), Small intestine (104), Duodenum (40), Small bowel (39), Jejunum (33), Unknown primary site (31), Gastric (28), Rectum (24), Ileum (18), Abdomen (17), Colon (8), Connective (7), Esophagus (7), Gastric cardia (4), Cardia (3), Gastroesophageal junction (3), Pancreas (3), Peritoneum (3), Retroperitoneum (2), Sigmoid colon (2), Appendix (1), Ascending colon (1), Descending colon (1), Esophagogastric junction (1), Rectosigmoid (1), Rectosigmoid colon (1), Rectosigmoid junction (1), Sigmoid (1), Other (50)	805	28.4	96.4%, (776/805)	99.9%, (775/776)	99.9%, (775/776)	198.4
Glio-blastoma	38	Frontal lobe (1119), Temporal lobe (929), Brain (618), Parietal lobe (558), Overlapping lesion of brain (174), Occipital lobe (145), Thalamus (50), Cerebellum (33), Cerebral meninges (2), Other (177)	3805	34.4	98.6%, (3753/3805)	99.8%, (3746/3753)	99.8%, (3745/3753)	208
Head and Neck Cancers	117	Base of tongue (284), Tonsil (255), Tongue (198), Oropharynx (187), Larynx (138), Head (124), Nasopharynx (108), Oral cavity (83), Supraglottis (83), Maxillary sinus (63), Hypopharynx (55), Nasal cavity (54), Unknown primary site (21), Connective (6), Skin (6), Parotid gland	2309	31.3	98.0%, (2263/2309)	98.8%, (2236/2263)	98.8%, (2235/2263)	201.2

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/μL)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
		(3), Lung (2), Other (639)						
Kidney Cancer	8	Kidney (1902), Renal pelvis (19), Bladder (1), Ureter (1), Urethra (1), Other (23)	1947	21.9	95.4%, (1857/1947)	98.9%, (1837/1857)	98.9%, (1836/1857)	190
Liver Hepato-cellular Carcinoma	3	Liver (682), Intrahepatic bile duct (2), Other (1)	685	18.9	96.2%, (659/685)	98.6%, (650/659)	98.9%, (652/659)	185
Low Grade Glioma	34	Frontal lobe (240), Brain (140), Temporal lobe (139), Parietal lobe (46), Cerebellum (31), Overlapping lesion of brain (20), Occipital lobe (13), Thalamus (10), Nasal cavity (1), Other (86)	726	23.2	97.0%, (704/726)	99.3%, (699/704)	99.3%, (699/704)	197.6
Lung Bronchioloalveolar carcinoma (BAC)	1	Lung (1)	1	8.2	100.0%, (1/1)	100.0%, (1/1)	100.0%, (1/1)	220
Lung Non-small cell lung cancer (NSCLC)	16	Lung (21508), Main bronchus (287), Bronchus (56), Unknown primary site (4), Bladder (1), Brain (1), Endometrium (1), Occipital lobe (1), Pleura (1), Other (36)	21896	23.2	96.0%, (21015/21896)	99.4%, (20888/21015)	99.4%, (20892/21015)	188.5
Lung Small Cell Cancer (SCLC)	5	Lung (874), Main bronchus (33), Bronchus (3), Unknown primary site (2), Other (1)	913	50.6	98.1%, (896/913)	99.8%, (894/896)	99.7%, (893/896)	205.2
Male Genital Tract Malignancy	13	Testis (63), Unknown primary site (2), Urethra (2), Lung (1), Penis (1), Other (31)	100	41.3	97.0%, (97/100)	99.0%, (96/97)	99.0%, (96/97)	199.4
Malignant Histiocytosis	13	Brain (2), Connective (2), Lung (1), Nasal cavity (1), Skin (1), Other (15)	22	21.5	95.5%, (21/22)	100.0%, (21/21)	100.0%, (21/21)	184.1
Malignant Solitary Fibrous Tumor of the Pleura (MSFT)	25	Connective (21), Pleura (10), Brain (5), Lung (4), Retroperitoneum (3), Unknown primary site (3), Frontal lobe (2), Occipital lobe (2),	74	35.3	90.5%, (67/74)	100.0%, (67/67)	100.0%, (67/67)	192.9

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/μL)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
		Parietal lobe (2), Abdomen (1), Cerebral meninges (1), Kidney (1), Meninges (1), Pancreas (1), Temporal lobe (1), Thigh (1), Other (15)						
Melanoma	117	Skin (3189), Unknown primary site (250), Vulva (40), Scalp (30), Nasal cavity (29), Vagina (25), Breast (21), Choroid (20), Connective (20), Rectum (15), Anorectum (12), Anal canal (9), Maxillary sinus (9), Anus (7), Thigh (4), Esophagus (3), Lung (3), Brain (2), Cervix (2), Nasopharynx (2), Bladder (1), Cecum (1), Colon (1), Duodenum (1), Exocervix (1), Gastroesophageal junction (1), Head (1), Jejunum (1), Oropharynx (1), Parotid gland (1), Small bowel (1), Stomach (1), Urethra (1), Other (265)	3970	32.1	97.7%, (3879/3970)	99.5%, (3859/3879)	99.4%, (3856/3879)	200.7
Merkel Cell Carcinoma (MCC)	26	Skin (121), Unknown primary site (28), Scalp (4), Breast (2), Connective (2), Parotid gland (2), Cecum (1), Colon (1), Head (1), Tongue (1), Other (22)	185	49.9	100.0%, (185/185)	99.5%, (184/185)	99.5%, (184/185)	205.6
Neuro-endocrine tumors	108	Unknown primary site (241), Pancreas (235), Lung (173), Bladder (74), Ileum (70), Adrenal gland (61), Prostate (59), Rectum (55), Cervix (45), Small intestine (42), Stomach (39), Small bowel (34), Esophagus (25), Colon (23), Liver (20), Cecum (19), Prostate gland (17),	1571	39.1	97.7%, (1535/1571)	98.9%, (1518/1535)	98.9%, (1518/1535)	199.4



Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/μL)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
		Ascending colon (14), Duodenum (14), Endometrium (14), Thymus (12), Vagina (12), Appendix (11), Breast (10), Jejunum (9), Thyroid (9), Anus (8), Ampulla of Vater (7), Cervix uteri (7), Ovary (7), Right colon (7), Ileocecal valve (6), Nasal cavity (6), Exocervix (5), Gastroesophageal junction (5), Kidney (5), Sigmoid colon (5), Transverse colon (5), Retroperitoneum (4), Ureter (4), Anal canal (3), Anorectum (3), Descending colon (3), Gastric (3), Gastric cardia (3), Larynx (3), Main bronchus (3), Overlapping lesion of cervix uteri (3), Overlapping lesion of colon (3), Uterus (3), Common bile duct (2), Endocervix (2), Esophagogastric junction (2), Maxillary sinus (2), Nasopharynx (2), Skin (2), Splenic flexure of colon (2), Urethra (2), Vulva (2), Abdomen (1), Base of tongue (1), Bile duct (1), Bronchus (1), Corpus uteri (1), Extrahepatic bile duct (1), Frontal lobe (1), Hepatic flexure of colon (1), Hypopharynx (1), Intrahepatic bile duct (1), Oropharynx (1), Parotid gland (1), Rectosigmoid colon (1), Renal pelvis (1), Salivary gland (1), Supraglottis (1), Tonsil (1), Other (88)						

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/μL)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
Non Epithelial Ovarian Cancer (non-EOC)	10	Ovary (318), Fallopian tube (2), Liver (1), Pleura (1), Retroperitoneum (1), Tubo-ovarian (1), Other (6)	330	70	99.4%, (328/330)	98.8%, (324/328)	98.8%, (324/328)	213.8
None Of These Apply	220	Skin (501), Cerebral meninges (319), Anus (194), Pleura (169), Anal canal (124), Femur (73), Meninges (65), Penis (63), Connective (56), Brain (53), Peritoneum (53), Cerebellum (45), Anorectum (43), Unknown primary site (34), Frontal lobe (33), Lung (30), Rectum (17), Scalp (11), Head (10), Liver (10), Nasal cavity (10), Vagina (10), Abdomen (7), Temporal lobe (7), Parietal lobe (6), Retroperitoneum (6), Adrenal gland (5), Breast (5), Occipital lobe (4), Overlapping lesion of brain (4), Testis (4), Vulva (4), Maxillary sinus (3), Pancreas (3), Stomach (3), Thigh (3), Appendix (2), Bladder (2), Esophagus (2), Ovary (2), Parotid gland (2), Prostate (2), Supraglottis (2), Bronchus (1), Colon (1), Descending colon (1), Extrahepatic bile duct (1), Ileum (1), Kidney (1), Nasopharynx (1), Oral cavity (1), Oropharynx (1), Rectosigmoid colon (1), Renal pelvis (1), Small intestine (1), Tongue (1), Urethra (1), Uterus (1), Other (639)	2655	34.6	97.6%, (2592/2655)	98.6%, (2557/2592)	98.6%, (2555/2592)	201.2
Ovarian Surface	29	Ovary (7218), Fallopian tube (1602), Peritoneum (709),	10321	46.3	98.7%, (10187/10321)	99.3%, (10120/10187)	99.4%, (10122/10187)	208.2

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/μL)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
Epithelial carcinomas		Tubo-ovarian (656), Unknown primary site (13), Endometrium (5), Retroperitoneum (4), Abdomen (2), Corpus uteri (1), Uterine (1), Uterus (1), Other (109)						
Pancreatic Adeno-carcinoma	9	Pancreas (5974), Pancreatic duct (64), Ampulla of Vater (6), Prostate (3), Prostate gland (2), Unknown primary site (2), Duodenum (1), Other (2)	6054	14.2	94.7%, (5735/6054)	99.7%, (5715/5735)	99.7%, (5715/5735)	179.7
Peripheral Nervous System Tumors	57	Adrenal gland (28), Retroperitoneum (9), Connective (6), Unknown primary site (6), Abdomen (5), Nasal cavity (3), Brain (2), Esophagus (1), Head (1), Lung (1), Rectum (1), Thigh (1), Transverse colon (1), Other (91)	156	39.6	98.7%, (154/156)	98.7%, (152/154)	98.7%, (152/154)	201.8
Pituitary carcinomas Oligodendrogloma	4	Other (30)	30	63.3	100.0%, (30/30)	100.0%, (30/30)	100.0%, (30/30)	214.3
Prostatic Adeno-carcinoma	5	Prostate (4166), Prostate gland (1900), Lung (1), Pancreas (1), Unknown primary site (1)	6069	19.7	96.2%, (5840/6069)	97.4%, (5691/5840)	97.5%, (5693/5840)	188.6
Retro-peritoneal or Peritoneal Sarcoma	37	Retroperitoneum (123), Connective (34), Kidney (7), Unknown primary site (6), Peritoneum (5), Abdomen (3), Small bowel (3), Stomach (3), Liver (2), Small intestine (2), Uterus (2), Ascending colon (1), Bladder (1), Breast (1), Colon (1), Corpus uteri (1), Gastric (1), Ileum (1), Lung (1), Rectum (1), Right colon (1), Sigmoid colon (1), Other (25)	226	27.1	98.2%, (222/226)	99.1%, (220/222)	99.5%, (221/222)	195.8

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/μL)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
Salivary Gland Tumors	51	Parotid gland (183), Salivary gland (81), Lung (13), Maxillary sinus (12), Nasal cavity (7), Unknown primary site (7), Base of tongue (6), Nasopharynx (5), Head (4), Main bronchus (4), Skin (3), Oropharynx (2), Tongue (2), Breast (1), Bronchus (1), Larynx (1), Oral cavity (1), Supraglottis (1), Other (177)	511	31.8	98.0%, (501/511)	99.2%, (497/501)	99.2%, (497/501)	197.9
Small Intestinal Malignancies	11	Duodenum (227), Ampulla of Vater (195), Jejunum (56), Small intestine (45), Small bowel (39), Appendix (37), Ileum (37), Peritoneum (1), Other (18)	655	30.3	98.8%, (647/655)	99.7%, (645/647)	99.7%, (645/647)	204
Soft Tissue Sarcoma - Well-Differentiated/Dedifferentiated Liposarcoma (WD-DDLS) for Retroperitoneal Sarcomas	30	Retroperitoneum (103), Connective (67), Peritoneum (7), Abdomen (4), Endometrium (2), Kidney (2), Breast (1), Pancreas (1), Parotid gland (1), Testis (1), Other (27)	216	20.2	93.1%, (201/216)	99.0%, (199/201)	99.0%, (199/201)	184.2
Soft Tissue Tumors	167	Connective (771), Breast (70), Skin (52), Unknown primary site (43), Thigh (41), Liver (32), Lung (30), Retroperitoneum (27), Uterus (16), Abdomen (13), Kidney (11), Scalp (10), Femur (8), Stomach (8), Endometrium (7), Prostate (7), Maxillary sinus (6), Nasal cavity (6), Ovary (6), Bladder (5), Nasopharynx (5), Frontal lobe (4), Small bowel (4), Vulva (4),	1550	29.2	95.4%, (1479/1550)	98.9%, (1462/1479)	98.9%, (1462/1479)	193.1

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/μL)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
		Adrenal gland (3), Cervix (3), Colon (3), Esophagus (3), Head (3), Penis (3), Rectum (3), Small intestine (3), Vagina (3), Brain (2), Cerebellum (2), Corpus uteri (2), Larynx (2), Oropharynx (2), Pancreas (2), Peritoneum (2), Pleura (2), Temporal lobe (2), Thyroid (2), Tubo-ovarian (2), Anus (1), Ascending colon (1), Cerebral meninges (1), Cervix uteri (1), Duodenum (1), Endocervix (1), Ileum (1), Meninges (1), Occipital lobe (1), Oral cavity (1), Parietal lobe (1), Prostate gland (1), Right colon (1), Splenic flexure of colon (1), Testis (1), Tongue (1), Uterine (1), Other (298)						
Thymic Carcinoma	4	Thymus (136), Lung (1), Unknown primary site (1), Other (5)	143	53.4	98.6%, (141/143)	100.0%, (141/141)	100.0%, (141/141)	189.6
Thyroid Carcinoma	4	Thyroid (758), Ovary (2), Larynx (1), Other (1)	762	34.8	97.2%, (741/762)	98.1%, (727/741)	98.1%, (727/741)	201.2
Uterine Neoplasms – Endometrial carcinoma	22	Endometrium (5222), Uterus (322), Corpus uteri (40), Uterine (26), Ovary (4), Cervix uteri (3), Peritoneum (2), Exocervix (1), Fallopian tube (1), Lung (1), Overlapping lesion of cervix uteri (1), Unknown primary site (1), Other (52)	5676	57.8	98.9%, (5614/5676)	99.6%, (5593/5614)	99.7%, (5595/5614)	211.3
Uterine Serous Carcinoma	11	Endometrium (1527), Uterus (109), Corpus uteri (10), Uterine (7), Tubo-ovarian (1), Other (16)	1670	62.4	99.3%, (1658/1670)	99.6%, (1652/1658)	99.7%, (1653/1658)	212.5

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration DNA Yield (ng/ $\mu$ L)	Extraction QC Pass (%)	DNA Total Reads QC Pass (%)	DNA Average Depth QC Pass (%)	Average Library DNA Input (ng)
Uveal Melanoma	9	Choroid (96), Cerebellum (1), Other (75)	172	24.3	97.7%, (168/172)	98.8%, (166/168)	98.8%, (166/168)	197.2
Vulvar Cancer (squamous cell carcinoma)	12	Vulva (315), Vagina (11), Skin (3), Other (46)	375	30	98.4%, (369/375)	99.7%, (368/369)	99.7%, (368/369)	204.9

## X. SUMMARY OF PRIMARY CLINICAL STUDIES

### Study Design

Eight clinical validation studies were completed to support the follow-on companion diagnostic (FCD) claims indicated in **Table 1** of the intended use statement. The safety and effectiveness of MI Cancer Seek was demonstrated through non-inferiority studies, in which the performance of MI Cancer Seek (FCD) was compared to FDA-approved comparator companion diagnostic (CCD) tests using remnant deidentified samples representative of the intended use population specific to each CCD. The CCD tests used to support MI Cancer Seek CDx claims and the study cohorts are summarized in **Table 27**.

The studies followed guidance from Li (2016) for non-inferiority statistical testing where the concordance study sample is not a random sample from the MI Cancer Seek intended use population and a reference standard is not available. Samples were tested once on MI Cancer Seek (FCD) and twice on the CCD (CCD1 and CCD2) following the schema in **Figure 3**. Repeats were permitted as needed and followed the applicable test instructions for use. Non-inferiority was evaluated by comparing conditional agreements between FCD, CCD1, and CCD2 based on the methods described by Li (2016).

Definitions and calculations used in non-inferiority analyses are provided below:

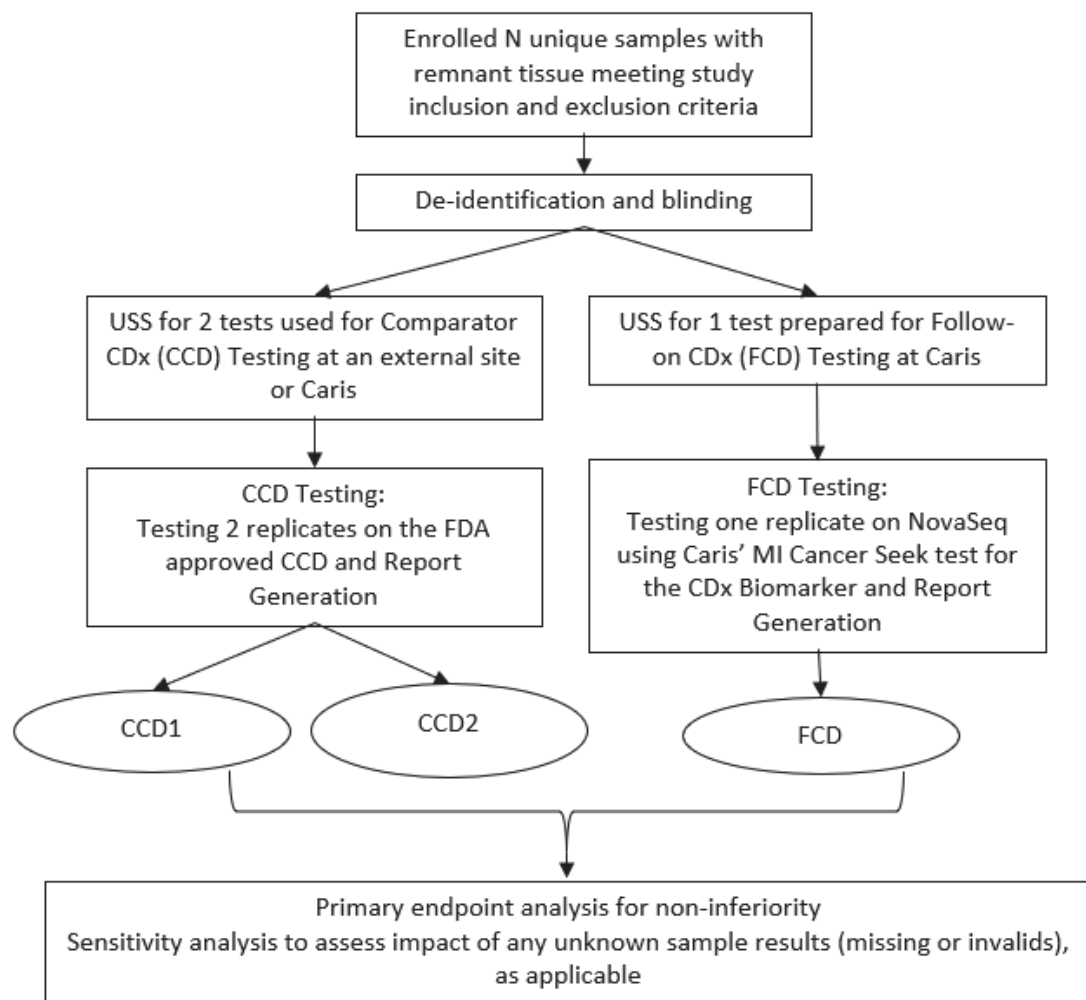
- $PPA_{C1C2}$  is the proportion of CCD1 positive results in which CCD2 is positive.
- $PPA_{C1F}$  is the proportion of CCD1 positive results in which FCD is positive.
- $PPA_{C2C1}$  is the proportion of CCD2 positive results in which CCD1 is positive.
- $PPA_{C2F}$  is the proportion of CCD2 positive results in which FCD is positive.
- $NPA_{C1C2}$  is the proportion of CCD1 negative results in which CCD2 is negative.
- $NPA_{C1F}$  is the proportion of CCD1 negative results in which FCD is negative.
- $NPA_{C2C1}$  is the proportion of CCD2 negative results in which CCD1 is negative.
- $NPA_{C2F}$  is the proportion of CCD2 negative results in which FCD is negative.

The differences in agreement (zetas) were calculated as follows:

- $\hat{\zeta}_{PPA1} = \widehat{PPA}_{C1C2} - \widehat{PPA}_{C1F}$
- $\hat{\zeta}_{PPA2} = \widehat{PPA}_{C2C1} - \widehat{PPA}_{C2F}$
- $\hat{\zeta}_{NPA1} = \widehat{NPA}_{C1C2} - \widehat{NPA}_{C1F}$
- $\hat{\zeta}_{NPA2} = \widehat{NPA}_{C2C1} - \widehat{NPA}_{C2F}$

Samples for clinical validation studies were not obtained from clinical trials and had limited demographic data available; however, baseline characteristics for age and gender were similar to the pivotal clinical trial populations for each original CDx. All studies based on non-inferiority passed the acceptance criteria specified in each study protocol.

**Figure 3. General Clinical Validation Schema**





**Table 27. Summary of Comparator CDx Tests and CV Study Cohorts**

<b>Biomarker and indication</b>	<b>FDA-approved Comparator Method</b>	<b>Unique Enrolled Samples</b>	<b>Eligible Samples (Tested with FCD &amp; CCD)</b>	<b>Samples with Complete Records</b>
MSI Status in Endometrial Carcinoma and Solid Tumor Types	MMR RxDx Panel	401	401	401
<i>BRAF</i> V600E/K in melanoma	THxID <i>BRAF</i> Kit	334	334	330
<i>BRAF</i> V600E in CRC	<i>therascreen BRAF</i> V600E RGQ PCR Kit	358	357	352
<i>KRAS</i> and <i>NRAS</i> Wild-Type in Advanced CRC	Praxis Extended RAS Panel	286	286	262
<i>EGFR</i> exon 19 deletions or L858R mutations in NSCLC	cobas <i>EGFR</i> Mutation Test V2	328	316	315
<i>PIK3CA</i> alterations in BC	<i>therascreen PIK3CA</i> RGQ PCR Kit	356	348	343

Data from these non-inferiority clinical concordance studies were the basis for this PMA approval decision. A summary of each clinical validation study is presented below.

**A. MI Cancer Seek Clinical Concordance Study for MSI in Endometrial Carcinoma and Solid Tumor Types**

The safety and effectiveness of MI Cancer Seek as an FCD was evaluated for the detection of MSI-H status in patients with solid tumors who may be eligible for treatment with FDA-approved therapies including KEYTRUDA (pembrolizumab) or JEMPERLI (dostarlimab-gxly) for patients with solid tumors or KEYTRUDA in combination with LENVIMA (lenvatinib) for endometrial carcinoma (EC). The study cohort was enriched to enroll an approximately equal number of positive and negative samples using comparator results (CCD1) from prior VENTANA MMR RxDx Panel testing. A total of 401 unique FFPE solid tumor samples with a positive CCD1 (VENTANA MMR RxDx Panel) test result were tested once on the VENTANA MMR RxDx Panel (CCD2) and once on MI Cancer Seek. Enrollment was based on both VENTANA MMR RxDx Panel's requirement criteria (minimum of 50 tumor cells) and MI Cancer Seek's requirements (20% tumor content and 25mm<sup>2</sup> tissue area). There were no missing samples or indeterminate results (i.e., results that are not reportable due to quantity not sufficient - QNS or sequencing depth not reached) results in the study for the FCD or CCD, therefore no sensitivity analysis to evaluate invalids was performed. In the clinical setting, data from Caris' commercial CLIA lab indicated a 4% QNS rate for MSI from 164,967 cases when using an earlier version that was shown to be equivalent to MCS (see

[Section IX.C.a\)](#) In case of an indeterminate MSI call, the patient report will carry the following message: “It was not possible to calculate the Microsatellite Instability (MSI) for this sample because the sequencing data did not reach the minimum depth of coverage required to provide a result on this biomarker.”

Solid tumor samples used for this study encompassed over 15 different tumor types which had similar age representation per MSI status. The demographics in the solid tumors cohort (mean age 66, 81% female) are comparable to the GARNET clinical trial population tested with the VENTANA MMR RxDx Panel (mean age 62, 77% female). To support the indications listed in **Table 1**, the cohort was analyzed two ways. The first analysis evaluated all solid tumor type samples and the second analysis used only the EC subset.

### **Solid Tumor CDx Indication**

In the 401 eligible samples tested on both MI Cancer Seek and CCD, no invalids were observed. All samples had complete records (i.e., valid results on each MI Cancer Seek, CCD1, and CCD2) and were available for the primary non-inferiority analysis. Concordance between MI Cancer Seek and the MMR RxDx Panel CCD is shown in **Table 28**.

**Table 28. Concordance Table with CCD1, CCD2 and MI Cancer Seek Results from Eligible MSI Solid Tumor Samples**

	CCD1+			CCD1-		
	CCD2+	CCD2-	Total	CCD2+	CCD2-	Total
MI Cancer Seek+	193	2	195	0	3	3
MI Cancer Seek-	5	1	6	3	194	197
Total	198	3	201	3	197	200

Concordance results from **Table 28** were used to determine the unadjusted and prevalence adjusted agreements in which the prevalence adjusted agreements were calculated using Bayes’s formula to be consistent with the sample selection strategy in this study as shown in **Table 29**.

**Table 29. Conditional Agreements for MSI Solid Tumor**

	% Agreement	
	Unadjusted	Adjusted (3.6% Prevalence)
PPA <sub>C1C2</sub>	98.5%	98.5%
PPA <sub>C1F</sub>	97.0%	97.0%
PPA <sub>C2C1</sub>	98.5%	71.0%
PPA <sub>C2F</sub>	96.0%	69.2%
NPA <sub>C1C2</sub>	98.5%	98.5%
NPA <sub>C1F</sub>	98.5%	98.5%
NPA <sub>C2C1</sub>	98.5%	99.9%
NPA <sub>C2F</sub>	97.5%	98.4%

Non-inferiority was measured by determining differences ( $\zeta$ ) between the adjusted PPAs and NPAs. The point estimates and corresponding upper and lower bounds of two-sided 95% CIs are shown in **Table 30**. The upper bound of the 95% CI was  $\leq 4\%$  for each non-inferiority hypothesis test. A sensitivity analysis was performed to evaluate the impact of prevalence at two additional levels and the collective results demonstrated that MI Cancer Seek is non-inferior to the FDA-approved VENTANA MMR RxDx Panel using pre-defined non-inferiority margins.

**Table 30. Concordance Analysis for Non-Inferiority Hypothesis Tests Based on Adjusted Prevalence for MSI Solid Tumor**

	Prevalence (3.6%)	
	Point Estimate (%)	95% two-sided CI* (%)
$\zeta_{PPA1}$	1.5	(-1.0, 4.0)
$\zeta_{PPA2}$	1.8	(0.4, 3.7)
$\zeta_{NPA1}$	0	(-2.5, 2.5)
$\zeta_{NPA2}$	15	(0.0, 3.5)
*Calculated using the percentile bootstrap method with 1000 bootstrap samples.		

### **Endometrial Carcinoma**

Concordance was determined for the 251 EC samples enrolled in the study in which the prevalence adjusted agreements were calculated using Bayes's formula to be consistent with the sample selection strategy in this study as shown in **Table 31**.

**Table 31. Concordance Table with CCD1, CCD2 and MI Cancer Seek Results from Eligible MSI EC Samples**

	CCD1+			CCD1-		
	CCD2+	CCD2-	Total	CCD2+	CCD2-	Total
MI Cancer Seek+	120	1	121	0	3	3
MI Cancer Seek-	2	0	2	2	123	125
Total	122	1	123	2	126	128

Concordance results from **Table 31** were used to determine the unadjusted and prevalence adjusted agreements in which the prevalence adjusted agreements were calculated using Bayes's formula to be consistent with the sample selection strategy in this study as shown in **Table 32**.

**Table 32. Conditional Agreements for MSI EC**

	% Agreement	
	Unadjusted	Adjusted (31.2% Prevalence)
PPA <sub>C1C2</sub>	99.2%	99.2%
PPA <sub>C1F</sub>	98.4%	98.4%
PPA <sub>C2C1</sub>	98.4%	96.6%
PPA <sub>C2F</sub>	96.8%	95.1%
NPA <sub>C1C2</sub>	98.4%	98.4%

	% Agreement	
	Unadjusted	Adjusted (31.2% Prevalence)
NPA <sub>C1F</sub>	97.7%	97.7%
NPA <sub>C2C1</sub>	99.2%	99.6%
NPA <sub>C2F</sub>	96.9%	97.3%

Non-inferiority was measured by determining differences ( $\zeta$ ) between the adjusted PPAs and NPAs in **Table 32**. The point estimates and corresponding upper and lower bounds of two-sided 95% CIs are shown in **Table 33**. The upper bound of the 95% CI was  $\leq 5.5\%$  for each non-inferiority hypothesis test. A sensitivity analysis was performed to evaluate the impact of prevalence at two additional levels and the collective results demonstrated that MI Cancer Seek is non-inferior to the MMR Rx Dx Panel using pre-defined non-inferiority margins.

**Table 33. Concordance Analysis for Non-Inferiority Hypothesis Tests Based on Adjusted Prevalence for MSI EC**

	Prevalence 31.2%	
	Point Estimate (%)	95% two-sided CI* (%)
$\zeta_{PPA1}$	0.8	(-1.6, 3.3)
$\zeta_{PPA2}$	1.5	(0.0, 3.9)
$\zeta_{NPA1}$	0.8	(-2.3, 3.9)
$\zeta_{NPA2}$	2.4	(0.0, 5.5)

\*Calculated using the percentile bootstrap method with 1000 bootstrap samples.

## **B. MI Cancer Seek Clinical Concordance Study for *BRAF* V600E or V600K Mutations in Melanoma**

The safety and effectiveness of MI Cancer Seek as an FCD was evaluated for the detection of *BRAF* V600E and V600K mutations in patients with melanoma who may be eligible for treatment with the FDA-approved therapies including trametinib (Mekinist®) or *BRAF/MEK* Inhibitor Combinations. The study cohort was enriched to enroll an approximately equal number of positive and negative samples using results from other NGS Assays which are independent of MI Cancer Seek. A total of 334 unique FFPE melanoma tissue samples were enrolled and tested twice on the THxID *BRAF* Kit (CCD) and once on MI Cancer Seek. The demographics for age and gender in this study (mean age 63, 38% female, 61% male) were comparable to the study population in the pivotal BREAK-3 trial of da*BRAF*enib using the bioMérieux THxID *BRAF* CDx Kit.

To support the indications listed in **Table 1**, the cohort was analyzed two ways. The first analysis treated V600E mutations as positive and V600K mutations as negative and the second analysis treated both V600E and V600K mutations as positive.

### **V600E as Positive, V600K as Negative**

In the 334 eligible samples tested on both MI Cancer Seek and CCD, there were four samples with invalid data, resulting in 330 samples with complete records available for

the primary non-inferiority analysis. The missing data points were evaluated in a sensitivity analysis that included best-case and worst-case scenarios using all eligible samples (N=334). Concordance between MI Cancer Seek and the THxID *BRAF* Kit CCD is shown in **Table 34**.

**Table 34. Concordance Table with CCD1, CCD2 and MI Cancer Seek Results from Eligible *BRAF* V600E Melanoma Samples**

	NGS Assays +				NGS Assays -			
	CCD1+		CCD1 -		CCD1+		CCD1-	
	CCD2 +	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-
MI Cancer Seek+	152	0	0	1	0	0	0	0
MI Cancer Seek-	1	0	0	0	1	2	0	173
Total	153	0	0	1	1	2	0	173

Concordance results from **Table 34**, excluding invalids, were used to determine the unadjusted and prevalence adjusted agreements in which the prevalence adjusted agreements were calculated using Bayes's formula to be consistent with the sample selection strategy in this study as shown in **Table 35**.

**Table 35. Conditional Agreements for *BRAF* V600E in Melanoma**

	% Agreement	
	Unadjusted	Adjusted (23.8% Prevalence)
PPA <sub>C1C2</sub>	98.7%	96.5%
PPA <sub>C1F</sub>	97.4%	94.2%
PPA <sub>C2C1</sub>	100.0%	100%
PPA <sub>C2F</sub>	98.7%	97.6%
NPA <sub>C1C2</sub>	100.0%	100%
NPA <sub>C1F</sub>	99.4%	99.8%
NPA <sub>C2C1</sub>	98.9%	98.9%
NPA <sub>C2F</sub>	99.4%	99.8%

Non-inferiority was measured by determining differences ( $\zeta$ ) between the adjusted PPAs and NPAs in **Table 35**. The point estimates and corresponding upper and lower bounds of two-sided 95% CIs are shown in **Table 36**. The upper bound of the 95% CI was  $\leq 6.4\%$  for each non-inferiority hypothesis test. A sensitivity analysis was performed to evaluate the impact of prevalence at two additional levels and the collective results demonstrated that MI Cancer Seek is non-inferior to the FDA-approved THxID *BRAF* Kit using the pre-defined non-inferiority margins.

**Table 36. Concordance Analysis for Non-Inferiority Hypothesis Tests Based on Adjusted Prevalence for *BRAF* V600E in Melanoma**

	Prevalence (23.8%)	
	Point Estimate (%)	95% two-sided CI* (%)
$\zeta_{PPA1}$	2.4	(0.0, 6.3)
$\zeta_{PPA2}$	2.4	(0.0, 6.4)
$\zeta_{NPA1}$	0.2	(0.0, 0.6)
$\zeta_{NPA2}$	-0.9	(-2.8, 0.4)

\*Calculated using the percentile bootstrap method with 1000 bootstrap samples.

### **Both V600E and V600K Variants as Positive**

In the 334 eligible samples tested on both MI Cancer Seek and CCD, there were four samples with invalid data, resulting in 330 samples with complete records (i.e., valid results on each FCD, CCD1, and CCD2) available for the primary non-inferiority analysis. The missing data points were evaluated in a sensitivity analysis that included best-case and worst-case scenarios using all eligible samples (N=334). Concordance between MI Cancer Seek and the THxID *BRAF* Kit CCD from *BRAF* V600E/K melanoma samples is shown in **Table 37**. In addition, concordance from *BRAF* V600K positive melanoma samples are shown separately in **Table 38**.

**Table 37. Concordance Table with CCD1, CCD2 and MI Cancer Seek Results from Eligible *BRAF* V600E/K Melanoma Samples**

	NGS Assays +				NGS Assays -			
	CCD1+		CCD1 -		CCD1+		CCD1-	
	CCD2+	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-
MI Cancer Seek+	173	0	0	1	0	0	0	0
MI Cancer Seek-	1	0	0	0	1	2	0	152
Total	174	0	0	1	1	2	0	152

**Table 38. Concordance Table with CCD1, CCD2 and MI Cancer Seek Results from Eligible *BRAF* V600K Melanoma Samples**

	NGS Assays +				NGS Assays -			
	CCD1+		CCD1 -		CCD1+		CCD1-	
	CCD2+	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-
MI Cancer Seek+	21	0	0	0	0	0	0	0
MI Cancer Seek-	0	0	0	0	0	1	0	308
Total	21	0	0	0	0	1	0	308

Concordance results from **Table 37**, excluding invalids, were used to determine the unadjusted and prevalence adjusted agreements in which the prevalence adjusted agreements were calculated using Bayes's formula to be consistent with the sample selection strategy in this study as shown in **Table 39**.

**Table 39. Conditional Agreements for *BRAF* V600E/K in Melanoma**

	% Agreement	
	Unadjusted	Adjusted (31% Prevalence)
PPA <sub>C1C2</sub>	98.9%	97.2%
PPA <sub>C1F</sub>	97.7%	95.3%
PPA <sub>C2C1</sub>	100.0%	100%
PPA <sub>C2F</sub>	98.9%	98.0%
NPA <sub>C1C2</sub>	100.0%	100%
NPA <sub>C1F</sub>	99.3%	99.7%
NPA <sub>C2C1</sub>	98.7%	98.7%
NPA <sub>C2F</sub>	99.4%	99.7%

Non-inferiority was measured by determining differences ( $\zeta$ ) between the adjusted PPAs and NPAs in **Table 39**. The point estimates and corresponding upper and lower bounds of two-sided 95% CIs are shown in **Table 40**. The upper bound of the 95% CI was  $\leq 5.3\%$  for each non-inferiority hypothesis test. A sensitivity analysis was performed to evaluate the impact of prevalence at two additional levels and the collective results demonstrated that MI Cancer Seek is non-inferior to the FDA-approved THxID *BRAF* kit using the pre-defined non-inferiority margins.

**Table 40. Concordance Analysis for Non-Inferiority Hypothesis Tests Based on Adjusted Prevalence for *BRAF* V600E/K in Melanoma**

	Prevalence (31%)	
	Point Estimate (%)	95% two-sided CI* (%)
$\zeta_{PPA1}$	1.9	(0.0, 5.2)
$\zeta_{PPA2}$	2.0	(0.0, 5.3)
$\zeta_{NPA1}$	0.3	(0.0, 0.8)
$\zeta_{NPA2}$	-1.0	(-3.1, 0.5)
*Calculated using the percentile bootstrap method with 1000 bootstrap samples.		

### C. MI Cancer Seek Clinical Concordance Study for *BRAF* V600E in CRC

The safety and effectiveness of MI Cancer Seek as an FCD was evaluated for the detection of *BRAF* V600E mutations in patients with CRC who may be eligible for treatment with FDA-approved therapies including encorafenib (*BRAFTOVI*®) in combination with cetuximab (*ERBITUX*®). The study cohort was enriched to enroll an approximately equal number of positive and negative samples using results from other NGS Assays which are independent of MI Cancer Seek. A total of 360 histologically confirmed FFPE CRC tissues samples were enrolled and tested twice on the *therascreen BRAF* V600E RGQ PCR Kit (CCD) and once on MI Cancer Seek. Two duplicate samples were enrolled, and one sample did not meet MI Cancer Seek testing requirements, therefore 357 unique samples were tested on MI Cancer Seek. The demographics for age and gender in this study (mean age 67, 58% female, 41% male) were comparable to the BEACON clinical trial population tested with the *therascreen BRAF* V600E RGQ PCR Kit (mean age 59.4, 48.7% female, 51.3% male).



In the 357 samples tested on both MI Cancer Seek and CCD, there were five samples with invalid data, resulting in 352 samples with complete records available for the primary non-inferiority analysis. The missing data points were evaluated in a sensitivity analysis that included best-case and worst-case scenarios using all eligible samples (N=357). Concordance between MI Cancer Seek and the *therascreen BRAF* V600E RGQ PCR Kit CCD is shown in **Table 41**.

**Table 41. Concordance Table with CCD1, CCD2, and MI Cancer Seek Results from Eligible *BRAF* V600E CRC Samples**

	NGS Assays +				NGS Assays -			
	CCD1+		CCD1 -		CCD1+		CCD1-	
	CCD2+	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-
MI Cancer Seek+	176	1	0	0	0	0	0	0
MI Cancer Seek-	0	0	0	0	1	0	0	174
Total	176	1	0	0	1	0	0	174

Concordance results from **Table 41**, excluding invalids, were used to determine the unadjusted and prevalence adjusted agreements in which the prevalence adjusted agreements were calculated using Bayes's formula to be consistent with the sample selection strategy in this study as shown in **Table 42**.

**Table 42. Conditional Agreements for *BRAF* V600E in CRC**

	% Agreement	
	Unadjusted	Adjusted (10% Prevalence)
PPA <sub>C1C2</sub>	99.4%	99.5%
PPA <sub>C1F</sub>	99.4%	95.1%
PPA <sub>C2C1</sub>	100%	100.0%
PPA <sub>C2F</sub>	99.4%	95.1%
NPA <sub>C1C2</sub>	100%	100.0%
NPA <sub>C1F</sub>	100.0%	100.0%
NPA <sub>C2C1</sub>	99.4%	99.9%
NPA <sub>C2F</sub>	99.4%	99.9%

Non-inferiority was measured by determining differences ( $\zeta$ ) between the adjusted PPAs and NPAs in **Table 42**. The point estimates and corresponding upper and lower bounds of two-sided 95% CIs are shown in **Table 43**. The upper bound of the 95% CI was  $\leq 13.4\%$  for the PPA non-inferiority hypothesis test and 0% for NPA. A sensitivity analysis was performed to evaluate the impact of prevalence at two additional levels and the collective results demonstrated that MI Cancer Seek is non-inferior to the FDA-approved *therascreen BRAF* V600E RGQ PCR Kit using the pre-defined non-inferiority margins.

**Table 43. Concordance Analysis for Non-Inferiority Hypothesis Tests Based on Adjusted Prevalence for *BRAF* V600E in CRC**

	Prevalence (10%)	
	Point Estimate (%)	95% two-sided CI* (%)
$\zeta_{PPA1}$	4.4	(-1.7, 13.2)
$\zeta_{PPA2}$	4.9	(0.0, 13.4)
$\zeta_{NPA1}$	0.0	(0.0, 0.0)
$\zeta_{NPA2}$	0.0	(0.0, 0.0)

\*Calculated using the percentile bootstrap method with 1000 bootstrap samples.

**D. MI Cancer Seek Clinical Concordance Study for *KRAS* and *NRAS* Wild-Type in CRC**

The safety and effectiveness of MI Cancer Seek as an FCD was evaluated for the detection of wild type status for *KRAS* (exons 2, 3, and 4) and *NRAS* (exons 2, 3, and 4) in patients with CRC who may be eligible for treatment with the FDA-approved VECTIBIX® (panitumumab). A total of 286 unique FFPE tissue samples histologically confirmed as CRC were enrolled and tested twice on the Praxis Extended RAS Panel (CCD) and once on MI Cancer Seek. The demographics for age and gender in this study (mean age 61, 55% female, 44% male) are comparable to the population tested with the Praxis Extended RAS Panel in the pivotal PRIME trial.

In the 286 samples tested on both MI Cancer Seek and CCD, there were 24 samples with invalid data, resulting in 262 samples with complete records available for the primary non-inferiority analysis. The missing data points were evaluated in a sensitivity analysis that included best-case and worst-case scenarios using all eligible samples (N=286). Concordance between MI Cancer Seek and the Praxis Extended RAS Panel CCD is shown in **Table 44**.

**Table 44. Concordance Table with CCD1\*, CCD2, and MI Cancer Seek Results from Eligible *KRAS/NRAS* CRC Samples**

	CCD1 +				CCD1 -			
	CCD2+	CCD2-	CCD2 Invalid	Total	CCD2+	CCD2-	CCD2 Invalid	Total
MI Cancer Seek+	118	0	5	123	0	4	1	5
MI Cancer Seek-	0	1	0	1	0	139	10	149
MI Cancer Seek Invalid	1	0	0	1	0	0	0	0
Total	119	1	5	125	0	143	11	154

\*There were 7 invalid samples for CCD1.

Concordance results from **Table 44**, excluding invalids, were used to determine the unadjusted agreements shown in **Table 45**.

**Table 45. Conditional Agreements for *KRAS/NRAS***

	Unadjusted Agreements
PPA <sub>C1C2</sub>	99.2%
PPA <sub>C1F</sub>	99.2%
PPA <sub>C2C1</sub>	100%
PPA <sub>C2F</sub>	100%
NPA <sub>C1C2</sub>	100.0%
NPA <sub>C1F</sub>	97.2%
NPA <sub>C2C1</sub>	99.3%
NPA <sub>C2F</sub>	97.2%

Non-inferiority was measured by determining differences ( $\zeta$ ) between the PPAs and NPAs in **Table 45**. The point estimates and corresponding upper and lower bounds of two-sided 95% CIs were calculated using the Wilson method from Newcombe, RG (2) and the results are shown in **Table 46**. The upper bound of the 95% CI was  $\leq 6.9\%$  for the non-inferiority hypothesis test. These results demonstrate MI Cancer Seek is non-inferior to the FDA-approved Praxis Extended RAS Panel using the pre-defined non-inferiority margins.

**Table 46. Concordance Analysis for Non-Inferiority Hypothesis Tests with *KRAS/NRAS* in CRC**

	Point Estimate (%)	95% two-sided CI* (%)
$\zeta_{PPA1}$	0.0	(-3.5, 3.5)
$\zeta_{PPA2}$	0.0	(-3.2, 3.2)
$\zeta_{NPA1}$	2.8	(-0.3, 6.9)
$\zeta_{NPA2}$	2.1	(-1.5, 6.3)
*Calculated using the percentile bootstrap method with 1000 bootstrap samples.		

#### **E. MI Cancer Seek Clinical Concordance Study for *EGFR* exon 19 Deletion or L858R Mutations in NSCLC**

The safety and effectiveness of MI Cancer Seek as an FCD was evaluated for the detection of *EGFR* exon 19 deletion or L858R mutations in patients with NSCLC who may be eligible for treatment with single-agent Tyrosine Kinase Inhibitors (TKIs) approved by the FDA. The study cohort was enriched to enroll an approximately equal number of positive and negative samples using results from other NGS Assays which are independent of MI Cancer Seek. A total of 328 unique FFPE tissues samples histologically confirmed as NSCLC were enrolled into the study and tested twice on the cobas *EGFR* Mutation Test V2 (CCD). Twelve samples did not meet MI Cancer Seek testing requirements; therefore 316 samples were tested once on MI Cancer Seek. The demographics for age and gender in this study (mean age 67, 56% female) are comparable to the EORTC clinical trial population tested with the Roche cobas *EGFR* Mutation Test V2 (mean age 64, 73% female).

In the 316 samples tested on both MI Cancer Seek and CCD, there was one sample with invalid data, resulting in 315 samples with complete records available for the primary non-inferiority analysis. The missing data point was evaluated in a sensitivity analysis that included best-case and worst-case scenarios using all eligible samples (N=316). Concordance between MI Cancer Seek and the cobas *EGFR* Mutation Test V2 CCD is shown in **Table 47**.

**Table 47. Concordance Table with CCD1, CCD2 and MI Cancer Seek Results from Eligible *EGFR* NSCLC Samples**

	NGS Assays +				NGS Assays -			
	CCD1+		CCD1 -		CCD1+		CCD1-	
	CCD2+	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-
MI Cancer Seek+	151	0	0	1	0	0	0	0
MI Cancer Seek-	3	0	0	0	0	0	0	160
Total	154	0	0	1	0	0	0	160

Concordance results from **Table 47**, excluding invalids, were used to determine the unadjusted and prevalence adjusted agreements in which the prevalence adjusted agreements were calculated using Bayes's formula to be consistent with the sample selection strategy in this study as shown in **Table 48**.

**Table 48. Conditional Agreements for *EGFR* in NSCLC**

	% Agreement	
	Unadjusted	Adjusted (10% Prevalence)
PPA <sub>C1C2</sub>	100.0%	100.0%
PPA <sub>C1F</sub>	98.1%	98.1%
PPA <sub>C2C1</sub>	100.0%	100.0%
PPA <sub>C2F</sub>	98.1%	98.1%
NPA <sub>C1C2</sub>	100.0%	100.0%
NPA <sub>C1F</sub>	99.4%	99.9%
NPA <sub>C2C1</sub>	100.0%	100.0%
NPA <sub>C2F</sub>	99.4%	99.9%

Non-inferiority was measured by determining differences ( $\zeta$ ) between the adjusted PPAs and NPAs in **Table 48**. The point estimates and corresponding upper and lower bounds of two-sided 95% CIs are shown in **Table 49**. The upper bound of the 95% CI was  $\leq 4.5\%$  for each non-inferiority hypothesis test. A sensitivity analysis was performed to evaluate the impact of prevalence at two additional levels and the collective results demonstrated that MI Cancer Seek is non-inferior to the FDA-approved cobas *EGFR* Mutation Test V2 using pre-defined non-inferiority margins.

**Table 49. Concordance Analysis for Non-Inferiority Hypothesis Tests Based on Adjusted Prevalence for *EGFR* in NSCLC**

	Prevalence (10%)	
	Point Estimate (%)	95% two-sided CI* (%)
$\hat{\zeta}_{PPA1}$	1.9	(0.0, 4.5)
$\hat{\zeta}_{PPA2}$	1.9	(0.0, 4.5)
$\hat{\zeta}_{NPA1}$	0.1	(0.0, 0.2)
$\hat{\zeta}_{NPA2}$	0.1	(0.0, 0.2)
*Calculated using the percentile bootstrap method with 1000 bootstrap samples.		

**F. MI Cancer Seek Clinical Concordance Study for *PIK3CA* Alterations in Breast Cancer**

The safety and effectiveness of MI Cancer Seek as an FCD was evaluated for the detection of *PIK3CA* mutations in patients with breast cancer who may be eligible for treatment with PIQRAY® (alpelisib) in accordance with the approved therapeutic product labeling. The study cohort was enriched to enroll an approximately equal number of positive and negative samples using results from other NGS Assays which are independent of MI Cancer Seek. A total of 356 unique FFPE tissue samples histologically confirmed as BC were enrolled and tested twice on the *therascreen PIK3CA* RGQ PCR Kit (CCD). One sample with CCD1 result was invalid. Eight samples did not meet MI Cancer Seek testing requirements; therefore, 348 samples were tested once on MI Cancer Seek. The demographics for age and gender in this study (mean age 60, 100% female) were comparable to the study population in the pivotal SOLAR-1 trial tested with the *therascreen PIK3CA* RGQ PCR Kit (mean age 63.3, 99.7% female).

In the 348 samples tested on both MI Cancer Seek and CCD, there were five sample with invalid data, resulting in 343 samples with complete records available for the primary non-inferiority analysis. The missing data points were evaluated in a sensitivity analysis that included best-case and worst-case scenarios using all eligible samples (N=348). Concordance between MI Cancer Seek and the *therascreen PIK3CA* RGQ PCR Kit CCD is shown in **Table 50**.

**Table 50. Concordance Table with CCD1, CCD2 and MI Cancer Seek Results from Eligible *PIK3CA* BC Samples**

	NGS Assays +				NGS Assays -			
	CCD1+		CCD1 -		CCD1+		CCD1-	
	CCD2+	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-	CCD2+	CCD2-
MI Cancer Seek+	174	0	0	0	0	0	0	0
MI Cancer Seek-	0	0	0	4	1	2	1	161
Total	174	0	0	4	1	2	1	161

Concordance results from the **Table 50**, excluding invalids, were used to determine the unadjusted and prevalence adjusted agreements in which the prevalence adjusted

agreements were calculated using Bayes's formula to be consistent with the sample selection strategy in this study as shown in **Table 51**.

**Table 51. Conditional Agreements for *PIK3CA* in BC**

	% Agreement	
	Unadjusted	Adjusted (36.4% Prevalence)
PPA <sub>C1C2</sub>	98.9%	97.9%
PPA <sub>C1F</sub>	98.3%	96.9%
PPA <sub>C2C1</sub>	99.4%	98.9%
PPA <sub>C2F</sub>	98.9%	97.9%
NPA <sub>C1C2</sub>	99.4%	99.4%
NPA <sub>C1F</sub>	100.0%	100.0%
NPA <sub>C2C1</sub>	98.8%	98.8%
NPA <sub>C2F</sub>	100.0%	100.0%

Non-inferiority was measured by determining differences ( $\zeta$ ) between the adjusted PPAs and NPAs in **Table 51**. The point estimates and corresponding upper and lower bounds of two-sided 95% CIs are shown in **Table 52**. The upper bound of the 95% CI was  $\leq 3.9\%$  for each non-inferiority hypothesis test. A sensitivity analysis was performed to evaluate the impact of prevalence at two additional levels and the collective results demonstrated that MI Cancer Seek is non-inferior to the FDA-approved cobas *therascreen PIK3CA* RGQ PCR Kit using the pre-defined non-inferiority margins.

**Table 52. Concordance Analysis for Non-Inferiority Hypothesis Tests Based on Adjusted Prevalence for *PIK3CA* in BC**

	Prevalence (36.4%)	
	Point Estimate	95% two-sided CI* (%)
$\zeta_{PPA1}$	1.0	(0.0, 3.9)
$\zeta_{PPA2}$	1.1	(0.0, 3.9)
$\zeta_{NPA1}$	-0.6	(-1.9, 0.0)
$\zeta_{NPA2}$	-1.2	(-3.0, 0.0)

\*Calculated using the percentile bootstrap method with 1000 bootstrap samples.

## **XI. FINANCIAL DISCLOSURE**

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

- Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0
- Significant payment of other sorts: 0

- Proprietary interest in the product tested held by the investigator: 0
- Significant equity interest held by investigator in sponsor of covered study: 1

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

## **XII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION**

Not applicable.

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

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

## **XIV. SAFETY AND EFFECTIVENESS RESULTS**

### **A. Effectiveness Conclusions**

Analytical performance studies were conducted with MI Cancer Seek using FFPE tissue or TNA extracted from FFPE tissue from a variety of cancer types. The performance for detecting the tested variants, when the test is used in accordance with the directions provided, has been characterized based on clinical and non-clinical studies conducted for the device as described above. The clinical benefit of MI Cancer Seek in the detection of alterations listed in the intended use was demonstrated through six clinical concordance studies using previously approved CDx tests as the comparator methods. All studies based on a non-inferiority (NI) statistical testing approach passed the acceptance criteria specified in each study protocol. The concordance observed between MI Cancer Seek and the approved CDx tests supports the effectiveness of MI Cancer Seek to identify patients whose tumors are positive for the alterations listed in the intended use and for which MI Cancer Seek results can be used to direct use of the associated therapeutics.

### **B. Safety Conclusions**

The risks of the device are based on analytical studies as well as data collected in clinical studies conducted to support PMA approval as described above. MI Cancer Seek is an IVD test, performed using TNA extracted from FFPE tumor tissue. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results and/or inappropriate patient management decisions in cancer treatment. Patients with false positive results may undergo treatment with one of the therapies listed in **Table 1** of the intended use statement without clinical benefit and may experience



adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy, from which they might have received meaningful clinical benefit. There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy.

### C. **Benefit-Risk Determination**

The probable benefits of the device are based on data collected in the analytical validation and clinical concordance studies conducted to support PMA approval. Treatment with the therapies listed in **Table 1** of the intended use provides meaningful clinical benefit to the patients selected for those therapies based on the finding of the associated genomic alterations. Based on data provided in each of the clinical concordance studies, which compared the ability of the MI Cancer Seek to detect the mutations to the corresponding comparator companion diagnostic assays, MI Cancer Seek is non-inferior to those comparator methods for patient selection. The performance of the MI Cancer Seek test with respect to the corresponding comparator companion diagnostic tests was also considered to be clinically acceptable, beyond passing the statistical acceptance criteria; therefore, there is probable benefit for the use of the MI Cancer Seek test for selection of patients with alterations, in the specific tumor types listed in **Table 1** above, for administration of the specific corresponding therapeutics. The concordance between the MI Cancer Seek and the comparator companion diagnostic tests was highest for alterations at allele frequencies above the LoD of the assay. For the results of the individual clinical concordance, using the non-inferiority study design, please see Section X above, for detailed performance for each biomarker. In addition, this test has significant probable clinical benefit, in that it interrogates a large panel of genomic alterations, namely detection of single nucleotide variants (SNVs) and insertions and deletions (indels) in 228 genes, copy number amplification (CNA) in one gene (*ERBB2*), and assessment of microsatellite instability (MSI) and quantitative detection of tumor mutation burden (TMB), for use in accordance with professional guidelines.

The risks associated with the use of this device, are mainly due to 1) false positives, false negatives, or failure to provide a result, and 2) incorrect interpretation of test results by the user. Risks associated with the MI Cancer Seek assay include the possibility of inaccurate results that may lead to mismanagement of patients and/or incorrect interpretation of test results by the user. Patients with false positive results (or false negative in the instance of *KRAS/NRAS*) may undergo treatment with one of the therapies listed in the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results (or false positive in the instance of *KRAS/NRAS*) may not be considered for treatment with the indicated therapy. There is also a risk of delayed results (perhaps greatest in the event of equivocal results), which may lead to delay of treatment with indicated therapy. These risks are mitigated by the analytical and clinical performance of the device. The MI Cancer Seek has demonstrated noninferiority to the companion diagnostics indicated in Section X above, and therefore the risk of this device is considered to be clinically acceptable for the indications listed.

Additional factors to be considered in determining probable risks and benefits for the MI Cancer Seek device were included analytical performance of the device and representation of the variants in the analytical and clinical studies. Additional analytical testing will be performed in the post approval setting.

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

#### **D. Patient Perspectives**

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

In conclusion, given the available information above, the data support that for MI Cancer Seek indications noted in the intended use statement, the probable benefits outweigh the probable risks.

#### **E. Overall Conclusions**

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indication for use. Data from the analytical and clinical validation studies support the performance of MI Cancer Seek as an aid in the identification of cancer patients for whom the therapies listed in **Table 1** of the Intended Use statement may be indicated.

#### **F. Pediatric Extrapolation**

Clinical data was leveraged to support the safety and effectiveness of the proposed device in the pediatric population aged 1-22 years old. Two pediatric samples (from individuals ages 18 and 21) were enrolled in clinical validation studies; one was concordant with the comparator CDx for MSI-H and one was reported as QNS (*ALK*) and did not have any slides remaining for re-extraction. Four pediatric samples (from individuals ages 10, 12, 18 and 19) were tested for variant accuracy in the largest analytical validation study, which enrolled and tested 500 unique samples.

Additionally, data from commercial testing on ~113,000 adults and 423 pediatric patients (minimum age is 1 year old) on the earlier version of MI Cancer Seek (see [Section IX.C.a](#) for the equivalency of the early version to MI Cancer Seek), demonstrates that the assay performance doesn't change across adult and pediatric samples from patients 1 year of age and older through comparison of quantity not sufficient (QNS) rates for adult vs. pediatric samples in lineages that are clinically applicable to pediatric patients. QNS rates for DNA are substantially lower in the pediatric cohort for Low Grade Glioma, Soft Tissue, and Thyroid cancers and are similar for Glioma. In cancer types less clinically relevant to pediatric patients, the QNS rates for DNA are slightly higher for the pediatric cohort.

Since there is no change in the assay workflow, including bioinformatics, based on the age of the patients, the validation data can be extrapolated to the pediatric population if a sample meets nucleic acid input requirements and run validity criteria which are the same in adult patients.

## **XV. CDRH DECISION**

CDRH issued an approval order on November 5, 2024. The final conditions of approval cited in the approval order are described below.

1. Caris Life Sciences must provide data evaluating the effects of endogenous interfering substances including high level presence of necrotic tissue, melanin and fatty acids. The samples for this assessment should represent a range of solid tumors across the intended use population including companion diagnostic and tumor profiling biomarkers. The data from this study must be adequate to demonstrate that the potential endogenous interfering substances in patients with solid tumor do not adversely impact companion diagnostic and tumor profiling biomarker detection.
2. Caris Life Sciences must provide the results from well-designed FFPE block and slide stability studies in which DNA yield, DNA quality, variant calling and invalid rates in aged samples are assessed by running samples through the entire assay workflow. The baseline or time zero (T0) must represent a freshly collected sample. The samples for this assessment should represent a range of solid tumors across the intended use population including companion diagnostic and tumor profiling biomarkers. Invalid rate and biomarker agreement rates must be assessed at each timepoint tested using the baseline measurement as reference. The data from this study must be adequate to support the MI Cancer Seek FFPE block and slide stability duration claims.

The final study protocols and study reports should be submitted within 12 months of the PMA supplement approval date.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

## **XVI. APPROVAL SPECIFICATIONS**

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

## **XVII. REFERENCES**

1. Li M. Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study. *Statistics in Biopharmaceutical Research*. 2016;8(3):355-63.
2. Newcombe RG. Improved confidence intervals for the difference between binomial proportions based on paired data. *Stat Med*. 1998;17(22):2635-50.