MI Cancer Seek™

TECHNICAL INFORMATION

Caris Life Sciences
4610 South 44th Place
Phoenix, AZ 85040

1. Intended 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	*PIK3CA* (C420R; E542K; E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, H1047Y)	PIQRAY® (alpelisib)
Colorectal Cancer (CRC)	*KRAS* wild-type (absence of mutations in exons 2, 3, and 4) and *NRAS* wild type (absence of mutations in exons 2, 3, and 4)	VECTIBIX® (panitumumab)
	BRAF V600E	BRAFTOVI® (encorafenib) in combination with ERBITUX ® (cetuximab)
Melanoma	*BRAF* V600E	*BRAF* Inhibitors approved by FDA*

Table 1. MI Cancer Seek Companion Diagnostic Indications		
Indication	**Biomarker**	**Therapy**
	BRAF V600E or V600K	MEKINIST® (trametinib) or *BRAF*/MEK Inhibitor Combinations approved by FDA*
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and exon 21 L858R alterations	EGFR 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: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools#Group_Labeling		

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

2. Contraindications

There are no known contraindications.

3. Warnings and Precautions

1. Biopsy may pose a risk to the patient when archival tissue is not available for use with the assay. The patient's physician should determine whether the patient is a candidate for biopsy.

4. Limitations

1. For *in vitro* diagnostic use.
2. CAUTION: Federal law restricts this device to sale by or on the order of a physician.
3. The acceptable preparation method for MI Cancer Seek specimens is FFPE. Other preparations have not been evaluated.
4. The test is designed to report out somatic variants and is not intended to report germline variants.
5. MI Cancer Seek requires a minimum tumor percentage of 20% for detection of alterations, with tumor content enrichment recommended for specimens with tumor percentage lower than 20%.
6. Genomic findings other than those listed in the Companion Diagnostic Indications table are not prescriptive or conclusive for labeled use of any specific therapeutic product. Confirmation of tumor mutation status using an FDA-approved CDx test is needed for therapeutic use.
7. A negative result does not rule out the presence of a mutation below the limits of detection of the assay.

8. MI Cancer Seek is only approved for use with Caris Life Sciences pre-qualified NovaSeq 6000 instruments.
9. The test is intended to be performed on specific serial numbered-controlled instruments by Caris Life Sciences.
10. MI Cancer Seek does not report TMB for values lower than 3 mut/Mb as the accuracy of TMB values below 3 mut/Mb are unreliable.
11. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.
12. Based on the low positive percent agreement (PPA) in the accuracy study for *ERBB2* copy number amplifications (CNAs) in breast cancer patients, this alteration may not be detected. 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.
13. Patients with breast cancer whose specimens have intermediate *ERBB2* CNA status should be tested with another FDA approved or cleared test to ascertain *ERRB2* CNA status in their tumor.
14. Test may have reduced sensitivity and may yield false negative results in samples where necrotic tissue is >15%, melanin is >5%, fat cells are >10%.

5. Test Principle

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 uses a custom bait panel to measure all coding regions of the exome 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

ABL1	*BRCA1*	*CYLD*	*FGFR1*	*JAK2*	*MPL*	*PDGFRA*	*RAF1*	*STAG2*
ACVR1	*BRCA2*	*DDR2*	*FGFR2*	*JAK3*	*MRE11*	*PDGFRB*	*RASA1*	*STAT3*
AIP	*BRIP1*	*DICER1*	*FGFR3*	*KDM5C*	*MSH2*	*PIK3CA*	*RB1*	*STK11*
AKT1	*BTK*	*DNMT3A*	*FGFR4*	*KDM6A*	*MSH3*	*PIK3CB*	*RET*	*SUFU*
AKT2	*CALR*	*EGFR*	*FH*	*KDR*	*MSH6*	*PIK3R1*	*RHOA*	*TCF7L2*
AKT3	*CARD11*	*EP300*	*FLCN*	*KEAP1*	*MTOR*	*PIK3R2*	*RNF43*	*TERT*
ALK	*CBFB*	*EPHA2*	*FLT1*	*KIT*	*MUTYH*	*PIM1*	*ROS1*	*TET2*
AMER1	*CCND1*	*ERBB2*	*FLT3*	*KLF4*	*MYC*	*PMS2*	*RUNX1*	*TMEM127*
APC	*CCND2*	*ERBB3*	*FOXA1*	*KMT2A*	*MYCN*	*POLD1*	*SDHA*	*TNFAIP3*
AR	*CCND3*	*ERBB4*	*FOXL2*	*KMT2C*	*MYD88*	*POLE*	*SDHAF2*	*TNFRSF14*

Table 2. MI Cancer Seek Reportable Gene List for SNVS and indels								
ARAF	*CD79B*	*ERCC2*	*FUBP1*	*KMT2D*	*NBN*	*POT1*	*SDHB*	*TP53*
ARID1A	*CDC73*	*ESR1*	*GATA3*	*KRAS*	*NF1*	*PPP2R1A*	*SDHC*	*TRAF7*
ARID2	*CDH1*	*EZH2*	*GNA11*	*LZTR1*	*NF2*	*PPP2R2A*	*SDHD*	*TSC1*
ASXL1	*CDK12*	*FANCA*	*GNA13*	*MAP2K1*	*NFE2L2*	*PRDM1*	*SETD2*	*TSC2*
ATM	*CDK4*	*FANCB*	*GNAQ*	*MAP2K2*	*NFKBIA*	*PRKACA*	*SF3B1*	*U2AF1*
ATRX	*CDKN1B*	*FANCC*	*GNAS*	*MAP2K4*	*NOTCH1*	*PRKAR1A*	*SMAD2*	*VHL*
AXIN2	*CDKN2A*	*FANCD2*	*H3F3A*	*MAP3K1*	*NPM1*	*PRKDC*	*SMAD4*	*WRN*
B2M	*CHEK1*	*FANCE*	*H3F3B*	*MAPK1*	*NRAS*	*PTCH1*	*SMARCA4*	*WT1*
BAP1	*CHEK2*	*FANCF*	*HIST1H3B*	*MAX*	*NSD1*	*PTEN*	*SMARCB1*	*XPO1*
BARD1	*CIC*	*FANCG*	*HNF1A*	*MED12*	*NSD2*	*PTPN11*	*SMARCE1*	*XRCC1*
BCL2	*CREBBP*	*FANCI*	*HOXB13*	*MEF2B*	*NTHL1*	*RAC1*	*SMO*	
BCL9	*CSF1R*	*FANCL*	*HRAS*	*MEN1*	*NTRK1*	*RAD50*	*SOCS1*	
BCOR	*CTCF*	*FANCM*	*IDH1*	*MET*	*NTRK2*	*RAD51B*	*SOS1*	
BLM	*CTNNA1*	*FAS*	*IDH2*	*MITF*	*NTRK3*	*RAD51C*	*SPEN*	
BMPR1A	*CTNNB1*	*FAT1*	*IRF4*	*MLH1*	*PALB2*	*RAD51D*	*SPOP*	
BRAF	*CXCR4*	*FBXW7*	*JAK1*	*MLH3*	*PBRM1*	*RAD54L*	*SRC*	

6. Summary and Explanation of Test

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 tumor specimens are sent to Caris Life Sciences and 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^2 aggregated across all unstained slides with ≥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 interferants as much as possible. H&E slides are annotated for manual microdissection and manual microdissection is performed to enrich tumor content and/or avoid areas with potential interferents such as necrotic tissue, fat cells, or melanin. While 25 mm^2 is the minimum, 120 mm^2 of aggregated tissue area is recommended for dissection as an optimal tissue input into TNA extraction.

TNA extraction is performed using the Biomek i7 and KingFisher Flex automated extraction instruments and the MI Cancer Seek Extraction Kit. 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 qualify for MI Cancer Seek testing. The optimal or maximum DNA input for the test to proceed with library construction is 220 ng.

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 fragment origin differentiation (RNA or DNA) by the pipeline. After end-repair, 3' adenylation, 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 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.

Sequencing results are analyzed by Caris' proprietary NGS bioinformatic pipeline and reports provided by Report Generation software. A physician report is generated identifying SNVs and indels detected within 228 genes outlined in Table 2, copy number amplifications in *ERBB2*, MSI and TMB.

MI Cancer Seek results are presented in three categories:

Level 1: CDx claims as noted in the Indications for Use Table 1
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.

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.

Instruments

The 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 3. Overview of MI Cancer Seek Instruments	
Instrument	**Vendor**
Biomek i7 Automated Workstation	Beckman
GloMax® Explorer Microplate Reader	Promega
BioMicroLab XL 100	BioMicroLab Inc
Bravo NGS workstation Option B	Agilent
NovaSeq 6000 Sequencing System	Illumina
LabElite ID Decapper	Hamilton
Fleet Control Software Thermocycler Server	Life Technologies
Qubit 4.0 Fluorometer	Life Technologies
Veriti (Pro) 96 Well Thermal Cycler	Life Technologies
VisionMate Sample Barcode Scanner	Thermo Fisher Scientific
KingFisher Flex	Thermo Fisher Scientific

7. Analytical Performance Characteristics

Performance Characteristics were established using Total Nucleic Acid (TNA) derived from a wide range of FFPE tissue types. Studies included CDx variants and cancer types as well as a broad range of representative alteration types, including substitutions (SNVs), indels (insertions, deletions) in various genomic contexts across a number of genes. Analysis of the genomic signatures for MSI and TMB were also performed. *ERBB2* CNA in samples from patients with breast cancer were also evaluated. Caris Life Sciences executed 16 analytical validation studies to support the CDx and tumor profiling claims indicated in the intended use statement. All studies passed the acceptance criteria specified in each study protocol.

1. Sample Coverage

The performance characteristics of the MI Cancer Seek Assay were determined by utilizing a combination of deidentified remnant clinical material and panel member samples having select biomarkers at various targeted

levels. The sample selection for each study aimed to capture a representative diversity of relevant markers, lineages and variant classes evaluated per study (**Table 4)**, supporting the intended clinical application of the MI Cancer Seek Assay. The studies assessed 1235 unique tumor samples, >10,000 individual test replicates, 38 tumor lineages, 236 reportable mutations, and 365 tumor profiling markers. The average depth across these samples ranged from 571x -1059x (mean of 866) and total DNA exome reads from 134 to 240 million with the mean of 193 million reads.

Table 4. Tumor Lineages, Targets, and Samples by Study

Study Title	Lineages		# Unique Samples	# of Total Samples	# of Unique Targets/ Study	# of Unique Samples / Variant Class					
						SNV	INDEL	ERBB2 CNA*	MSI-H	Not MSI-H	TMB
Carry-over & Cross-contamination (*Negative/WT Samples Counted*)	5	Breast Carcinoma, Colorectal Adenocarcinoma, Lung Non-small Cell Lung Cancer (NSCLC), Melanoma, Prostatic Adenocarcinoma	23(7 Additional**)	305(71Additional**)	33 (7 Additional**)	2 - SNV 2 - WT	3 - INDEL 2 - WT	2 - Amplified 2 - Not Amp	3	7	10
Limit of Detection (Variant Frequency)	9	Breast Carcinoma, Colorectal Adenocarcinoma, Glioblastoma, Low Grade Glioma, Lung Non-small cell lung cancer (NSCLC), Melanoma, Ovarian Surface Epithelial Carcinomas, Prostatic Adenocarcinoma, Uterine Neoplasms - Endometrial carcinoma	29	1695	29	17	12				
Limit of Detection (%Tumor Content)	8	Breast Carcinoma, Colorectal Adenocarcinoma, Gastric Adenocarcinoma, Kidney Cancer, Lung Non-small cell lung cancer (NSCLC), Ovarian Surface Epithelial Carcinomas, Pancreatic Adenocarcinoma, Prostatic Adenocarcinoma, Uterine Neoplasms - Endometrial carcinoma	12	1100	21			2	8	1	10

Table 4. Tumor Lineages, Targets, and Samples by Study											
Study Title	Lineages		# Unique Samples	# of Total Samples	# of Unique Targets/ Study	# of Unique Samples / Variant Class					
						SNV	INDEL	ERBB2 CNA*	MSI-H	Not MSI-H	TMB
DNA Input	8	Colorectal Adenocarcinoma, Melanoma, Breast Carcinoma, Lung Non-small cell lung cancer (NSCLC), Small Intestinal Malignancies, Bladder cancer - urothelial, Uterine Neoplasms - Endometrial carcinoma, Female Genital Tract Malignancy	16	325	23	8	4	2	4		5
Precision	14	Colorectal Adenocarcinoma, Melanoma, Breast Carcinoma, Lung Non-small cell lung cancer (NSCLC), Small Intestinal Malignancies, Prostatic Adenocarcinoma, Glioblastoma, Uterine Serous Carcinoma, Gastrointestinal Stromal Tumors (GIST), Uterine Neoplasms - Endometrial carcinoma, Ovarian Surface Epithelial Carcinomas, Female Genital Tract Malignancy, Salivary Gland Tumors, Soft Tissue Tumors	49	3096	53	21	19	2	4	2	5
Interfering Substances - Exogenous	8	Breast Carcinoma, Cholangiocarcinoma, Colorectal Adenocarcinoma, Liver Hepatocellular Carcinoma, Lung Non-small cell lung cancer (NSCLC), Melanoma, Ovarian Surface Epithelial Carcinomas, Small Intestinal Malignancies	17	435	48	8	4	2	6	11	17
Interfering Substances - Endogenous	10	Breast Carcinoma, Cholangiocarcinoma, Colorectal Adenocarcinoma, Lung Non-small cell lung cancer (NSCLC), Melanoma, Pancreatic Adenocarcinoma, Prostatic Adenocarcinoma, Small Intestinal Malignancies, Thyroid Carcinoma, Uterine	23	250	64	11	6	1	7	16	23

Table 4. Tumor Lineages, Targets, and Samples by Study											
Study Title	Lineages		# Unique Samples	# of Total Samples	# of Unique Targets/ Study	# of Unique Samples / Variant Class					
						SNV	INDEL	ERBB2 CNA*	MSI-H	Not MSI-H	TMB
		Neoplasms - Endometrial carcinoma									
Process Hold Times	5	Breast Carcinoma, Non-Small Cell Lung Cancer (NSCLC), Pancreatic Adenocarcinoma, Uterine Neoplasms - Endometrial carcinoma, Uterine Serous Carcinoma	6	504	14	2	1	1	2		6
Reagent Interchangeability	7	Breast Carcinoma, Melanoma, Uterine Neoplasms - Endometrial carcinoma, Lung Non-small cell lung cancer (NSCLC), Ovarian Surface Epithelial Carcinomas, Colorectal Adenocarcinoma, Glioblastoma	11	241	21	5	2	1	2		11
Closed-Kit Reagent Stability)	7	Breast Carcinoma, Colorectal Adenocarcinoma, Glioblastoma, Lung Non-small cell lung cancer (NSCLC), Melanoma, Small Intestinal Malignancies, Uterine Neoplasms - Endometrial carcinoma	21	132 (*on going*)	56	6	4	4	3	18	21
Open-Kit Reagent Stability	9	Breast Carcinoma, Colorectal Adenocarcinoma, Kidney Cancer, Lung Non-small cell lung cancer (NSCLC), Melanoma, Ovarian Surface Epithelial Carcinomas, Pancreatic Adenocarcinoma, Uterine Neoplasms – Endometrial carcinoma	11	165 (*on going*)	30	3	3	2	4	7	11

Table 4. Tumor Lineages, Targets, and Samples by Study

Study Title	Lineages		# Unique Samples	# of Total Samples	# of Unique Targets/ Study	# of Unique Samples / Variant Class					
						SNV	INDEL	ERBB2 CNA*	MSI-H	Not MSI-H	TMB
Extracted TNA Stability	34	Various***	451	451	1329	300	113	14	40	411	451
Extracted TNA Freeze Thaw Stability	7	Breast Carcinoma, Colorectal Adenocarcinoma, Lung Non-small cell lung cancer (NSCLC), Melanoma, Pancreatic Adenocarcinoma, Prostatic Adenocarcinoma, Uterine Neoplasms - Endometrial carcinoma	13	130	41	10	4	1	3	10	13
Guard banding	5	Colorectal Adenocarcinoma, Melanoma, Breast Carcinoma, Lung Non-small cell lung cancer (NSCLC). Lung Small Cell Cancer (SCLC)	7	420	17	1	1	1	1	6	7
Accuracy	38	Various***	500	500	1677	923 (3 MNV)	251				500
Limit of Blank	15	Melanoma, Extrahepatic Bile Duct Adenocarcinoma, Lung Non-small cell lung cancer (NSCLC), Esophageal Carcinoma, Colorectal Adenocarcinoma, Bladder cancer - urothelial, Uterine Serous Carcinoma, Ovarian Surface Epithelial Carcinomas, Kidney Cancer, Thyroid Carcinoma, Liver Hepatocellular Carcinoma, Gastric Adenocarcinoma, Small Intestinal Malignancies, Pancreatic Adenocarcinoma, Breast Carcinoma	39	408							

**ERBB2* CNA correspond to samples from breast cancer patients only.

**Additional samples were included that contained markers in addition to the listed variant classes and were used to analyze Carry-over and Cross-contamination.

***Various include the following:

Only in TNA stability

Male Genital Tract Malignancy, None Of These Apply

Only in TMB accuracy

Malignant Solitary Fibrous Tumor of the Pleura (MSFT), Non Epithelial Ovarian Cancer (non-EOC), Peripheral Nervous System Tumors, Retroperitoneal or Peritoneal Sarcoma, Soft Tissue Sarcoma - Well-Differentiated/Dedifferentiated Liposarcoma (WD-DDLS) for Retroperitoneal Sarcomas, Uveal Melanoma

Common between Accuracy, TMB accuracy and extracted TNA stability

Bladder cancer - non-urothelial, Bladder cancer - urothelial, Breast Carcinoma, Cancer of Unknown Primary, Cervical Cancer, Cholangiocarcinoma, Colorectal Adenocarcinoma, Esophageal Carcinoma, Esophagogastric Junction Carcinoma, Extrahepatic Bile Duct Adenocarcinoma, Female Genital Tract Malignancy, Gastric Adenocarcinoma, Gastrointestinal Stromal Tumors (GIST), Glioblastoma, Head and Neck Cancers, Kidney Cancer, Liver Hepatocellular Carcinoma, Low Grade Glioma, Lung Non-small cell lung cancer (NSCLC), Lung Small Cell Cancer (SCLC), Melanoma, Neuroendocrine tumors, Ovarian Surface Epithelial Carcinomas, Pancreatic Adenocarcinoma, Prostatic Adenocarcinoma, Salivary Gland Tumors, Small Intestinal Malignancies, Soft Tissue Tumors, Thyroid Carcinoma, Uterine Neoplasms - Endometrial carcinoma, Uterine Serous Carcinoma, Vulvar Cancer (squamous cell carcinoma)

2. Accuracy

a. Comparison to an Orthogonal Method for Tumor Profiling (SNVs, MNVs 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 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 on 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 on an externally validated Whole Exome Sequencing Assay and were processed using the lab's proprietary, CLIA-approved extraction and processing protocols. 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.

SNV 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 the 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 analysis due to either sample quality or sample swap 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 performance is further stratified by FDA's biomarker class level for tumor profiling in **Table 5**. 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.

Table 5. 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]**
All Variants	170636	1177	215	54	77467298	95.61% (1177/1231) [94.31-96.69]	100.00% (77467298/77467513 [100.00-100.00]
All SNVs	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]
All MNVs	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]
All Insertions	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]

Table 5. 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]**
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]
All Deletions	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]

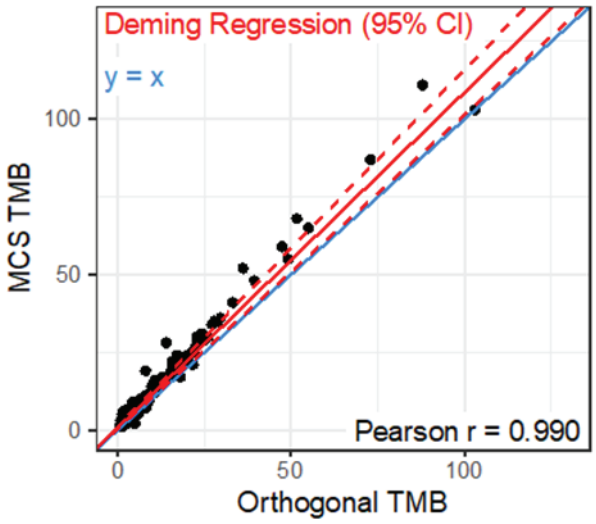
Table 5. Agreement Summary for SNVs, MNVs, Insertions and Deletions for Tumor Profiling							
Variant Type	Total Unique Variants	MI Cancer Seek (+) Comparator (+)	MI Cancer Seek (+) Comparator (-)	MI Cancer Seek (-) Comparator (+)	MI Cancer Seek (-) Comparator (-)	PPA (n/N) [95% CI]	NPA (n/N) [95% CI]
Deletions >25bp	8161	3	4	0	3705087	100.00% (3/3) [29.24-100.00]	100.00% (3705087/3705091) [100.00-100.00]

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 lineages. A total of three samples were excluded from analysis due to sample quality or sample swap (there was 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 model was completed using Deming regression analysis to estimate the 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 ≥50x are evaluated.

Figure 1: TMB Accuracy Using Deming Regression Analysis



Comparison to an Orthogonal Fluorescence In Situ Hybridization (FISH) Assay for *ERBB2* CNAs in samples from patients with breast cancer.

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 twice with the FDA approved fluorescence in situ hybridization (FISH), PathVysion HER2 DNA Probe Kit, and once on 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 have 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 6** and **Table 7**.

Table 6. 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 7**, treating the MI Cancer Seek "Intermediate" result as positive or negative result were used to determine agreements shown in **Table 8**. 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 7. 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%)

*Wilson 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 Clinical Performance Studies section.

3. Precision – Repeatability and Reproducibility

This study was performed to confirm LoD and evaluate MI Cancer Seek repeatability (within-run) and reproducibility (total within-lab) under varied conditions including different reagent lots, instruments, operators, and non-consecutive run days across. A total of 49 panel member samples were created, representing 14 different tumor types, to evaluate CDx markers, challenging tumor profiling variant types including short/long indels in non-homopolymer/homopolymer regions, and the other variant classes detected 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 panels members). In total there were 86 samples evaluated in the precision study. The study included 3 operator teams, 3 instrument sets, 3 reagent lots and was minimally executed across 3 non-consecutive days over a 20-day span. This study design resulted in 36 data points per sample. 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 9. 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 8. Agreements for Targeted CDx and Tumor Profiling Variants reported by MI Cancer Seek

Panel Member	Specimen 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%)

Table 8. Agreements for Targeted CDx and Tumor Profiling Variants reported by MI Cancer Seek							
Panel Member	**Specimen Lineage**	**Variant**	**Bio-marker Type**	**Fold LoD Level*** **	**Average VAF, MSI Score, or ERBB2 Copies**	**Number Positive / Number Expected**	**Agreement (95% CI)**
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%)
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	*ERBB2* CNA	Tumor Profiling	2-3x	11.627	36/36	100% (90.3%, 100.0%)
19	Breast Carcinoma	*ERBB2* CNA	Tumor Profiling	1-1.5x	7.877	35/36	97.2% (85.5%, 99.9%)
20	Breast Carcinoma	*PALB2* 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	*SMAD2* 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	*MLH1* 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	*MSH2* 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	*MSH2* 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	*MSH6* 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%)

Table 8. Agreements for Targeted CDx and Tumor Profiling Variants reported by MI Cancer Seek							
Panel Member	Specimen Lineage	Variant	Bio-marker Type	Fold LoD Level***	Average VAF, MSI Score, or ERBB2 Copies	Number Positive / Number Expected	Agreement (95% CI)
26	Colorectal Adenocarcinoma	*PIK3R1* 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	*PMS2* 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)	*KIT* 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)	*NF1* 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	*TERT* 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)	*ARID1A* 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)	*EGFR* 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%)
33	Lung Non-small cell lung cancer (NSCLC)	*EGFR* 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)	*ERBB2* 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	*TERT* 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	*BRCA1* 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	*BRCA2* 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	*BRCA2* 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	*CTNNB1* 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		*APC* T1556fs		1-1.5x	0.108	36/36	100% (90.3%, 100.0%)

Table 8. Agreements for Targeted CDx and Tumor Profiling Variants reported by MI Cancer Seek							
Panel Member	**Specimen Lineage**	**Variant**	**Bio-marker Type**	**Fold LoD Level*** **	**Average VAF, MSI Score, or ERBB2 Copies**	**Number Positive / Number Expected**	**Agreement (95% CI)**
	Small Intestinal Malignancies		Tumor Profiling	2-3x	0.208	36/36	100% (90.3%, 100.0%)
39	Small Intestinal Malignancies	*KRAS* 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	*TP53* 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	*ARID1A* 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	*TP53* 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	*TP53* F113fs c.336_337delCT	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	*TP53* 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%)
45	Uterine Neoplasms - Endometrial carcinoma	Not MSI-H	CDx	High Negative	30.056	34/36	94.4% (81.3%, 99.3%)

*Stop codon
**Panel Member 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/indel, *ERBB2* CNA, and MSI) and the results are summarized per variant class in **Table 9** and **Table 10**. The positive agreement rates were 100% for SNV, MNV, and *ERBB2* CNA at alteration levels 2-3x LoD, 97.2% for MSI and CNA (1-1.5x LoD), and >99% for indel. The negative call rates were 100% for *ERBB2* CNA and >97% for all others. 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.

Table 9. 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%)
ERBB2 CNA	1-1.5x LoD	36	35	1	0	97.2%	(85.5%, 99.9%)	97.2%	(85.5%, 99.9%)
ERBB2 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 10. 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%)

Table 10. 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)**
INDEL	1-1.5x LoD	153091656	167	149770010	3321479	100%	(100.0%, 100.0%)	97.8%	(97.8%, 97.8%)
CNA	1-1.5x LoD	72	0	72	0	100%	(95.0%, 100.0%)	100%	(95.0%, 100.0%)
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.									

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 the sample selection in the study) assessing agreement based on variant type stratified by VAF (≥0%, ≥5%, ≥8%, ≥10%, and ≥15%) is presented in Table 12 below. Positive call rates near the reporting threshold of ≥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 VAF≥8%, all variant types but Long Indel in Homopolymer Region had ≥99.2% positive call rates. Performance of this challenging variant type at VAF≥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 11. 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)

Table 11. 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**
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)
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)

Table 11. 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**
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 analyses was performed for 45 samples using the underlying quantitative metric for the tracked variant (i.e., VAF for SNV/ indel, copies for *ERBB2* CNA, MSI value for MSI). Mean values were determined using all valid data points per sample (36). 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.

The entire precision study sample set of 49 was evaluated for TMB at each dilution level. Samples had mean TMB scores ranging from 2 to 30 mutations/megabase (mb). The results demonstrated <12% CV between operators/instruments 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. Thus, MI Cancer Seek results are reproducible for all variant classes when samples are processed with multiple operators, instruments, and reagent lots within lab.

4. TNA Extraction

Two separate retrospective analyses were performed across multiple tumor types to evaluate extraction performance of MI Cancer Seek. The first analysis included 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) and a total of 1,038 sample replicates were available for analysis. 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 performance in **Table 12** and TMB in **Table 13**. Overall concordance for all variant classes was high, as most were >99% and the average SD and %CV were low for TMB. These data indicate that DNA extraction performance is robust for all variants (Levels 1- 3), MSI, and TMB across the pan-tumor setting when testing across multiple reagents lots & instruments.

Table 12. DNA Extraction Performance for Variants and MSI in 14 Tumor Types

Variant Class	Samples per Variant Class*	Total Replicates	AVG 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%)
Level 1 MSI – H	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 13. 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

5. 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 non-tumor wild-type (WT) samples across multiple tissue types. The study used 28 clinical WT (non-tumor) FFPE samples across multiple tissue types and each sample was tested using 2 lots of reagents (3 replicates per reagent lot), which generated a total of 168 data points to determine the LoB. 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 14**.

Table 14. LoB Study Summary Results

Category	Per Position False Positive Rate	Per Sample False Positive Rate*
Level 1: *BRAF* V600E	0%	0%
Level 1: *BRAF* V600K	0%	0%
Level 1: *EGFR* Exon 19 deletions	0%	0%
Level 1: *EGFR* Exon 21 substitution mutations	0%	0%
Level 1: *PIK3CA* C420R	0%	0%
Level 1: *PIK3CA* E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R	0%	0%
Level 1: *PIK3CA* H1047L, H1047R, H1047Y	0%	0%
Level 1: *KRAS* SNVs	0%	0%
Level 1: *NRAS* SNVs	0%	0%
Level 1: MSI-H	N/A	0%
Level 2: *ERBB2* 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 15**.

Table 15. Supplemental LoB Study Results Per Position and Sample

Level & Variant Class	Per Position False Positive Rate	Per Sample False Positive Rate*
Level 1: *BRAF* V600E	0%	0%
Level 1: *BRAF* V600K	0%	0%
Level 1: *EGFR* Exon 19 Dels	0%	0%
Level 1: *EGFR* L858R	0%	0%
Level 1: *PIK3CA* C420R	0%	0%
Level 1: *PIK3CA* E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R	0%	0%
Level 1: *PIK3CA* 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: *ERBB2* Amplifications	N/A	0%
*Calculated from 2 reagent lots (n=240 data points)		

b. Limit of Detection for SNVs and INDELs

This study was designed to determine the Limit of Detection (LoD) for SNVs and indels for CDx biomarkers and tumor profiling claims for MI Cancer Seek assay using a representative approach. A total of 29 panel member samples were tested at minimal DNA input, including 12 panel members to support CDx biomarker claims and 17 panel members 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 panel member for the 17 tumor profiling markers. The LoD for each panel member was determined as the lowest Variant Allele Frequency (VAF) level with ≥ 95% detection (Hit rate =0.95) based on the positivity threshold of 5%. The LoD for SNVs and Insertions ranged from 6-11% VF, and LoD for Deletions ranged from 6-8%VF.

The LoD for CDx biomarkers and tumor profiling markers are shown in **Table 16** and **Table 17**, respectively.

Table 16. LoD for CDx Biomarkers			
Variant Type	**Mutation**	**Tumor Type**	**LoD (%VAF)**
SNV	*BRAF* V600E	Colorectal Adenocarcinoma	11%
SNV	*BRAF* V600E	Melanoma	7%
SNV	*BRAF* V600K	Melanoma	8%
Indel	EGFR L747_T751del	NSCLC)	7%
SNV	*EGFR* L858R	NSCLC	7%
SNV	*KRAS* A146T	Colorectal Adenocarcinoma	8%
SNV	*KRAS* G12C	Colorectal Adenocarcinoma	7%
SNV	*KRAS* Q61H	Colorectal Adenocarcinoma	10%
SNV	*NRAS* A146T	Colorectal Adenocarcinoma	11%
SNV	*NRAS* G12A	Colorectal Adenocarcinoma	7%
SNV	*NRAS* Q61R	Colorectal Adenocarcinoma	10%
SNV	*PIK3CA* E542K	Breast Carcinoma	11%

Table 17. LoD for Tumor Profiling Alterations			
Variant Type	**Mutation**	**Tumor Type**	**LoD (%VAF)**
Insertion (Long homopolymer)	*ARID1A* A330fs	Uterine Neoplasms - Endometrial carcinoma	8%
SNV	*ARID1A* Q575*	Uterine Neoplasms - Endometrial carcinoma	6%
Deletion (Long non-homopolymer)	*ASXL1* E635fs	Ovarian Surface Epithelial Carcinomas	7%
SNV	*BRCA1* M1775R	Breast Carcinoma	7%
SNV	*BRCA2* S1882*	Prostatic Adenocarcinoma	8%
Insertion	*BRCA2* T2125fs	Prostatic Adenocarcinoma	11%
Insertion	*EGFR* A767_V769dup	NSCLC	9%
Insertion	*ERBB2* G776delinsVC	NSCLC	9%
Insertion (Long non-homopolymer)	*FANCA* D944fs	Colorectal Adenocarcinoma	8%
Insertion	*NF1* I679fs	Uterine Neoplasms - Endometrial carcinoma	6%
SNV	*NOTCH1* Y550fs	Low Grade Glioma	7%
Deletion (Long homopolymer)	*PIK3R1* N453_Y463del	Colorectal Adenocarcinoma	8%

Table 17. LoD for Tumor Profiling Alterations			
Variant Type	**Mutation**	**Tumor Type**	**LoD (%VAF)**
Insertion (Short homopolymer)	*SMAD2* L175fs	Breast Carcinoma	9%
SNV	*SMARCA4* R1005*	Colorectal Adenocarcinoma	7%
Deletion (Short homopolymer)	*SUFU* T13fs	Melanoma	6%
SNV	*TERT* c.-146C>T	Glioblastoma	11%
SNV	*TP53* G266V	Ovarian Surface Epithelial Carcinomas	6%

c. Limit of Detection for *ERBB2* CNA

The LoD for *ERBB2* CNA was also established relative to the positivity threshold for the biomarker like SNV/indel and samples were run at minimum DNA input. Two *ERBB2* panel member samples were tested 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 LoD for each panel member was determined to be the lowest level of copies (CNA) with ≥ 95% detection (Hit rate = 0.95), and these data established 8.3 copies as the LoD for *ERBB2* CNA.

d. Analytical Sensitivity – Tumor Purity

The lowest level of tumor content that supports robust performance of MI Cancer Seek was evaluated. A total of 12 unique panel member samples from nine lineages were prepared and tested at minimal DNA input across different variant classes including CNA (2 samples), MSI (9 samples), and TMB (10 samples). At least five dilution levels were prepared per panel member resulting in a total of 100 data points (ten replicates x five dilution levels x two reagent lots) per panel member 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 the performance of MI Cancer Seek assay at six DNA input levels to not only verify performance at the minimum (50 ng) and maximum (targeted) DNA input (220ng) requirement, but also to 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 panel member samples near LoD (1-3x). A minimum of 3 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%. These results indicate performance of MI Cancer Seek is robust at both the minimum and maximum DNA input requirements.

6. 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, CNA, MSI, and TMB using one reagent lot at minimum DNA input. A total of 425 data points (17 FFPE clinical samples x five replicates x five conditions) were generated across all interferant 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 was reported at 100% for all conditions and NPA was 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 exogenous substances. TMB included 0 for the 95% 1-sided confidence interval around the mean difference between all TMB test and control condition.

b. Interfering Substances – Endogenous

This study evaluated the impact of endogenous interfering substances (hemoglobin, colloid, calcium/calcium phosphate, mucin, and conjugated bile acids), on performance of the MI Cancer Seek assay in their respective tumor tissue samples for both CDx and tumor profiling claims. A total of 23 unique clinical FFPE samples (mutation positive) from 10 lineages, selected at 2-3x LoD from the tissue types associated with the endogenous interfering substances were used. MSI and TMB were evaluated

across all the samples tested in the study. For each sample, five replicates of each test (spiked with the potential interfering substance) and corresponding control condition (without the potential interfering substance) were processed through the complete assay workflow, from extraction through sequencing with and without endogenous substances, using one reagent lot at minimum DNA input. A total of 130 data points were generated for both endogenous interferent positive and endogenous interferent negative (control) replicates yielding a total of 260 data points generated.

For CNA, Expression, indel, and SNV, both NPA and PPA were 100% and no deleterious effect between control and test condition were observed when tested with endogenous 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 result demonstrated that comparison conditions 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. Using the same method described above for TMB, this condition passes, showing that it is sample selected, rather than colloid being 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/or 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 generated for carryover using 30 unique FFPE samples (14 high positive and 16 negative) from five lineages 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 > 10 mut/MB. The results demonstrated that NPA for all DNA variant classes including SNV/indel, MSI, TMB, and *ERBB2* Amplification, 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 studies. The results demonstrated there was <0.00% index cross-contamination in all 20 flowcells evaluated. Additionally, all 300 samples had <0.00% index 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 a 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.

7. 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, CNA, and TMB). Two replicates of each sample at 1-3x LoD were tested at minimum DNA input for all timepoints starting

from extraction. All five MI Cancer Seek reagents were stored at their specified storage temperatures and unopened kits were 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, T0 and T2 timepoints. PPA was 100% for SNV, indel, 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 early version of MI Cancer Seek (concordance between the early version and MI Cancer Seek is provided under Section 8.a) 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.

8. Guard Banding/Robustness

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, 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 MI Cancer Seek performance, as both PPA and NPA were 100% for SNV/indel, CNA, and MSI across all conditions evaluated in the study, and TMB included 0 for the 95% 1-sided CI around the mean difference between all test and control conditions.

9. 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 lineages, 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%. The 95% 1-sided CI around the mean difference between the mean TMB test conditions and control condition included zero. This demonstrated 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.

10. Reagent Interchangeability

The study demonstrated that different lots of the MI Cancer Seek assay kit reagents are interchangeable. This study evaluated the performance of 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 3 different lots and each kit tested with varying combinations across 11 sequencing runs, and two technical replicates each 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 ≤20% CV when the average TMB was >6 mut/Mb. When the average TMB was ≤6 mut/Mb for a given sample, the standard deviation was ≤1 mut/Mb.

8. Additional Studies

a. Comparison to Early Version of MI Cancer Seek

Concordance between MI Cancer Seek and its early version, MI Tumor Seek Hybrid V2, was demonstrated for variants, MSI, and TMB in 111 clinical FFPE samples representing 14 different tumor types tested from extraction through sequencing and results are summarized in **Table 18** and **Figure 2** below. Concordance for *ERBB2* CNAs was demonstrated using 32 clinical FFPE breast cancer samples. These data indicate there is a high degree of concordance between the MI Cancer Seek and its early version, thereby justifying the

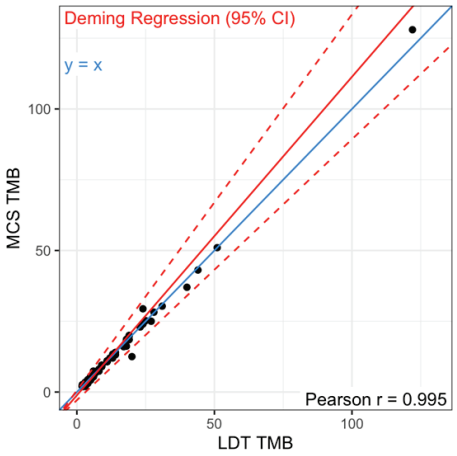
use of data from the early version of the test to support MI Cancer Seek performance claims for Extracted TNA Stability Study (Section 7.7.c) and Pan-Tumor Comparability Study (Section 8.b).

Table 18. Summary of Concordance Between MI Cancer Seek and the early version of MI Cancer Seek*

Alteration Type	MCS[1]+ EV[2]+ (TP)	MCS[1]- EV[2]- (TN)	MCS[1]+ EV[2]- (FP)	MCS[1]- EV[2]+ (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]
ERBB2 CNAs, Level 2	12	20	0	0	100% (12/12) [0.713, 1.037]	100% (20/20) [0.806, 1.027]
MSI	19	91	1	0	95% (19/20) [0.743, 1.007]	100% (91/91) [0.95, 1.008]

*Early Version of the test was considered the reference method.
Abbreviations:
[1]MCS= MI Cancer Seek
[2]EV: Early version of the test

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 early version of MI Cancer Seek, MI Tumor Seek Hybrid V2, for 119,952 samples across 46 solid tumor types (**Table 19**). A concordance analysis provided above provides evidence that these two assays have high concordance and are deemed to be comparable. The concordance analysis between the early version and CDx versions considered the same sample testing criteria (for TNA input and other requirements such as percent tumor content) used for the MI Seek Cancer Seek (CDx version).

Table 19. Pan Tumor QC Pass Rates by Tumor Type

Tumor Type	Total Sites Tested	Sites Tested	N	Average Concentration (ng/uL)	Extraction Pass (%)	DNA Total Reads Pass (%)	DNA Average Depth 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
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

Table 19. Pan Tumor QC Pass Rates by Tumor Type								
Tumor Type	**Total Sites Tested**	**Sites Tested**	**N**	**Average Concentration (ng/uL)**	**Extraction Pass (%)**	**DNA Total Reads Pass (%)**	**DNA Average Depth Pass (%)**	**Average Library DNA Input (ng)**
Cholangiocarcinoma	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 Adenocarcinoma	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 (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)	16442	36.4	98.3%, (16159/16442)	99.5%, (16075/16159)	99.5%, (16078/16159)	205.3
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
Esophagogastric Junction Carcinoma	10	Esophagus (1823), Gastroesophageal junction (601), Esophagogastric junction (189), Cardia (21),	2662	23.7	97.9%, (2607/2662)	99.7%, (2599/2607)	99.7%, (2599/2607)	199.2

Table 19. Pan Tumor QC Pass Rates by Tumor Type								
Tumor Type	**Total Sites Tested**	**Sites Tested**	**N**	**Average Concentration (ng/uL)**	**Extraction Pass (%)**	**DNA Total Reads Pass (%)**	**DNA Average Depth Pass (%)**	**Average Library DNA Input (ng)**
		Stomach (17), Gastric cardia (7), Gastric (1), Pyloric antrum (1), Sigmoid colon (1), Other (1)						
Extrahepatic Bile Duct Adenocarcinoma	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 Adenocarcinoma	13	Stomach (1654), Cardia (108), Gastric (105), Gastric cardia (104), Pyloric antrum (77), Gastroesophageal junction (27), Esophagus (4), Esophagogastric junction (3), Other (70)	2152	21.5	97.6%, (2101/2152)	99.8%, (2097/2101)	99.8%, (2096/2101)	194.2
Gastrointestinal 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	805	28.4	96.4%, (776/805)	99.9%, (775/776)	99.9%, (775/776)	198.4

Table 19. Pan Tumor QC Pass Rates by Tumor Type								
Tumor Type	**Total Sites Tested**	**Sites Tested**	**N**	**Average Concentration (ng/uL)**	**Extraction Pass (%)**	**DNA Total Reads Pass (%)**	**DNA Average Depth Pass (%)**	**Average Library DNA Input (ng)**
		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)						
Glioblastoma	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 (3), Lung (2), Other (639)	2309	31.3	98.0%, (2263/2309)	98.8%, (2236/2263)	98.8%, (2235/2263)	201.2
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 Hepatocellular 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

Table 19. Pan Tumor QC Pass Rates by Tumor Type								
Tumor Type	**Total Sites Tested**	**Sites Tested**	**N**	**Average Concentration (ng/uL)**	**Extraction Pass (%)**	**DNA Total Reads Pass (%)**	**DNA Average Depth Pass (%)**	**Average Library DNA Input (ng)**
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), Parietal lobe (2), Abdomen (1), Cerebral meninges (1), Kidney (1), Meninges (1), Pancreas	74	35.3	90.5%, (67/74)	100.0%, (67/67)	100.0%, (67/67)	192.9

Table 19. Pan Tumor QC Pass Rates by Tumor Type								
Tumor Type	**Total Sites Tested**	**Sites Tested**	**N**	**Average Concentration (ng/uL)**	**Extraction Pass (%)**	**DNA Total Reads Pass (%)**	**DNA Average Depth Pass (%)**	**Average Library DNA Input (ng)**
		(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
Neuroendocrine 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), Ascending colon (14),	1571	39.1	97.7%, (1535/1571)	98.9%, (1518/1535)	98.9%, (1518/1535)	199.4

Table 19. Pan Tumor QC Pass Rates by Tumor Type								
Tumor Type	**Total Sites Tested**	**Sites Tested**	**N**	**Average Concentration (ng/uL)**	**Extraction Pass (%)**	**DNA Total Reads Pass (%)**	**DNA Average Depth Pass (%)**	**Average Library DNA Input (ng)**
		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),						

Table 19. Pan Tumor QC Pass Rates by Tumor Type								
Tumor Type	**Total Sites Tested**	**Sites Tested**	**N**	**Average Concentration (ng/uL)**	**Extraction Pass (%)**	**DNA Total Reads Pass (%)**	**DNA Average Depth Pass (%)**	**Average Library DNA Input (ng)**
		Renal pelvis (1), Salivary gland (1), Supraglottis (1), Tonsil (1), Other (88)						
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),	2655	34.6	97.6%, (2592/2655)	98.6%, (2557/2592)	98.6%, (2555/2592)	201.2

Table 19. Pan Tumor QC Pass Rates by Tumor Type								
Tumor Type	**Total Sites Tested**	**Sites Tested**	**N**	**Average Concentration (ng/uL)**	**Extraction Pass (%)**	**DNA Total Reads Pass (%)**	**DNA Average Depth Pass (%)**	**Average Library DNA Input (ng)**
		Renal pelvis (1), Small intestine (1), Tongue (1), Urethra (1), Uterus (1), Other (639)						
Ovarian Surface Epithelial Carcinomas	29	Ovary (7218), Fallopian tube (1602), Peritoneum (709), Tubo-ovarian (656), Unknown primary site (13), Endometrium (5), Retroperitoneum (4), Abdomen (2), Corpus uteri (1), Uterine (1), Uterus (1), Other (109)	10321	46.3	98.7%, (10187/10321)	99.3%, (10120/10187)	99.4%, (10122/10187)	208.2
Pancreatic Adenocarcinoma	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, Oligodendroglioma	4	Other (30)	30	63.3	100.0%, (30/30)	100.0%, (30/30)	100.0%, (30/30)	214.3
Prostatic Adenocarcinoma	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
Retroperitoneal or	37	Retroperitoneum (123), Connective (34), Kidney (7), Unknown primary	226	27.1	98.2%, (222/226)	99.1%, (220/222)	99.5%, (221/222)	195.8

Table 19. Pan Tumor QC Pass Rates by Tumor Type								
Tumor Type	**Total Sites Tested**	**Sites Tested**	**N**	**Average Concentration (ng/uL)**	**Extraction Pass (%)**	**DNA Total Reads Pass (%)**	**DNA Average Depth Pass (%)**	**Average Library DNA Input (ng)**
Peritoneal Sarcoma		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)						
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 Retroperito	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

Table 19. Pan Tumor QC Pass Rates by Tumor Type								
Tumor Type	**Total Sites Tested**	**Sites Tested**	**N**	**Average Concentration (ng/uL)**	**Extraction Pass (%)**	**DNA Total Reads Pass (%)**	**DNA Average Depth Pass (%)**	**Average Library DNA Input (ng)**
neal Sarcomas								
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), 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)	1550	29.2	95.4%, (1479/1550)	98.9%, (1462/1479)	98.9%, (1462/1479)	193.1

Table 19. Pan Tumor QC Pass Rates by Tumor Type								
Tumor Type	**Total Sites Tested**	**Sites Tested**	**N**	**Average Concentration (ng/uL)**	**Extraction Pass (%)**	**DNA Total Reads Pass (%)**	**DNA Average Depth Pass (%)**	**Average Library DNA Input (ng)**
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
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

9. Clinical Performance Characteristics

Caris Life Sciences executed six clinical validation studies and a supplemental real-world clinical data concordance analysis to support the MI Cancer Seek CDx claims indicated in **Table 1** of the intended use statement. These studies compared the performance of MI Cancer Seek, a follow-on CDx (FCD) to an FDA-approved CDx test (called comparator CDx or CCD) using remnant deidentified samples representative of the intended use population specific to each CCD. Samples were tested twice on the CCD (CCD1 and CCD2) and results were compared to a single replicate tested on MI Cancer Seek (FCD). Non-inferiority performance was measured by comparing the agreement between CCD1 and CCD2 to the agreements of FCD to CCD1 and FCD to CCD2. CDx claims were based on a non-inferiority (NI) statistical testing approach from Li (2016), when the concordance study sample is not a random

sample from the companion diagnostic (MI Cancer Seek) intended use population and a reference standard is not available. The CCD tests used to support MI Cancer Seek CDx claims and the study cohorts are summarized in **Table 20**. 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 demonstrated that MI Cancer Seek is non-inferior to the FDA approved CCD for the intended use of the assay.

Table 20. Summary of Comparator CDx Tests and CV Study Cohorts

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

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

   Clinical validity of MI Cancer Seek as a CDx was evaluated for the detection of MSI status in patients with solid tumors who may be eligible for treatment with FDA-approved therapies including KEYTRUDA® (pembrolizumab) or JEMPERLI (dostarlimab-gxly) for Solid Tumors or KEYTRUDA® (pembrolizumab) in combination with LENVIMA® (lenvatinib) for EC. The study cohort was enriched to enroll an approximately equal number of positive and negative samples using comparator results (CCD1) from prior MMR RxDx Panel testing. A total of 401 unique FFPE tissue samples across 16 different tumor types from patients histologically confirmed as a solid tumor cancer were enrolled and tested twice on MMR RxDx Panel (CCD) and once on MI Cancer Seek; there were no invalids for this study. The distribution of demographics in the study was comparable to the GARNET clinical trial population tested with the MMR RxDx Panel. 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.

   a. **Analysis for Solid Tumors**

Clinical concordance between MI Cancer Seek and MMR RxDx Panel (CCD) with 401 eligible samples is shown in **Table 21**.

Table 21. Concordance Table with CCD1, CCD2 and MI Cancer Seek Results with Eligible Samples for Solid Tumor

	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

Agreement between MI Cancer Seek and MMR RxDx Panel (CCD) calculated using CCD1 and CCD2 consensus calls as a reference standard showed high concordance:

PPA is 97.5% (193/198) (95% CI [94%, 99.1%])

NPA is 98.5% (194/197) (95% CI [95.4%, 99.7%])

b. Analysis for Endometrial Carcinoma

Clinical concordance between MI Cancer Seek and the MMR RxDx Panel (CCD) for the EC with 251 (out of 401) eligible samples is shown in **Table 22**.

Table 22. Concordance Table with CCD1, CCD2 and MI Cancer Seek Results with Eligible Samples for EC

	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

Agreement between MI Cancer Seek and MMR RxDx Panel (CCD) calculated using CCD1 and CCD2 consensus calls as a reference standard showed high concordance:

PPA: 98.4% (120/122) (95% CI [93.8%, 99.9%])

NPA: 97.6% (123/126) (95% CI [92.8%, 99.5%])

This study clinically validates MI Cancer Seek for the MSI solid tumor CDx claim listed in **Table 1.**

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

Clinical validity of MI Cancer Seek as a CDx was evaluated for the detection of *BRAF* V600E and V600K mutations in melanoma patients 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 tissue samples from patients histologically confirmed as melanoma were enrolled and tested twice on the THxID *BRAF* Kit (CCD) and once on MI Cancer Seek. The distribution of demographics (gender and average age) in this study were comparable to the study population in the pivotal BREAK-3 trial of dabrafenib 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 variants positive, V600K variants 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 23**.

Table 23. 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

Agreement between MI Cancer Seek and THxID *BRAF* kit (CCD) calculated using CCD1 and CCD2 consensus calls as a reference standard showed high concordance:

PPA: 98.7% (152/154) (95% CI [95.0%, 99.9%])

NPA: 99.4% (173/174) (95% CI [96.4%, 100.2%])

Both V600E and V600K variants 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 is shown in **Table 24**.

Table 24. 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 25. Concordance Table with CCD1, CCD2 and MI Cancer Seek Results with Eligible Samples for BRAF V600K as Positives

	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

Agreement between MI Cancer Seek and THxID *BRAF* kit (CCD) using CCD1 and CCD2 consensus calls as a reference standard showed high concordance:

PPA: 98.9% (173/175) (95% CI [95.6%, 99.9%])

NPA: 99.3% (152/153) (95% CI [95.9%, 100.2%])

This study clinically validates MI Cancer Seek for the *BRAF V600E/K* melanoma CDx claim listed in **Table 1.**

3. MI Cancer Seek Clinical Concordance Study for *BRAF* V600E Mutations in CRC

Clinical Validity of MI Cancer Seek as a CDx was evaluated for the detection of *BRAF V600E* mutations in CRC patients 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 FFPE tissues samples from patients histologically confirmed as CRC were enrolled and tested twice on the *therascreen BRAF* V600E RGQ PCR Kit (CCD) and once on MI Cancer Seek. Two overlapping samples were enrolled and one sample did not meet MI Cancer Seek testing requirements.

Therefore, 357 unique samples were tested on MI Cancer Seek, of which five samples had 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). The distribution of demographics in this study (age and gender) were comparable to the BEACON clinical trial population tested with the *therascreen BRAF* V600E RGQ PCR Kit.

Concordance between MI Cancer Seek and the *therascreen BRAF* V600E RGQ PCR Kit CCD with 352 eligible samples is shown in **Table 26**.

Table 26. 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

Agreements between MI Cancer Seek and *therascreen* BRAF V600E RGQ PCR Kit (CCD) calculated using CCD1 and CCD2 consensus calls as a reference standard showed high concordance:

PPA: 99.4% (176/177) (95% CI [96.5%, 100.2%])

NPA:100% (174/174) (95% CI [97.3%, 100.4%])

This study clinically validates MI Cancer Seek for the *BRAF* V600E CRC CDx claim listed in **Table 1**.

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

Clinical Validity of MI Cancer Seek as a CDx was evaluated for the detection of wild type status for *KRAS* (exons 2, 3, and 4) and *NRAS* (exons 2, 3, and 4) in CRC patients who may be eligible for treatment with the FDA-approved VECTIBIX® (panitumumab). A total of 286 unique FFPE tissue samples from patients histologically confirmed as CRC were enrolled and tested twice on the Praxis Extended RAS Panel (CCD) and once on MI Cancer Seek. 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). The distribution of demographics in this study (age and gender) were comparable to the population tested with the Praxis Extended RAS Panel in the pivotal PRIME trial. Concordance between MI Cancer Seek and the Praxis Extended RAS Panel CCD is shown in **Table 27**.

Table 27. 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.								

Agreement between MI Cancer Seek and the Praxis Extended *RAS* Panel (CCD) calculated using CCD1 and CCD2 consensus calls as a reference standard showed high concordance:

PPA is 100.0% (118/118) [95% CI 96.1%, 100.6%]

NPA is 97.2% (139/143) [95% CI 92.7%, 99.1%].

This study clinically validates MI Cancer Seek for the *KRAS*/*NRAS* CRC CDx claim listed in **Table 1**.

5. **MI Cancer Seek Clinical Concordance Study for *EGFR* exon 19 deletion or L858R mutations in NSCLC**

Clinical validity of MI Cancer Seek as a CDx was evaluated for the detection of *EGFR* exon 19 deletion or L858R mutations in NSCLC patients who may be eligible for treatment with the 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 from patients histologically confirmed as NSCLC were enrolled into the study and tested twice on the cobas *EGFR* Mutation Test V2 (CCD) and once on MI Cancer Seek. Twelve samples did not meet MI Cancer Seek testing requirements; therefore 316 samples were tested on MI Cancer Seek. The distribution of demographics in the study (gender and age) was comparable to the EURTAC clinical trial population tested with the cobas *EGFR* Mutation Test V2.

Clinical concordance analysis between MI Cancer Seek and the cobas *EGFR* Mutation Test V2 (CCD) with 315 eligible samples is shown in **Table 28**.

Table 28. 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

Agreements between FCD and CCD calculated using CCD1 and CCD2 consensus calls as a reference standard showed high concordance:

PPA: 98.1% (151/154) (95% CI [94.1%, 99.6%])

NPA: 99.4% (160/161) (95% CI [96.1%, 100.2%])

This study clinically validates MI Cancer Seek for the *EGFR* NSCLC CDx claim listed in **Table 1**.

6. MI Cancer Seek Clinical Concordance Study for *PIK3CA* Alterations in BC

Clinical Validity of MI Cancer Seek as a CDx was evaluated for the detection of *PIK3CA* mutations in BC patients who may be eligible for treatment with PIQRAY (alpelisib). 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 tissues samples from patients histologically confirmed as BC were enrolled into the study and tested twice on the *therascreen PIK3CA* RGQ PCR kit (CCD) and once on MI Cancer Seek. Eight samples did not meet MI Cancer Seek testing requirements; therefore 348 samples were tested on MI Cancer Seek. The distribution of demographics in this study (age and gender) were comparable to the SOLAR-1 clinical trial population tested with the *therascreen* PIK3CA RGQ PCR Kit.

Clinical concordance between MI Cancer Seek and the *therascreen PIK3CA* RGQ PCR Kit (CCD) for 343 eligible samples is shown in **Table 29**.

Table 29. 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

Agreements between MI Cancer Seek and *therascreen PIK3CA* RGQ PCR Kit (CCD) using CCD1 and CCD2 consensus calls as a reference standard showed high concordance:

PPA: 99.4% (174/175) (95% CI [96.4%, 100.2%])

NPA: 100.0% (165/165) (95% CI [97.2%, 100.4%])

This study clinically validates MI Cancer Seek for the *PIK3CA* BC CDx claim listed in **Table 1.**

10. Variant Classification for Companion Diagnostic (CDx) Biomarkers

Table 30 describes the biomarker rules for CDx claims in **Table 1** of the intended use reported by MI Cancer Seek.

Table 30. Biomarker Rules for Companion Diagnostic Claims Reported by MI Cancer Seek

Indication	Biomarker	Reportable Mutations
Breast Cancer	*PIK3CA* SNVs	C420R E542K; E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R H1047L, H1047R, H1047Y
Colorectal Cancer (CRC)	*KRAS* wild-type (exons 2, 3, and 4) and NRAS wild-type (exons 2, 3, and 4)	Wild-type as determined by a "Variant Not Detected" result in the following locations: KRAS Exon 2: G12A, G12C, G12D, G12F, G12N, G12R, G12S, G12V, G12W, G13C, G13D (c.38G>A; c.38_39delinsAT), G13E, G13R, G13V KRAS Exon 3: A59G, A59T, Q61E, Q61H (c.183A>C; c.183A>T), Q61K, Q61L, Q61R KRAS Exon 4: A146P, A146T, A146V, K117N (c.351A>C; c.351A>T) NRAS Exon 2: G12A, G12C, G12D, G12F, G12N, G12R, G12S, G12V, G12W, G13C, G13D, G13E (c.38_39delinsAA; c.38_39delinsAG), G13R, G13V NRAS Exon 3: A59G, A59T, Q61E, Q61H (c.183A>T, c.183A>C), Q61K, Q61L, Q61R NRAS Exon 4: A146V, A146T, A146P, K117N (c.351G>C; c.351G>T)
	BRAF SNV	V600E
Melanoma	*BRAF* SNV	V600E, V600K
NSCLC	*EGFR* activating mutations (*EGFR* 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,

Table 30. Biomarker Rules for Companion Diagnostic Claims Reported by MI Cancer Seek		
Indication	**Biomarker**	**Reportable Mutations**
		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.		

11. Additional MI Cancer Seek Variant Details

Table 31. MI Cancer Seek Number of Reportable Alterations Per Gene					
Gene Name	**Number of Variants**	**Gene Name**	**Number of Variants**	**Gene Name**	**Number of Variants**
ABL1 (NM_005157.5)	36	FGFR1 (NM_023110.2)	13	PDGFRA (NM_006206.5)	74
ACVR1 (NM_001105.4)	9	FGFR2 (NM_000141.4)	89	PDGFRB (NM_002609.3)	28
AIP (NM_003977.3)	64	FGFR3 (NM_000142.4)	30	PIK3CA (NM_006218.3)	588
AKT1 (NM_005163.2)	51	FGFR4 (NM_002011.4)	10	PIK3CB (NM_006219.2)	11
AKT2 (NM_001626.5)	1	FH (NM_000143.3)	241	PIK3R1 (NM_181523.2)	2965
AKT3 (NM_005465.4)	2	FLCN (NM_144997.6)	122	PIK3R2 (NM_005027.3)	3
ALK (NM_004304.4)	47	FLT1 (NM_002019.4)	1	PIM1 (NM_002648.3)	45
AMER1 (NM_152424.3)	855	FLT3 (NM_004119.2)	20	PMS2 (NM_000535.6)	405
APC (NM_000038.5)	8033	FOXA1 (NM_004496.3)	464	POLD1 (NM_001256849.1)	5
APC (NM_001127511.2)	7	FOXL2 (NM_023067.3)	3	POLE (NM_006231.3)	80
AR (NM_000044.4)	6	FUBP1 (NM_003902.4)	647	POT1 (NM_015450.2)	549
ARAF (NM_001654.4)	4	GATA3 (NM_001002295.1)	849	PPP2R1A (NM_014225.5)	30
ARID1A (NM_006015.5)	3367	GNA11 (NM_002067.4)	17	PPP2R2A (NM_002717.3)	135
ARID2 (NM_152641.3)	2773	GNA13 (NM_006572.5)	90	PRDM1 (NM_001198.3)	186
ASXL1 (NM_015338.5)	973	GNAQ (NM_002072.4)	12	PRKACA (NM_002730.3)	3
ATM (NM_000051.3)	4092	GNAS (NM_000516.5)	15	PRKAR1A (NM_001276289.1)	268
ATRX (NM_000489.4)	2622	H3F3A (NM_002107.4)	11	PRKDC (NM_006904.6)	1445
AXIN2 (NM_004655.3)	484	H3F3B (NM_005324.4)	1	PTCH1 (NM_000264.4)	270
B2M (NM_004048.2)	449	HIST1H3B (NM_003537.3)	1	PTEN (NM_000314.6)	4376
BAP1 (NM_004656.3)	2105	HNF1A (NM_000545.6)	288	PTPN11 (NM_002834.4)	77
BARD1 (NM_000465.3)	426	HOXB13 (NM_006361.5)	1	RAC1 (NM_006908.4)	10
BCL2 (NM_000633.2)	51	HRAS (NM_005343.3)	41	RAD50 (NM_005732.3)	673
BCL9 (NM_004326.3)	11	IDH1 (NM_005896.3)	11	RAD51B (NM_133509.3)	143
BCOR (NM_017745.5)	1339	IDH2 (NM_002168.3)	14	RAD51C (NM_058216.2)	151
BLM (NM_000057.3)	622	IRF4 (NM_002460.3)	6	RAD51D (NM_002878.3)	124
BMPR1A (NM_004329.2)	300	JAK1 (NM_002227.3)	747	RAD54L (NM_003579.3)	209
BRAF (NM_004333.5)	49	JAK2 (NM_004972.3)	787	RAF1 (NM_002880.3)	26
BRCA1 (NM_007294.3)	2374	JAK3 (NM_000215.3)	7	RASA1 (NM_002890.2)	743

Table 31. MI Cancer Seek Number of Reportable Alterations Per Gene					
Gene Name	**Number of Variants**	**Gene Name**	**Number of Variants**	**Gene Name**	**Number of Variants**
BRCA2 (NM_000059.3)	3442	KDM5C (NM_004187.3)	1263	RB1 (NM_000321.2)	4308
BRIP1 (NM_032043.2)	633	KDM6A (NM_021140.3)	2934	RET (NM_020975.5)	10
BTK (NM_000061.2)	2	KDR (NM_002253.3)	5	RHOA (NM_001664.3)	33
CALR (NM_004343.3)	5	KEAP1 (NM_012289.3)	1509	RNF43 (NM_017763.5)	1508
CARD11 (NM_032415.5)	23	KIT (NM_000222.2)	508	ROS1 (NM_002944.2)	11
CBFB (NM_001755.2)	285	KLF4 (NM_004235.5)	3	RUNX1 (NM_001754.4)	693
CCND1 (NM_053056.2)	114	KMT2A (NM_005933.3)	1830	SDHA (NM_004168.3)	227
CCND2 (NM_001759.3)	7	KMT2C (NM_170606.2)	4646	SDHAF2 (NM_017841.2)	76
CCND3 (NM_001760.4)	32	KMT2D (NM_003482.3)	6841	SDHB (NM_003000.2)	130
CD79B (NM_000626.3)	7	KRAS (NM_004985.4)	247	SDHC (NM_003001.3)	84
CDC73 (NM_024529.4)	377	LZTR1 (NM_006767.3)	584	SDHD (NM_003002.3)	62
CDH1 (NM_004360.4)	2483	MAP2K1 (NM_002755.3)	81	SETD2 (NM_014159.6)	2951
CDK12 (NM_016507.3)	1393	MAP2K2 (NM_030662.3)	23	SF3B1 (NM_012433.3)	42
CDK4 (NM_000075.3)	7	MAP2K4 (NM_003010.3)	624	SMAD2 (NM_005901.5)	475
CDKN1B (NM_004064.4)	629	MAP3K1 (NM_005921.1)	1903	SMAD4 (NM_005359.5)	2365
CDKN2A (NM_000077.4)	2963	MAPK1 (NM_002745.4)	5	SMARCA4 (NM_001128844.1)	2330
CHEK1 (NM_001114122.2)	213	MAX (NM_002382.4)	172	SMARCB1 (NM_003073.4)	348
CHEK2 (NM_007194.3)	474	MED12 (NM_005120.2)	59	SMARCE1 (NM_003079.4)	269
CIC (NM_015125.4)	1151	MEF2B (NM_001145785.1)	5	SMO (NM_005631.4)	9
CREBBP (NM_004380.2)	2006	MEN1 (NM_130799.2)	764	SOCS1 (NM_003745.1)	95
CSF1R (NM_005211.3)	8	MET (NM_001127500.2)	427	SOS1 (NM_005633.3)	19
CTCF (NM_006565.3)	522	MITF (NM_000248.3)	1	SPEN (NM_015001.2)	1204
CTNNA1 (NM_001903.4)	420	MLH1 (NM_000249.3)	955	SPOP (NM_003563.3)	87
CTNNB1 (NM_001904.3)	174	MLH3 (NM_001040108.1)	313	SRC (NM_005417.4)	4
CXCR4 (NM_001008540.2)	19	MPL (NM_005373.2)	8	STAG2 (NM_001282418.1)	559
CYLD (NM_015247.2)	523	MRE11 (NM_005590.3)	378	STAT3 (NM_139276.2)	14
DICER1 (NM_177438.2)	989	MSH2 (NM_000251.2)	970	STK11 (NM_000455.4)	2100

Table 31. MI Cancer Seek Number of Reportable Alterations Per Gene					
Gene Name	**Number of Variants**	**Gene Name**	**Number of Variants**	**Gene Name**	**Number of Variants**
DNMT3A (NM_022552.4)	961	MSH3 (NM_002439.4)	394	SUFU (NM_016169.3)	228
EGFR (NM_005228.4)	489	MSH6 (NM_000179.2)	1000	TCF7L2 (NM_030756.4)	517
EP300 (NM_001429.3)	1607	MTOR (NM_004958.3)	62	TERT (NM_198253.2)	7
EPHA2 (NM_004.4)	733	MUTYH (NM_001128425.1)	269	TET2 (NM_001127208.2)	1343
ERBB2 (NM_004448.3)	135	MYC (NM_002467.5)	12	TMEM127 (NM_017849.3)	47
ERBB3 (NM_001982.3)	50	MYCN (NM_005378.5)	5	TNFAIP3 (NM_001270507.1)	202
ERBB4 (NM_005235.2)	18	MYD88 (NM_002468.4)	5	TNFRSF14 (NM_003820.3)	120
ERCC2 (NM_000400.3)	445	NBN (NM_002485.4)	574	TP53 (NM_000546.5)	11498
ESR1 (NM_001122742.1)	49	NF1 (NM_001042492.2)	5607	TRAF7 (NM_032271.2)	19
EZH2 (NM_004456.4)	251	NF2 (NM_000268.3)	1403	TSC1 (NM_000368.4)	1216
FANCA (NM_000135.3)	297	NFE2L2 (NM_006164.4)	176	TSC2 (NM_000548.4)	1273
FANCB (NM_001018113.2)	195	NFKBIA (NM_020529.2)	243	U2AF1 (NM_006758.2)	10
FANCC (NM_000136.2)	233	NOTCH1 (NM_017617.4)	2804	VHL (NM_000551.3)	1136
FANCD2 (NM_033084.4)	652	NPM1 (NM_002520.6)	3	WRN (NM_000553.5)	255
FANCE (NM_021922.2)	227	NRAS (NM_002524.4)	79	WT1 (NM_024426.5)	284
FANCF (NM_022725.3)	133	NSD1 (NM_022455.4)	1257	XPO1 (NM_003400.3)	3
FANCG (NM_004629.1)	245	NSD2 (NM_133335.3)	1	XRCC1 (NM_006297.2)	143
FANCI (NM_001113378.1)	414	NTHL1 (NM_002528.6)	93		
FANCL (NM_018062.3)	240	NTRK1 (NM_002529.3)	17		
FANCM (NM_020937.3)	756	NTRK2 (NM_006180.4)	1		
FAS (NM_000043.5)	127	NTRK3 (NM_002530.3)	7		
FAT1 (NM_005245.3)	2753	PALB2 (NM_024675.3)	868		
FBXW7 (NM_033632.3)	1452	PBRM1 (NM_018313.4)	2605		

MI Cancer Seek

PHYSICIAN INFORMATION

Caris Life Sciences
4610 South 44th Place
Phoenix, AZ 85040

For the most current information on the association of the biomarker and therapeutic outcomes, refer to the therapeutic labels available at Drugs@FDA on the FDA website.

1. 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	*PIK3CA* (C420R; E542K; E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, H1047Y)	PIQRAY® (alpelisib)
Colorectal Cancer (CRC)	*KRAS* wild-type (absence of mutations in exons 2, 3, and 4) and *NRAS* wild type (absence of mutations in exons 2, 3, and 4)	VECTIBIX® (panitumumab)
	BRAF V600E	BRAFTOVI® (encorafenib) in combination with ERBITUX® (cetuximab)

Table 1. MI Cancer Seek Companion Diagnostic Indications		
Indication	**Biomarker**	**Therapy**
Melanoma	*BRAF* V600E	*BRAF* Inhibitors approved by FDA*
	BRAF V600E or V600K	MEKINIST® (trametinib) or *BRAF*/MEK Inhibitor Combinations approved by FDA*
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and exon 21 L858R alterations	EGFR 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: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools#Group_Labeling		

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

2. Contraindications

There are no known contraindications.

3. Warnings and Precautions

1. Biopsy may pose a risk to the patient when archival tissue is not available for use with the assay. The patient's physician should determine whether the patient is a candidate for biopsy.

4. Limitations

1. For *in vitro* diagnostic use.
2. CAUTION: Federal law restricts this device to sale by or on the order of a physician.
3. The acceptable preparation method for MI Cancer Seek specimens is FFPE. Other preparations have not been evaluated.
4. The test is designed to report out somatic variants and is not intended to report germline variants.
5. MI Cancer Seek requires a minimum tumor percentage of 20% for detection of alterations, with tumor content enrichment recommended for specimens with tumor percentage lower than 20%.

6. Genomic findings other than those listed in the Companion Diagnostic Indications table are not prescriptive or conclusive for labeled use of any specific therapeutic product. Confirmation of tumor mutation status using an FDA-approved CDx test is needed for therapeutic use.
7. A negative result does not rule out the presence of a mutation below the limits of detection of the assay.
8. MI Cancer Seek is only approved for use with Caris Life Sciences pre-qualified NovaSeq 6000 instruments.
9. The test is intended to be performed on specific serial numbered-controlled instruments by Caris Life Sciences.
10. MI Cancer Seek does not report TMB for values lower than 3 mut/Mb as the accuracy of TMB values below 3 mut/Mb are unreliable.
11. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.
12. Based on the low positive percent agreement (PPA) in the accuracy study for *ERBB2* copy number amplifications (CNAs) in breast cancer patients, this alteration may not be detected. 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.
13. Patients with breast cancer whose specimens have intermediate *ERBB2* CNA status should be tested with another FDA approved or cleared test to ascertain *ERRB2* CNA status in their tumor.
14. Test may have reduced sensitivity and may yield false negative results in samples where necrotic tissue is >15%, melanin is >5%, fat cells are >10%.

5. FDA Evidence Levels

Genomic findings other than those listed in the Intended Use are not prescriptive or conclusive for labeled use of any specific therapeutic product. Test results should be interpreted in the context of pathological evaluation of tumors, treatment history, clinical findings, and other laboratory data. The test report includes genomic findings reported in the following levels (**Table 2** below).

Table 2. Classification Criteria for FDA Evidence Levels

Level	Criteria
Level 1	Biomarker is FDA-approved as a companion diagnostic as part of MI Cancer Seek
Level 2	Cancer alterations that are well-established with strong clinical evidence that the clinician must know according to professional consensus guidelines in the specific tumor type.

Table 2. Classification Criteria for FDA Evidence Levels	
Level	**Criteria**
Level 3	Cancer alterations with potential clinical significance, e.g., biomarkers deemed useful for directing patients to a clinical trial or simply for informational purposes.
	i. Clinical data such as case reports, single or several case series, or Phase I/II clinical trial data that support the utility of specific biomarker alteration to direct a patient to clinical trials, or ii. Pre-clinical and/or *in vitro* studies provide structural analysis of the mutation, fusion, or isoform to confirm pathogenicity (tumor-promoting), sensitivity, or resistance through colony forming assays, growth inhibition or drug sensitivity assays, etc.

CARIS | MI CANCER SEEK™

Patient

Name: First Name Last Name
Date of Birth: Mo/Da/Yr
Sex: Female
Case Number: TN24-1111111
CDx Report Generated: 11/02/2024

Specimen Information

Diagnosis and Tumor Type:
Infiltrating duct carcinoma, NOS, Breast, NOS
Specimen Site: Axillary lymph node
Specimen ID: A1B1C1-1234
Specimen Type: Formalin-fixed paraffin-embedded
Specimen Collected: 00/00/0000

Ordered By

Physician Name
Institution
Physician Location

CDx Associated Findings

Genomic Findings Detected	FDA-approved Therapeutic Options
Findings did not yield any biomarker results with Companion Diagnostic (CDx) Claims.	
Microsatellite Instability (MSI)	Not MSI-H
PIK3CA *C420R; E542K; E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, H1047Y)*	Not Detected

Tumor Profiling Results

MI Cancer Seek is FDA-approved to provide tumor mutation profiling results for previously diagnosed oncology patients with solid tumors.

Other Alterations and Biomarkers Identified

Results reported in this section are not prescriptive or conclusive for labeled use of any specific therapeutic product.
Confirmation of tumor mutation status using an FDA-approved CDx test is needed for therapeutic use.

Tumor Mutational Burden (TMB)	6 mut/Mb
ERBB2	Amplified
PTEN	I253fs

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director •

MI Cancer Seek™

Intended 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	*PIK3CA* (C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, H1047Y)	PIQRAY® (alpelisib)
Colorectal Cancer (CRC)	*KRAS* wild-type (absence of mutations in exons 2, 3, and 4) and *NRAS* wild-type (absence of mutations in exons 2, 3, and 4)	VECTIBIX® (panitumumab)
	BRAF V600E	BRAFTOVI® (encorafenib) in combination with ERBITUX® (cetuximab)
Melanoma	*BRAF* V600E	BRAF Inhibitors approved by FDA*
	BRAF V600E or *BRAF* V600K	Mekinist® (trametinib) or BRAF/MEK Inhibitor Combinations approved by FDA*
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and exon 21 L858R alterations	EGFR 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 therapeutic products in this group, go to: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools#Group_Labeling		

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

Contraindications:

There are no known contraindications.

Warnings and Precautions:

Biopsy may pose a risk to the patient when archival tissue is not available for use with the assay. The patient's physician should determine whether the patient is a candidate for biopsy.

Limitations:

- For *in vitro* diagnostic use.
- CAUTION: Federal law restricts this device to sale by or on the order of a physician.
- The acceptable preparation method for MI Cancer Seek CDx specimens is FFPE. Other preparations have not been evaluated.
- The test is designed to report out somatic variants and is not intended to report germline variants.
- MI Cancer Seek requires a minimum tumor percentage of 20% for detection of alterations, with tumor content enrichment recommended for specimens with tumor percentage lower than 20%.
- Genomic findings other than those listed in the Companion Diagnostic Indications table are not prescriptive or conclusive for labeled use of any specific therapeutic product. Confirmation of tumor mutation status using an FDA-approved CDx test is needed for therapeutic use.
- A negative result does not rule out the presence of a mutation below the limits of detection of the assay.
- MI Cancer Seek is only approved for use with Caris Life Sciences pre-qualified Illumina NovaSeq 6000 instruments.
- The test is intended to be performed on specific serial number-controlled instruments by Caris Life Sciences.
- MI Cancer Seek does not report TMB for values lower than 3 mut/Mb as the accuracy of TMB values below 3 mut/Mb are unreliable.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.
- Based on the low positive percent agreement (PPA) in the accuracy study for ERBB2 copy number amplifications (CNAs) in breast cancer patients, this alteration may not be detected. 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.
- Patients with breast cancer whose specimens have intermediate ERBB2 CNA status should be tested with another FDA approved or cleared test to ascertain ERRB2 CNA status in their tumor.
- Test may have reduced sensitivity and may yield false negative results in samples where necrotic tissue is >15%, melanin is >5%, fat cells are >10%.

Test Principle:

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 uses a custom bait panel to measure all coding regions of the exome 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

ABL1	*BCL9*	*CDKN2A*	*ESR1*	*FLT1*	*JAK3*	*MET*	*NRAS*	*PPP2R2A*	*RUNX1*	*STK11*
ACVR1	*BCOR*	*CHEK1*	*EZH2*	*FLT3*	*KDM5C*	*MITF*	*NSD1*	*PRDM1*	*SDHA*	*SUFU*
AIP	*BLM*	*CHEK2*	*FANCA*	*FOXA1*	*KDM6A*	*MLH1*	*NSD2*	*PRKACA*	*SDHAF2*	*TCF7L2*
AKT1	*BMPR1A*	*CIC*	*FANCB*	*FOXL2*	*KDR*	*MLH3*	*NTHL1*	*PRKAR1A*	*SDHB*	*TERT*
AKT2	*BRAF*	*CREBBP*	*FANCC*	*FUBP1*	*KEAP1*	*MPL*	*NTRK1*	*PRKDC*	*SDHC*	*TET2*
AKT3	*BRCA1*	*CSF1R*	*FANCD2*	*GATA3*	*KIT*	*MRE11*	*NTRK2*	*PTCH1*	*SDHD*	*TMEM127*
ALK	*BRCA2*	*CTCF*	*FANCE*	*GNA11*	*KLF4*	*MSH2*	*NTRK3*	*PTEN*	*SETD2*	*TNFAIP3*
AMER1	*BRIP1*	*CTNNA1*	*FANCF*	*GNA13*	*KMT2A*	*MSH3*	*PALB2*	*PTPN11*	*SF3B1*	*TNFRSF14*
APC	*BTK*	*CTNNB1*	*FANCG*	*GNAQ*	*KMT2C*	*MSH6*	*PBRM1*	*RAC1*	*SMAD2*	*TP53*
AR	*CALR*	*CXCR4*	*FANCI*	*GNAS*	*KMT2D*	*MTOR*	*PDGFRA*	*RAD50*	*SMAD4*	*TRAF7*
ARAF	*CARD11*	*CYLD*	*FANCL*	*H3F3A*	*KRAS*	*MUTYH*	*PDGFRB*	*RAD51B*	*SMARCA4*	*TSC1*
ARID1A	*CBFB*	*DDR2*	*FANCM*	*H3F3B*	*LZTR1*	*MYC*	*PIK3CA*	*RAD51C*	*SMARCB1*	*TSC2*
ARID2	*CCND1*	*DICER1*	*FAS*	*HIST1H3B*	*MAP2K1*	*MYCN*	*PIK3CB*	*RAD51D*	*SMARCE1*	*U2AF1*
ASXL1	*CCND2*	*DNMT3A*	*FAT1*	*HNF1A*	*MAP2K2*	*MYD88*	*PIK3R1*	*RAD54L*	*SMO*	*VHL*
ATM	*CCND3*	*EGFR*	*FBXW7*	*HOXB13*	*MAP2K4*	*NBN*	*PIK3R2*	*RAF1*	*SOCS1*	*WRN*
ATRX	*CD79B*	*EP300*	*FGFR1*	*HRAS*	*MAP3K1*	*NF1*	*PIM1*	*RASA1*	*SOS1*	*WT1*
AXIN2	*CDC73*	*EPHA2*	*FGFR2*	*IDH1*	*MAPK1*	*NF2*	*PMS2*	*RB1*	*SPEN*	*XPO1*
B2M	*CDH1*	*ERBB2*	*FGFR3*	*IDH2*	*MAX*	*NFE2L2*	*POLD1*	*RET*	*SPOP*	*XRCC1*
BAP1	*CDK12*	*ERBB3*	*FGFR4*	*IRF4*	*MED12*	*NFKBIA*	*POLE*	*RHOA*	*SRC*	
BARD1	*CDK4*	*ERBB4*	*FH*	*JAK1*	*MEF2B*	*NOTCH1*	*POT1*	*RNF43*	*STAG2*	
BCL2	*CDKN1B*	*ERCC2*	*FLCN*	*JAK2*	*MEN1*	*NPM1*	*PPP2R1A*	*ROS1*	*STAT3*	

FDA Evidence Levels:

Genomic findings other than those listed in the Intended Use are not prescriptive or conclusive for labeled use of any specific therapeutic product. Test results should be interpreted in the context of pathological evaluation of tumors, treatment history, clinical findings, and other laboratory data. The test report includes genomic findings reported in the following levels (Table 3).

Table 3. Classification Criteria for FDA Evidence Levels

LEVEL	CRITERIA
Level 1	Biomarker is FDA-approved as a companion diagnostic as part of MI Cancer Seek™
Level 2	Cancer alterations that are well-established with strong clinical evidence that the clinician must know according to professional consensus guidelines in the specific tumor type.
Level 3	Cancer alterations with potential clinical significance, e.g., biomarkers deemed useful for directing patients to a clinical trial or simply for informational purposes.
	i. Clinical data such as case reports, single or several case series, or Phase I/II clinical trial data that support the utility of specific biomarker alteration to direct a patient to clinical trials, or ii. Pre-clinical and/or *in vitro* studies provide structural analysis of the mutation, fusion, or isoform to confirm pathogenicity (tumor-promoting), sensitivity, or resistance through colony forming assays, growth inhibition or drug sensitivity assays, etc.

