SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Circulating Tumor DNA, Molecular Residual Disease, Somatic Variant Detection System, Solid Tumors

Device Trade Name: Signatera™ CDx

Device Procode: PQP

Applicant's Name and Address: Natera, Inc.
201 Industrial Road, Suite 410
San Carlos, CA 94070

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P260004

Date of FDA Notice of Approval: May 15, 2026

Breakthrough Device: Granted breakthrough device status on December 6, 2022.

II. INDICATIONS FOR USE

Signatera™ CDx is a personalized, tumor-informed, multiplex-PCR and next-generation sequencing (NGS)–based assay that detects circulating tumor DNA (ctDNA) molecular residual disease (MRD) from plasma of peripheral whole blood collected in Streck Cell-Free DNA Blood Collection Tubes (BCTs), using bespoke assays designed to track somatic variants identified from sequencing of a patient's formalin-fixed, paraffin-embedded (FFPE) tumor specimen.

Signatera™ CDx is intended to identify patients with ctDNA MRD positive status who may benefit from treatment listed in **Table 1**, in accordance with the approved therapeutic product labeling.

Table 1: Signatera™ CDx Indicated Use and Associated Therapy

Biomarker	Indication	Therapy
ctDNA MRD	Muscle Invasive Bladder Cancer (MIBC)	TECENTRIQ® (atezolizumab), TECENTRIQ HYBREZA® (atezolizumab and hyaluronidase-tqjs)

III. CONTRAINDICATIONS

Testing cannot be performed on patients who:

1. Have a history of allogenic bone marrow transplant.

IV. WARNINGS AND PRECAUTIONS

1. Only Blood Collection Tubes (BCTs) listed must be used. Do not freeze the contents of the BCTs. Do not transfer blood collected with other anticoagulants into the Signatera CDx BCTs, as this may inhibit the performance of the assay. Heparin collection tubes should not be used for blood collection since heparin can interfere with assay results. Do not reuse the BCTs.
2. Treat all samples as biohazardous, using appropriate handling and disposal procedures in accordance with local safety regulations.
3. This assay requires both tissue and blood samples.
4. These instructions are intended only for trained medical professionals.
5. Use only recommended materials, equipment, and procedures specified in the IFU.
6. Always wear appropriate personal protective equipment (PPE) such as gloves, lab coats, and eye protection.

LIMITATIONS

1. For in vitro diagnostic use.
2. For prescription use only.
3. Signatera CDx should be performed at least 6 weeks after cystectomy in muscle invasive bladder cancer (MIBC) patients.
4. A negative result does not preclude the presence of ctDNA MRD. Patients with negative results should continue with serial testing until a positive result or completion of the recommended 12-month testing window.
5. Signatera CDx is intended to detect ctDNA MRD from MIBC.
6. The assay has decreased ability to detect ctDNA MRD in samples with low levels of ctDNA (below 0.1 mean tumor molecules (MTM)/mL).
7. Signatera CDx is not intended to be used for standalone diagnostic purposes.
8. Signatera CDx does not report germline variants and does not infer hereditary cancer risk.
9. Decisions on patient care and treatment should be based on the independent medical judgment of the treating physician, taking into account all clinicopathological factors, in accordance with the standard of care.
10. The potential impact on the assay results of excessive anticoagulant from the Streck Cell-Free DNA BCTs or underfilling of the Streck Cell-Free DNA BCTs with less than 10 mL of blood has not been evaluated.
11. Incomplete or overmixing of the Streck Cell-Free DNA BCTs impacts assay performance.
12. The assay is intended to be performed on specific serial number-controlled instruments at Natera, Inc. clinical laboratories.

V. DEVICE DESCRIPTION

Signatera CDx is a personalized, tumor-informed, multiplex-PCR and next-generation sequencing (NGS)-based assay using tumor-specific single nucleotide variants (SNVs) identified in a patient's formalin-fixed paraffin-embedded (FFPE) tumor tissue and detected in plasma isolated from anticoagulated peripheral whole blood from patients previously diagnosed with muscle invasive bladder cancer (MIBC).

Signatera CDx is intended to be used as an *in vitro* diagnostic device to detect circulating tumor DNA (ctDNA) molecular residual disease (MRD) after curative-intent treatment (cystectomy) in patients with resectable MIBC to aid physician decision-making of adjuvant treatment in conjunction with other clinicopathological factors.

The device includes two workflows: (1) tumor variant identification and selection using whole exome sequencing (WES) of a FFPE tumor tissue sample and patient-matched whole blood sample to identify and select 16 bespoke tumor-specific SNVs, and (2) tumor variant detection using multiplex PCR and ultra-deep next-generation sequencing for detection of the tumor-associated SNVs in a patient's plasma-derived cell-free DNA (cfDNA) for determination of ctDNA MRD status. Serial testing with the plasma workflow can be performed starting at least 6 weeks after cystectomy, every 6 weeks for 9 months with a final test at one year. A patient's plasma sample is considered ctDNA MRD-positive when at least two (2) SNVs out of 16 are detected. Otherwise, the sample is considered ctDNA MRD-negative. Tumor variant identification and selection is performed once on FFPE tissue samples, and tumor variant detection in the patient's plasma is performed at multiple longitudinal time points.

Briefly, DNA isolated from FFPE tumor tissue and matched whole blood collected in K2EDTA blood collection tubes (BCTs) are processed for tumor variant identification using WES. Through the tumor variant identification and selection process, tumor-specific somatic variants are identified following elimination of mutations from the germline and clonal hematopoiesis of indeterminate potential (CHIP) by subtraction of the patient-matched whole blood-derived genomic sequence. From these candidate variants, a panel of 16 bespoke tumor SNVs is selected based primarily on tumor variant allele frequency (VAF) as a proxy for clonality, predicted noise profile in the plasma, and multiplex PCR compatibility between primers. A patient-specific 16-plex primer set is manufactured to be used to detect ctDNA MRD in cfDNA isolated from the plasma component of whole blood samples collected in Streck BCTs.

Assay Output

Signatera CDx is a qualitative assay that reports "Signatera CDx Positive" when at least two (2) of the 16 bespoke tumor SNVs are detected and reports "Signatera CDx Negative" when fewer than two (2) bespoke tumor SNVs are detected.

Instruments

Signatera CDx testing is performed with serial number-controlled instruments as indicated below in **Table 2**. All instruments are qualified by Natera, Inc.

Table 2. Signatera CDx Instrument List (Instruments Qualified by Natera)

Instrument	Manufacturer	Function	Workflow Component (WES or Plasma)
NovaSeq 6000	Illumina	WES library sequencing platform	WES
NovaSeq 6000	Illumina	Plasma library sequencing platform	Plasma
Biomek i7 Automated Workstation	Beckman Coulter	WES Library Preparation	WES
Veriti 96-well thermal cycler	ThermoFisher	Library Preparation	Plasma
QIAsymphony SP	QIAGEN	Whole Blood gDNA Extraction and cfDNA extraction	WES and Plasma

Reagents

All assay reagents included in the Signatera CDx are purchased from Natera qualified vendors, manufactured and/or qualified by Natera, Inc. Reagents, materials, and equipment needed to perform the test are used exclusively in Natera clinical laboratories. The reagents required to perform the assay are listed below.

The following reagents are manufactured by Natera:

- WES Workflow
    - Positive run controls used in patient-matched whole blood sequencing
    - Oligonucleotide adapters and primers
- Plasma Workflow
    - Process and run controls
    - Library preparation ligation buffer
    - Oligonucleotide primers

The following reagents are purchased from qualified vendors and are qualified by Natera under its Quality System:

- WES Workflow
    - DNA extraction reagents
    - DNA purification reagents
    - DNA quantitation dye
    - Library preparation enzyme mix and buffers
    - Library enrichment probes

    - Positive and negative run control used in FFPE tissue sequencing
    - Negative run control used in patient-matched whole blood sequencing
    - Next generation sequencing kit
- Plasma Workflow
    - cfDNA extraction reagents
    - cfDNA purification reagents
    - cfDNA quantitation dye
    - DNA purification reagents
    - Library preparation enzyme mix and buffers
    - Library enrichment reagents
    - No Template Control
    - Next generation sequencing kit

The materials required for specimen collection, but not provided, include five slide boxes, one Becton Dickinson (BD) IVD K2EDTA BCT (#367863), and two Streck IVD cell-free DNA BCTs (#230471).

Assay Process

A. Signatera CDx WES Workflow

The WES workflow is performed at Natera, Inc. clinical laboratory located in CA, USA.

Specimen Collection and Preparation

The tumor tissue samples are shipped at ambient temperature as FFPE blocks (with a surface area >25 mm^2) or FFPE slides (10-20 slides with 5µm thickness or 5-10 slides with 10µm thickness). FFPE tumor tissue blocks are sectioned into 6 slides of 10µm thickness. Prior to starting the assay, a Hematoxylin and Eosin (H&E) stained slide is prepared, and then reviewed by a board-certified pathologist to confirm disease ontology and confirm tumor content is sufficient (≥ 20% nucleated tumor cells) to proceed with the assay.

Whole blood from the same patient is collected in one K2EDTA BCT (6 mL) and used for germline/CHIP subtraction.

DNA Extraction

Tumor DNA is extracted from pathology-confirmed, macro-dissected (if applicable), unstained FFPE tissue slides. DNA extraction from tissue is performed on Biomek liquid handlers using Omega Bio-tek Mag-Bind® FFPE DNA/RNA Kit qualified by Natera.

The patient-matched whole blood DNA is extracted from peripheral blood, using an automated workflow on the QIAsymphony platform with QIAsymphony qualified by Natera.

DNA Purification & Quantification

Each tissue DNA and patient-matched whole blood (matched normal) genomic DNA sample is quantified using a fluorescence-based assay detecting double-stranded DNA following the manufacturer's instructions. Samples yielding ≥50 ng of gDNA mass for the tissue and ≥100 ng for patient-matched whole blood are used in library preparation for the WES workflow.

Library Construction

The normalized tissue DNA and matched normal gDNA are sheared and subjected to end-repair, A-tailing, and adapter ligation. The ligated products are then purified, PCR amplified using barcoded primers and further purified to obtain high-quality libraries. Both tissue and matched normal libraries are quantified using a fluorescence-based assay detecting double-stranded DNA. Samples meeting quality control requirements proceed to hybrid capture.

Hybrid Capture

Hybrid capture is performed using amplified libraries from tissue and matched normal samples. Libraries from the same sample type are combined, purified, and eluted in a hybridization mix containing target-specific capture probes. Following hybridization, captured libraries are purified, enriched through PCR amplification, and subjected to additional purification steps to obtain high-quality sequencing pools. The enriched pools are then quantified, normalized to appropriate loading concentration, and denatured prior to sequencing.

Next Generation Sequencing

Libraries are sequenced on the NovaSeq 6000 platform, qualified by Natera, at 2x150bp to achieve the on-target coverage, as specified in the assay protocol.

Sequence Analysis

Raw sequencing data are converted to FASTQ format and reads are mapped to the hg19 reference genome resulting in two .bam files, one for the tumor sample and one for the patient-matched blood. The mapped sequencing reads go through a quality control (QC) process to flag samples that did not meet pre-specified QC metrics. Reads are aligned to the hg19 human reference genome. Sample contamination and/or sample swaps are detected by assessing genotype concordance between tumor and matched normal samples. Somatic SNVs are identified by comparing tumor and patient-matched whole blood data, excluding known population polymorphisms, variants in repetitive regions and mutations arising from CHIP. High-confidence somatic variants are prioritized primarily based on VAF and background error rate modeling. Top-ranked variants are selected for custom assay design. Primer pairs targeting tumor-specific variants are generated using automated primer design algorithms, evaluated for specificity and primer-primer interaction potential, and optimized to produce a final set of non-overlapping, high-quality amplicons for downstream analysis.

WES workflow sample-level sequencing control metrics are summarized in **Table 3** below.

Table 3. Sample-Level Sequencing Control Metrics for WES Workflow

Metric	Purpose / Threshold
Data Quality	Ensures the sequencing run generates sufficient high-quality sequence reads / ≥ 80% Reads with Base Q30.
NGS Coverage	Ensures adequate read depth within a run***
Contamination and sample-swap QC	Ensures sample contamination and sample-swaps are identified***
Designability	Ensures a 16-plex primer set can be designed / Ability to design a 16-plex assay (identification of at least 16 somatic variants meeting all criteria to be included in the plasma panel)

*** Pre-specified threshold values. Data not shown.

B. Signatera CDx Plasma Workflow

The plasma workflow is performed at Natera, Inc. clinical laboratory located in TX, USA.

Specimen Collection and Preparation

Whole blood samples are collected in two 10 mL peripheral whole blood collection tubes (Streck Cell-Free BCT) and shipped at ambient temperature.

Plasma Isolation

Upon receipt at the testing laboratory, blood samples are accessioned, centrifuged, and plasma is isolated using an automated process within a defined time frame following collection.

DNA Extraction

Plasma samples (10 mL) are processed for cfDNA extraction using an automated workflow, and the purified cfDNA is eluted into a DNA suspension buffer.

DNA Purification & Quantification

Each cfDNA sample is purified using a magnetic bead–based cleanup method and quantified with a fluorescence-based assay detecting double-stranded DNA. Samples yielding ≥10 ng of cfDNA are used in library preparation.

Library Construction

cfDNA meeting minimum input requirements is used as input into library preparation. The cfDNA is end-repaired, A-tailed, and ligated with custom adapters. The purified ligation product is amplified and purified prior to further use.

Multiplex PCR

An aliquot of each cfDNA library is used as input for a multiplex PCR reaction targeting tumor-specific variants. Amplified libraries are labeled with sample-specific index barcodes and pooled with appropriate positive and negative controls and further purified to enrich for target fragments, then quantified using a fluorescence-based assay detecting double-stranded DNA. Prior to sequencing, the pooled libraries are normalized to appropriate loading concentration and denatured.

Next Generation Sequencing

Paired-end sequencing is performed using the Illumina NovaSeq 6000, qualified by Natera.

Sequence Analysis

Raw data files are converted to FASTQ format and demultiplexed based on sample-specific index barcodes. Paired reads are merged, and low-quality bases or mismatches between read pairs are filtered to minimize sequencing errors. The processed reads are aligned to the hg19 reference genome. Mapped reads undergo QC to remove off-target or low-confidence sequences, and high-quality reads are assigned to their corresponding amplicons and samples. Only on-target reads with high mapping Q30 reads are included for further analysis. For each of the 16 SNV targets, the variant is considered detected if supporting variant reads are above the pre-specified confidence threshold.

The sample-level sequencing control metrics are documented in **Table 4**.

Table 4. Sample Level Sequencing Control Metrics for Plasma Workflow

Metric	Purpose / Threshold
Total Filtered Target Number of Reads	Ensures adequate number of sequencing reads are obtained for each sample***
Median Amplicon Depth of Reads	Ensures adequate read depth across all 16-plex panel targets for reliable variant detection***
Working Targets	Ensures that a minimum number of 16-plex panel targets are successfully amplified and sequenced / Fail below 11

Median Sequencing Error Rates	Ensures sequencing quality by measuring the frequency of transition and transversion errors***
Contamination and sample-swap QC	Ensures sample contamination and sample-swaps are identified***

*** Pre-specified threshold values. Data not shown.

C. Report Generation

A patient's plasma sample is considered ctDNA MRD-positive when at least two (2) SNVs out of 16 are detected. Otherwise, the sample is considered ctDNA MRD-negative. Signatera CDx is a qualitative test, reporting the presence or absence of ctDNA MRD, as "Signatera CDx-Positive" or "Signatera CDx-Negative".

Samples failing any QC metric are automatically held and no report is released. A patient sample that has failed a QC metric may be rerun by using stored intermediate products. A "Test Not Performed" (TNP) result is issued for samples unable to pass QC metrics.

D. Internal Process Controls

WES workflow

Positive Controls: A positive control sample is included for both the tissue and patient-matched normal whole blood (MN) workflows. The tissue control contains a FFPE reference standard and is introduced at the gDNA extraction stage. The MN control contains a genomic DNA reference standard and is added during sample normalization. Quality control metrics for the positive controls are evaluated at the batch level to ensure consistency and performance across runs.

Negative Controls: A no-template control is included in both the tissue and matched normal workflows at the sample normalization stage to monitor for potential contamination at the batch level. Negative control samples are processed alongside clinical samples through library preparation and quantification steps. Batches in which negative controls meet predefined quality controls are cleared to proceed to hybridization and subsequent sequencing analysis.

Plasma workflow

The workflow includes batch-level run controls and sample-level endogenous controls to ensure run and sample analysis integrity. Each sample batch includes a well-characterized reference cell line as a ctDNA MRD-negative sample and a well-characterized cell line blend as ctDNA MRD-positive samples, one blended at a high VAF and one at a low VAF. A no-template control is included to assess run-to-run or carryover contamination. Batches in which positive and negative cell-line controls and no-template control meet predefined quality controls proceed to subsequent sample-level analysis.

E. Software System

The Signatera CDx software system consists of data analysis platforms and supporting laboratory management systems that integrate end-to-end laboratory and analytical workflows. It connects laboratory operations, data management, and computational analysis through a centralized information management platform and cloud-based services, ensuring standardized, traceable, and compliant processing of laboratory and analytical data.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no FDA-approved or -cleared companion diagnostic (CDx) alternatives for the detection of ctDNA MRD in this intended use population.

VII. MARKETING HISTORY

Signatera CDx has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate assignment of treatment with TECENTRIQ (atezolizumab) or TECENTRIQ HYBREZA (atezolizumab and hyaluronidase-tqjs). Patients with false positive results may receive treatment with TECENTRIQ (atezolizumab) or TECENTRIQ HYBREZA (atezolizumab and hyaluronidase-tqjs) without clinical benefit and may experience adverse reactions and exposure to potential toxicity associated with the therapy. Patients with false negative results may not be considered for treatment with TECENTRIQ (atezolizumab) or TECENTRIQ HYBREZA (atezolizumab and hyaluronidase-tqjs). There is also a risk of delayed test results, which may lead to delays of treatment.

For the specific adverse events related to the approved therapeutics, please see the FDA approved package insert for TECENTRIQ and TECENTRIQ HYBREZA which is available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Device Cutoff Determination

1. Sample-level Device Cutoff

A patient's plasma sample is considered ctDNA MRD-positive when at least two (2) SNVs out of 16 patient-specific SNVs are detected. Otherwise, the sample is considered ctDNA MRD-negative. This device cutoff was set through an in-silico analysis that balanced specificity and sensitivity, while prioritizing specificity during initial or longitudinal testing. This in-silico

analysis modeled a varying number of SNVs detectable in plasma and the detection of two SNVs yielded 99.7% specificity and 90% sensitivity at a sample-level VAF of 0.01%. Consequently, the device cutoff for a positive ctDNA MRD result was set to the threshold of at least two (2) SNVs detected out of 16 patient-specific SNVs.

2. Plasma Workflow Variant-level Calling Threshold

Signatera CDx variant-level calling confidence thresholds of the plasma workflow were established using 1,368 ctDNA MRD-negative clinical samples from various cancer types. A proprietary background error model was employed to evaluate and select variant-specific calling confidence thresholds by calculating false positive rates for each base substitution class. The calling thresholds were chosen to achieve ≥99.5% sample-level specificity with a minimum of two (2) positive variants required for a sample-level positive call.

B. Laboratory Studies

Signatera CDx performance characteristics were established using clinical samples from patients with MIBC. The clinical samples consisted of FFPE tumor tissue, patient-matched whole blood, and plasma extracted from peripheral whole blood. Studies included patient-specific SNVs representative of the intended use population with various genomic contexts across the device's reportable range. Due to limitations in clinical sample availability and the requirement for three different specimen types (i.e., FFPE tumor tissue, whole blood and plasma) from each patient to perform the assay, contrived samples were utilized in some non-clinical studies. These contrived samples were comprised of MIBC tumor-derived fragmented FFPE DNA spiked into healthy donor plasma samples. A contrived sample functional characterization (CSFC) study was conducted to demonstrate comparable performance between contrived samples and clinical cfDNA samples prior to their use in nonclinical studies. Since Signatera CDx includes both upfront tumor tissue characterization through WES to design a patient-specific 16-plex primer set and plasma cfDNA sequencing to detect ctDNA MRD, several studies were performed to assess the performance of each workflow component in addition to end-to-end workflow assessments.

1. Analytical Accuracy / Concordance with an Orthogonal Method

Analytical accuracy of Signatera CDx was determined through concordance with an externally validated orthogonal assay. The study used 66 sample sets, each consisting of MIBC tumor tissue, patient-matched whole blood, and plasma from the same patient. The specimens were split into two equal amounts to be tested on both assays for assessment of positive and negative agreement. The positive percent agreement (PPA) and negative percent agreement (NPA) values were evaluated and presented in **Table 5**.

Table 5. Sample-level Agreement between Signatera CDx and the Comparator Assay

	Comparator		Total
Signatera CDx	**Positive**	**Negative**	
Positive	31	6[1]	37
Negative	2	13	15
No Call	9	5	14
Total	42	24	66
Point Estimate (n/N) [95% CI] (Excluding No Calls)	**PPA** = 93.9% (31/33) [80.4%, 98.3%], **NPA** = 68.4% (13/19) [46.0%, 84.6%],		
Point Estimate (n/N) [95% CI] (Including No Calls as Discordance)	**PPA** = 73.8% (31/42) [58.9%, 84.7%], **NPA** = 54.2% (13/24) [35.1%, 72.1%],		

CI: Wilson Score Confidence Interval

[1] Upon retesting to determine whether the discordance was repeatable, two initially comparator negative samples returned positive calls when rerun with the comparator assay.

Discordance results may be attributed to variability in calls around the LoD of the assays. There are two (2) samples with a positive result by the comparator assay and a negative result by Signatera CDx, and six (6) samples with a negative result by the comparator assay and a positive result by Signatera CDx. These discordant samples all have low ctDNA levels.

Variant-level agreement analyses were performed to calculate the overlap of the 16-plex variants between Signatera CDx and the orthogonal assay, and the concordance (PPA and NPA) for these overlapping variants. For the 52 samples with valid ctDNA MRD results from both assays, 53.5% (445/832) of the 16-plex variants overlapped between the two assays, with a median of 9 overlapping targets across samples (**Table 6**). Out of the 445 overlapping 16-plex variants, 45 variants yielded no result on the orthogonal assay. The point estimates for PPA and NPA for the 400 overlapping 16-plex variants with a valid result on the orthogonal assay were 88.8% (143/161) and 87% (208/239), respectively **(Table 7)**. Lower variant-level agreement is expected relative to patient-level MRD determination, as the test is designed to detect the overall presence of ctDNA rather than any single specific mutation.

Table 6. Overlap of Designed 16-plex Between Signatera CDx and the Comparator Assay

	Min	1st Quantile	Median	3rd Quantile	Max	Mean
Overlap of 16-plex	4	7	9	10	12	8.6

Table 7. Variant-level Agreement between Signatera CDx and the Comparator Assay

	Comparator			Total
Signatera CDx	**Positive**	**Negative**	**No Result**	
Positive	143	30	29	202
Negative	15	208	15	238
No Result	3	1	1	5
Total	161	239	45	445
Point Estimate (n/N) [95% CI]	**PPA** = 88.8% (143/161) [83.0%, 92.8%] **NPA** = 87.0% (208/239) [82.2%, 90.7%]			

A post market study is planned (listed in Conditions of Approval in Section XV below) to supplement the analytical accuracy of Signatera CDx in low positive clinical specimens.

2. Analytical Sensitivity

a. Limit of Blank (LoB) Studies

WES Workflow LoB

The limit of blank for SNV calls in non-cancerous bladder tissues was evaluated using a tissue replicate pairing approach, in which each non-cancerous bladder FFPE tissue replicate was analyzed against sequencing data from the germline DNA replicate from the same individual acting as the matched whole blood comparator within the standard WES workflow. Across 180 WES pipeline runs (30 comparisons per sample across six samples), zero (0/180) runs generated a 16-plex panel, demonstrating high specificity for 16-plex assay design. The average number of variants identified as potential 16-plex targets was 1.7, corresponding to a WES variant specificity of >99.99% across the 45 MB region assessed.

An additional study was conducted to evaluate the risk of detecting false variants by the Signatera CDx WES workflow in MIBC tumor tissues, using a cohort of 391 clinical MIBC FFPE tissue specimens from the intended use population. A validated orthogonal WES assay was performed to characterize the somatic SNVs in these specimens. The Signatera CDx WES workflow identified a total of 138,868 somatic SNVs, of which 122,621 (88.3%) were also detected by the orthogonal WES assay. Out of the 16,247 SNVs detected exclusively by Signatera CDx WES workflow, 176 SNVs were selected as 16-plex targets, representing 0.13% of the 138,868 SNVs detected by Signatera CDx and 2.8% of the 6,256 (391×16) Signatera CDx 16-plex targets generated for these patients.

Plasma Workflow LoB

The LoB of the plasma workflow was established by evaluating whole blood samples from healthy (cancer-free) donors across two sets of operators, reagent lots, and instruments. Bespoke 16-plex primer sets were generated from 103 unique clinical MIBC FFPE tumor tissue and patient-matched whole blood sample pairs. Three (3) different cfDNA samples were derived from pooled plasma samples from a unique set of healthy donors. A total of 69 healthy donor samples were utilized in this study. Each of the 103 primer pools was tested with the three (3) cfDNA samples for a total of 309 independent assessments. Samples were tested using 45-66 ng cfDNA input, at the challenging, high end of the cfDNA input range for Signatera CDx. All combinations were called ctDNA MRD-negative by Signatera CDx, resulting in a sample-level false positive rate of 0% (0/309). Seven (7) positive SNV target calls were identified in the target-level analysis, for a target-level false positive rate of 0.14% (7/4928).

b. Limit of Detection (LoD) Studies

WES Workflow LoD

The WES LoD was established using four (4) clinical MIBC FFPE tumor tissue and patient-matched whole blood sample pairs diluted to six target levels using gDNA from non-cancerous bladder FFPE tissue samples. The study processed seven (7) replicates per sample at each dilution level for each of three (3) reagent lots, for a total of 21 replicates per sample per dilution level, run at the assay's lowest DNA input of 50 ng. A representative set of SNVs were selected across the four unique samples, taking consideration of variant prevalence, genomic context and base substitution class. For each pre-selected somatic SNV, the LoD was estimated using probit regression analysis. If more than one dilution level had a detection rate < 10% or > 90% on either end of the dilution series, a probit model could not be fit and the LoD for that SNV was established as the lowest dilution level with ≥ 95% empirical detection rate. The summary of LoD estimates for each SNV and SNV category for the WES workflow is presented in **Table 8**. The range of SNV-level LoD estimates was 3.8% - 11.3% VAF, with a median of 5.3% VAF.

Table 8. Summary Results of WES LoD Establishment for Each SNV and SNV Category

SNV Category	Target ID	LoD Estimates per SNV	Range of LoD Estimate per SNV Category
Commonly Targeted MIBC Genes[1]	chr4_1804642_C_G (Gene FGFR3)	5.9% VAF	3.9-9.0% VAF
	chr4_1806089_G_T (Gene FGFR3)	5.0% VAF	

SNV Category	Target ID	LoD Estimates per SNV	Range of LoD Estimate per SNV Category
	chr17_7579716_G_A (Gene TP53)	9.0% VAF	
	chr4_1803568_C_G (Gene FGFR3)	3.9% VAF	
Selected into 16-plex	chr7_138602109_G_C	5.3% VAF	4.5-6.9% VAF
	chr2_21225180_G_C	5.7% VAF	
	chr13_30351259_A_T	4.5% VAF	
	chr1_183085681_C_G	6.9% VAF	
Difficult to Sequence[2]	chr6_5086204_C_G	4.8% VAF	4.8-6.7% VAF
	chr2_42396803_C_A	5.2% VAF	
	chr12_53454502_C_T	6.7% VAF	
	chr2_170493815_G_T	5.3% VAF	
Not Difficult to Sequence	chr3_39374376_C_G	3.8% VAF	3.8-7.4% VAF
	chr2_201473694_G_A	5.2% VAF	
	chr7_20826418_C_T	7.4% VAF	
	chr7_138602109_G_C	5.3% VAF	
GC Rich (> 65% GC Content)	chr6_5086204_C_G	4.8% VAF	4.8-7.7% VAF
	chr2_42396803_C_A	5.2% VAF	
	chr12_53454502_C_T	6.7% VAF	

SNV Category		Target ID	LoD Estimates per SNV	Range of LoD Estimate per SNV Category
		chr3_48629139_C_T	7.7% VAF	
GC Depleted (< 25% GC Content)		chr2_26068393_C_A	11.3% VAF	4.6-11.3% VAF
		chr5_38972013_A_G	5.3% VAF	
		chr2_175245466_C_G	4.6% VAF	
		chr12_81751874_C_A	5.9% VAF	
High Coverage[3]		chr3_39374376_C_G	3.8% VAF	3.8-7.3 % VAF
		chr1_111781401_G_C	5.4% VAF	
		chr7_98988657_G_C	7.3% VAF	
		chr14_69077745_C_T	5.0% VAF	
Low Coverage[3]		chr1_53333244_C_T	5.2% VAF	5.2-5.7 % VAF
		chr11_103092850_C_G	5.4% VAF	
		chr2_21225180_G_C	5.7% VAF	
		chr10_122649395_C_G	5.2% VAF	
Base Substitution Class	A>C / T>G	chr16_27246550_A_C	5.4% VAF	
	A>G / T>C	chr13_43181019_T_C	5.7% VAF	
	A>T / T>A	chr4_55573411_A_T	5.8% VAF	
	C>A / G>T	chr2_42396803_C_A	5.2% VAF	

SNV Category		Target ID	LoD Estimates per SNV	Range of LoD Estimate per SNV Category
	C>G / G>C	chr3_39374376_C_G	3.8% VAF	
	C>T / G>A	chr2_201473694_G_A	5.2% VAF	
Overall Assay LoD Estimate			3.8-11.3 % VAF	

[1] SNV targets for the IMvigor011 study population most commonly occurred on five genes: TP53, FGFR3, KDM6A, DMD, or HUWE1. However, because the 16-plex are highly specific for each patient, SNVs within these genes only represented 0.3% of all potential 16-plex targets among the study subjects in the clinical validation study. No eligible SNVs from KDM6A, DMD and HUWE1 were found in the samples for this study.
[2] SNVs identified in the difficult to sequence whole genome sequencing (WGS) panel generated by the consortium Genome in a Bottle (NIST, Genome in a Bottle Project, 2024).
[3] High Coverage: ≥ 75th percentile of tumor total Depth of Read (DOR) per sample; Low Coverage: ≤ 25th percentile of tumor total DOR per sample

LoD of the WES workflow was confirmed in the WES precision study using 10 undiluted clinical MIBC samples representative of the intended use population at the minimum DNA input (i.e., 50 ng tissue DNA, 100 ng matched normal DNA). The samples were tested in triplicate across three (3) operators and three (3) instruments/reagent lot combinations, for a total of 27 replicates per sample. For each SNV, the mean observed VAF was calculated as the average of its VAF values across the samples that passed QC. For each SNV with mean VAF in the 1-1.5x LoD range (5.3-7.95% VAF), a SNV-specific hit rate was calculated and then aggregated into a single overall SNV hit rate estimate. The LoD for the Signatera CDx WES workflow was confirmed with an overall hit rate of 97.74% (95% CI: [97.58%, 97.89%]) across a total of 1323 unique SNVs (refer to **Precision of WES Workflow** and **Table 20**).

Plasma Workflow LoD Establishment

LoD of the Signatera CDx plasma workflow was established as sample-level mean VAF of the 16-plex targets, which is correlated with the number of targets detected in each sample. The study evaluated ten (10) ctDNA MRD-positive, MIBC clinical samples representative of the intended use population and covering different SNV categories. Clinical cfDNA samples were diluted with cfDNA from healthy donor plasma to six target sample-level mean VAF levels (0.066, 0.033, 0.017, 0.008, 0.004, and 0.002%, equivalent to 0.2, 0.1, 0.05, 0.025, 0.013, and 0.0063 mean tumor molecules (MTM)/mL, respectively). Each sample was tested at the minimum cfDNA input mass of 10 ng with the associated bespoke 16-plex primer sets designed from that patient's matched tumor tissue and

whole blood samples. The LoD estimate based on probit models was 0.0214% VAF. An LoD of 0.043% sample-level mean VAF was conservatively established based on a consistent ≥ 95% hit rate across reagent lots, which is equivalent to 0.13 MTM/mL at cfDNA input of 10ng extracted from 10mL of plasma (**Table 9)**.

Table 9. Summary of Plasma Workflow LoD Establishment Results

Intended VAF %		**0.066**	**0.033**	**0.017**	**0.008**	**0.004**	**0.002**	**0**
Intended MTM/mL[1]		**0.2**	**0.1**	**0.05**	**0.025**	**0.013**	**0.0063**	**0**
Mean Observed VAF %	Lot 1	0.0717	0.0383	0.0168	0.008	0.0028	0.0018	0
	Lot 2	0.0801	0.0426	0.0164	0.0078	0.0029	0.0004	0
	Lot 3	0.0769	0.0388	0.0169	0.0087	0.0019	0.0019	0
Mean Observed MTM/mL	Lot 1	0.2174	0.116	0.051	0.0243	0.0084	0.0054	0
	Lot 2	0.2426	0.1292	0.0497	0.0238	0.0088	0.0011	0
	Lot 3	0.2329	0.1176	0.0512	0.0264	0.0057	0.0057	0
Hit Rate; (n/N)	Lot 1	100.0% (20/20)	100.0% (20/20)	85.0% (17/20)	65.0% (13/20)	25.0% (5/20)	20.0% (4/20)	0.0% (0/20)
	Lot 2	100.0% (20/20)	100.0% (20/20)	80.0% (16/20)	55.0% (11/20)	30.0% (6/20)	5.0% (1/20)	0.0% (0/20)
	Lot 3	100.0% (20/20)	100.0% (20/20)	85.0% (17/20)	65.0% (13/20)	20.0% (4/20)	20.0% (4/20)	0.0% (0/20)
Mean # Positive Targets	Lot 1	9.20	6.50	3.50	2.20	0.90	0.90	0
	Lot 2	10.05	6.50	3.40	1.70	1.00	0.55	0.1
	Lot 3	10.45	6.70	3.60	2.15	1.00	0.65	0.15

[1]MTM/mL = (sample-level mean VAF x cfDNA mass (ng) x 1000 (pg/ng)) / (3.3 (pg) x plasma volume (mL)

Exploratory variant level analysis supports that the assay's sensitivity of detecting individual variants is around 0.33% VAF. A post market study is planned (listed in Conditions of Approval in Section XV below) to supplement the LoD of Signatera CDx plasma workflow for individual variants.

Plasma Workflow LoD Confirmation

Signatera CDx plasma workflow LoD was confirmed at the established LoD using three ctDNA MRD-positive clinical MIBC samples representative of the intended use population tested at levels spanning the assay's cfDNA input range of 10 - 66 ng. Twenty replicates were tested per sample at 0.1 MTM/ml at each cfDNA input level, corresponding to a sample-level mean VAF of 0.033% at 10 ng DNA input, 0.010% at 33 ng DNA input and 0.005% at 66 ng DNA input, using a single reagent lot. All three cfDNA input levels tested achieved ≥ 95% hit rates, confirming the assay LoD at different DNA input levels (**Table 10**).

Table 10. Summary of Plasma Workflow LoD Confirmation Results at Different Input Levels

cfDNA input (ng)	Intended VAF (%)	Mean Observed VAF %	Hit Rate (n/N)	95% CI	Equivalent MTM/mL	Mean # Positive Targets
10	0.033	0.0442%	100% (60/60)	[94.0%, 100%]	0.1340	6.48
33	0.010	0.0122%	96.6% (57/59)	[88.5%, 99.1%]	0.1224	5.31
66	0.005	0.0057%	96.7% (58/60)	[88.6%, 99.1%]	0.1141	4.63

3. Analytical Specificity

a. Interfering Substances

The impact of potentially interfering substances on the performance of Signatera CDx was evaluated using four (4) ctDNA MRD-positive clinical MIBC cfDNA samples diluted with healthy donor plasma to 2-3 x LoD. Samples were processed at the minimum cfDNA input of 10 ng, with 3 replicates each per condition. Interference was evaluated separately for each condition based on sample-level PPA**.** The number of detected positive targets from the 16-plex and sample-level mean VAF were also evaluated. All nine potentially interfering substances demonstrated PPA of 100% (95% CI: [75.75%, 100%]), and there were no significant differences in the number of detected positive targets or sample-level mean VAF values between conditions (**Table 11)**.

Table 11. Summary Results for Potentially Interfering Substances

Interferent	Test Concentration	PPA (%) (n/N)	95% CI	Mean # Positive Targets	Mean Sample-level Mean VAF
Nominal	N/A	N/A	N/A	10.9	0.093
Albumin	60 g/L	100 (12/12)	[75.75%, 100%]	10.8	0.096
Bilirubin Conjugated	475 µmol/L (40 mg/dL)	100 (12/12)	[75.75%, 100%]	11.2	0.106
Bilirubin Unconjugated	684 µmol/L (40 mg/dL)	100 (12/12)	[75.75%, 100%]	10.8	0.085
Ethanol	6.00E+02 mg/dL (1.30E+05 µmol/L)	100 (12/12)	[75.75%, 100%]	10.3	0.091
Hemoglobin	10 g/L (1000 mg/dL)	100 (12/12)	[75.75%, 100%]	11.2	0.090
Index Barcodes	Standard +30%	100 (12/12)	[75.75%, 100%]	10.2	0.096
Proteinase K	2x Standard Amount	100 (12/12)	[75.75%, 100%]	10.2	0.093
Staphylococcus epidermidis	1x10^6 CFU/mL	100 (12/12)	[75.75%, 100%]	10.1	0.088
Triglycerides	16.94 mol/L (1,500 mg/dL)	100 (12/12)	[75.75%, 100%]	10.5	0.087

b. *In silico* Specificity Analysis

Hybrid Capture Bait Specificity:

Hybrid capture bait specificity was assessed using sequencing data from WES processing of 77 clinical MIBC FFPE tumor tissue and patient-matched whole blood samples. For the reads in regions where a variant was called, the reads that also mapped to a probe region were measured and reported as "on-variant, on-probe" reads. The proportion of high-quality reads that were on-variant, on-probe reads was calculated to evaluate hybrid capture probe specificity.

All 77 tumor samples produced valid outputs. The average proportion of high-quality reads that were on-variant, on-probe among all high-quality reads was > 99.99%.

Multiplex PCR Primer Specificity:

Primer specificity was assessed using plasma sequencing data from the full workflow testing of 77 clinical MIBC patient samples representative of the intended use population. In this study, BAM files from plasma samples were processed to extract on-variant and off-variant reads. The

proportion of on-variant reads was calculated to evaluate multiplex PCR primer pool specificity.

All 77 plasma BAM files completed analysis with no QC or computational failures. The mean proportion of high-quality plasma reads aligning to the intended amplicon regions was >99%.

4. Contrived Sample Functional Characterization (CSFC)

Comparable performance between clinical cfDNA samples and contrived samples was confirmed by demonstrating physical and functional equivalency between the contrived and clinical samples. Ten unique clinical specimens from patients with MIBC were used to create contrived cfDNA-like clinical specimens. This contrived material was serially diluted with cfDNA extracted from plasma of healthy donors to target six levels consistent with the LoD establishment study design and tested at 10 ng DNA input using three reagent lots, each lot with two replicates per sample per dilution level for a total of 360 data points. Physical equivalency was evaluated through electropherogram profiles of clinical and contrived DNA fragment size distributions. The analytical sensitivity in contrived samples was established by fitting a probit regression model. The contrived sample results were compared to the LoD establishment results for clinical cfDNA samples at the lowest assay cfDNA input mass of 10 ng to demonstrate functional equivalency. For each sample type, the regression model coefficients for intercept and slope, and the predicted estimates for C5, C25, C50, C75, and C95 were calculated.

Electropherogram profiles of clinical and contrived DNA showed similar peak and size distribution demonstrating physical equivalency. The probit regression yielded closely aligned models for clinical and contrived samples across the range of tested VAF levels. None of the calculated absolute differences between regression model estimates for clinical and contrived samples were statistically significant, supporting comparability between the clinical and contrived samples.

5. Precision Studies

The precision of Signatera CDx was evaluated through three different precision studies encompassing the end-to-end device workflow, as well as the WES workflow and the plasma workflow, respectively, as detailed below.

a. Precision of Signatera CDx End-to-End Workflow

The end-to-end precision of the entire Signatera CDx workflow was demonstrated using clinical patient sample trios of tumor FFPE tissue, whole blood, and plasma. For each patient, the tumor tissue and whole blood pair was run in triplicate using three reagent lot combinations and three sequencing runs, yielding three 16-plex primer designs. At least one WES replicate was run at the minimum DNA input. Each primer design

was then used in the plasma workflow with a corresponding plasma sample that was run in triplicate using three reagent lot combinations and three sequencing runs. All plasma sample replicates were run at the 10ng minimum cfDNA input level.

A total of 37 unique sample trios were evaluated in this study. Precision of the WES workflow was assessed for 35 unique samples. Two samples were excluded due to QC failures leaving only one evaluable replicate. A total of 25,014 unique variants were identified by the WES workflow, with an overall concordance of 91.6% (95% CI: [91.4%, 91.8%]). The number of unique variants identified in each sample ranged from 43 to 2,332 with the variant positive call rate aggregated within a sample ranging from 54.5% - 96.8%. The specimen with the lowest variant positive call rate had 14 targets consistently overlapping across replicates, supporting that the SNVs with low positive call rates in this specimen were not prioritized for selection as 16-plex targets. Across replicates, the average number of 16-plex target overlap ranged from 9 - 15.3 (**Table 12**).

Table 12. Summary of Precision Results per Sample—WES Workflow Output

Specimen	# Valid WES Outputs	Total Unique Variants Detected Across WES Replicates	Variant Positive Call Rate (n/N) [two-sided 95% CI]	Average Overlapping 16-plex [Range]
1	2	1,185	88.9% (2,106/2,370) [87.5%, 90.1%]	13.0 [13, 13]
2	3	627	83.4% (1,568/1,881) [81.6%, 85.0%]	14.3 [14, 15]
3	2	1,393	96.7% (2,694/2,786) [96.0%, 97.3%]	13.0 [13, 13]
5	3	570	90.7% (1,551/1,710) [89.2%, 92.0%]	11.3 [11, 12]
7	3	276	91.8% (760/828) [89.7%, 93.5%]	10.3 [9, 12]
8	3	196	93.2% (548/588) [90.9%, 95.0%]	9.7 [9, 11]
9	3	446	54.5% (729/1,338) [51.8%, 57.1%]	14.0 [14, 14]
10	3	260	87.4% (682/780) [84.9%, 89.6%]	15.0 [15, 15]
11	3	197	87.5% (517/591) [84.6%, 89.9%]	13.3 [12, 15]
12	3	959	73.8% (2,124/2,877) [72.2%, 75.4%]	12.3 [11, 13]

Specimen	# Valid WES Outputs	Total Unique Variants Detected Across WES Replicates	Variant Positive Call Rate (n/N) [two-sided 95% CI]	Average Overlapping 16-plex [Range]
13	3	2,332	84.7% (5,923/6,996) [83.8%, 85.5%]	13.3 [12, 15]
14	3	303	74.9% (681/909) [72.0%, 77.6%]	15.3 [15, 16]
15	3	1,688	96.6% (4,890/5,064) [96.0%, 97.0%]	10.3 [9, 12]
16	3	418	74.2% (931/1,254) [71.8%, 76.6%]	13.3 [12, 15]
17	3	198	66.8% (397/594) [63.0%, 70.5%]	14.7 [14, 15]
18	3	694	89.9% (1,872/2,082) [88.5%, 91.1%]	15.0 [15, 15]
19	2	638	88.9% (1,135/1,276) [87.1%, 90.6%]	12.0 [12, 12]
20	3	278	85.6% (714/834) [83.1%, 87.8%]	12.3 [12, 13]
21	3	584	74.7% (1,309/1,752) [72.6%, 76.7%]	13.0 [12, 14]
22	3	317	93.1% (885/951) [91.3%, 94.5%]	11.0 [10, 12]
23	3	192	92.9% (535/576) [90.5%, 94.7%]	14.3 [14, 15]
24	3	214	84.6% (543/642) [81.6%, 87.2%]	11.0 [10, 12]
25	3	427	64.6% (828/1,281) [62.0%, 67.2%]	13.0 [13, 13]
26	3	694	96.2% (2,002/2,082) [95.2%, 96.9%]	14.3 [14, 15]
27	3	937	93.0% (2,615/2,811) [92.0%, 93.9%]	9.0 [8, 10]
28	3	714	94.2% (2,018/2,142) [93.1%, 95.1%]	14.3 [14, 15]
29	3	1,481	91.4% (4,063/4,443) [90.6%, 92.2%]	12.0 [12, 12]
30	3	43	74.4% (96/129) [66.3%, 81.2%]	14.7 [14, 15]

Specimen	# Valid WES Outputs	Total Unique Variants Detected Across WES Replicates	Variant Positive Call Rate (n/N) [two-sided 95% CI]	Average Overlapping 16-plex [Range]
31	3	660	91.8% (1,817/1,980) [90.5%, 92.9%]	10.3 [10, 11]
32	3	599	86.8% (1,560/1,797) [85.2%, 88.3%]	13.7 [13, 14]
33	3	1,570	96.8% (4,557/4,710) [96.2%, 97.2%]	13.7 [13, 14]
34	3	699	95.9% (2,011/2,097) [95.0%, 96.7%]	13.3 [12, 14]
35	2	740	92.4% (1,368/1,480) [91.0%, 93.7%]	10.0 [10, 10]
36	3	1,357	60.3% (2,454/4,071) [58.8%, 61.8%]	11.0 [10, 12]
37	3	1,128	85.1% (2,879/3,384) [83.8%, 86.2%]	11.7 [11, 13]

Thirty-four (34) sample trios (254 replicates) produced sufficient WES and Plasma results and were included in the analysis of ctDNA MRD concordance. Three samples were excluded due to failed QC.

Sample-level ctDNA MRD concordance was 96.9% (246/254), and the overall variant-level concordance was 91.6% (3,671/4,007) (**Table 13)**. Lower variant-level precision is expected relative to patient-level MRD determination due to stochastic sampling variability, as the test is designed to detect the overall presence of ctDNA rather than any single specific mutation.

Five WES and plasma reagent lot sets were included in the study with each sample evaluated using three unique lot sets. Sample-level within-lot precision ranges from 95.7% to 100% for WES workflow reagents and 97.2% to 100% for plasma workflow reagents (**Table 14**).

Table 13. Summary of End-to-End Workflow Precision—Plasma Workflow Output

Samples	# of samples	Total # of replicates	Sample-Level Call Concordance (n/N) [95% CI]	Variant-Level Call Concordance (n/N) [95% CI]
All	34	254	96.9% (246/254) [93.9%, 98.4%]	91.6% (3671/4007) [90.7%, 92.4%]

Positive above LoD	14	94	100% (94/94) [96.1%, 100%]	89.1% (1323/1485) [87.4%, 90.6%]
Positive below LoD	5	40	90.0% (36/40) [76.9%, 96.0%]	76.0% (479/630) [72.5%, 79.2%]
Negative with 1 Positive Target	2	18	83.3% (15/18) [60.8%, 94.2%]	96.8% (276/285) [94.1%, 98.3%]
Negative with 0 Positive Targets	13	102	99.0% (101/102) [94.7%, 99.8%]	99.1% (1593/1607) [98.5%, 99.5%]

Table 14. Summary of Precision Results by Reagent Lot

Reagent Lot	**Within-Lot Precision**	
	% (n/N) [95% CI]	**% Per Specimen[1] (Min, Max)**
WES Lot 1	100% (34/34) [89.8%, 100%]	(100%, 100%)
WES Lot 2	100% (33/33) [89.6%, 100%]	(100%, 100%)
WES Lot 3	96.4% (81/84) [90.0%, 98.8%]	(50%, 100%)
WES Lot 4	96.0% (48/50) [86.5%, 98.9%]	(67%, 100%)
WES Lot 5	95.7% (45/47) [85.8%, 98.8%]	(67%, 100%)
Plasma Lot 1	100% (42/42) [91.6%, 100%]	(100%, 100%)
Plasma Lot 2	97.7% (85/87) [92.0%, 99.4%]	(50%, 100%)
Plasma Lot 3	100% (46/46) [92.3%, 100%]	(100%, 100%)
Plasma Lot 4	97.2% (35/36) [85.8%, 99.5%]	(67%, 100%)
Plasma Lot 5	97.2% (35/36) [85.8%, 99.5%]	(67%, 100%)

[1] Each lot was tested across a minimum of 12 specimens that yielded 2-3 evaluable plasma replicates.

The majority (30/34) of samples were 100% ctDNA MRD concordant across replicates. For the remaining four samples which were primarily

challenging samples below LoD or borderline negative with one positive target detected on average, the concordance ranges from 66.7% - 88.9%. Variant-level concordance aggregated within a sample ranged from 68.1% - 100%, with the lowest concordance observed in an ctDNA MRD positive sample with 100% sample-level concordance (**Table 15**).

Table 15. Summary of Precision Results per Sample—Plasma Workflow Output

Specimen	Valid Plasma Outputs	Modal Call Status[1]	Sample-level Call Concordance (n/N) [95% CI]	# Positive Targets		Sample-level Mean VAF (%)		Variant-level Call Concordance (n/N) [95% CI]
				Mean (SD)	CV %	Mean (SD)	CV %	
1	6/6	Positive Above LoD	100% (6/6) [61.0%, 100%]	15.67 (0.52)	3.3	9.7599 (0.3324)	3.41	100% (94/94) [96.1%, 100%]
2	9/9	Positive Above LoD	100% (9/9) [70.1%, 100%]	15.89 (0.33)	2.1	6.4776 (0.1573)	2.43	100% (143/143) [97.4%, 100%]
3	6/6	Positive Above LoD	100% (6/6) [61.0%, 100%]	16 (0)	0	3.2779 (0.1372)	4.18	100% (96/96) [96.2%, 100%]
4	3/3	Positive Above LoD	100% (3/3) [43.9%, 100%]	16 (0)	0	1.5413 (0.0397)	2.58	100% (48/48) [92.6%, 100%]
5	6/9	Positive Above LoD	100% (6/6) [61.0%, 100%]	15.33 (0.82)	5.32	1.1013 (0.0985)	8.94	100% (92/92) [96.0%, 100%]
6	3/3	Positive Above LoD	100% (3/3) [43.9%, 100%]	16 (0)	0	1.096 (0.1324)	12.08	100% (48/48) [92.6%, 100%]
7	6/9	Positive Above LoD	100% (6/6) [61.0%, 100%]	16 (0)	0	0.814 (0.054)	6.63	100% (96/96) [96.2%, 100%]
8	3/9	Positive Above LoD	100% (3/3) [43.9%, 100%]	16 (0)	0	0.4827 (0.0468)	9.7	100% (37/37) [90.6%, 100%]
9	9/9	Positive Above LoD	100% (9/9) [70.1%, 100%]	15.78 (0.44)	2.79	0.4607 (0.0417)	9.04	99.3% (142/143) [96.1%, 99.9%]
10	9/9	Positive Above LoD	100% (9/9) [70.1%, 100%]	12.33 (0.5)	4.05	0.0829 (0.0102)	12.36	78.5% (113/144) [71.1%, 84.4%]
11	9/9	Positive Above LoD	100% (9/9) [70.1%, 100%]	11.11 (1.36)	12.28	0.0821 (0.0115)	14.04	79.9% (115/144) [72.6%, 85.6%]
12	7/9	Positive Above LoD	100% (7/7) [64.6%, 100%]	10 (1.29)	12.91	0.0699 (0.0111)	15.9	78.6% (88/112) [70.1%, 85.2%]
13	9/9	Positive Above LoD	100% (9/9) [70.1%, 100%]	8.89 (1.96)	22.11	0.0424 (0.0106)	25.05	73.6% (106/144) [65.9%, 80.1%]

Specimen	Valid Plasma Outputs	Modal Call Status[1]	Sample-level Call Concordance (n/N) [95% CI]	# Positive Targets		Sample-level Mean VAF (%)		Variant-level Call Concordance (n/N) [95% CI]
				Mean (SD)	CV %	Mean (SD)	CV %	
14	9/9	Positive Above LoD	100% (9/9) [70.1%, 100%]	6.33 (1.73)	27.35	0.0389 (0.0118)	30.47	72.9% (105/144) [65.1%, 79.5%]
15	9/9	Positive Below LoD	100% (9/9) [70.1%, 100%]	9 (2.65)	29.4	0.0335 (0.0119)	35.68	68.1% (98/144) [60.1%, 75.1%]
16	9/9	Positive Below LoD	100% (9/9) [70.1%, 100%]	5.89 (1.17)	19.81	0.0266 (0.0044)	16.7	71.5% (103/144) [63.7%, 78.3%]
17	9/9	Positive Below LoD	100% (9/9) [70.1%, 100%]	4.78 (1.48)	31.01	0.0172 (0.0054)	31.08	75.0% (108/144) [67.3%, 81.4%]
18	9/9	Positive Below LoD	66.7% (6/9) [35.4%, 87.9%]	2.67 (2.18)	81.73	0.0152 (0.0117)	77.27	84.4% (114/135) [77.4%, 89.6%]
19	4/6	Positive Below LoD	75.0% (3/4) [30.1%, 95.4%]	1.75 (0.5)	28.57	0.0063 (0.0064)	101.13	88.9% (56/63) [78.8%, 94.5%]
20	9/9	Negative 1 Positive Target	66.7% (6/9) [35.4%, 87.9%]	1.22 (0.67)	54.55	0.0025 (0.0038)	150.52	97.9% (138/141) [93.9%, 99.3%]
21	9/9	Negative 1 Positive Target	100% (9/9) [70.1%, 100%]	0.67 (0.5)	75	0[2] (0)	NE[3]	95.8% (138/144) [91.2%, 98.1%]
22	9/9	Negative 0 Positive Targets	100% (9/9) [70.1%, 100%]	0.33 (0.5)	150	0 (0)	NE	97.9% (141/144) [94.1%, 99.3%]
23	9/9	Negative 0 Positive Targets	100% (9/9) [70.1%, 100%]	0.33 (0.5)	150	0 (0)	NE	97.9% (141/144) [94.1%, 99.3%]
24	9/9	Negative 0 Positive Targets	100% (9/9) [70.1%, 100%]	0.33 (0.5)	150	0 (0)	NE	97.9% (141/144) [94.1%, 99.3%]
25	9/9	Negative 0 Positive Targets	88.9% (8/9) [56.5%, 98.0%]	0.33 (0.71)	212.13	0.0005 (0.0016)	300	97.8% (132/135) [93.7%, 99.2%]
26	9/9	Negative 0 Positive Targets	100% (9/9) [70.1%, 100%]	0.22 (0.44)	198.43	0 (0)	NE	98.6% (142/144) [95.1%, 99.6%]
27	3/9	Negative	100% (3/3) [43.9%, 100%]	0 (0)	NE	0 (0)	NE	100% (36/36) [90.4%, 100%]

Specimen	Valid Plasma Outputs	Modal Call Status[1]	Sample-level Call Concordance (n/N) [95% CI]	# Positive Targets		Sample-level Mean VAF (%)		Variant-level Call Concordance (n/N) [95% CI]
				Mean (SD)	CV %	Mean (SD)	CV %	
		0 Positive Targets						
28	9/9	Negative 0 Positive Targets	100% (9/9) [70.1%, 100%]	0 (0)	NE	0 (0)	NE	100% (143/143) [97.4%, 100%]
29	9/9	Negative 0 Positive Targets	100% (9/9) [70.1%, 100%]	0 (0)	NE	0 (0)	NE	100% (144/144) [97.4%, 100%]
30	6/9	Negative 0 Positive Targets	100% (6/6) [61.0%, 100%]	0 (0)	NE	0 (0)	NE	100% (95/95) [96.1%, 100%]
31	9/9	Negative 0 Positive Targets	100% (9/9) [70.1%, 100%]	0 (0)	NE	0 (0)	NE	100% (144/144) [97.4%, 100%]
32	6/9	Negative 0 Positive Targets	100% (6/6) [61.0%, 100%]	0 (0)	NE	0 (0)	NE	100% (96/96) [96.2%, 100%]
33	9/9	Negative 0 Positive Targets	100% (9/9) [70.1%, 100%]	0 (0)	NE	0 (0)	NE	100% (144/144) [97.4%, 100%]
34	6/9	Negative 0 Positive Targets	100% (6/6) [61.0%, 100%]	0 (0)	NE	0 (0)	NE	100% (94/94) [96.1%, 100%]

[1] Modal Call Status of a specimen is the Signatera CDx results for majority of the replicates.
[2] Sample-level mean VAF of ctDNA MRD-Negative results is set to 0%.
[3] NE: Not estimable.

b. Precision of WES Workflow

This study evaluated the Signatera CDx WES workflow within-run and between-run / within-laboratory precision for identifying tumor-derived somatic variants under varied conditions including different reagent lots, instruments, and operators across multiple days. Ten clinical MIBC tumor FFPE samples representative of the intended use population were tested at the minimum WES DNA input (50 ng tissue DNA, 100 ng patient-matched whole blood DNA). Twenty-seven replicates were tested per sample for a total of 270 data points across three operators and three instruments/reagent lot combinations.

For each specimen, all variants identified at least once across replicates (range: 301 -3,118) with an average VAF across replicates of at least 2.5% were evaluated. Within-run precision was defined as agreement with the within-run majority call while between-run / within-laboratory precision was defined as agreement with the majority call among all the replicates of a given SNV. The overall estimates were taken as the average across all samples and variants, resulting in a within-run precision of 98.54% and between-run / within-laboratory precision of 98.25% (**Table 16**). Across individual variants, the mean within-run precision is 96.3%, ranging from 66.7% to 100%, and the mean between-run / within-laboratory precision is 95.2%, ranging from 51.9% to 100% (**Table 17**). Within-run precision of SNV calls was also calculated for each of the nine batches. The analysis of within-run precision across instrument sets and reagent lots is presented in **Table 18**.

Table 16. Summary of Precision of Signatera CDx WES Workflow

Number of Unique SNVs in Range[1]	Within-run Precision [95% CI]	Between-run / Within-laboratory Precision [95% CI]
11,403	98.54% [97.47%, 99.07%]	98.25% [96.95%, 98.90%]

[1]Analysis includes all SNVs identified at least once across replicates with an average VAF above 2.5%.

Table 17. Distribution of WES Precision across Variants[1]

	Min	Q1	Median	Mean	Q3	Max
Within-run Precision	66.7 %	100%	100%	96.3%	100%	100%
Between-run / Within-laboratory Precision	51.9%	96.3%	100%	95.2%	100%	100%

[1]Analysis includes all SNVs identified at least once across replicates with an average VAF above 2.5%.

Table 18. Precision of SNV Agreement by Instrument Set and Reagent Lot[1]

Reagent	Precision [95% CI]			
	Instrument Set 1	Instrument Set 2	Instrument Set 3	Overall
Reagent Lot 1	98.59% [97.53%, 99.11%]	98.45% [97.25%, 99.06%]	98.73% [97.80%, 99.24%]	98.59% [97.53%, 99.13%]

Reagent Lot 2	98.43% [97.07%, 99.02%]	98.63% [97.72%, 99.09%]	98.29% [96.99%, 98.91%]	98.45% [97.27%, 99.00%]
Reagent Lot 3	98.47% [97.42%, 99.03%]	98.62% [97.58%, 99.10%]	98.64% [97.77%, 99.14%]	98.58% [97.60%, 99.08%]
Overall	98.50% [97.35%, 99.05%]	98.57% [97.52%, 99.08%]	98.55% [97.53%, 99.09%]	98.54% [97.47%, 99.07%]

[1]Analysis includes all SNVs identified at least once across replicates with an average VAF above 2.5%.

Variant level positive call rates were calculated as the detection rate in the unique variants across all sample replicates, which ranged from 90.5% to 99.3% across the samples. Among the 27 16-plex assays per specimen, an average of 9.6-14.3 targets overlapped between replicate pairs. The highest rate of target overlap was observed in the specimen with the lowest overall SNV positive call rate, as the SNVs with low positive call rates in this specimen were not prioritized for selection as 16-plex targets (**Table 19**). **Table 20** summarizes the positive call rates stratified by SNV category and VAF.

Table 19. Summary Results of WES Precision Per Sample

Specimen	# Replicates	Total Unique SNVs[1]	Variant Positive Call Rate (n/N) [95% CI]	Average Overlapping 16-plex [range]
1	27	446	98.6% (11,870/12,042) [98.3%, 98.8%]	13.7 [12, 16]
2	27	334	98.6% (8,893/9,018) [98.4%, 98.8%]	12.7 [10, 15]
3	27	303	94.9% (7,763/8,181) [94.4%, 95.3%]	13.4 [10, 15]
4	27	485	93.5% (12,246/13,095) [93.1%, 93.9%]	13.9 [12, 16]
5	27	1711	99.3% (45,865/46,197) [99.2%, 99.4%]	10.9 [7, 14]

6	27	3118	98.0% (82,544/84,186) [98.0%, 98.1%]	9.6 [7, 13]
7	27	301	90.5% (7,351/8,127) [89.8%, 91.1%]	14.3 [12, 16]
8	27	1031	99.1% (27,574/27,837) [98.9%, 99.2%]	12.9 [10, 16]
9	27	2567	99.2% (68,735/69,309) [99.1%, 99.2%]	12.3 [10, 16]
10	27	1107	98.1% (29,315/29,889) [97.9%, 98.2%]	14.0 [12, 16]

[1]Analysis includes all SNVs identified at least once across replicates with an average VAF above 2.5%.

Table 20. Summary Results of WES Precision by SNV Category and VAF Levels

SNV Category	VAF % Bin (Range)[4]	# Unique SNVs	Mean VAF %	Variant Positive Call Rate (n/N) [95% CI]
Overall	[2.5, 5.3)	937	3.7233	85.28% (21575/25299) [84.84%, 85.71%]
	[5.3, 7.95)	1323	6.9540	97.74% (34913/35721) [97.58%, 97.89%]
	[7.95, 11.925)	2239	9.8830	99.23% (59989/60453) [99.16%, 99.30%]
	[11.925, 17.8875)	2663	14.6386	99.46% (71514/71901) [99.41%, 99.51%]
	[17.8875, 100)	4241	30.8147	99.70% (114158/114507) [99.66%, 99.73%]
Commonly Targeted MIBC Genes[1]	[2.5, 5.3)	0	NE	NE
	[5.3, 7.95)	1	6.3528	96.30% (26/27) [81.72%, 99.34%]
	[7.95, 11.925)	0	NE	NE
	[11.925, 17.8875)	2	12.7047	100% (54/54) [93.36%, 100%]
	[17.8875, 100)	19	48.4895	99.22% (509/513) [98.01%, 99.70%]
Difficult to Sequence[2]	[2.5, 5.3)	319	3.7646	84.99% (7320/8613) [84.22%, 85.73%]
	[5.3, 7.95)	418	6.8842	97.34% (10986/11286) [97.03%, 97.62%]
	[7.95, 11.925)	677	9.9350	99.00% (18096/18279) [98.84%, 99.13%]
	[11.925, 17.8875)	883	14.5776	99.38% (23694/23841) [99.28%, 99.48%]

	[17.8875, 100)	1310	30.7971	99.62% (35235/35370) [99.55%, 99.68%]
Not Difficult to Sequence[2]	[2.5, 5.3)	618	3.7020	85.43% (14255/16686) [84.89%, 85.96%]
	[5.3, 7.95)	905	6.9862	97.92% (23927/24435) [97.73%, 98.09%]
	[7.95, 11.925)	1562	9.8605	99.33% (41893/42174) [99.25%, 99.41%]
	[11.925, 17.8875)	1780	14.6688	99.50% (47820/48060) [99.43%, 99.56%]
	[17.8875, 100)	2931	30.8226	99.73% (78923/79137) [99.69%, 99.76%]
GC Rich (> 65% GC Content)	[2.5, 5.3)	234	3.7603	88.45% (5588/6318) [87.63%, 89.21%]
	[5.3, 7.95)	286	6.9820	99.42% (7677/7722) [99.22%, 99.56%]
	[7.95, 11.925)	464	9.9140	99.48% (12463/12528) [99.34%, 99.59%]
	[11.925, 17.8875)	645	14.6332	99.66% (17355/17415) [99.56%, 99.73%]
	[17.8875, 100)	992	30.7219	99.68% (26698/26784) [99.60%, 99.74%]
GC Depleted (< 25% GC Content)	[2.5, 5.3)	11	4.0082	81.82% (243/297) [77.03%, 85.79%]
	[5.3, 7.95)	16	6.6877	89.81% (388/432) [86.60%, 92.32%]
	[7.95, 11.925)	27	10.0193	98.08% (715/729) [96.80%, 98.85%]
	[11.925, 17.8875)	29	14.6474	99.87% (782/783) [99.28%, 99.98%]
	[17.8875, 100)	52	32.2239	99.15% (1392/1404) [98.51%, 99.51%]
High Coverage[3]	[2.5, 5.3)	260	3.8131	92.85% (6518/7020) [92.22%, 93.43%]
	[5.3, 7.95)	358	6.9746	99.51% (9619/9666) [99.35%, 99.63%]
	[7.95, 11.925)	591	9.8107	99.71% (15910/15957) [99.61%, 99.78%]
	[11.925, 17.8875)	647	14.6451	99.85% (17443/17469) [99.78%, 99.90%]
	[17.8875, 100)	970	31.1293	99.83% (26146/26190) [99.77%, 99.87%]
Low Coverage[3]	[2.5, 5.3)	235	3.7646	73.70% (4676/6345) [72.60%, 74.76%]
	[5.3, 7.95)	329	6.8764	93.08% (8268/8883) [92.53%, 93.59%]
	[7.95, 11.925)	563	9.9280	97.99% (14896/15201) [97.76%, 98.20%]
	[11.925, 17.8875)	667	14.6296	98.63% (17763/18009) [98.45%, 98.79%]

	[17.8875, 100)	1094	32.1506	99.49% (29386/29538) [99.40%, 99.56%]
Base Substitution: A>C/T>G	[2.5, 5.3)	38	3.6501	77.68% (797/1026) [75.03%, 80.12%]
	[5.3, 7.95)	32	7.0213	95.83% (828/864) [94.29%, 96.98%]
	[7.95, 11.925)	67	9.4166	98.40% (1780/1809) [97.71%, 98.88%]
	[11.925, 17.8875)	49	14.3914	99.77% (1320/1323) [99.34%, 99.92%]
	[17.8875, 100)	80	32.8918	99.81% (2156/2160) [99.52%, 99.93%]
Base Substitution: A>G/T>C	[2.5, 5.3)	134	3.8292	83.53% (3022/3618) [82.28%, 84.70%]
	[5.3, 7.95)	264	6.8985	97.04% (6917/7128) [96.62%, 97.41%]
	[7.95, 11.925)	284	9.4034	98.96% (7588/7668) [98.70%, 99.16%]
	[11.925, 17.8875)	151	14.6656	99.17% (4043/4077) [98.84%, 99.40%]
	[17.8875, 100)	340	34.1939	99.55% (9139/9180) [99.39%, 99.67%]
Base substitution: A>T/T>A	[2.5, 5.3)	45	3.6209	83.37% (1013/1215) [81.18%, 85.36%]
	[5.3, 7.95)	44	6.7378	94.36% (1121/1188) [92.90%, 95.53%]
	[7.95, 11.925)	53	9.8047	99.23% (1420/1431) [98.63%, 99.57%]
	[11.925, 17.8875)	69	14.6117	98.39% (1833/1863) [97.71%, 98.87%]
	[17.8875, 100)	110	31.0764	99.63% (2959/2970) [99.34%, 99.79%]
Base Substitution: C>A/G>T	[2.5, 5.3)	128	3.7348	85.91% (2969/3456) [84.71%, 87.03%]
	[5.3, 7.95)	168	6.9789	97.93% (4442/4536) [97.47%, 98.30%]
	[7.95, 11.925)	207	9.7307	99.07% (5537/5589) [98.78%, 99.29%]
	[11.925, 17.8875)	233	15.0060	98.92% (6223/6291) [98.63%, 99.15%]
	[17.8875, 100)	350	30.5028	99.41% (9394/9450) [99.23%, 99.54%]
Base Substitution: C>G/G>C	[2.5, 5.3)	169	3.7351	85.38% (3896/4563) [84.33%, 86.38%]
	[5.3, 7.95)	170	7.0366	98.08% (4502/4590) [97.64%, 98.44%]
	[7.95, 11.925)	545	10.2124	99.41% (14628/14715) [99.27%, 99.52%]
	[11.925, 17.8875)	614	14.4183	99.67% (16524/16578) [99.58%, 99.75%]

	[17.8875, 100)	840	29.3716	99.88% (22652/22680) [99.82%, 99.91%]
Base Substitution: C>T/G>A	[2.5, 5.3)	423	3.6991	86.49% (9878/11421) [85.85%, 87.10%]
	[5.3, 7.95)	645	6.9598	98.21% (17103/17415) [98.00%, 98.40%]
	[7.95, 11.925)	1083	9.9049	99.30% (29036/29241) [99.20%, 99.39%]
	[11.925, 17.8875)	1547	14.6770	99.53% (41571/41769) [99.46%, 99.59%]
	[17.8875, 100)	2521	30.8058	99.69% (67858/68067) [99.65%, 99.73%]
SNVs in 16-plex	[2.5, 5.3)	3	4.2448	80.25% (65/81) [70.30%, 87.46%]
	[5.3, 7.95)	2	6.4727	98.15% (53/54) [90.23%, 99.67%]
	[7.95, 11.925)	1	11.7453	100% (27/27) [87.54%, 100%]
	[11.925, 17.8875)	32	15.7336	99.65% (861/864) [98.98%, 99.88%]
	[17.8875, 100)	262	37.1043	99.80% (7060/7074) [99.67%, 99.88%]

[1] SNV targets for the IMvigor011 study population most commonly occurred on five genes: TP53, FGFR3, KDM6A, DMD, or HUWE1. SNVs within these genes represented 0.3% of all potential 16-plex targets among the study subjects in the clinical validation study. No eligible SNVs from KDM6A, DMD and HUWE1 were found in the samples for this study.
[2] SNVs identified in the difficult to sequence whole genome sequencing (WGS) panel generated by the consortium Genome in a Bottle (NIST, Genome in a Bottle Project, 2024).
[3] High Coverage: ≥ 75th percentile of tumor total DOR per sample; Low Coverage: ≤ 25th percentile of tumor total DOR per sample.
[4] Analysis includes all SNVs identified at least once across replicates with an average VAF above 2.5%.

c. Precision of Plasma Workflow

The study evaluated within-run and between-run / within-laboratory precision of the Signatera CDx plasma workflow under varied conditions including different reagent lots, instruments, and operators.

The precision of ctDNA MRD-positive samples was evaluated using six ctDNA MRD-positive clinical MIBC cfDNA samples, diluted using healthy donor cfDNA to 1.5x LoD. Each sample was tested using three replicates across three reagent lots, three instrument sets, and three operators, for a total of 81 replicates per sample and 486 total data points. The study generated a total of 463 data points after QC exclusions. The precision from ctDNA MRD-negative samples was evaluated using three pools of cfDNA from healthy donors tested using two replicates for each combination of variance components generated across three reagent lots, three instrument sets, and three operators, using three 16-plex primer pools from MIBC clinical samples used in the same precision study, for a total of 54 replicates per sample and 162 total data points. The study generated

a total of 156 data points after QC exclusions. Both sample types were processed at the 10ng minimum cfDNA input.

The point estimate for within-run precision was calculated as the percentage of sample-level replicates that agreed with the majority ctDNA MRD call within a sequencing run across all sample-run combinations. The point estimate for between-run / within-laboratory precision was calculated as the percentage of sample-level replicates that agreed with the majority ctDNA MRD call among all replicates of that sample across all runs. The point estimates for sample-level within-run and between-run / within-laboratory precision were both 100% overall (**Table 21**) and by factor (**Table 22**).

Table 21. Summary of Plasma Workflow Precision Study (Sample Level)

Sample Type	Total Sample Replicates (N)	Within-run Precision (%) (n/N) [95% CI]	Between-run / Within-laboratory Precision (%) (n/N) [95% CI]
ctDNA MRD Positive	463	100% (463/463) [99.2%, 100%]	100% (463/463) [99.2%, 100%]
ctDNA MRD Negative	156	100% (156/156) [97.6%, 100%]	100% (156/156) [97.6%, 100%]

Table 22. Summary of Plasma Workflow Precision by Study Factor (Sample Level)

Factor	Total Sample Replicates (N)	Within-run Precision (%) (n/N)	Between-run / Within-laboratory Precision (%) (n/N)
Reagent Lot 1	159	100% (159/159) [97.6%, 100%]	100% (159/159) [97.6%, 100%]
Reagent Lot 2	161	100% (161/161) [97.7%, 100%]	100% (161/161) [97.7%, 100%]
Reagent Lot 3	143	100% (143/143) [97.4%, 100%]	100% (143/143) [97.4%, 100%]
Instrument Set 1	161	100% (161/161) [97.7%, 100%]	100% (161/161) [97.7%, 100%]
Instrument Set 2	161	100% (161/161) [97.7%, 100%]	100% (161/161) [97.7%, 100%]

Factor	Total Sample Replicates (N)	Within-run Precision (%) (n/N)	Between-run / Within-laboratory Precision (%) (n/N)
Instrument Set 3	141	100% (141/141) [97.3%, 100%]	100% (141/141) [97.3%, 100%]
Operator 1	162	100% (162/162) [97.7%, 100%]	100% (162/162) [97.7%, 100%]
Operator 2	158	100% (158/158) [97.6%, 100%]	100% (158/158) [97.6%, 100%]
Operator 3	143	100% (143/143) [97.4%, 100%]	100% (143/143) [97.4%, 100%]

Precision for individual samples is summarized in **Table 23**. Precision was 100% across patients' samples, with variant level concordance aggregated within a sample ranging from 62.4% - 71.3% across the positive samples and 98.4% - 99.8% across the negative samples. Across individual variants, the mean within-run precision is 79.2%, ranging from 70.1% to 100%, and the mean between-run / within-laboratory precision is 65.7%, ranging from 49.4% to 100% (**Table 24**). Variant-level precision across a range of genomic contexts demonstrated negative call rates for modal negative variants (as defined by majority call across replicates) ranging from 59.1% (C>T/G>A) to 74.3% (A>C/T>G) across variant classifications and positive call rates for modal positive variants ranging from 55.8% (C>A/G>T) to 73.9% (A>T/T>A) (**Table 25**).

Table 23. Summary of Plasma Workflow Precision Study per Sample

Specimen	Modal Call Status	# Replicates	Sample Call Concordance (n/N) [95% CI]	# Positive Targets		Sample Mean VAF		Variant Call Concordance (n/N) [95% CI]
				Mean (SD)	%CV	Mean (SD)	%CV	
1	Positive	78	100% (78/78) [95.3, 100]	8.0 (2.1)	26.0	0.0653 (0.0223)	34.1	67.4% (841/1248) [64.7%, 69.9%]
2	Positive	77	100% (77/77) [95.2, 100]	7.4 (2.3)	31.0	0.0603 (0.0201)	33.3	62.4% (769/1232) [59.7%, 65.1%]
3	Positive	77	100% (77/77) [95.2, 100]	8.3 (2.0)	23.5	0.0614 (0.0183)	29.9	62.4% (769/1232) [59.7%, 65.1%]

Specimen	Modal Call Status	# Replicates	Sample Call Concordance (n/N) [95% CI]	# Positive Targets		Sample Mean VAF		Variant Call Concordance (n/N) [95% CI]
				Mean (SD)	%CV	Mean (SD)	%CV	
4	Positive	77	100% (77/77) [95.2, 100]	6.4 (2.1)	32.3	0.0534 (0.0196)	36.7	71.3% (878/1232) [68.7%, 73.7%]
5	Positive	76	100% (76/76) [95.2, 100]	7.5 (1.9)	25.0	0.0463 (0.0188)	40.5	64.1% (779/1216) [61.3%, 66.7%]
6	Positive	78	100% (78/78) [95.3, 100]	7.6 (2.0)	25.8	0.0574 (0.0181)	31.5	66.7% (833/1248) [64.1%, 69.3%]
7	Negative	52	100% (52/52) [93.1, 100]	0.0 (0.2)	NE	0 (0)	NE	99.2% (825/832) [98.3%, 99.6%]
8	Negative	52	100 (52/52) [93.1, 100]	0.1 (0.3)	NE	0 (0)	NE	98.4% (819/832) [97.3%, 99.1%]
9	Negative	52	100 (52/52) [93.1, 100]	0.0 (0.1)	NE	0 (0)	NE	99.8% (830/832) [99.1%, 99.9%]

Table 24. Distribution of Plasma Precision across Variants

	Min	Q1	Median	Mean	Q3	Max
Within-run Precision	70.1 %	74.8%	77.9%	79.2%	81.9%	100%
Between-run / Within-laboratory Precision	49.4%	55.8%	61.5%	65.7%	72.7%	100%

Table 25. Summary of Plasma Workflow Precision by Variant VAF and Categories[1]

Variant Type	Modal Target Call	Mean VAF Range	# Unique SNVs	Variant Call Rate (n/N) [95% CI]
All	Negative		54	65.7% (2736/4163) [64.3%, 67.1%]
	Positive	(0-0.0545]	11	55.5% (472/850) [52.2%, 58.8%]
		(0.0545-0.0672]	10	61.0% (470/771) [57.5%, 64.3%]

Variant Type	Modal Target Call	Mean VAF Range	# Unique SNVs	Variant Call Rate (n/N) [95% CI]
		(0.0672-0.0904]	10	63.8% (492/771) [60.4%, 67.1%]
		(0.0904-0.2211]	10	81.2% (629/775) [78.3%, 83.8%]
Difficult to Sequence[2]	Negative		13	65.4% (656/1003) [62.4%, 68.3%]
	Positive	[0.0384, 0.1259]	10	60.2% (465/772) [56.7%, 63.6%]
Not Difficult to Sequence[2]	Negative		41	65.8% (2080/3160) [64.2%, 67.5%]
	Positive	[0.0354, 0.2211]	31	66.7% (1598/2395) [64.8%, 68.6%]
GC Rich (> 65% GC Content)	Negative		10	67.2% (519/772) [63.8%, 70.4%]
	Positive	[0.0384, 0.1259]	9	60.3% (419/695) [56.6%, 63.9%]
GC Depleted (< 25% GC Content)	Negative		2	62.3% (96/154) [54.5%, 69.6%]
High Coverage[3]	Negative		16	64.3% (795/1237) [61.6%, 66.9%]
	Positive	[0.0486, 0.2211]	18	70.0% (974/1392) [67.5%, 72.3%]
Low Coverage[3]	Negative		17	64.4% (843/1310) [61.7%, 66.9%]
	Positive	[0.0590, 0.2187]	6	66.3% (307/463) [61.9%, 70.5%]
A>C/T>G	Negative		5	74.3% (286/385) [69.7%, 78.4%]
	Positive	[0.0525, 0.0904]	3	61.0% (141/231) [54.6%, 67.1%]
A>T/T>A	Negative		10	66.1% (510/772) [62.6%, 69.3%]
	Positive	[0.0577, 0.2205]	6	73.9% (342/463) [69.7%, 77.7%]
C>A/G>T	Negative		1	62.3% (48/77) [51.2%, 72.3%]
	Positive	[0.1259, 0.1259]	1	55.8% (43/77) [44.7%, 66.4%]
C>G/G>C	Negative		36	64.9% (1801/2775) [63.1%, 66.7%]
	Positive	[0.0354, 0.1995]	28	63.2% (1366/2162) [61.1%, 65.2%]
C>T/G>A	Negative		2	59.1% (91/154) [51.2%, 66.5%]

Variant Type	Modal Target Call	Mean VAF Range	# Unique SNVs	Variant Call Rate (n/N) [95% CI]
	Positive	[0.0741, 0.2211]	3	73.1% (171/234) [67.1%, 78.4%]

[1] Target replicates which yielded no call are included in analysis and account for differences in total positive and negative percentages from 100.
[2] SNVs identified in the difficult to sequence whole genome sequencing (WGS) panel generated by the consortium Genome in a Bottle (NIST, Genome in a Bottle Project, 2024).
[3] High Coverage: ≥ 75th percentile of tumor total DOR per sample; Low Coverage: ≤ 25th percentile of tumor total DOR per sample

Plasma Workflow Extraction Precision

To evaluate precision of the entire Signatera CDx plasma workflow from plasma samples, seven (7) contrived ctDNA-positive plasma samples prepared from healthy donor plasma spiked with MIBC tumor-derived fragmented FFPE DNA targeting approximately 2–3× LoD and eight (8) negative healthy donor plasma samples without spike-in DNA were evaluated at the minimum cfDNA input of 10 ng. Samples were processed in duplicates on four different days, for a total of eight (8) replicates per sample. Within-day precision was estimated from duplicate extraction replicates generated from the same sample within each study day. Between-day / within-laboratory precision was estimated across extraction replicates across all study days. This study integrated eight (8) operators, two (2) instruments, three (3) extraction reagent lots, three (3) elution buffer lots and two (2) PBS saline solution lots. The study generated a total of 120 observations across 60 replicate pairs. A total of nine (9) replicates did not have a valid ctDNA MRD call and were excluded from analysis of between-day / within-laboratory precision. Seven (7) out of the 60 replicate pairs were removed from within-day precision analysis as they did not have a valid ctDNA MRD call for both replicate pairs. Both within-day and between-day / within-laboratory precision achieved point estimates of 100% on the sample level (**Table 26**). Variant-level within-day precision was 77.12% (654/848) (95% CI: [74.18%, 79.82%]), ranging from 70.00% to 81.25% across variant categories. Variant-level between-day / within-laboratory precision was 85.87% (1525/1776) (95% CI: [84.17%, 87.41%]), ranging from 70.00% to 90.62% across variant categories.

Table 26. Summary Results of Plasma Workflow Precision Study from Plasma Samples

Sample Type	Sample-level Within-Day Precision		Sample-level Between-Day / Within Laboratory Precision	
	Total Sample Replicate Pairs	%, (n/N) [95% CI]	Total Sample Replicates	%, (n/N) [95% CI]
ctDNA MRD Positive	27	100% (27/27) [87.54%, 100%]	55	100% (55/55) [93.47%, 100%]

ctDNA MRD Negative	26	100% (26/26) [87.13%, 100%]	56	100% (56/56) [93.58%, 100%]

A post market study is planned (listed in Conditions of Approval in Section XV below) to supplement the plasma workflow precision and the lot-to-lot precision of the Streck cfDNA BCTs.

6. Cross-contamination and Carryover

The study evaluated the risk of cross-contamination (intra-plate, within-run) and carryover contamination (inter-plate, between-runs) in the Signatera CDx plasma workflow. Contrived ctDNA MRD-positive samples diluted into healthy donor cfDNA (to test challenging samples with high VAF) and ctDNA MRD-negative samples from MIBC patients and healthy donors were tested.

For cross-contamination, five ctDNA MRD-positive MIBC contrived samples were diluted with healthy donor cfDNA to a target 10x LoD. Each sample was tested in four replicates per plate across two plates. On both plates, 20 negative sample replicates were positioned adjacent to positive samples in all directions.

Carryover between sequencing runs was assessed by sequentially processing two plates containing 20 negative sample replicates placed in the same positions as high VAF positive samples from the cross-contamination test plates using the same instrumentation and reagents. The first carryover test plate was processed immediately after the first cross-contamination test plate containing positive samples, and the second carryover test plate was processed immediately after the second cross-contamination test plate containing positive samples.

Three negative carryover contamination samples failed post-sequencing QC (unrelated to contamination) and were removed from the analysis. Both evaluations achieved 100% sample-level NPA and no carryover or cross-contamination was observed in the study.

7. Tumor Content for WES Workflow

The minimum acceptable tumor content for Signatera CDx was assessed using three (3) unique MIBC-patient FFPE tumor tissues and patient-matched whole blood sample pairs at three tumor content levels of ≤ 10%, 20%, and 30%. Eight (8) replicates per sample pair were tested at the WES minimum input (50 ng tissue DNA and 100 ng whole blood DNA). The study verified acceptable minimum tumor content by assessing the sample-level QC pass rates of the WES workflow, including successful generation of a full 16-plex primer set. The overall pass rate was 66.7% (16/24) when tumor content was ≤ 10%, while pass rates of 100% (24/24) were achieved at the 20% and 30% tumor content levels. A minimum of 20% tumor content was validated for Signatera CDx.

8. DNA Input

a. DNA Input for WES Workflow

The minimum acceptable tumor tissue DNA input for the WES workflow of Signatera CDx is 50 ng, and the minimum acceptable whole blood DNA input is 100 ng. The robustness of the WES workflow at different input levels was validated using eight (8) unique MIBC-patient FFPE tissues and patient-matched whole blood sample pairs across a range of tumor content, with a minimum of 20%, processed with one lot of reagents. FFPE tumor tissue samples were tested at five DNA input levels: 25, 50, 100, 250 and 375 ng with three replicates per input level. Whole blood samples were tested at 100 ng DNA input with guardbanding below the minimum input at 75 and 90 ng with three replicates per input. Sample-level QC pass rates of the WES workflow, including successful generation of 16-plex primer set per DNA input of tissue samples were assessed. Variant-level concordance of all detected SNVs across replicates at each DNA input level were also evaluated.

For FFPE tumor tissue DNA input, an overall sample-level pass rate of 100% was achieved across all input levels (**Table 27)**. The variant-level PPAs were ≥95% for all DNA input levels, except 25ng input for which the variant-level PPA was 94.4%. The minimum FFPE tumor tissue DNA input was validated at 50 ng.

Table 27. Summary of Pass Rates and Variant-level Concordance Across DNA Input Levels Derived from FFPE Tumor Tissue

Tissue DNA Input (ng)	Matched Normal Whole Blood DNA Input (ng)	Total Number of Sample Pair Replicates (Tissue and MN)	Overall Pass Rate (%, n/N)	Variant-level PPA[1](n/N) [95% CI]
25	100	24	100%, 24/24	94.4% (3477/3682) [93.6%, 95.1%]
50	100	24	100%, 24/24	95.6% (3520/3682) [94.9%, 96.2%]
100	100	24	100%, 24/24	95.7% (3525/3682) [95.0%, 96.3%]
250	**100**	**24**	**100%, 24/24**	**Reference**
375	100	24	100%, 24/24	95.6% (3521/3682) [94.9%, 96.2%]

[1] Variant level PPA was calculated across all detected SNVs using 250ng input as reference.

For guardbanding of patient-matched whole blood DNA input, an overall pass rate of >95% and variant-level PPA of ≥95% was achieved across all input levels (**Table 28).** The minimum patient-matched whole blood DNA input was validated at 100 ng.

Table 28. Summary of Pass Rates and Variant-level Concordance Across DNA Input Levels Derived from Patient-Matched Whole Blood

Matched Normal Whole Blood DNA Input (ng)	Tissue DNA Input (ng)	Total Number of Sample Pairs (Tissue and MN)	Overall Pass Rate %, n/N	Variant-level PPA[1](n/N) [95% CI]
100	**50**	**24**	**100%, 24/24**	**Reference**
90	50	24	100%, 24/24	97.8% (3625/3706) [97.3%, 98.2%]
75	50	24	95.8%, 23/24	97.5% (3613/3706) [96.9%, 97.9%]

[1]Variant level PPA was calculated across all detected SNVs using 100ng input as reference.

b. cfDNA Input for Plasma Workflow

The purpose of this study was to evaluate the impact of potential variation in the measurement of cfDNA concentration on the Signatera CDx assay plasma workflow performance. The study used two clinical and two contrived samples for establishing minimum and maximum cfDNA input. The conditions tested included the minimum input of 10 ng, 7.5 ng, and 5 ng and the maximum input of 66 ng, 82.5 ng, and 99 ng. For minimum input testing, samples were diluted to 1.5x LoD and tested across 10 replicates per sample at each cfDNA input condition, for a total of 40 replicates per input. For maximum input testing, samples were diluted to 1.5x LoD and tested across six replicates per sample at each cfDNA input condition, for a total of 24 replicates per input. Signatera CDx performance was evaluated based on test failure (Test Not Performed: TNP) rates and ctDNA MRD positive detection rates for varying cfDNA input levels. Number of detected targets, sample-level mean VAF and variant level concordance were also evaluated. TNP rates of ≤ 5% were observed for all cfDNA inputs. ctDNA MRD positive detection rates of 100% were observed for all cfDNA inputs (**Table 29**). Number of detected targets, sample-level mean VAF and variant level concordance across cfDNA inputs are presented in **Table 30** and **Table 31**. The results support the robustness of the plasma workflow of Signatera CDx with DNA input variations of 25% and 50% below minimum input (10 ng cfDNA), and 25% and 50% above maximum input (66 ng cfDNA).

Table 29. TNP and ctDNA MRD Positive Detection Rates Across Samples by cfDNA Input

cfDNA Input (ng)	TNP[1] Rate (%) (n/N)	ctDNA MRD Positive Detection Rate (%) (n/N)
5	2.5 (1/40)	100 (39/39)
7.5	0 (0/40)	100 (40/40)
10	**0 (0/40)**	**100 (40/40)**
66	**0 (0/24)**	**100 (24/24)**
82.5	4.2 (1/24)	100 (23/23)
99	0 (0/24)	100 (24/24)

[1] TNP: Test not performed (assay failure).

Table 30. Number of Detected Positive Targets, Sample-level Mean VAF and Variant-level Concordance per Sample by cfDNA Input (Low Input Levels)

Specimen	cfDNA Input (ng)	Mean # Positive Targets	Mean Sample-level Mean VAF (%)	Variant-level Concordance (n/N) [95% CI][1]
1 (Clinical)	5	7.5	0.0544	62.0% (93/150) [54.0%, 69.4%]
	7.5	8.6	0.0541	68.0% (102/150) [60.2%, 74.9%]
	10	7.1	0.0560	64.7% (97/150) [56.7%, 71.9%]
2 (Clinical)	5	7.4	0.0531	48.6% (70/144) [40.6%, 56.7%]
	7.5	7.6	0.0466	57.5% (92/160) [49.8%, 64.9%]
	10	9.6	0.0517	66.2% (106/160) [58.6%, 73.1%]
3 (Contrived)	5	7.5	0.0485	54.4% (87/160) [46.6%, 61.9%]
	7.5	9.1	0.0469	56.9% (91/160) [49.1%, 64.3%]
	10	8.9	0.0444	68.1% (109/160) [60.6%, 74.8%]
4 (Contrived)	5	8.7	0.0578	55.6% (89/160) [47.9%, 63.1%]
	7.5	10.1	0.0569	60.6% (97/160) [52.9%, 67.9%]
	10	9.6	0.0612	63.8% (102/160) [56.1%, 70.8%]

[1] Compared to modal target detection status at 10 ng input

Table 31. Number of Detected Positive Targets, Sample-level VAF and Variant-level Concordance per Sample by cfDNA Input (High Input Levels)

Specimen	cfDNA Input (ng)	Mean # Positive Targets	Mean Sample-level Mean VAF (%)	Variant-level Concordance (n/N) [95% CI] [1]
1 (Clinical)	66	6.0	0.008	71.1% (64/90) [61.0%, 79.5%]
	82.5	6.2	0.0077	64.0% (48/75) [52.7%, 73.9%]
	99	6.3	0.0109	66.7% (60/90) [56.4%, 75.5%]
2 (Clinical)	66	7.5	0.0087	69.8% (67/96) [60.0%, 78.1%]
	82.5	8.5	0.0081	63.5% (61/96) [53.6%, 72.5%]
	99	8.0	0.0088	60.4% (58/96) [50.4%, 69.6%]
3 (Contrived)	66	4.3	0.0061	72.9% (70/96) [63.3%, 80.8%]
	82.5	9.3	0.0136	50.0% (48/96) [40.2%, 59.8%]
	99	8.2	0.0098	51.0% (49/96) [41.2%, 60.8%]
4 (Contrived)	66	6.0	0.0083	66.7% (64/96) [56.8%, 75.3%]
	82.5	7.8	0.0098	59.4% (57/96) [49.4%, 68.7%]
	99	9.7	0.0105	50.0% (48/96) [40.2%, 59.8%]

[1]Compared to modal target detection status at 66 ng input

9. Sample Stability Studies

a. Whole Blood and Transport Stability

Whole blood stability was evaluated for samples collected in K2EDTA BCTs for the WES workflow and Streck cell-free DNA BCTs for the plasma workflow under ambient storage conditions (18–25°C) and simulated summer and winter shipping conditions.

For K2EDTA BCTs, 14 whole blood samples from healthy donors were stored under ambient, simulated summer, and simulated winter temperature profiles and evaluated in duplicate at multiple time points (baseline, 14, 15, 30 and 31 days for ambient profile; baseline, 14 days, and 15 days for summer and winter profile). Sample-level quality control (QC) pass rates were assessed at each time point.

For Streck cfDNA BCTs, 8 whole blood samples from healthy donors and 6 contrived ctDNA MRD-positive whole blood samples targeting a VAF of 2-3x LoD were stored in duplicate under ambient, simulated summer,

and simulated winter conditions. Sample-level ctDNA MRD status at each time point (ambient profile: 7, 8, 9, 10, and 11 days; summer and winter profiles: 7 days, 8 days) was compared to the baseline (T0) reference.

Overall, these studies demonstrated that whole blood collected in K2EDTA BCTs is stable for up to 30 days at ambient temperature (18-25 °C), including stability during simulated summer and winter shipping conditions. The sample-level QC passing rates ranged from 92.9% to 100.0% at all evaluated time points. Whole blood collected in Streck cfDNA BCTs was demonstrated to be stable for up to 10 days at ambient temperature including stability during simulated summer and winter shipping conditions. All passing QC samples achieved point estimates of 100.0% PPA and NPA of sample-level ctDNA MRD results relative to T0 across all conditions and time points.

Confirmation of whole blood stability in Streck cfDNA BCTs using clinical MIBC samples is ongoing.

A post market study will be performed (listed in Conditions of Approval in Section XV below), to evaluate the potential impact of underfilling of the Streck Cell-Free DNA BCTs on performance of Signatera CDx.

b. <u>FFPE Cut-slide Stability</u>

The stability of FFPE cut tissue slides from 12 unique MIBC patients, stored at ambient temperature (18-25°C), was evaluated for multiple timepoints (baseline, 3 months and 4 months). FFPE tumor tissue blocks from two replicates of each MIBC patient were sectioned into six slides and stored under ambient conditions prior to testing. Patient-matched whole blood samples were used to support the Signatera CDx WES workflow for this study.

Stability was assessed by evaluating standard QC metrics applied during the WES workflow, including successful generation of a valid 16-plex primer set, total number of SNVs detected and VAF levels. At each time point, > 95.0% of replicates generated a valid 16-plex primer pool. The overlapping of variants in the 16-plex ranged from 8 to 15 across samples and timepoints, with no observable decrease over the tested interval. In addition, both the number of SNVs detected and median SNV VAFs were consistent across timepoints. These results demonstrate that FFPE cut slides remain stable and are suitable for use in the Signatera CDx WES workflow for up to three (3) months when stored at ambient temperature (18–25 °C).

c. <u>Plasma Stability</u>

The stability of plasma samples following isolation from whole blood was evaluated under refrigerated and frozen storage conditions. Plasma samples were stored at 2-8°C and tested at multiple timepoints (baseline,

5, 7 and 8 days) and at -80°C for longer-term storage (baseline and 4 months). Stability was assessed using plasma from eight (8) healthy donors and seven (7) contrived ctDNA MRD-positive samples targeting a VAF of 2-3x LoD prepared in duplicate.

Across all evaluated storage conditions and time points, 100.0% agreement was observed at the sample level between stored samples and the baseline reference, as measured by PPA and NPA. There was no observable decrease in the number of targets detected across evaluated timepoints. These results demonstrate that plasma is stable when stored at 2-8°C for up to 7 days and supports interim stability at -80°C for at least 3 months.

Evaluation of extended stability at -80°C is ongoing. Both storage conditions will be confirmed with clinical MIBC samples.

d. <u>cfDNA and cfDNA Library Stability</u>

The stability of extracted MIBC cfDNA samples and cfDNA libraries stored at -20 °C was evaluated using four (4) ctDNA MRD-positive and three (3) ctDNA MRD-negative extracted cfDNA samples, and ten (10) ctDNA MRD-positive and three (3) ctDNA MRD-negative prepared cfDNA libraries from residual clinical MIBC samples. Stability was assessed by comparing sample-level ctDNA MRD status at each storage time point relative to the corresponding baseline reference and PPA and NPA were calculated.

For extracted cfDNA, the PPA is 100% and the NPA is 75% compared to baseline ctDNA MRD status at the evaluated storage time point (4 months). When analysis was limited to ctDNA MRD-positive samples above the LoD and ctDNA MRD-negative samples with no single observed variants above threshold (SOVAT) at T0, the PPA and NPA both achieved 100.0%. There was no significant decrease in the number of targets detected at 4 months.

The cfDNA libraries demonstrated complete agreement with baseline status across all evaluated time points (7 and 14 months), with point estimates of PPA and NPA achieving 100.0%. There was no observable decrease in the number of targets detected across time points.

These results support interim stability of extracted cfDNA for up to 3 months and cfDNA libraries for up to 13 months when stored at −20 °C.

Multiple freeze-thaw cycles of the cfDNA are not permitted in the Signatera CDx workflow. Extracted cfDNA remains in a continuous frozen storage and is not refrozen after thawing.

Evaluation of extended stability is ongoing.

e. Plasma Workflow Intermediate Stability

The stability of Signatera CDx plasma workflow intermediates, including enriched sequencing pools (EPOLs) and multiplex PCR (mPCR) libraries, was evaluated following storage at -20 °C. The study utilized residual clinical MIBC samples and evaluated six (6) ctDNA MRD-positive specimens in triplicate and five (5) ctDNA MRD-negative specimens in duplicate, contained in three (3) EPOLs. The mPCR libraries consisted of evaluation of single replicates of 16 positive and 10 negative samples.

Stability was assessed by comparing sample-level ctDNA MRD status for each replicate at each storage time point to the corresponding baseline reference using PPA and NPA. EPOLs were assessed at baseline, 8, 14 and 15 days, and mPCR libraries were assessed at baseline, 3-4 and 4-5 months.

For both EPOLs and mPCR libraries, complete agreement with baseline ctDNA MRD status was observed across all evaluated time points for sample replicates meeting QC requirements, with PPA and NPA achieving 100%. There was no observable decrease in the number of targets detected across timepoints. For EPOLs, the final evaluated time point supports stability for up to 14 days when stored at -20°C. For mPCR libraries, results support stability for up to 4 months when stored at -20°C.

10. Reagent Stability Studies

a. Shelf-life Stability of WES Workflow Reagents

The study evaluated the shelf-life of Signatera CDx WES workflow reagents, including those formulated in-house and those manufactured by external vendors and processed further at Natera. Three (3) production lots of reagents were evaluated using six (6) MIBC tissue samples and their corresponding matched whole blood samples in six (6) replicates. Testing was performed using minimum DNA input requirements for both tissue and blood. Reagent performance was assessed at baseline and at multiple time points following manufacture (0.5-3.1 months and 3.2-4.8 months). At each time point, run-level and sample-level QC metrics were evaluated for both tissue and blood workflows, including successful generation of a full 16-plex primer set, as well as the total number of SNVs detected and VAF levels. All evaluated reagent lots met all predefined run-level and sample-level QC acceptance criteria at the tested time points, achieving pass rates of 100% at both timepoints. No failures attributable to reagent degradation were observed. In addition, both the number of SNVs detected and median SNV VAFs were consistent across timepoints.

Evaluation of extended stability is ongoing.

b. In-Use Stability of WES Workflow Reagents

The study established the in-use stability of critical reagents and master mixes utilized in the Signatera CDx WES workflow at ambient temperature (18-25°C). The evaluation covered reagents used in library preparation, library amplification, hybrid capture, and capture enrichment steps. Five (5) MIBC patient tumor tissue samples and matched normal whole blood samples were tested at three (3) time points (baseline, 2 and 4 hours) with sample replicates using the minimum DNA input for tissue and matched normal whole blood.

Across all evaluated time points, ≥ 95.0% of run- and sample-level QC metrics passed with successful generation of a full 16-plex primer set. The overlapping of variants in the 16-plex ranged from 11 to 15 across samples and timepoints, with no observable decrease over the tested interval. In addition, both the number of SNVs detected and median SNV VAFs were consistent across timepoints.

c. Multiplex Primer Stability

The study evaluated the stability of the bespoke 16-plex primer sets using four positive and four negative clinical MIBC samples. For each sample, primers were prepared in duplicate, aliquoted into two storage tube types, and stored at 2-8°C for five months and -20°C for five months with 15 freeze-thaw cycles. Stability was assessed for each condition and timepoint by calculating PPA and NPA relative to the baseline for all samples with valid baseline status.

PPA and NPA for samples tested at 5 months for both temperature conditions, with up to 15 freeze/thaw cycles in the -20°C arm, relative to the baseline, were 100.0% across all study arms and tube types. There was no observable decrease in the number of targets detected across timepoints. These interim data support stability of the 16-plex primer sets for four months when stored at 2–8°C and -20°C regardless of storage tube type.

Evaluation of extended stability is ongoing.

d. Shelf-life Stability of Plasma Workflow Reagents

This study evaluated the shelf-life of Signatera CDx plasma workflow reagents under intended storage conditions. Reagents were grouped into three categories: pre-sequencing reagents, controls and control primer pools, and sequencing reagents. Three production-grade reagent lots from each reagent category were stored under their intended storage conditions and tested at multiple time intervals after manufacturing (baseline and 3.0-6.5 months). Testing used 10 replicates each of three (3) contrived ctDNA MRD-positive samples and three (3) healthy donor cfDNA samples. Stability was assessed using sample-level PPA and NPA, reagent-specific QC metrics and run-level QC metrics at each timepoint.

For pre-sequencing reagents, QC pass rates were ≥ 90% across all reagent-grouped lots and sample types. For controls and control primers, the pass rate of each reagent and lot was 100.0%. For sequencing reagents, all runs (100%) within each reagent lot passed run-level metrics. Both PPA and NPA achieved 100.0%, and there was no significant decrease in the number of targets detected across timepoints.

Evaluation of extended stability is ongoing.

e. In-Use Stability of Plasma Workflow Reagents

The study assessed the in-use stability of critical reagents (beads, master mixes, and normalized sample pool) used in the Signatera CDx Plasma workflow at room temperature (18 to 25°C). Four contrived ctDNA MRD-positive samples near LoD and one healthy donor cfDNA sample were utilized. Each of the four contrived samples was tested in triplicate with its corresponding 16-plex primer sets. The single healthy donor cfDNA was tested in triplicate with each of the four different contrived sample 16-plex primer sets. In total, 12 replicates of contrived samples and 12 healthy donor replicates were tested at each time point (baseline, one hour and two hours), for each reagent type.

Stability was evaluated by assessing the concordance of the sample-level calls relative to baseline. Samples that failed sequencing QC metrics for reasons unrelated to the in-use stability were removed from the analysis. The agreement rates were 100.0% for all reagent types at one hour, and 100.0% for beads and the normalized sample pools and ≥90% for the master mix at two hours. There was no observable decrease in the number of targets detected across time points.

C. Animal Studies

No animal studies were conducted using Signatera CDx.

D. Additional Studies

No additional nonclinical studies were performed.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The clinical performance of Signatera CDx for selection of MIBC patients who are ctDNA MRD-positive following cystectomy and may benefit from treatment with TECENTRIQ (atezolizumab) was established with clinical data generated from the IMvigor011 (NCT04660344) clinical study. The diagnostic clinical bridging study was performed to evaluate the concordance between the Signatera Clinical Trial Assay (CTA) used for enrollment of the study and Signatera CDx to establish the clinical validity of the device.

IMvigor011 Therapeutic Study

IMvigor011 is a multi-center, randomized, double blind, placebo controlled trial for the adjuvant treatment of patients with MIBC after cystectomy who had circulating tumor DNA molecular residual disease (ctDNA MRD).

The trial enrolled patients with histologically confirmed MIBC who underwent radical cystectomy with lymph node dissection. Eligible patients had pathologic tumor staging of pT2-4a and/or positive lymph nodes following cystectomy, with no evidence of residual disease or metastasis on imaging, confirmed within 28 days before randomization. Patients were eligible regardless of whether they had received prior neoadjuvant chemotherapy (NAC) or not. Patients were excluded if they had received any anti-cancer therapy within 3 weeks prior to trial enrollment or had a history of autoimmune disease.

Serial ctDNA MRD testing was performed starting at least 6 weeks after cystectomy, every 6 weeks for 9 months with a final test at one year. Patients who did not develop ctDNA MRD within the testing period were monitored without study treatment, while those determined to be ctDNA MRD-positive were screened for the treatment phase by confirming that they remained free of radiographic disease. The ctDNA MRD status was determined using either Signatera CTA or a local clinical trial assay performed in China for those patients enrolled in China.

A total of 250 patients were randomized in a 2:1 ratio into the treatment phase, 225 of which were tested with the Signatera CTA and 25 by the local clinical trial assay in China. The major efficacy outcome measure was investigator assessed disease-free survival (DFS). Overall Survival (OS) was an additional efficacy measure. The study demonstrated statistically significant improvements in DFS and OS for patients randomized to the TECENTRIQ arm compared with the placebo. Efficacy results are presented in **Table 32**.

Table 32. Efficacy Results from IMvigor011 Clinical Study

	TECENTRIQ N=167	**Placebo N=83**
Investigator-assessed DFS		
Number of events (%)	112 (67%)	66 (80%)
Median[1], months	9.9	4.8

(95% CI)	(7.2, 12.7)	(4.1, 8.3)
Hazard ratio[2,3] (95% CI)	0.64 (0.47, 0.87)	
p-value[3,4]	0.0047	
Overall survival[3]		
Number of deaths (%)	60 (36%)	36 (43%)
Median[1], months	32.8	21.1
(95% CI)	(27.7, NE)	(14.7, NE)
Hazard ratio[2,3] (95% CI)	0.59 (0.39, 0.90)	
p-value[3,4]	0.0131	

CI=confidence interval; NE=not estimable

[1]Based on Kaplan-Meier estimates

[2]Based on stratified Cox proportional hazards model

[3]Stratified by programmed death-ligand 1 (PD-L1) status, nodal status, tumor stage after cystectomy

[4]Based on stratified log-rank test

Clinical Bridging Study

The IMvigor011 study used Signatera CTA for study participant enrollment into surveillance and treatment arms. During the study, Signatera CTA underwent design changes as it evolved into Signatera CDx, the final diagnostic device. Therefore, the safety and effectiveness of Signatera CDx for detecting ctDNA MRD in MIBC patients was evaluated through testing of the clinical trial samples in a clinical bridging study. The study evaluated concordance between the Signatera CTA and CDx assays and estimated treatment efficacy using Signatera CDx to assess its effectiveness in identifying MIBC.

A. Study Design

Banked and/or leftover samples from subjects enrolled to IMvigor011 were used to assess the safety and effectiveness of Signatera CDx. The clinical bridging study tested all available ctDNA MRD-positive samples from subjects randomized in the clinical study and an equal number of randomly selected subjects who remained serially ctDNA MRD-negative in the study. CTA-negative cases were selected from the last negative timepoint of subjects who were repeatedly negative for up to 1 year following cystectomy while CTA-positive cases were determined based on the initial CTA positive timepoint that triggered randomization in IMvigor011. Clinical efficacy (DFS and OS) for Signatera CDx ctDNA MRD-positive subjects, was compared between treatment arms (TECENTRIQ (atezolizumab) vs. placebo), starting at the time of randomization based on ctDNA MRD positive status.

1. Clinical Bridging Study Inclusion and Exclusion Criteria

Inclusion Criteria

- The IMvigor011 study subjects who maintained consent for sample use, including all ctDNA MRD-positive randomized subjects and a random subset of surveillance subjects who never tested ctDNA MRD-positive.
- Subjects must have had sufficient banked tumor tissue, whole blood, and plasma samples or remnant sample material to obtain a ctDNA MRD result on Signatera CDx for each subject. The inclusion requirements for each sample type were as follows:
    - Signatera CDx WES retesting requirements included banked FFPE cut slides and patient-matched whole blood to the fullest extent possible. Residual leftover extracted DNA was used when banked clinical trial samples were not available.
    - Signatera CDx plasma retesting requirements included banked frozen plasma or residual cfDNA libraries from Signatera CTA testing when banked plasma was not available. For randomized subjects, the time point that led to randomization was tested. For surveillance subjects who never tested positive with the Signatera CTA assay, the last valid time point was tested.
    - When there was sufficient leftover plasma but insufficient tumor tissue or residual gDNA from tissue or matched normal whole blood, WES data generated by Signatera CTA was reanalyzed by the Signatera CDx bioinformatics pipeline for primer design.

Exclusion Criteria

- Consented subjects enrolled in IMvigor011 study but not randomized due to invalid CTA assay results or assay failures (TNP).
- CTA-positive subjects enrolled in IMvigor011 study but not randomized due to additional inclusion or exclusion criteria in IMvigor011 for the treatment phase of the study.
- Participants enrolled in China.

2. Follow-up Schedule

Signatera CDx bridging study involved only retrospective testing of IMvigor011 clinical study samples and no additional patient follow-up was conducted.

3. Clinical Endpoints

The primary objectives were to determine the degree of concordance between Signatera CDx and Signatera CTA and to estimate the efficacy of TECENTRIQ (atezolizumab) treatment in Signatera CDx-positive subjects.

The concordance between Signatera CTA and Signatera CDx was assessed as PPA and NPA.

The primary efficacy endpoint was the difference in DFS between the treatment and control groups among Signatera CDx positives. Investigator-assessed DFS was defined as the time from randomization to the first occurrence of a DFS event, including (1) local recurrence of urothelial carcinoma (UC), (2) urinary tract recurrence of UC, (3) distant metastasis of UC, or (4) death from any cause. For subjects without a DFS event, data were censored at the last date the subject was confirmed alive and free of recurrence. If no post-baseline disease or death assessment was available, data were censored at the randomization date. Multiple imputations were performed for missing values of Signatera CDx ctDNA MRD status. The primary efficacy endpoint, DFS, was estimated in the Signatera CDx-positive population. DFS results were compared to those determined among the CTA-positive randomized subjects.

The difference in OS between the treatment and control groups among Signatera CDx positives was also evaluated as an additional efficacy measure.

B. Accountability of Study Cohort

A total of 656 subjects were enrolled into the IMvigor011 clinical study using Signatera CTA, including 225 CTA-positive subjects randomized to the treatment phase, 317 CTA-negative subjects, 109 CTA-positive subjects with treatment phase screen failures, and 5 subjects with no CTA result. The bridging study included testing samples from 216 CTA-positive randomized subjects (96.0% of CTA-positive randomized subjects) and an equal number of randomly selected samples from CTA-negative subjects (**Table 33**).

Table 33. Patient Disposition

Population	Overall n (%)
Enrolled Population	656 (100.0)
CTA-Positive Population	334 (50.9)
Screen failed to meet criteria unrelated to CTA	109 (32.6)
Randomized, Material not available for CDx testing	9 (2.7)
Randomized, Material available for CDx testing	216 (64.7)

Population	Overall n (%)
CTA-Negative Population	317 (48.3)
Not CDx tested	101 (31.9)
Randomly selected for CDx testing	216 (68.1)
CTA-TNP (Assay Failure)	5 (0.8)

C. **Study Population Demographics and Baseline Parameters**

Demographics and baseline clinical characteristics were evaluated in the study population, with subgroups determined by whether or not a Signatera CDx result was evaluable, shown in **Table 34**. CDx-evaluable subjects (N = 429) and CDx-unevaluable subjects (N = 227) are generally comparable.

Table 34. Demographics and Baseline Characteristics in the IMvigor011 population[1]

Characteristic	CDx Evaluable Subjects N = 429	CDx Unevaluable Subjects N = 227	Total N = 656
Age at enrollment, years			
Mean (SD)	68.49 (9.08)	68.63 (8.97)	68.54 (9.03)
Median	69.26	69.36	69.29
Min, Max	36.64, 90.04	41.46, 86.42	36.64, 90.04
Age group at enrollment, n (%)			
<65 Years	139 (32.4)	75 (33.0)	214 (32.6)
≥65 Years	290 (67.6)	152 (67.0)	442 (67.4)
Sex, n (%)			
Female	91 (21.2)	57 (25.1)	148 (22.6)
Male	338 (78.8)	170 (74.9)	508 (77.4)
Ethnicity, n (%)			

Hispanic Or Latino	52 (12.1)	21 (9.3)	73 (11.1)
Not Hispanic Or Latino	352 (82.1)	189 (83.3)	541 (82.5)
Not Reported	22 (5.1)	17 (7.5)	39 (5.9)
Unknown	3 (0.7)	0 (0.0)	3 (0.5)
Race, n (%)			
American Indian Or Alaska Native	8 (1.9)	3 (1.3)	11 (1.7)
Asian	97 (22.6)	65 (28.6)	162 (24.7)
Black Or African American	8 (1.9)	2 (0.9)	10 (1.5)
Native Hawaiian Or Other Pacific Islander	1 (0.2)	1 (0.4)	2 (0.3)
White	289 (67.4)	141 (62.1)	430 (65.5)
Multiracial[2]	2 (0.5)	0 (0.0)	2 (0.3)
Unknown	24 (5.6)	15 (6.6)	39 (5.9)
Region, n (%)			
APAC	97 (22.6)	64 (28.2)	161 (24.5)
CASA	41 (9.6)	16 (7.0)	57 (8.7)
EU	286 (66.7)	144 (63.4)	430 (65.5)
NA	5 (1.2)	3 (1.3)	8 (1.2)
Tumor PD-L1 expression, n (%)			
<5% (IC0/1)	250 (58.3)	129 (57.1)	379 (57.9)
≥5% (IC2/3)	179 (41.7)	97 (42.9)	276 (42.1)
Missing	0	1	1

Nodal status, n (%)			
Negative	261 (60.8)	141 (62.1)	402 (61.3)
Positive	168 (39.2)	86 (37.9)	254 (38.7)
Tumor stage after surgery, n (%)			
≤T2	156 (36.4)	81 (35.7)	237 (36.1)
T3/T4	273 (63.6)	146 (64.3)	419 (63.9)
Time since initial diagnosis to enrollment, months			
Mean (SD)	10.95 (15.50)	10.13 (12.72)	10.67 (14.60)
Median	7.62	7.72	7.66
Min, Max	1.41, 144.50	1.91, 123.70	1.41, 144.50
Missing	16	12	28
Time since surgery to enrollment, months			
Mean (SD)	3.29 (0.93)	3.28 (0.91)	3.29 (0.92)
Median	3.15	3.12	3.15
Min, Max	1.38, 7.49	1.64, 8.05	1.38, 8.05
Missing	1	0	1
Type of urinary diversion, n (%)			
Continent Urinary Diversion	1 (0.2)	0 (0.0)	1 (0.2)
Cutaneous Tube Ureterostomy (Ctu)	9 (2.1)	11 (4.8)	20 (3.1)
Cutaneous Urinary Diversion (Unspecified)	2 (0.5)	1 (0.4)	3 (0.5)
Ileal Conduit Urinary Diversion (Bricker)	328 (76.6)	173 (76.2)	501 (76.5)

Neobladder	68 (15.9)	33 (14.5)	101 (15.4)
Nephrostomy	3 (0.7)	2 (0.9)	5 (0.8)
Unknown	17 (4.0)	7 (3.1)	24 (3.7)
Missing	1	0	1
Number of lymph nodes resected, n (%)			
< 10	103 (24.7)	48 (21.4)	151 (23.6)
≥10	314 (75.3)	176 (78.6)	490 (76.4)
Missing	12	3	15
Prior neoadjuvant chemotherapy (NAC), n (%)			
No	204 (47.6)	115 (50.7)	319 (48.6)
Yes	225 (52.4)	112 (49.3)	337 (51.4)

SD = Standard deviation. N = Number of patients; n (%) = Number (percent) of patients in this category.
[1] Excludes subjects enrolled in China.
[2] Subjects were classified as multiracial if they selected more than one race.

D. Safety and Effectiveness Results

1. Safety Results

There were no safety endpoints for the clinical bridging study, and no adverse events were reported in the conduct of the study.

For a complete safety information on the TECENTRIQ and TECENTRIQ HYBREZA, please refer to Drugs@FDA.

2. Effectiveness Results

Concordance Between Signatera CTA and Signatera CDx

In the primary concordance analysis, 429 of 432 tested subject samples had evaluable CDx results for comparison to the CTA (**Table 35**). Among 216 CTA-positive subjects, 214 had valid CDx results of which 171 were CDx-positive and 43 were CDx-negative for a PPA of 79.9%. Among 216 CTA-negative subjects, 215 had valid CDx results of which 4 were CDx-positive and 211 were CDx-negative for a NPA of 98.1%.

Table 35. Concordance Between Signatera CTA and Signatera CDx

CDx	CTA		
	Positive	**Negative**	**Total**
Positive	171	4	175
Negative	43	211	254
Total	214	215	429
Performance	**n/N**	**% (95% CI)**	
PPA	171/214	79.9% (74.0%, 84.7%)	
NPA	211/215	98.1% (95.3%, 99.3%)	

A data-informed analysis was conducted to estimate the likelihood of longitudinal ctDNA MRD-positive detection among the 43 discordant CTA+/CDx- cases. Data were analyzed to determine the number of subsequent tests performed while the patient was on-study and DFS-event free. Patients randomized to the atezolizumab arm were matched to placebo counterparts as closely as possible based on the week of ctDNA MRD positivity, MTM/mL level at the end of surveillance, and the study stratification factors. A similar procedure was used to estimate the projected probability that an ctDNA MRD-positive result would be returned. To prevent over-estimating the duration of time these patients would have been DFS-event free had surveillance been continued without the administration of atezolizumab, only remaining surveillance visits within the timeframe their matched placebo patient remained on-study and DFS-event free was considered. Analysis was obtained for 37 of the 43 patients. The study PPA estimate would be expected to increase from the observed 79.9% (171/214) to 97.2% (208/214).

A post market study will be performed (listed in Conditions of Approval in Section XV below) to test samples of the 43 CTA+/CDx- patients collected from later available time points within 12 months to supplement concordance between Signatera CDx and the CTA and cumulative sensitivity of Signatera CDx for ctDNA MRD positivity.

Efficacy Analyses

The differences in DFS between the treatment and placebo groups were statistically significant in both the CTA-positive study population and CDx-

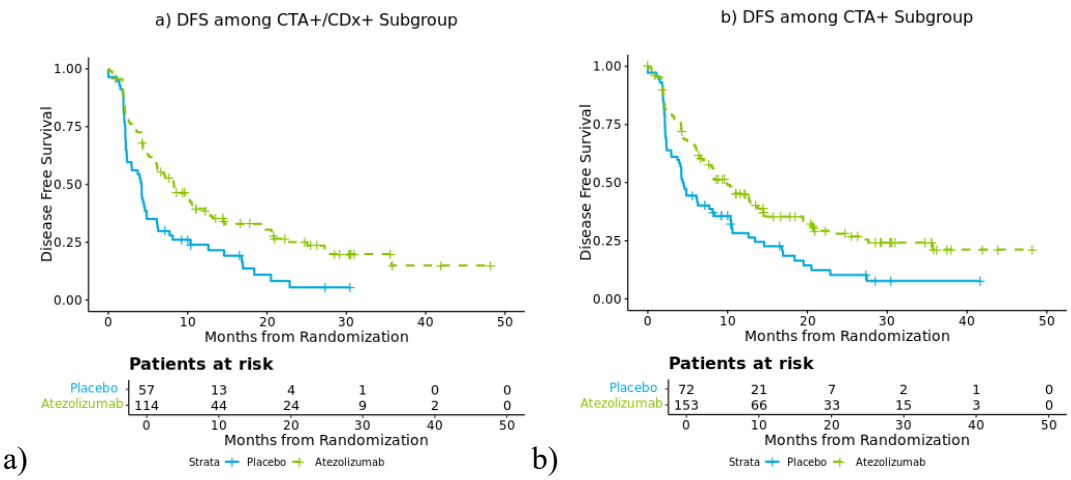
positive subgroup with hazard ratios of 0.58 for all CTA-positive subjects and 0.51 for the CDx-positive subgroup (**Table 36** and **Figure 1**).

Table 36. Disease Free Survival (DFS) from Time of Randomization in Efficacy Population

	CDx+/CTA+ (N = 171)		**CDx-/CTA+ (N = 43)**		**CDx Unevaluable/CTA+ (N=11)**		**CTA+ (N = 225)**	
Arm	**Atezolizumab (N = 114)**	**Placebo (N = 57)**	**Atezolizumab (N = 31)**	**Placebo (N = 12)**	**Atezolizumab (N = 8)**	**Placebo (N = 3)**	**Atezolizumab (N = 153)**	**Placebo (N = 72)**
Investigator-assessed DFS, Months								
No. of events (%)	83 (72.8%)	50 (87.7%)	16 (51.6%)	9 (75%)	4 (50%)	1 (33.3%)	103 (67.3%)	60 (83.3%)
Median (95% CI)	8.3 (6.1, 10.6)	4.2 (2.3, 4.8)	14.8 (8.3, NE)	10.6 (10.4, NE)	12.7 (10.5, NE)	NE (2.2, NE)	9.9 (7.9, 13.2)	4.4 (3.7, 8.3)
Hazard ratio[1] (95% CI)	0.51 (0.35, 0.75)		0.55 (0.20, 1.50)		2.03 (0.20, 20.33)		0.58 (0.42, 0.80)	
p-value[2]	0.0004		0.2370		0.5408		0.0009	

CI=confidence interval; NE=not estimable; DFS = disease free survival
[1]Based on stratified[2] log rank test
[2]Stratified by programmed death-ligand 1 (PD-L1) status, nodal status, tumor stage after cystectomy

Figure 1. Disease Free Survival (DFS) by treatment group among a) CTA+/CDx+ and b) CTA+ subgroups



a) b)

The overall DFS hazard ratio (HR) in the CDx-positive population was estimated under varying assumptions about the treatment effect in CTA-negative/CDx-positive subjects (Li, 2015). The hazard ratio estimated for the CDx ranged from 0.55 to 0.60 across a range of assumptions for the unknown treatment effect, with and without imputation for missing CDx values.

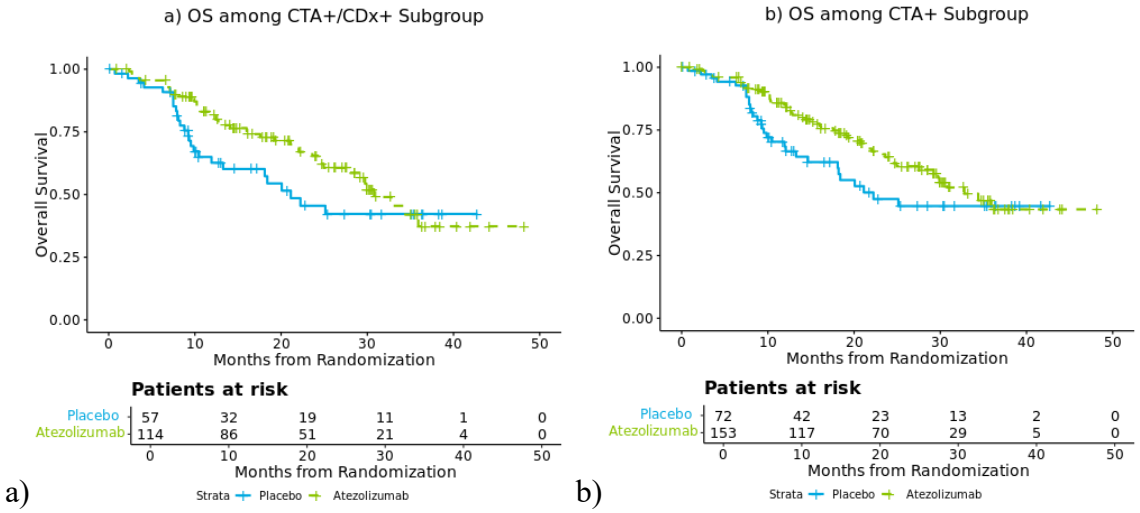
The difference in overall survival (OS) in the treatment and placebo groups was also evaluated and found to be statistically significant in both the CTA-positive study population and CDx-positive subgroup, with similar hazard ratios of 0.58 for all CTA-positive subjects and 0.55 for the CDx-positive subgroup (**Table 37** and **Figure 2**).

Table 37. Overall Survival (OS) from Time of Randomization in Efficacy Population

	CDx+/CTA+ (N = 171)		CDx-/CTA+ (N = 43)		CDx Unevaluable/CTA+ (N=11)		CTA+ (N = 225)	
Arm	**Atezolizumab (N = 114)**	**Placebo (N = 57)**	**Atezolizumab (N = 31)**	**Placebo (N = 12)**	**Atezolizumab (N = 8)**	**Placebo (N = 3)**	**Atezolizumab (N = 153)**	**Placebo (N = 72)**
OS, Months								
No. of events (%)	43 (37.7 %)	26 (45.6 %)	10 (32.3 %)	4 (33.3 %)	1 (12.5 %)	0 (0.0%)	54 (35.3 %)	30 (41.7%)
Median (95% CI)	30.8 (27.7, NE)	21.1 (13.3, NE)	NE (20.8, NE)	18.2 (14.7, NE)	NE (NE, NE)	NE (NE, NE)	32.8 (29.1, NE)	22.2 (18.1, NE)
Hazard ratio[2] (95% CI)	0.55 (0.33, 0.92)		0.23 (0.04, 1.55)		NE (NE, NE)		0.58 (0.37, 0.92)	
p-value[1]	0.0209		0.1164		NE		0.0188	

CI=confidence interval; NE=not estimable; OS = overall survival
[1]Based on stratified[2] log rank test
[2]Stratified by programmed death-ligand 1 (PD-L1) status, nodal status, tumor stage after cystectomy

Figure 2. Overall Survival (OS) by treatment group among a) CTA+/CDx+ and b) CTA+ subgroups



The OS HR in the CDx-positive population was estimated under varying assumptions about the treatment effect in CTA-negative/CDx-positive subjects (Li, 2015). The hazard ratio estimated for the CDx ranged from 0.68 to 0.70 across a range of assumptions for the unknown treatment effect, with and without imputation for missing CDx values.

3. Subgroup Analyses

None.

4. Pediatric Extrapolation

Data from adult patients in the IMvigor011 study is considered generalizable to the pediatric population aged 18-22 years. The youngest patient enrolled in the study was 36 years old. Additionally, since there is no change in the specimen type (plasma and tissue) to be used or assay workflow prescribed in the Instructions for Use based on the age of the patient, the validation data from patients aged 36 and above can be extrapolated to the pediatric population and can be relied upon to establish safety and effectiveness within the 18-22 years age group.

XI. FINANCIAL DISCLOSURE

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

- Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: Zero
- Significant payment of other sorts: Two full-time employment
- Proprietary interest in the product tested held by the investigator: Zero
- Significant equity interest held by investigator in sponsor of covered study: Two

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

XII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

None.

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

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

XIV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The analytical sensitivity, specificity, and precision of Signatera CDx for the detection of ctDNA MRD are reported in Section IX. The clinical benefit of Signatera CDx in detecting ctDNA MRD in patients with MIBC was demonstrated and is summarized in Section X.

B. Safety Conclusions

The risks of the device are based on analytical studies as well as data collected in clinical studies conducted to support PMA approval as described above.

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions in cancer treatment. Patients with false positive results may undergo treatment with TECENTRIQ (atezolizumab) and TECENTRIQ HYBREZA (atezolizumab and hyaluronidase-tqjs) without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy.

C. Benefit-Risk Determination

The probable benefit of the Signatera CDx for the identification of patients with MIBC, post cystectomy, with ctDNA MRD positive disease, for treatment with TECENTRIQ (atezolizumab) or TECENTRIQ HYBREZA (atezolizumab and hyaluronidase-tqjs), are based on data collected in the IMvigor011 clinical trial and the clinical bridging study. The clinical benefit of the Signatera CDx assay for the selection of MIBC patients with ctDNA MRD positive status was demonstrated in a retrospective bridging study using samples from patients enrolled in IMvigor011.

As assessed by investigators using the DFS criteria, clinical efficacy of ctDNA MRD-positive patients by Signatera CDx indicated a stratified HR of 0.51 with 95% CI [0.35, 0.75] and p-value of 0.0004, which was numerically similar to the stratified HR of 0.58 with 95% CI [0.42, 0.80] and p-value of 0.0009 in the CTA-positive

population for patients with MIBC in the IMvigor011 study, which provides a meaningful clinical benefit in this population. Stratification factors for the hazard ratio included PD-L1 status, nodal status, and tumor stage after cystectomy. The median DFS for the Signatera CDx-positive MIBC population was 8.3 months (95% CI: 6.1, 10.6) for patients treated with atezolizumab compared to 4.2 months (95% CI: 2.3, 4.8) for patients receiving placebo, representing a clinically meaningful improvement. In addition, sensitivity analysis was performed under varying assumptions, accounting for the CTA-negative and CDx-positive subjects (which were not present in the study); the HR was estimated to be between 0.55 to 0.60 across a range of assumptions, for this population. For overall survival (OS), the stratified HR for Signatera CDx-positive patients was 0.55 with 95% CI [0.33, 0.92] and p-value of 0.0209, with median OS of 30.8 months (95% CI: 27.7, NE) for atezolizumab-treated patients compared to 21.1 months (95% CI: 13.3, NE) for placebo patients. Thus, patients identified with the Signatera CDx as ctDNA MRD positive, show clinically meaningful improvement in disease-free survival and overall survival when treated with TECENTRIQ (atezolizumab), which is salient considering the context and clinical severity of the disease.

Though the Signatera CDx successfully identified responders to atezolizumab, the bridging concordance comparison to the enrolling Signatera CTA, revealed a significant risk of false negativity of this device for ctDNA MRD-positivity. The PPA of the CDx positives, conditional on the CTA positives was only 79.9%, while the NPA of the CDx negatives conditional on the CTA negatives was 98.1% for the MIBC patient population from the IMvigor011 study. This indicates that a subset of patients may be missed by Signatera CDx. However, the 43 discordant CTA-positive/CDx-negative subjects, generally, had low ctDNA levels below LoD and represented borderline ctDNA MRD-positive cases. A data-informed analysis estimated that with serial testing, at remaining timepoints, as performed in the IMvigor011 study, the PPA would be expected to increase from 79.9% to 97.2%, supporting the use of serial/longitudinal ctDNA MRD testing, over the course of one year, as was done in the trial, to improve cumulative sensitivity for ctDNA MRD positive status, such that the risk of false negativity with the Signatera CDx device was deemed to be acceptable, for the indications of use.

However, there is still potential risk associated with the use of this device, mainly due to 1) false positives, false negatives, or failure to provide a result, and 2) incorrect interpretation of test results by the user. The risks of the Signatera CDx assay are associated with the potential mismanagement of patients resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial which may lead to adverse events or may have delayed access to treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial drug. The risk of false results is partially mitigated by the longitudinal testing strategy and the clinical and analytical performance of the device, as described above, in this SSED.

The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with reasonable accuracy, when performed longitudinally, mitigating the potential for false results. In addition, to

supplement and further confirm the aforementioned pre-market analytical and clinical data, post-market studies are planned as summarized in Section XV, below.

1. Patient Perspective

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

In conclusion, given all the available information above and the totality of the analytical and clinical data, the probable benefits for the use of the Signatera CDx device, to select post-cystectomy MIBC patients, with ctDNA MRD positive status, for treatment with TECENTRIQ (atezolizumab) or TECENTRIQ HYBREZA (atezolizumab and hyaluronidase-tqjs), are deemed to exceed the probable risks.

D. Overall Conclusions

The totality of data in this application supports the reasonable assurance of safety and effectiveness of Signatera CDx when used in accordance with the indications for use. The provided studies support use of Signatera CDx as an aid in identifying patients with MIBC with Signatera CDx detected ctDNA MRD who may be considered for treatment with TECENTRIQ (atezolizumab), TECENTRIQ HYBREZA (atezolizumab and hyaluronidase-tqjs).

XV. CDRH DECISION

CDRH issued an approval order on 5/15/2026. The final conditions of approval cited in the approval order are listed below.

1. Natera, Inc. must provide data from testing available samples of the 43 CTA+/CDx- patients collected from later time points within 12 months from cystectomy in the IMvigor011 clinical study. The data from this study must be adequate to supplement concordance between Signatera CDx and the CTA and cumulative sensitivity of Signatera CDx for ctDNA MRD positivity. This information must be provided to supplement the observed clinical performance of the Signatera CDx.
2. Natera, Inc. must provide data from an additional study to supplement the analytical accuracy of Signatera CDx in low positive clinical specimens. The orthogonal method for this study should have a comparable or higher sensitivity to the sensitivity of Signatera CDx. The clinical specimens should appropriately represent the intended use population of Signatera CDx and include an adequate number of low positive, below LoD, as well as negative specimens.
3. Natera, Inc. must provide additional data to supplement characterization of plasma workflow limit of detection (LoD) on the variant level. This study should evaluate a set of clinical samples with variants that represent the intended use population of the Signatera CDx.
4. Natera, Inc. must provide data from testing additional ctDNA MRD-positive and ctDNA MRD-negative plasma samples to supplement lot-to-lot precision of the Streck Cell-Free DNA BCTs. The samples should adequately represent the intended use population of Signatera CDx.

5. Natera, Inc. must provide data evaluating potential interference caused by underfilling of the Streck Cell-Free DNA BCTs. The data from this study must be adequate to support that underfilling of the BCTs does not adversely impact Signatera CDx results.

The final study data, study conclusions, and labeling revisions should be submitted within 12 months of the PMA approval date.

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

XVI. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XVII. REFERENCES

Li M. Statistical consideration and challenges in bridging study of personalized medicine. J Biopharm Stat. 2015;25(3):397-407. doi: 10.1080/10543406.2014.920340. PMID: 24897254.