510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K200009

B Applicant

Adaptive Biotechnologies Corporation

C Proprietary and Established Names

Adaptive Biotechnologies clonoSEQ Assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QDC	Class II (Special Controls)	21 CFR 866.6100	88 − Pathology

II Submission/Device Overview:

A Purpose for Submission:

Expansion of indications for use to include chronic lymphocytic leukemia (CLL)

B Measurand:

Rearranged IgH (VDJ), IgH (DJ), IgK, and IgL receptor gene sequences, as well as translocated BCL1/IgH (J) and BCL2/IgH (J) sequences.

C Type of Test:

Multiplex polymerase chain reaction and next generation sequencing-based in vitro diagnostic to measure minimal residual disease

III Intended Use/Indications for Use:

A Indications for Use:

The clonoSEQ Assay is an in vitro diagnostic that uses multiplex polymerase chain reaction (PCR) and next-generation sequencing (NGS) to identify and quantify rearranged IgH (VDJ), IgH (DJ), IgK and IgL receptor gene sequences, as well as translocated BCL1/IgH (J) and BCL2/IgH (J) sequences in DNA extracted from bone marrow from patients with B-cell acute lymphoblastic leukemia (ALL) or multiple myeloma (MM), and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL).

The clonoSEQ Assay measures minimal residual disease (MRD) to monitor changes in burden of disease during and after treatment. The test is indicated for use by qualified healthcare professionals in accordance with professional guidelines for clinical decision-making and in conjunction with other clinicopathological features.

The clonoSEQ Assay is a single-site assay performed at Adaptive Biotechnologies Corporation in Seattle, Washington.

B Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For *in vitro* diagnostic use

C Special Instrument Requirements:

Illumina NextSeq™ 500 and 550 Systems (qualified by Adaptive Biotechnologies)

IV Device/System Characteristics:

A Device Description:

A description of required equipment, software, reagents, vendors, and storage conditions were provided, and are described in the product labeling. With the exception of specimen collection tubes, Adaptive Biotechnologies assumes responsibility for all reagents, materials, and equipment needed to perform the assay, and are used exclusively at the Adaptive Biotechnologies single laboratory site. The clonoSEQ Assay is intended to be performed with serial number-controlled instruments qualified by Adaptive. An ambient temperature sample shipper kit is available for use through Adaptive Clinical Services Team if requested by the ordering healthcare provider.

1. Sample Preparation

The minimum genomic DNA (gDNA) sample input requirement is 500 ng. Shipment of 1 mL of bone marrow aspirate (BMA) is requested to obtain sufficient extracted gDNA and extraction methods have been validated using volumes ranging from 250 µL to 1 mL. Shipment of 2 mL of whole blood is requested to obtain sufficient extracted gDNA and extraction methods have been validated using volumes ranging from 500 µL to 2 mL. For both sample types, the extraction method isolates gDNA by first lysing cells and denaturing proteins after which the DNA is bound to a substrate. Once the DNA is bound, a series of wash steps removes impurities. Following the wash steps the DNA is eluted from the substrate. DNA is quantified using a spectrophotometer; the measured DNA concentration is used to add a target of 20 µg of amplifiable gDNA to the assay. The MRD test can be performed with 500 ng – 20 µg gDNA. Internal controls in the PCR and sequencing steps are used to confirm that sufficient gDNA has been amplified and that amplification was successful.

2. Library Preparation

Genomic DNA is amplified using locus-specific multiplex PCR using V, D and J gene primers containing molecular barcodes to amplify IgH (VDJ), IgH (DJ), IgK, IgL, BCL1/IgH (J), BCL2/IgH (J) and housekeeping gene (HKG) sequences. Reaction-specific index barcodes for sample identification are added to the amplified receptor sequences by PCR. Sequencing libraries are prepared by pooling barcoded amplified DNA. qPCR is used to verify the adequacy of the pooled amplified DNA library concentration.

3. Sequencing and Data Analysis

Sequencing is conducted with the Illumina NextSeqTM 500 or 550 Systems. The sequencing process incorporates multiple quality checks. Sequencing output is then processed by the bioinformatics pipeline software as follows:

a. **Flowcell Level Metrics.** The analysis pipeline performs quality control (QC) checks on the flowcell data. The pipeline evaluates the percentage of reads that pass the Illumina quality filter (%PF), which must be greater than 70% of reads. The system uses spike-in PhiX templates to evaluate the error rate. The pipeline evaluates the proportion of PhiX reads, which must be greater than 2%, and the associated error rate as computed by the Illumina RTA software, which must be less than 3%.

b. **Demultiplexing and FASTQ Generation.** The pipeline uses Illumina software to demultiplex reads from the instrument output run folder. The analysis pipeline performs a QC check to evaluate whether unexpected barcodes are observed and raises a flag if more than 30,000 reads carry a barcode not specified in the input sample sheet.

c. **Read Assignment to Receptors**. The pipeline assigns reads to rearranged receptors for each sample after demultiplexing.

d. **Clonal Sequence Determination.** After assigning reads to receptor loci, the pipeline then clusters reads into clonal receptor sequences.

e. **Sample Level QC Checks.** The pipeline performs a series of sample level QC checks including assessment that sequencing data is sufficient and acceptable based on amplification of sets of internal synthetic controls, assessment that sufficient gDNA is sampled, and a final screen of the calculated values for biologic relevance. One set of internal synthetic controls are evaluated for sufficient read quantity per molecule and read coverage across receptor loci. Another set of internal controls' presence or absence is used to screen for the expected degradation of residual primers. The estimated mass of input gDNA based on an optical density measurement and the estimated number of sampled nucleated cells based on amplification of a set of internal reference gene are used as metrics to check if sufficient material is sampled. The pipeline also checks that the detected numbers of total and B cells are within a biologically relevant range, and screens for clone sharing by evaluating if sequences are shared across samples that are processed together.

f. **Calibrations**. Clonal sequences are assessed for their suitability as ID sequences (to be used for subsequent tracking) by first aggregating highly similar sequences and requiring that the frequency of the sequence is at least 3% as a percentage of all sequences in the locus. The clone must also have a frequency of at least 0.2% of all nucleated cells in the sample and must have sufficient abundance and differentiation from a polyclonal background. Each sequence that is being considered for MRD tracking is compared against a B cell repertoire database and assigned a uniqueness value that, together with its abundance relative to other sequences, is used to assign the sequence to a sensitivity bin which will be used in the estimation of the reported limit of detection (LoD) and limit of quantitation (LoQ).

g. **Tracking.** When a previous calibration test has identified suitable ID sequences for tracking, they are compared to sequences in the most recent tracking sample in order to assess residual disease. After approximate matching, which allows for mutations in the sample clones as compared to the ID sequences, sequence proportions in the sample are assessed and compared to the LoD and LoQ values. The analysis pipeline then reports whether ID sequences were detected above the LoQ, above LoD but below LoQ, below LoD, or not detected.

h. **Control Materials.** The following controls are used to measure the success of DNA extraction, PCR amplification and sequencing:

   i. **Synthetic Internal Controls.** Each sample includes two sets of internal synthetic controls. The controls are panels of synthetic analogues of somatically rearranged B-cell receptor (BCR) immune receptor molecules. The composition of the reference template pools before and after amplification is measured and used for QC. One set of synthetic templates is added to every pre-amp PCR well as a positive control; these synthetic templates are used to measure primer performance, including identification and correction of amplification bias, and to screen for sufficient sequencing coverage. Another set is added after a step used to remove

residual primers; the lack of amplification of these molecules is used to confirm the success of primer removal.

ii. **DNA Extraction Process Controls:** Each extraction is performed with Positive and Negative Extraction Controls. The Extraction Negative Control is used to confirm lack of contamination during the extraction process. The Extraction Negative Control is subsequently amplified and sequenced in the same fashion as test samples. The Extraction Positive Control is included to assess effectiveness of the extraction process (it is required to be above a pre-set threshold for DNA recovery). If readily available, source material for Extraction Positive Controls is matched to the specimen source type. Exception: The Extraction Positive Control for bone marrow specimens consists of frozen human whole blood.

iii. **PCR Amplification Process Controls:** Each PCR amplification is performed with an Amplification Positive and Negative Control and subsequently sequenced in the same manner as test samples. The Amplification Positive Control consists of gDNA derived from peripheral blood mononuclear cells (PBMCs) and serves as an additional check to confirm successful product amplification. Buffer (1x TE) is used as the negative control.

iv. **Sequencing Process Controls:** To every sequencing flow cell, two sequencing controls are added. Both a PhiX control purchased from Illumina and a well-characterized amplified library (Sequencing Positive Control) are loaded with test samples.

i. **Result Reporting:** The pipeline renders results into a PDF-formatted patient report. The report displays any ID sequences identified in the sample that can be used for tracking with their quantitation and sample-level metrics. For tracking tests, the report includes a result (ID sequences detected above LoD, below LoD, or not detected) and quantitation for the tracked sequences within the most recent sample.

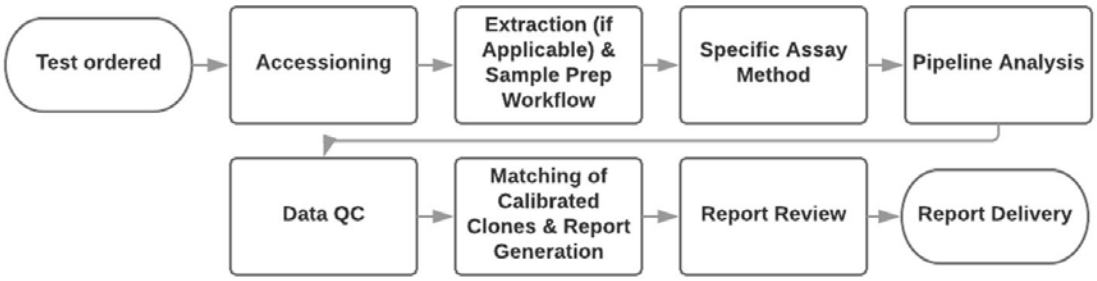
B Principle of Operation:

The clonoSEQ Assay is a next-generation sequencing (NGS) based assay that identifies rearranged IgH (VDJ), IgH (DJ), IgK, and IgL receptor gene sequences, as well as translocated BCL1/IgH (J) and BCL2/IgH (J) sequences. The assay also includes primers that amplify specific genomic regions present as diploid copies in normal genomic DNA (gDNA) to allow determination of total nucleated cell content.

Testing begins with gDNA extracted from the specimen supplied (Figure 1). Extracted gDNA quality is assessed and rearranged immune receptors are amplified using a multiplex PCR. Reaction-specific index barcode sequences for sample identification are added to the amplified receptor sequences by PCR. Sequencing libraries are prepared from barcoded amplified DNA, which are then sequenced by synthesis using NGS. Raw sequence data are uploaded from the sequencing instrument to the Adaptive analysis pipeline. These sequence data are analyzed in a

multi-step process: first, a sample's sequence data are identified using the sample index sequences. Next, data are processed using a proprietary algorithm with in-line controls to remove amplification bias. When the clonoSEQ Clonality (ID) assessment is conducted, the immune repertoire of the sample is checked for the presence of DNA sequences specific to "dominant" clone(s) consistent with the presence of a lymphoid malignancy. Each sequence that is being considered for MRD tracking is compared against a B cell repertoire database and assigned a uniqueness value that, together with its abundance relative to other sequences, is used to assign the sequence to a sensitivity bin which will be used in the estimation of the reported LoD and LoQ on the patient report. During clonoSEQ Tracking (MRD) assessment, the complete immunoglobulin receptor repertoire is again assessed, and the previously identified dominant clonotype sequence(s) are detected and quantified to determine the sample MRD level. The clonoSEQ Assay MRD assessment measures residual disease in a biologic sample.

Figure 1: clonoSEQ Assay Workflow



Following completion of these data processing steps, a report is issued. A Clonality (ID) report indicates the presence of dominant sequences residing within a presumed malignant lymphocyte clonal population, as identified in the baseline (diagnostic or high disease burden) sample from a patient. After one or more dominant sequence(s) have been identified in a baseline sample, subsequent samples from the same patient can be assessed for MRD after which a Tracking (MRD) report is generated. The MRD is expressed as a frequency that quantifies the level of residual disease based on the number of remaining copies of the initially dominant sequence(s) relative to the total number of nucleated cells in the sample.

C Instrument Description Information:

1. Instrument Name:
   Illumina NextSeq™ 500 and 550 Systems (qualified by Adaptive Biotechnologies)

2. Specimen Identification: Refer to Device Description section above.

3. Specimen Sampling and Handling: Refer to Device Description section above.

4. Calibration: Clonal sequences are assessed for their suitability as ID sequences (to be used for subsequent tracking) by first aggregating highly similar sequences and requiring that the frequency of the sequence is at least 3% as a percentage of all sequences in the locus. The clone must also have a frequency of at least 0.2% of all nucleated cells in the sample and must have sufficient abundance and differentiation from a polyclonal background. Each

sequence that is being considered for MRD tracking is compared against a B cell repertoire database and assigned a uniqueness value that, together with its abundance relative to other sequences, is used to assign the sequence to a sensitivity bin which will be used in the estimation of the reported LoD and LoQ.

5. Quality Control: Refer to Device Description section above.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Adaptive Biotechnologies clonoSEQ® Assay

B Predicate 510(k) Number(s):
DEN170080

C Comparison with Predicate(s):

Device & Predicate Device(s):	**DEN170080**	**K200009**
Device Trade Name	Adaptive clonoSEQ® Assay	Same
General Device Characteristic Similarities		
Intended Use/Indications for Use	The clonoSEQ Assay is an in vitro diagnostic that uses multiplex polymerase chain reaction (PCR) and next-generation sequencing (NGS) to identify and quantify rearranged IgH (VDJ), IgH (DJ), IgK and IgL receptor gene sequences, as well as translocated BCL1/IgH (J) and BCL2/IgH (J) sequences in DNA extracted from bone marrow from patients with B-cell acute lymphoblastic leukemia (ALL) or multiple myeloma (MM). The clonoSEQ Assay measures minimal residual disease (MRD) to monitor changes in burden of disease during and after treatment. The test is indicated for use by qualified healthcare professionals in accordance with professional guidelines for clinical decision-making and in	The clonoSEQ Assay is an in vitro diagnostic that uses multiplex polymerase chain reaction (PCR) and next-generation sequencing (NGS) to identify and quantify rearranged IgH (VDJ), IgH (DJ), IgK and IgL receptor gene sequences, as well as translocated BCL1/IgH (J) and BCL2/IgH (J) sequences in DNA extracted from bone marrow from patients with B-cell acute lymphoblastic leukemia (ALL) or multiple myeloma (MM), and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL). The clonoSEQ Assay measures minimal residual disease (MRD) to monitor changes in burden of disease during and after treatment. The test is indicated for use by qualified healthcare professionals in

Device & Predicate Device(s):	DEN170080	K200009
	conjunction with other clinicopathological features. The clonoSEQ Assay is a single-site assay performed at Adaptive Biotechnologies Corporation.	accordance with professional guidelines for clinical decision-making and in conjunction with other clinicopathological features. The clonoSEQ Assay is a single-site assay performed at Adaptive Biotechnologies Corporation in Seattle Washington.
Test Sample	Bone marrow aspirate for ALL and MM	Blood or bone marrow aspirate for CLL
Genomic DNA input	500 ng to 20 µg	Same
Type of Test	Quantitative	Same
Stability/Shelf Life	Specimens assessed by the clonoSEQ Assay were stable for the following storage conditions: • At -15 °C to -25 °C for up to 18 months for BMA • At 2 °C to 8 °C for up to 7 days for BMA • At 15 °C to 25 °C for up to 3 days for BMA • Up to 3 freeze/thaw cycles for BMA Specimens assessed by the clonoSEQ Assay were stable within the clonoSEQ shipper for ambient, summer and winter shipping conditions: • For up to 4 days for BMA	Specimens assessed by the clonoSEQ Assay were stable for the following storage conditions: • At -15 °C to -25 °C for up to 18 months for BMA and up to 3 months for blood • At 2 °C to 8 °C for up to 7 days for BMA and up to 14 days for blood • At 15 °C to 25 °C for up to 3 days for BMA and up to 5 days for blood • Up to 3 freeze/thaw cycles for BMA and blood Specimens assessed by the clonoSEQ Assay were stable within the clonoSEQ shipper for ambient, summer and winter shipping conditions: • For up to 4 days for BMA • For up to 5 days for blood
Instrument (qualified by Adaptive Biotechnologies)	NextSeq 500	NextSeq 500 and 550 series

VI Standards/Guidance Documents Referenced:

CLSI guideline EP06-A Evaluation of the Linearity of Quantitative Measurement Procedures- A Statistical Approach, and CLSI Guideline EP05-A3.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility of MRD Frequency:
   The precision of the assay in CLL was evaluated for both blood and bone marrow specimens. Precision studies were performed by 3 operators using 4 reagent lots, and 4 instrument sets (2 thermal cycler/liquid handlers and 2 NextSeq instruments). Each operator performed a minimum of 3 runs across 21 calendar days. The study design for blood used both NextSeq 500 and 550s. The study designs for bone marrow aspirates (BMA) is summarized in Figure 2 and for blood in Figure 3.

   Figure 2. BMA Precision Study Design Schematic

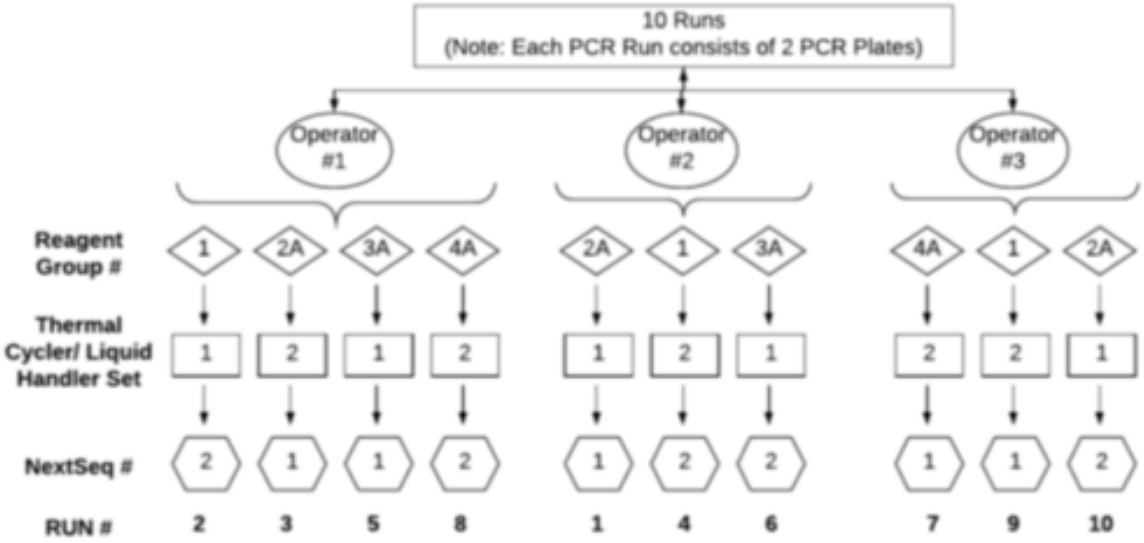
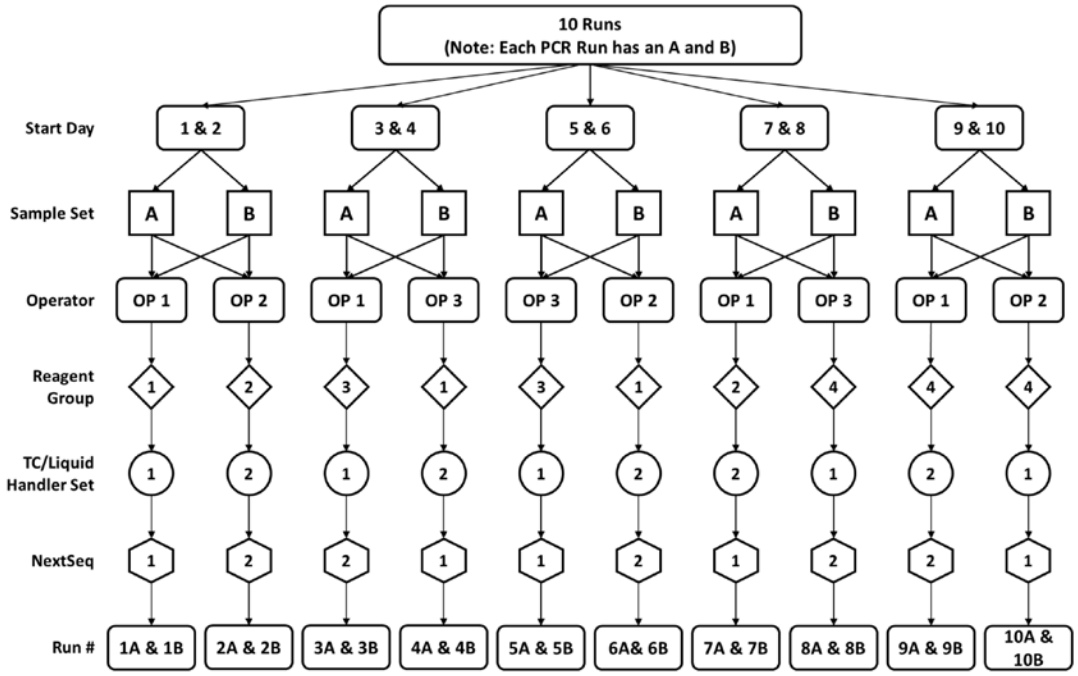


Figure 3: Blood Precision Study Design Schematic



a) *Bone Marrow:*
Precision studies in BMA derived samples tested gDNA extracted from clinical specimens from 22 patients with CLL. The gDNA from these specimens were used to contrive specific MRD levels by pooling and blending them into gDNA extracted from the BMA of healthy donors. The study included 3 DNA inputs (500 ng, 2 µg, and 20 µg) for each of 6 MRD levels for each patient sample. Each run of the assay was tested in 18 combinations of DNA input and MRD frequency in duplicate. In all, 360 contrived samples were tested. Of these, one plate with 18 samples was invalid due to sample QC failures; the plate-level failure rate was therefore 1/20 = 0.05. An additional two contrived samples (44 MRD measurements) failed sample QC due to insufficient sequencing coverage. While normal operating procedures permit re-sequencing, for this analysis these two samples were classified as failures. The analysis used the remaining 340 contrived samples with up to 22 MRD measurements per sample, for a total of 7,480 MRD measurements.

The precision of the clonoSEQ Assay is largely dependent upon the number of malignant cells that are being evaluated rather than the MRD frequency. Consequently, the same MRD frequency is expected to have lower precision at lower gDNA inputs. For these studies, precision estimates were calculated based on the MRD frequency per gDNA input and estimates of imprecision of the absolute number of malignant cells detected were calculated. Precision analysis, including variation from instrument set, operator, processing day, processing run, and reagent lot, is reported as %CV for each tested MRD frequency at each DNA input. Precision ranged from 18.5% to 60.1% CV. MRD frequency range refers to the central 95% range of MRD estimates that were observed across all of the patient samples tested at each DNA input and frequency condition. These data were used to define the 95% confidence intervals that are used in patient reports. The data is shown in Table 1.

Table 1. Precision of the clonoSEQ Assay in CLL BMA Samples

DNA Input	Target MRD Frequency	Measurements	%CV	Mean MRD Frequency	Frequency Range (95% CI)
500 ng	$2.8x10^{-5}$	396	57.9	4.4×10^{-5}	$0 - 1.0 \times 10^{-4}$
	$8.0x10^{-5}$	396	42.5	1.0×10^{-4}	$3.4 \times 10^{-5} - 2.0 \times 10^{-4}$
	$2.8x10^{-4}$	396	28.1	3.4×10^{-4}	$1.7 \times 10^{-4} - 5.6 \times 10^{-4}$
	$8.0x10^{-4}$	440	24.3	9.2×10^{-4}	$5.6 \times 10^{-4} - 1.5 \times 10^{-3}$
	$2.8x10^{-3}$	440	21.2	2.9 x 10-3	$1.9 \times 10^{-3} - 4.4 \times 10^{-3}$
	$8.0x10^{-3}$	440	19.5	7.1×10^{-3}	$4.5 \times 10^{-3} - 1.0 \times 10^{-2}$
2 µg	$7.0x10^{-6}$	396	60.2	1.0×10^{-5}	$0 - 2.4 \times 10^{-5}$
	$2.0x10^{-5}$	396	44.7	2.6×10^{-5}	$9.3 \times 10^{-6} - 5.4 \times 10^{-5}$
	$7.0x10^{-5}$	396	28.9	8.5×10^{-5}	$4.5 \times 10^{-5} - 1.4 \times 10^{-4}$
	$2.0x10^{-4}$	440	23.5	2.3×10^{-4}	$1.4 \times 10^{-4} - 3.8 \times 10^{-4}$
	$7.0x10^{-4}$	440	21.4	8.2×10^{-4}	$5.1 \times 10^{-4} - 1.3 \times 10^{-3}$
	$2.0x10^{-3}$	440	19.2	2.2×10^{-3}	$1.4 \times 10^{-3} - 3.2 \times 10^{-3}$
20 µg	$7.0x10^{-7}$	396	59.9	1.1×10^{-6}	$0 - 2.6 \times 10^{-6}$
	$2.0x10^{-6}$	396	41.2	2.9×10^{-6}	$1.0 \times 10^{-6} - 5.6 \times 10^{-6}$
	$7.0x10^{-6}$	396	27.2	9.1×10^{-6}	$5.4 \times 10^{-6} - 1.5 \times 10^{-5}$
	$2.0x10^{-5}$	440	22.8	2.6×10^{-5}	$1.6 \times 10^{-5} - 3.9 \times 10^{-5}$
	$7.0x10^{-5}$	440	20.1	8.4×10^{-5}	$5.2 \times 10^{-5} - 1.2 \times 10^{-4}$
	$2.0x10^{-4}$	396	26.7	2.3×10^{-4}	$1.6 \times 10^{-4} - 3.3 \times 10^{-4}$

* These frequencies correspond to an estimated 2.14, 6.13, 21.44, 61.26, 214.40 and 612.56 malignant cells tested at each DNA dilution (95% confidence intervals on malignant cell count not shown).

b) *Blood*:

Precision studies in blood-derived samples were contrived in a similar process, using gDNA extracted from 15 CLL patient specimens, pooled and blended into gDNA from health donor blood to create 6 specific MRD levels for each of 3g DNA mass inputs (500 ng, 2 µg, and 20 µg). Each run of the assay tested 16 combinations of gDNA input and MRD frequency, in duplicate, for a total of 320 contrived samples tested. There were no plate failures. One sample replicate was invalid due to sample QC failure, leaving 319 contrived samples and a total of 4,785 MRD measurements in the final analysis (15 MRD measurements x 319 samples). Precision analysis, including variation from instrument set, operator, processing day, processing run, and reagent lot, is reported as %CV for each tested MRD frequency at each DNA input. Data was calculated and presented as described for bone marrow. Precision ranged from 18.7% to 54.9% CV in CLL. The data is shown in Table 2.

Table 2. Precision of the clonosSEQ Assay in CLL Blood.

DNA Input	Target MRD Frequency*	Measurements	%CV	Mean MRD Frequency	Frequency Range (95% CI)
500 ng	$4.0x10^{-5}$	300	54.9	$5.3x10^{-5}$	$1.1x10^{-5}$ - $1.2x10^{-4}$
	$1.0x10^{-4}$	300	38.3	$1.2x10^{-4}$	$4.1x10^{-5}$ - $2.2x10^{-4}$
	$4.0x10^{-4}$	300	28.9	$4.4x10^{-4}$	$2.3x10^{-4}$ - $7.1x10^{-4}$
	$1.0x10^{-3}$	285	24.4	$1.1x10^{-3}$	$6.4x10^{-4}$ - $1.7x10^{-3}$
	$4.0x10^{-3}$	300	21.9	$4.3x10^{-3}$	$2.8x10^{-3}$ - $6.3x10^{-3}$
2 µg	$1.0x10^{-5}$	300	51.6	$1.2x10^{-5}$	$2.4x10^{-6}$ - $2.7x10^{-5}$
	$2.5x10^{-5}$	300	37.3	$2.9x10^{-5}$	$1.1x10^{-5}$ - $5.2x10^{-5}$
	$1.0x10^{-4}$	300	26.5	$1.1x10^{-4}$	$6.1x10^{-5}$ - $1.7x10^{-4}$
	$2.5x10^{-4}$	300	23.0	$2.7x10^{-4}$	$1.7x10^{-4}$ - $4.0x10^{-4}$
	$1.0x10^{-3}$	300	20.8	$1.1x10^{-3}$	$6.8x10^{-4}$ - $1.6x10^{-3}$
20 µg	$1.0x10^{-6}$	300	49.2	$1.3x10^{-6}$	$2.9x10^{-7}$ - $2.8x10^{-6}$
	$2.5x10^{-6}$	300	36.4	$2.9x10^{-6}$	$1.2x10^{-6}$ - $5.3x10^{-6}$
	$1.0x10^{-5}$	300	25.9	$1.1x10^{-5}$	$6.6x10^{-6}$ - $1.8x10^{-5}$
	$2.5x10^{-5}$	300	23.0	$2.7x10^{-5}$	$1.7x10^{-5}$ - $4.3x10^{-5}$
	$1.0x10^{-4}$	300	19.5	$1.1x10^{-4}$	$7.5x10^{-5}$ - $1.6x10^{-4}$
	$2.5x10^{-4}$	300	18.7	$2.7x10^{-4}$	$1.9x10^{-4}$ - $3.9x10^{-4}$

*These frequencies correspond to an estimated 3.06, 7.66, 30.63, 76.67, 306.28, and 765.70 malignant cells tested at each DNA dilution. (95% confidence intervals on malignant cell count not shown).

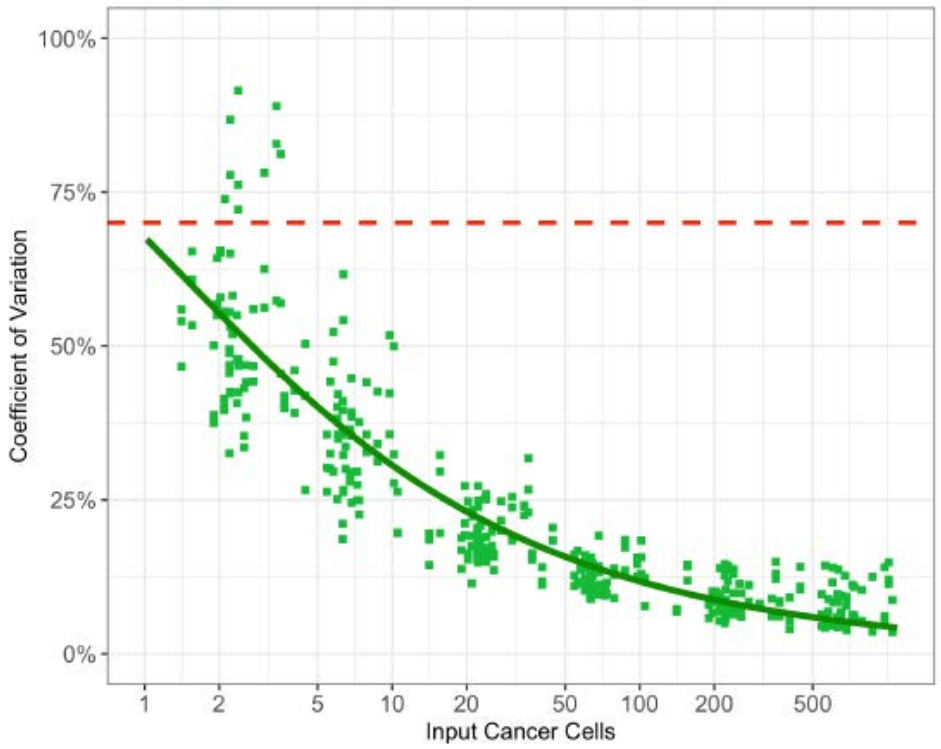
2. Precision/Reproducibility of Malignant Cells

a) Bone marrow:

The precision of malignant cells detected in bone marrow was evaluated across the range of tested malignant cells (estimated to be 2.14 – 612.56 based on gDNA concentration and read depths of internal controls). For this analysis, the results from all of the DNA inputs were pooled into a single analysis. The precision was primarily influenced by cell numbers being evaluated. Precision ranged from 59% CV at 2.14 cells to 20% CV at 612.56 cells. The tested factors (Operator, Instrument Sets, Reagent Lots, Day, and Run) minimally contributed to variability with attributable %CV ranging from 0% to 5% (data not shown).

The precision for each sample at each tested condition across all DNA inputs is summarized in a Sadler's precision profile (Figure 4). The Sadler's precision profile visualizes the relationship between the number of sampled malignant cells and precision as measured by %CV. This analysis demonstrates that the precision of the clonoSEQ Assay is largely dependent on the number of malignant cells that are being evaluated by the assay.

Figure 4. Sadler's Precision Profile (Coefficient of Variation) of the clonoSEQ Assay as a Function of Input CLL Cancer Cells in BMA

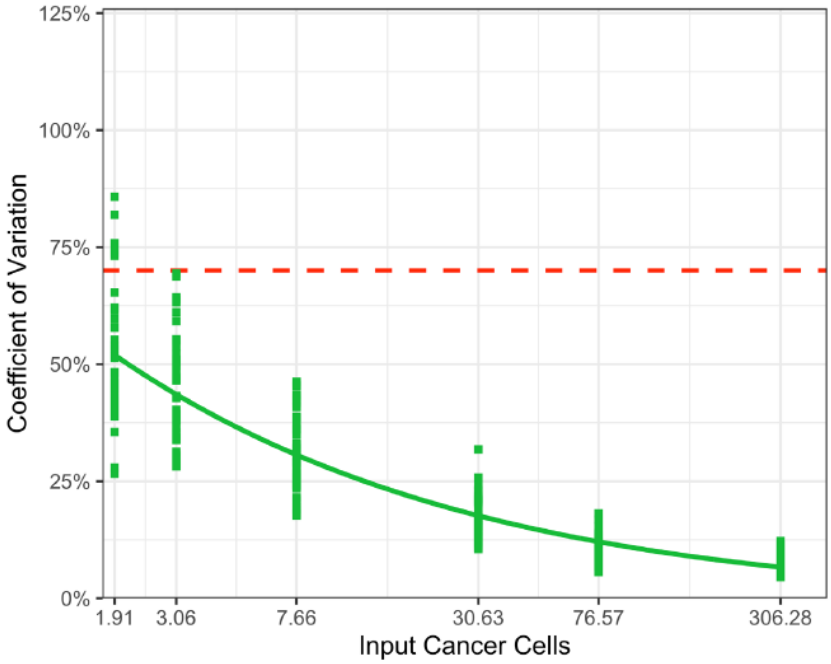


b) Blood

The precision of malignant cells detected in blood was evaluated across the estimated range of tested malignant cells (3.10 – 765.70). Precision ranged from 53% CV at 3.10 cells to 19% CV at 765.70 cells. DNA input mass contributed to variability with attributable %CV ranging from 8% to 10%, and the pre-specified test factors (Operator, Instrument Sets, Reagent Lots, Day, and Run) minimally contributed to variability, with attributable %CV ranging from 0% to 2% (data not shown).

The precision for each sample at each tested condition across all DNA inputs is summarized in a Sadler's precision profile (Figure 5). The Sadler's precision profile visualizes the relationship between the number of sampled malignant cells and precision as measured by %CV (note the inclusion of a lower input to align with the BMA plot). This analysis demonstrates that, like BMA, the precision of the clonoSEQ Assay in CLL blood is largely dependent on the number of malignant cells that are being evaluated by the assay.

Figure 5. Sadler's Precision Profile (Coefficient of Variation) of the clonoSEQ Assay as a Function of Input CLL Cancer Cells in Blood



3. Precision of Sequencing
   The clonoSEQ assay reports the sequence information for dominant clones which are then tracked in future measurements. The repeatability of sequences was evaluated in DEN170080.

4. Detection Limit:

   a) *Limit of Blank*
   The LoB was determined by measuring the specificity of a patient's trackable immunoglobulin (Ig) sequences. These sequences were identified from 22 samples from patients diagnosed CLL. The LoB was determined by searching for the presence and abundance of these trackable sequences in healthy bone marrow samples. The 95th percentile of sample MRD frequencies for these trackable sequences was zero at 500 ng, 20 µg, and 40 µg of gDNA input. Therefore, the LoB was zero, demonstrating that trackable Ig sequences are highly patient-specific.

   Similarly, the LoB in samples derived from blood was verified by searching for the presence and abundance of the trackable sequences from 15 samples from patients with CLL. The presence and abundance of these trackable sequences in healthy blood was assessed. The LoB was confirmed as zero in blood, based on the 95th percentile of sample MRD frequencies at 500 ng, 20 ug and 40 ug inputs.

   b) *Limit of Detection (LoD) /Limit of Quantitation (LoQ)*
   For bone marrow samples, the LoD and LoQ were determined by blending gDNA extracted from 22 specimens from patients with CLL into 500 ng and 20 µg of gDNA from bone marrow. A dilution series of 22.97, 10.72, 4.59, 2.14 and 0.94 malignant cell equivalents was made for each patient at each DNA input level. Each sample was tested in duplicate for each of 4 reagent lots resulting in 8 results for each of the 22

samples at each dilution condition. A probit approach was used to determine the LoD to be 1.630 malignant cells (95% CI; 1.42 – 1.87) based on the combined data from both DNA inputs. The LoQ was defined as the lowest absolute number of malignant cells whose frequency can be quantitatively determined with an accuracy of 70% relative total error. The LoQ was found to be 2.427 malignant cells (95% CI; 1.63 – 8.91).

The LoD and LoQ were similarly determined for the background matrix of blood. The gDNA extracted from 15 specimens from patients with CLL was mixed into 500 ng and 20 µg of gDNA from healthy donors of blood to achieve targeted disease levels. A dilution series of 30.63, 7.66, 3.06, 1.91 and 0.77 malignant cell equivalents was made for each patient at each DNA input level. Each sample was tested in duplicate in each run, for each of 4 reagent lots, 3 operators and 2 sequencing instruments, resulting in twenty results for each of the 15 samples at each dilution condition. A probit approach was used to determine the LoD to be based on the combined data for all indications from both DNA inputs. The LoQ was calculated by applying the Sadler's precision profile model to all non-zero MRD frequency results for each indication.

The LoD and LoQ for MRD measurements of CLL in the background matrix of blood was compared to the LoD and LoQ for measurements in BMA specimens for ALL, MM and CLL using bootstrapping of BMA data. The LoD and LoQ of CLL in blood were lower or within the 95% CI of bootstrapped prior data and thus were not significantly higher than the prior established values.

The clonoSEQ Assay can use a range of DNA inputs from 500 ng to 40 µg of DNA. The LoD/LoQ by MRD frequency will vary based on the DNA input and the total nucleated cells that are evaluated by the assay. The estimated LoD/LoQ at 500 ng and 20 µg of DNA input for both blood and bone marrow are shown in Table 3.

Table 3. LoD/LoQ in BMA and blood by MRD Counts and by MRD frequency with 95% confidence intervals

	Measure	**Malignant Cells***	**500 ng DNA Input Frequency (95% CI)**	**20 µg DNA Input Frequency (95% CI)**
BMA	LoD	1.630 (1.42 - 1.87)	$2.13x10^{-5}$ ($1.74x10^{-5}$ - $2.61x10^{-5}$)	$5.28x10^{-7}$ ($4.39x10^{-7}$4 – $6.3x10^{-7}$)
	LoQ	2.427 (1.63 - 8.91)	$2.63x10^{-5}$ ($2.13x10^{-5}$ – $6.07x10^{-5}$)	$1.58 x 10^{-6}$ ($5.28x10^{-7}$ – $4.59x10^{-6}$)
Blood	LoD	1.32 (1.20 – 1.48)	$2.66x10^{-5}$ ($2.32x10^{-5}$ – $3.2x10^{-5}$)	$4.62x10^{-7}$ ($4.11x10^{-7}$ – $5.47x10^{-7}$)
	LoQ	2.5 (1.4 – 3.88)	$3.53x10^{-5}$ ($2.85x10^{-5}$ – $4.15x10^{-5}$)	$1.49 x 10^{-6}$ ($3.83x10^{-7}$ – $2.71x10^{-6}$)

*Calculated from samples with 500 ng and 20 µg of DNA input

5. Linearity:

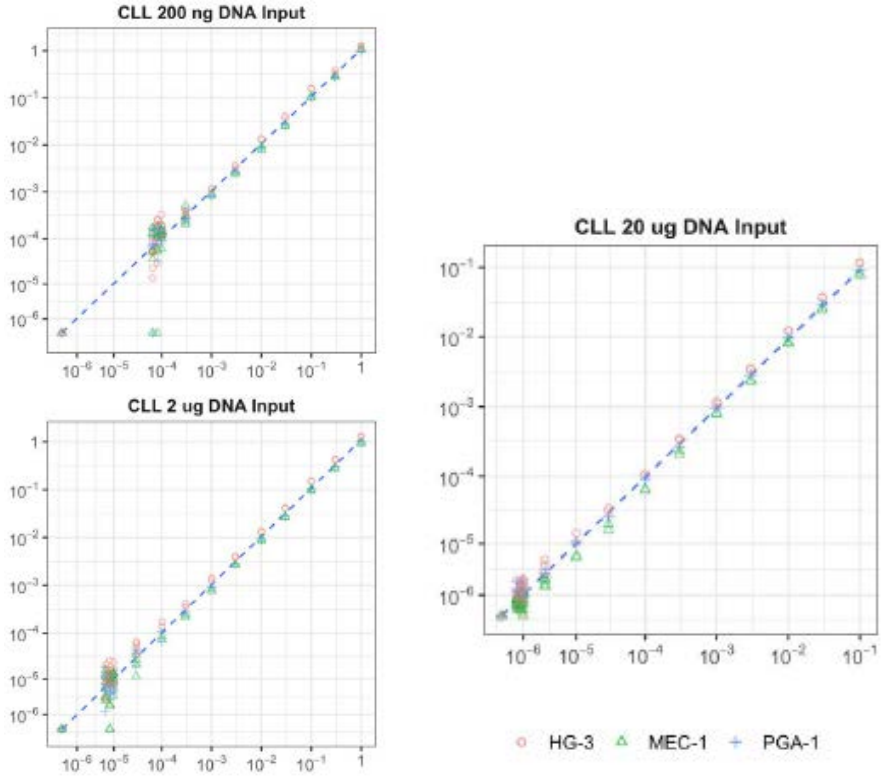
a) *Linearity with cell lines:* Linearity of the clonoSEQ Assay using 3 CLL cell lines (HG-3, MEC-1, and PGA-1) was evaluated by blending cell line gDNA with gDNA from healthy subjects using DNA inputs of 200 ng, 2 µg and 20 µg gDNA and tested at zero and across

11 MRD frequencies at each DNA input. This study was performed to measure the linearity of the clonoSEQ Assay across the indicated range of reporting. The frequency range of $6.0x10^{-5}$ to 1.0 was tested at the 200 ng DNA input. The frequency range of $6.5x10^{-6}$ to 1.0 was tested at the 2 µg DNA input. The frequency range of $6.6x10^{-7}$ to 0.1 was tested at 20 µg DNA input. The linear range of the assay was determined by finding the input range where the maximum deviation from linearity (based on a quadratic or cubic fit to the data) was less than 5%. Linearity was established for each sample input across the entire tested range (Table 4), with data shown in Figure 6. This study demonstrated linearity of MRD frequencies across several orders of magnitude.

Table 4. Linearity of the clonoSEQ Assay in BMA using Cell lines

Input DNA	Tested Range	Combined Analysis			Summary of Individual Patient Analyses	
		Linear Range	Slope	Intercept	Slope Range	Intercept Range
200 ng	0 to 1	0 to 1	0.994	0.018	0.978 to 1.011	-0.045 to 0.129
2 ug	0 to 1	0 to 1	1.004	0.034	0.998 to 1.016	-0.045 to 0.161
20 ug	0 to 0.1	0 to 0.1	0.994	-0.033	0.974 to 1.019	-0.159 to 0.111

Figure 6. Linearity of the clonoSEQ Assay in BMA. Expected (x-axis) and Observed (y-axis) MRD Frequency of 3 Cell Lines.



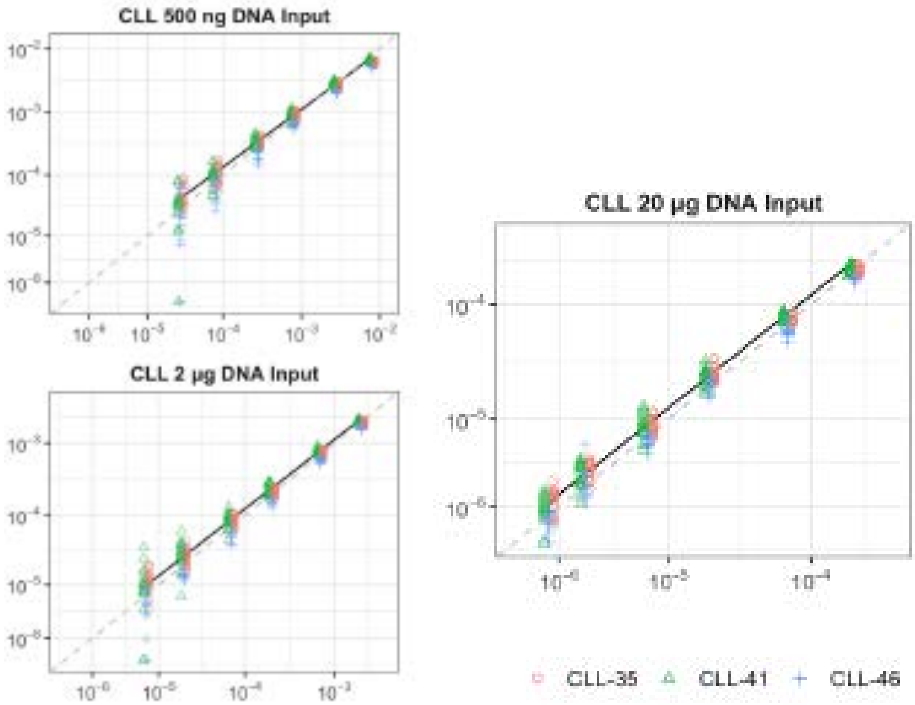
b) Linearity using Clinical BMA Specimens

Linearity was confirmed using clinical samples from the precision study (Section 2.4), which evaluated blended gDNA extracted from 22 CLL specimens at 3 DNA inputs and 6 MRD frequencies per DNA input. These data were re-analyzed to confirm linearity at the lower frequency range of the assay. The linear range of the assay was determined by finding the input range where the maximum deviation from linearity (based on a quadratic or cubic fit to the data) was less than 5%. Results are summarized in Table 5. The slopes and intercepts are reported as the average and range of values across all clinical specimens that were tested at each DNA input by disease indication. Results from 3 representative specimens for CLL are shown in Figure 7. This study demonstrated linearity across a wide range of MRD frequencies using clinical specimens.

Table 5. Linearity of clonoSEQ Assay in BMA using Clinical Specimens

Input DNA	Tested Range	Combined Analysis			Summary of Individual Patient Analyses	
		Linear Range	Slope	Intercept	Slope Range	Intercept Range
500ng	$2.8x10^{-5}$ to 0.008	$2.8x10^{-5}$ to 0.008	0.916	-0.216	0.847 to 1.004	-0.450 to 0.011
2ug	$7.0x10^{-6}$ to 0.002	$7x10^{-6}$ to 0.002	0.964	-0.057	0.877 to 1.043	-0.358 to 0.248
20ug	$7.0x10^{-7}$ to $2.0x10^{-4}$	$7.0x10^{-7}$ to $2.0x10^{-4}$	0.984	0.013	0.924 to 1.048	-0.417 to 0.272

Figure 7. Linearity of clonoSEQ Assay in BMA. The Expected (x-axis) and Observed (y-axis) MRD Frequency of 3 Clinical Samples



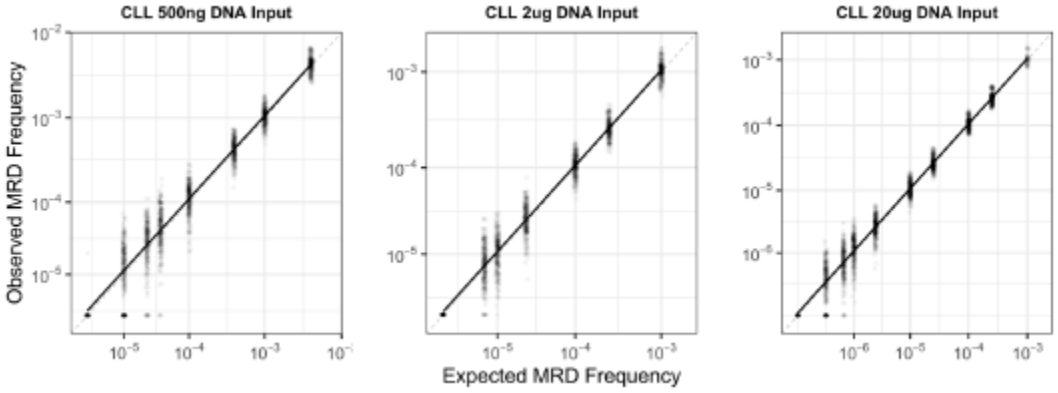
c) Linearity using Clinical Blood Specimens

Linearity was confirmed in blood using clinical samples from the precision study (Section 2.4), which evaluated blended gDNA extracted from 15 CLL specimens at 3 DNA inputs and 6 MRD frequencies per DNA input. The linear range of the assay was determined by finding the input range where the maximum deviation from linearity (based on a quadratic or cubic fit to the data) was less than 5%. Results are summarized in Table 6. The slopes and intercepts are reported as the average and range of values across all clinical specimens that were tested at each DNA input. Results are shown in Figure 8. This study demonstrated linearity across a wide range of MRD frequencies using clinical blood specimens.

Table 6. Linearity using Clinical CLL Blood Specimens

Input DNA	Tested Range	Combined Analysis			Summary of Individual Patient Analyses	
		Linear Range	Slope	Intercept	Slope Range	Intercept Range
20ug	0 to $1x10^{-3}$	0 to $1x10^{-3}$	0.997	-0.009	0.942 to 1.044	-0.515 to 0.51
2ug	0 to $1x10^{-3}$	0 to $1x10^{-3}$	0.995	-0.030	0.93 to 1.049	-0.78 to 0.53
500ng	0 to $4x10^{-3}$	0 to $4x10^{-3}$	0.989	-0.075	0.941 to 1.092	-0.628 to 0.872

Figure 8. Linearity of the clonoSEQ Assay in CLL Blood. The Expected (x-axis) and Observed (y-axis) MRD Frequency of 15 Clinical Samples



6. Analytical Specificity/Interference:

Interference of the assay was evaluated in CLL specimens. Testing was performed to characterize the effects of 5 endogenous (Table 7) and 3 exogenous (Table 8) substances in BMA and blood samples on the clonoSEQ Assay to identify potential interfering substances. BMA was tested with K2EDTA, Chloroform and Heparin and blood was tested with K2EDTA, K3EDTA, and Heparin. The potential exogenous and endogenous substances were spiked separately into 250 µL aliquots of bone marrow or 2 µL blood from 4 different donors. Each condition was replicated for a total of eight times (4 donors with 2 replicates each) and all conditions passed the pre-specified MRD frequency equivalence margin of ± 30%. For each endogenous and exogenous substance, two-one sided t-tests demonstrated a mean MRD frequency difference > -30% and < 30%, when comparing MRD frequency with

and without interferent substances. These results support the claim that each interfering substance tested does not have an effect on the clonoSEQ output for a given sample.

This study concluded that MRD results were not substantially influenced by the presence of the tested interfering substances in either blood or BMA.

An additional assessment of four CLL clinical blood specimens was performed with inclusion of K3EDTA at low and high (3x) concentrations. The MRD results of both high and low K3EDTA were within the confidence intervals of the baseline MRD measurement for all four CLL specimens. The assay performance with K3EDTA in clinical samples was within assay variation at the baseline measurement.

Table 7. Endogenous Interfering Substances Tested

Substance Name	Reference Level (Low)	Recommended Concentration (High)	Acceptance Criteria: Pass/Fail
Bilirubin conjugated	3.4 µmol/l	342 µmol/l	Pass
Bilirubin unconjugated*	21 µmol/l	342 µmol/l	Pass
Hemoglobin	1 g/l	2 g/l	Pass
Cholesterol*	5.2 mmol/l	13 mmol/l	Pass
Triglycerides	3.7 mmol/l	37 mmol/l	Pass

* Chloroform was used as solvent to resuspend bilirubin (unconjugated) and cholesterol.

Table 8. Exogenous Interfering Substances Tested

Substance Name	Concentration (Low)	Concentration (High)	Acceptance Criteria: Pass/Fail
K_2EDTA*	1.8 mg/ml	3.6 mg/ml	Pass
K_3EDTA^{Y}	1.8 mg/ml	3.6 mg/ml	Pass
K_3EDTA$^{¥}$	1.8 mg/ml	5.4 mg/ml	N/A$^{¥}$
Heparin	15 USP U/µ	30 USP U/ml	Pass
Chloroform (solvent)†	2.5 µl	N/A	Pass

* BMA and blood samples were shipped to Adaptive containing 1.8 mg/ml EDTA ("Low" concentration) for anti-coagulation purposes. Additional EDTA was spiked in to achieve the High K2EDTA level.

† Chloroform inhibition was tested at a single spiked-in volume (2.5 µl), in only bone marrow.

^{Y}Assessed for healthy blood samples.

$^{¥}$Assessed only for CLL patient blood samples. Assay performance with interferent was within assay variation at the baseline measurement.

7. Carry-Over:

The assessment of cross-contamination included multiple studies; one study to measure contamination of ID samples during automated DNA extraction of BMA and BMMCs, one study to measure contamination causing false ID or false MRD results in samples during

automated DNA extraction of blood, and one study to measure contamination of DNA from MRD samples during PCR, library pooling, and sequencing with the clonoSEQ Assay.

Cross-contamination of ID samples during automated DNA extraction was assessed using a panel of lymphoid malignancy cell lines (3 ALL and 3 MM) each spiked to 10% of total cells in a BMA pool of 2 healthy subjects or a bone marrow mononuclear cell (BMMC) pool of 4 healthy subjects. PBS (blank) samples were included in this study. Samples were evaluated as to whether they correctly calibrated. There were no false calibrations for run-to-run with 0/44 BMA and 0/44 BMMC false calibrations. There was one false calibration for the well-to-well study with 1/44 BMA and 0/44 BMMC samples falsely calibrating. The falsely calibrated sequence was found in a PBS sample with 83 total templates and the sequence was not associated with any of the 6 cell lines. The PBS sample provided a sensitive test for contamination since there was no background DNA and a contamination of 83 templates would not be expected to cause false calibration of a clinical specimen.

Cross contamination during extraction of blood samples leading to incorrectly calling samples ID calibrated or MRD positive was determined using a panel of lymphoid malignancy cell lines (2 MM cell lines and 4 ALL cell lines), either individually spiked into background normal healthy blood at a 10% frequency, or by pooling the clinical cell line samples and spiking this pool into a background normal blood sample at a target of 10^{-5} PBS (blank) samples were included in this study. Samples were evaluated for ID calibration results and MRD results. There were no contamination or clone-sharing events that resulted in false positive ID or MRD results.

Cross contamination of incorrectly calling samples MRD positive was assessed using gDNA from blood from healthy subjects as MRD-negative specimens and blends of cell line gDNA and gDNA from blood of healthy subjects spiked to a concentration of 5%. The 5% level was used to simulate a patient with clinical relapse. This study evaluated for the presence of a clonal sequence and molecular barcode simultaneously. There were no run-to-run contamination events observed in 0/36 tests. Well-to-well cross contamination was observed in 8/712 comparisons; this was likely caused by contamination of a primer barcode plate sourced from a vendor. All contamination events were below $4x10^{-6}$. Cross contamination between samples from the same patient is prevented by process controls that disallow co-processing of samples from the same patient.

8. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) In-Use Reagent Stability

An in-use stability study was executed to determine stability needs of the clonoSEQ Assay for reaction mixes and intermediate steps. The following critical steps were evaluated: pre-amp and PCR primer mix stability, master mix stability, complete reaction stability, and process pause stability. gDNA was tested using seven replicates for all conditions tested. Acceptance criteria were based on sequencing results meeting all QC metrics; all of the conditions tested met the pre-specified acceptance criteria and the clonoSEQ Assay in-use stability needs.

b) Real Time Stability of Pre-Amp and PCR Mixes

The real-time reagent stability studies used the primer QC processes to assess primer performance and determine primer stability. The primer QC process uses a set of synthetic double-stranded molecules representing rearrangements of the targeted exons to determine whether each manufactured lot of pre-amp PCR primers and PCR primers are performing within specification. The priming sites on synthetic molecules are identical to biologic priming sites on targeted exons. Data from these molecules were analyzed and assessed for the ability of the primers to amplify each identified exon at acceptable levels and the presence of primer sequences. These data were used to confirm that the performance of the pre-amp and PCR primers was adequate and consistent with previous primer lots. The performance of the amplification of the synthetic molecules met the pre-specified acceptance criteria.

This real-time reagent stability study established a 15-month shelf life of pre-amp and PCR primer mixes when stored at -20 ± 5 °C. These data were confirmed by assessing the equivalence of MRD frequency in 40 clinical samples amplified with primer lots of different ages, and by tracking the stability of MRD measurements of synthetic molecules over time. The conditions tested in the real time stability study met the pre-specified acceptance criteria of a pairwise equivalence test of clinical specimens to be within ± 30% MRD frequency.

c) Sample Stability

Sample Storage and Shipment Stability
Stability of CLL specimens was assessed for the current indicated time and temperatures. The study evaluated the stability of clinical CLL blood samples including at -15°C to -25°C, 2°C to 8°C, 15°C to 25°C and up to 3 freeze thaw cycles. Sample stability at -15°C to -25°C was assessed up to 6 months, 2 °C to 8 °C for up to 14 days, and 15°C to 25°C up to 5 days. Study results demonstrated that samples are stable based on an allowable 30% MRD frequency variation.

Specimens assessed by the clonoSEQ Assay were stable for the following storage conditions:
• At -15 °C to -25 °C for up to 18 months for BMA and up to 6 months for blood
• At 2 °C to 8 °C for up to 7 days for BMA and up to 14 days for blood
• At 15 °C to 25 °C for up to 3 days for BMA and up to 5 days for blood
• Up to 3 freeze/thaw cycles for BMA and blood

Specimens assessed by the clonoSEQ Assay were stable within the clonoSEQ shipper for ambient, summer and winter shipping conditions:
• For up to 4 days for BMA
• For up to 5 days for blood

9. Accuracy (Instrument):

a) Concordance with multi-parameter flow cytometry (mpFC) in Blood Clinical Samples

Clinical accuracy of the clonoSEQ Assay was assessed by comparing concordance in two ways: (1) concordance of MRD positive or negative calls and (2) concordance of quantitative MRD frequency. This study compared MRD results reported by the clonoSEQ Assay to multi-parameter flow cytometry (mpFC). The flow study included 299 matched samples. In a comparison of qualitative calls between mpFC and the clonoSEQ Assay, MRD negativity was defined as $< 10^{-4}$ for flow and MRD < LoD for

the clonoSEQ Assay. The positive percent agreement (PPA) between the clonoSEQ Assay and flow was 98.9%, with a 95% confidence bound of 94.3%-100%. The negative percent agreement (NPA) was 47.5%, with a 95% confidence bound of 40.5%-54.6% (Table 9). A PPA > 98% demonstrates the high concordance of positive calls between flow and the clonoSEQ Assay, while an NPA < 50% reflects the greater sensitivity of the clonoSEQ Assay, with 107 samples being called MRD positive by clonoSEQ and MRD negative by flow (Table 9). These results demonstrate the high concordance between the MRD calls of these technologies.

Table 9. Summary of mpFC vs. the clonoSEQ Assay Concordance Data for CLL

	flow MRD+	flow MRD-	PPA (95% CI)	NPA (95% CI)
clonoSEQ MRD+	94	107	98.9% (94.3-100%)	47.5% (40.5-54.6%)
clonoSEQ MRD-	1	97		

B Comparison Studies:

1. Method Comparison with Predicate Device:

   N/A

2. Matrix Comparison:

   Comparison of paired blood and bone marrow sample by the clonoSEQ Assay

   MRD values by sample type were compared by evaluating paired CLL blood and bone marrow samples from patients using the clonoSEQ Assay. For all patients and MRD time points for which paired blood and bone marrow samples were processed by the clonoSEQ Assay, the relative MRD frequencies in these two sample types were compared. The PPA, NPA, OPA, PPV, and NPV with (95% score confidence limits) for detectable MRD (sample MRD frequency > 0) in blood relative to bone marrow were calculated from the values in Table 10

Table 10. Two-by-two table of MRD detection by clonoSEQ from blood with bone marrow

		Bone Marrow		
		Detected	Undetected	Total
Blood	Detected	19	1	20
	Undetected	3	3	6
	Total	22	4	26

The comparison of MRD frequencies between paired blood and bone marrow samples is shown in Figure 9. Deming and Passing-Bablok regressions analysis (Table 11) were performed on the log10 based frequencies between these two samples types (blood vs bone marrow).

Figure 9. Comparison of MRD detection in clonoSEQ blood and bone marrow

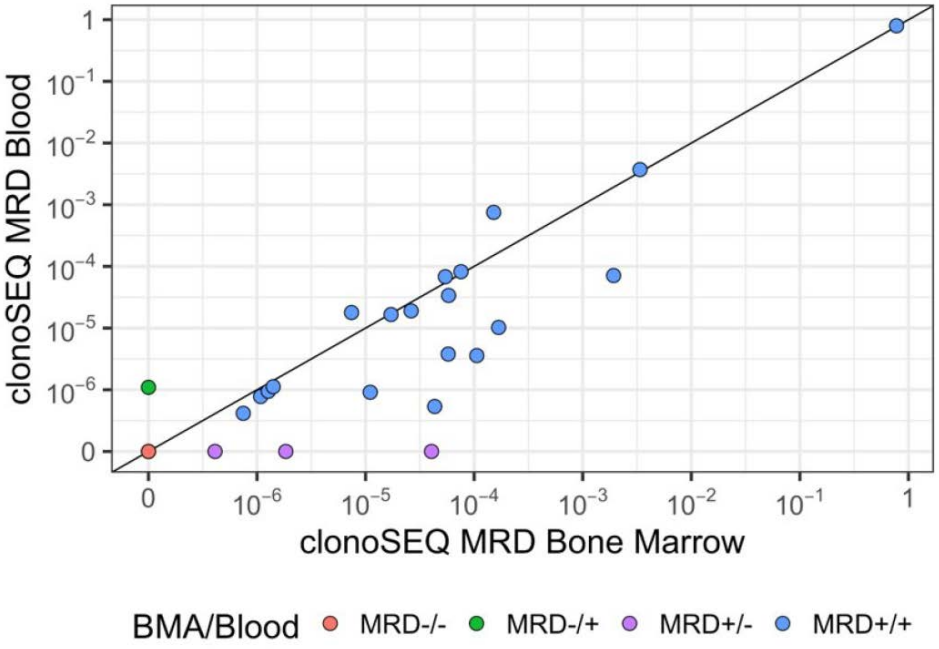


Table 11. Regression of clonoSEQ MRD levels compared between blood and bone marrow specimens

		Intercept			Slope			
Method	n	Estimate	LCL	UCL	Estimate	LCL	UCL	Pearsons r
Deming	26	-0.30	-1.60	0.54	1.03	0.79	1.21	0.87
Passing Bablok	26	-0.15	-1.00	0.00	1.01	0.84	1.23	0.87

Note that prior to model fitting, MRD frequencies are log transformed, with a pseudo frequency of 10^{-6} added to all values (0 cannot be log transformed). For Passing- Bablok regression, MRD frequencies were converted to frequencies per million nucleated cells (i.e. MRD frequency10^6).

CLL is a disease with compartmental effect. MRD status in blood may not be reflected in bone marrow.

C Clinical Studies:

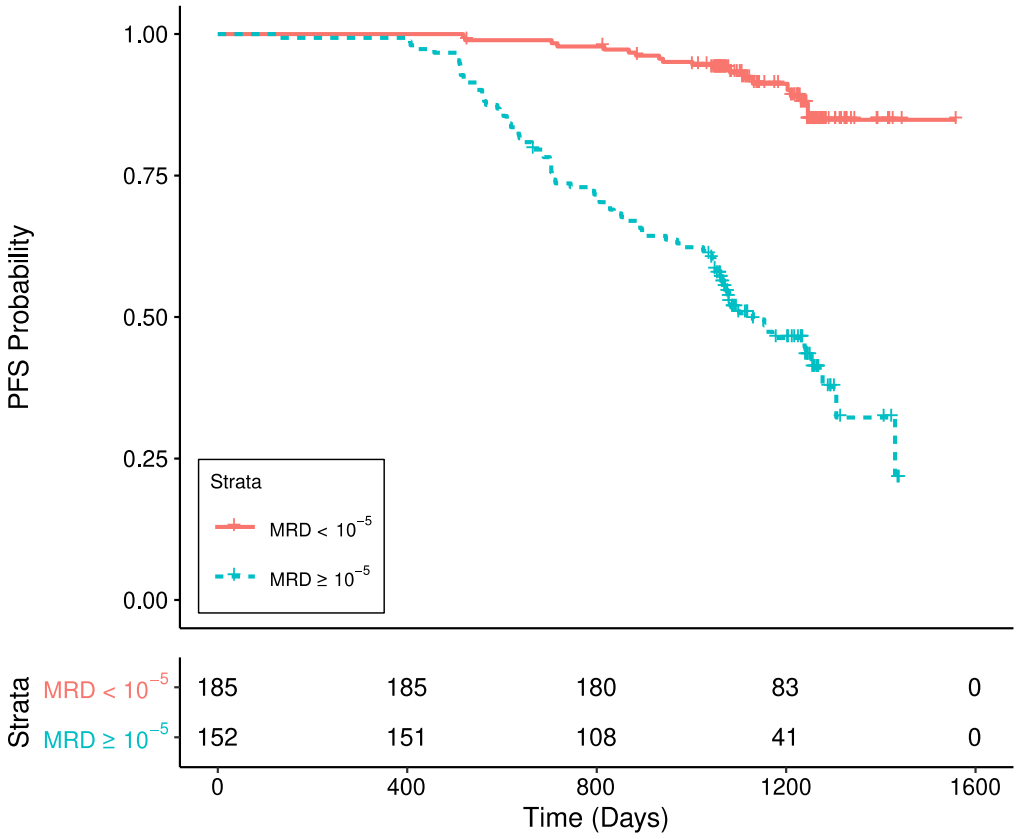
Two separate studies were analyzed to support that MRD as estimated with the clonoSEQ Assay is prognostic of patient outcomes in CLL, including data from clinical trials NCT02242942 and NCT00759798.[1, 2, 3]

The primary objective of the first study was to evaluate the ability of the clonoSEQ Assay to predict progression-free survival (PFS) at the MRD threshold of 10^{-5} using available blood samples from patients accrued under clinical trial NCT02242942, protocol BO25323, "A Prospective, Open-Label, Multicenter Randomized Phase III Trial to Compare The Efficacy and Safety of A Combined Regimen of Obinutuzumab and Venetoclax (GDC-0199/ABT-199) Versus Obinutuzumab and Chlorambucil in Previously Untreated Patients With CLL and Coexisting Medical Conditions."[1] The study was also designed to evaluate the clinical validity of the clonoSEQ Assay using continuous MRD measures and monitoring MRD across multiple time points.

Clinical trial NCT02242942 is a Phase III randomized trial to compare, in previously untreated patients, the efficiency and safety of a combined regimen of obinutuzumab and venetoclax (GDC-0199/ABT-199) versus obinutuzumab and chlorambucil. Samples and outcomes data were collected from 445 patients. For the clinical trial, blood was collected at multiple timepoints during and after treatment; for this study, only samples collected three months following treatment (FUM3) or later were included in analyses. While all available specimens from this trial were tested with the clonoSEQ Assay, 359 of the 445 patients originally enrolled in clinical trial NCT02242942 had both clinical outcomes data and sample material from the FUM3 timepoint available for analysis. Of these, one patient's sample failed QC, leaving 358 with usable clonoSEQ Assay MRD data. Twenty-one patients progressed prior to the FUM3 timepoint, leaving 337 patients for primary analysis.

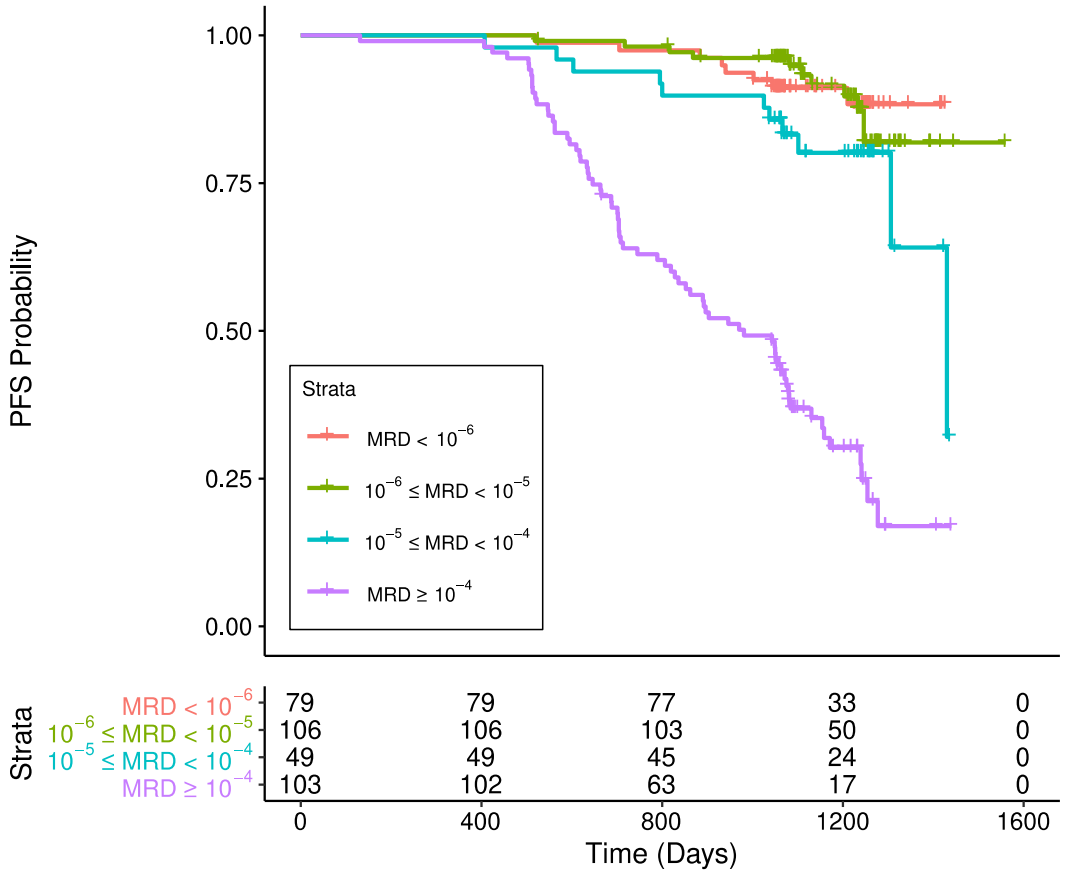
The ability of MRD measurements from the clonoSEQ Assay at FUM3 to predict PFS was evaluated in 337 patients. MRD negativity at $< 10^{-5}$ by the clonoSEQ Assay significantly predicted PFS (likelihood ratio $P = 3.075 \times 10^{-19}$; log-rank $P = 7.38 \times 10^{-12}$, Figure 10), with a 6.64-fold higher event risk in MRD positive patients (MRD $\geq 10^{-5}$) compared to MRD negative patients (95% CI: 3.65-12.1). Cox regression analysis of PFS using continuous MRD values demonstrated that the clonoSEQ Assay is significantly associated with PFS (likelihood ratio $P = 2.96 \times 10^{-27}$) and that each 10-fold increase in MRD level is associated with a 2.35-fold increase in event risk (95% CI: 1.86-2.48). The results were also analyzed for confounding by other variables and indicated that the MRD level at FUM3 is a stronger predictor of PFS than age, sex, geographic region, Binet stage, or treatment arm of the clinical trial. Together, these results demonstrate the clinical validity of MRD measurement in CLL.

Figure 10. Kaplan-Meier Survival Curves for PFS given clonoSEQ Assay MRD Calls at a Threshold of 10^{-5} at FUM3



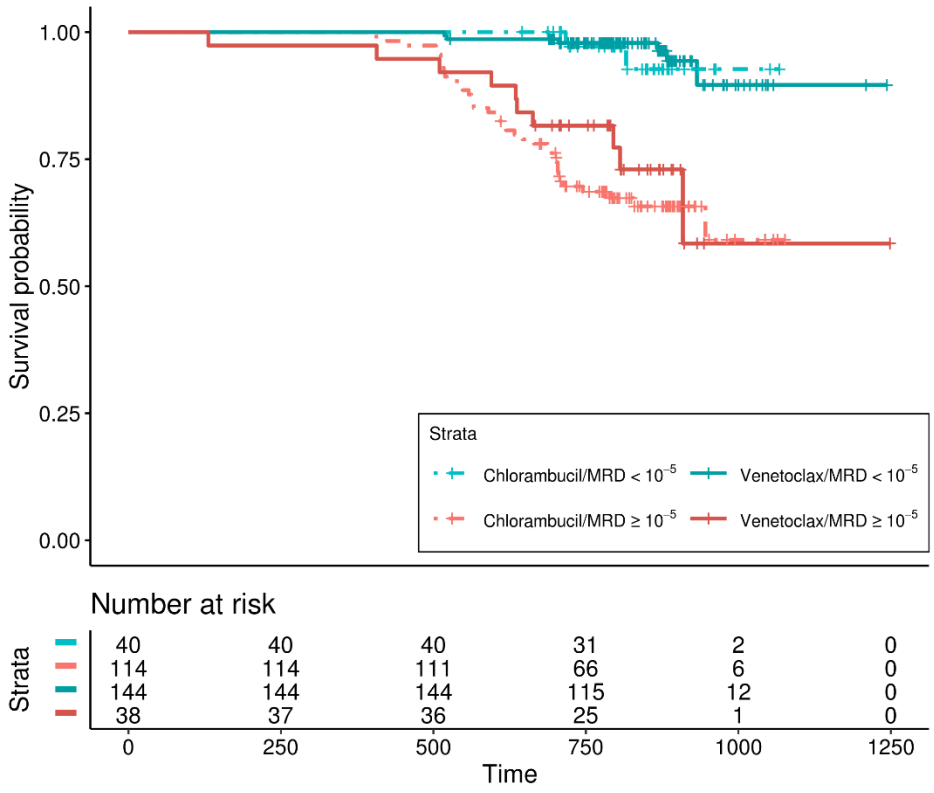
The clonoSEQ Assay was also used to assess MRD at various disease burden thresholds to determine the correlation of MRD level with PFS. Patients with clonoSEQ MRD $\leq 10^{-6}$ or between 10^{-6} and 10^{-5} had longer PFS, followed by patients with MRD between 10^{-5} and 10^{-4} and patients with MRD $\geq 10^{-4}$ (log-rank P = 4.902 x 10-31, Figure 11). These data demonstrate that patients with MRD $\leq 10^{-5}$ have better outcomes than patients with MRD > 10^{-5}, and that increasing MRD levels above 10^{-5} are associated with an increased risk of progression within the follow-up time of this study.

Figure 11. Kaplan-Meier Survival Curves for PFS using the clonoSEQ Assay with 4 MRD Bins in CLL at FUM3: $<10^{-6}$, $10^{-6} – 10^{-5}$, $10^{-5} – 10^{-4}$, $\geq 10^{-4}$



The association with treatment arm reflects the relative efficacy of the different drugs used in this trial. Patients on the chlorambucil arm of the trial were less likely to meet the criteria for primary analysis, which is consistent with the results of the trial: patients receiving chlorambucil had lower PFS than those on Venetoclax. To assess whether this uneven sampling from the two treatment arms might have affected the results, the primary analysis of MRD was stratified at 10^{-5} by treatment arm. This analysis is shown in Figure 12, which confirms that the prognostic value of MRD is not confounded by therapy regimen: conditional on MRD status (positive or negative), PFS did not differ between treatment arms.

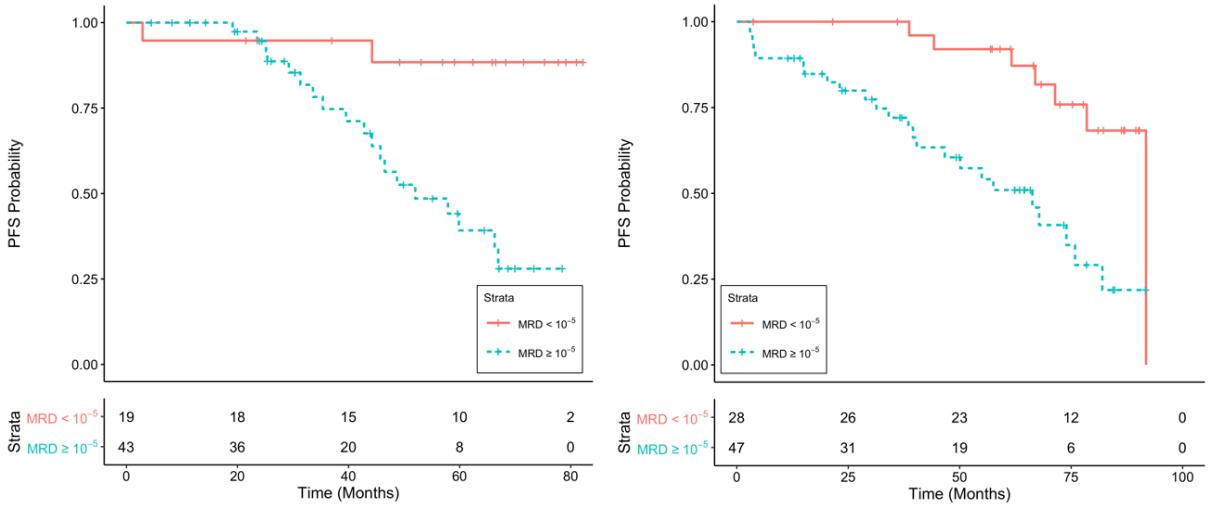
Figure 11. Kaplan-Meier Survival Curves for PFS given clonoSEQ Assay MRD Calls at a Threshold of 10^{-5} at FUM3, Stratified by Treatment Arm. The strata in the table of sample counts are shown in order of (1) chlorambucil/MRD < 10^{-5}, (2) chlorambucil/MRD ≥ 10^{-5}, (3) Venetoclax/MRD < 10^{-5}, (4) Venetoclax/MRD ≥ 10^{-5}.



The second study, Thompson et al, was a prospective, phase 2 clinical trial that evaluated six cycles of fludarabine, cyclophosphamide, and rituximab (FCR) in 111 front-line chronic lymphocytic leukemia (CLL) patients with clonoSEQ ID samples and a corresponding 137 clonoSEQ MRD samples also evaluated by 4-color flow cytometry at an MRD threshold of 10-4 (NCT00759798) and with pertinent co-variate data. Within this cohort of 111 patients with flow MRD results, bone marrow was available for 75 patients and blood was available for 62 patients, of which 26 patients provided both blood and bone marrow. Due to some missing clinical covariates, 3 patients that provided bone marrow only, were excluded from analyses requiring these covariates.

There was an association between PFS and continuous clonoSEQ MRD measurement in both blood and bone marrow, after end of treatment, where PFS is defined as the time from start of treatment until death, disease progression, or last time of disease assessment ($p = 9.66 \times 10^{-4}$ for blood, $p = 2.13 \times 10^{-4}$ for bone marrow). Additionally, patients who were MRD negative at a threshold $\leq 10^{-5}$ had superior progression-free survival compared to patients with MRD > 10^{-5} ($p = .02$ for blood and $p = 8.17 \times 10^{-5}$ for bone marrow, Figure 13).

Figure 23. Kaplan-Meier Survival Curves for PFS given clonoSEQ Assay MRD Calls at a Threshold of 10^{-5} post-treatment (Left: Blood, Right: Bone Marrow)



This association was also demonstrated at a threshold of 10^{-4} and 10^{-6} Figures 14 and 15)

Figure 14. Kaplan-Meier Survival Curves for PFS given clonoSEQ Assay MRD Calls at a Threshold of 10^{-4} post-treatment (Left: Blood, Right: Bone Marrow)

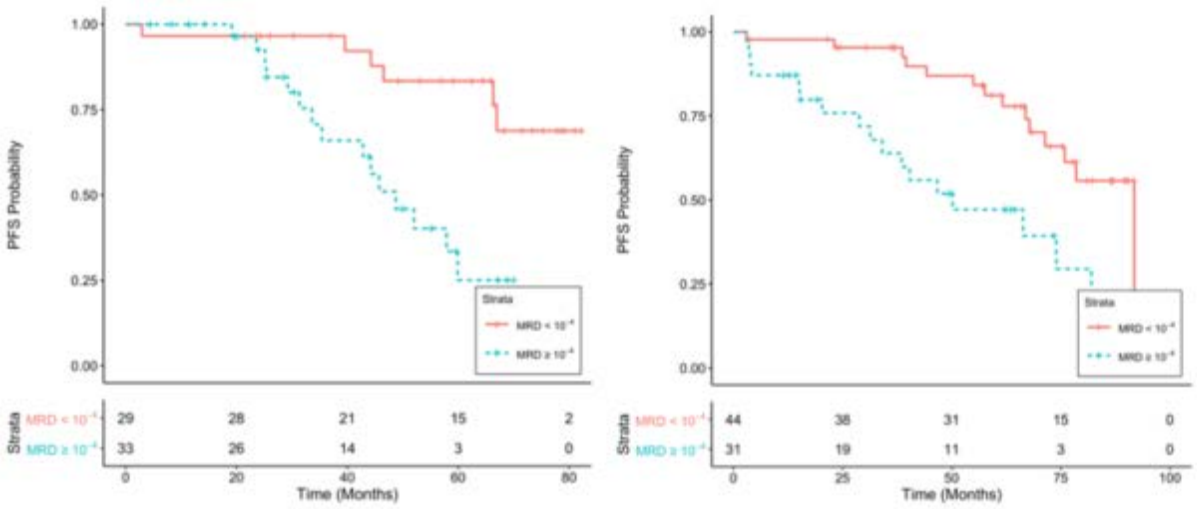
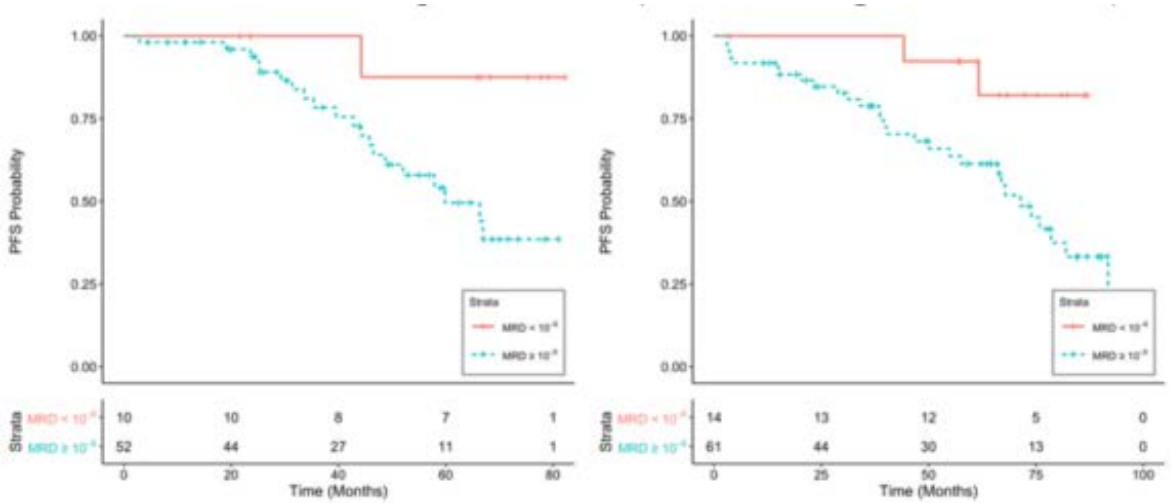


Figure 15. Kaplan-Meier Survival Curves for PFS given clonoSEQ Assay MRD Calls at a Threshold of 10^{-6}



Taken together these results support the use of the clonoSEQ assay in CLL patients. Limiting statements for testing in CLL included in the labeling are described below:

- MRD is based on measurements of tumor cells detected in peripheral blood and/or bone marrow. However, patients may have significant residual disease in unassessed compartments and U-MRD in one compartment cannot fully rule out the presence of disease in the other compartment, for example, U-MRD in blood may not be the same in bone marrow. Therefore, assessment of MRD in CLL should employ a multimodal approach including clinical examination, patient medical history, and other findings.
- Outcome for patients with MRD detectable in bone marrow but not in peripheral blood (PB-/BM+) may differ according to type of therapy.
- This assay is capable of monitoring specific tumor clonotypes. The association between MRD assessments and patient clinical status for the purpose of monitoring changes in disease (e.g., relapse, remission, stable disease) has not been demonstrated.
- The value of MRD in CLL for previously untreated or "watch and wait" patients is not established.
- CLL is a heterogeneous disease. MRD values and expectations for outcome may not be generalizable across treatments. Changes in MRD should be interpreted with caution when used to evaluate disease burden in therapies that have not been validated.
- Regardless of MRD status, cytogenetics plays an independent role in patient risk status and its impact on PFS/OS.

References

- Thompson P. et al, Undetectable MRD Using Next Generation Sequencing is Associated with Improved PFS After Treatment with FCR for CLL. Blood 2019 28;134(22):1951-1959
- Fischer K. et al, Venetoclax and Obinutuzumab in Patients with CLL and Coexisting Conditions. New England Journal of Medicine.2019; 380:2225-2236.

- Prospective Identification of Significant Prognostic Factors in Patients Treated with Fludarabine, Cyclophosphamide, and Rituximab (FCR) as Initial Therapy for Chronic Lymphocytic Leukemia (MDA2008-0431), NCT00759798

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

Not applicable.

F Other Supportive Instrument Performance Characteristics Data:

Performance between the Nextseq500 and 550 instruments were assessed through analytical studies on precision and linearity.

The sponsor provided a summary of literature to include 24 articles published between 2007 and 2020 that supported the prognostic impact of MRD in CLL using both flow based and nucleic acid based technologies, further confirming the role of these assessments in CLL patients.

VIII Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type.

VIII. Patient Perspectives

This submission did include patient perspective on the role of MRD testing. Oncologists specializing in the management of CLL patients also shared perspectives regarding patient preferences.

IX. Conclusion:

The submitted information in these 510(k) notifications supports the addition of CLL to the Indications for Use for the clonoSEQ assay and demonstrates that clonoSEQ assay is as safe and effective as the predicate device and therefore supports a substantial equivalence conclusion.