

clonoSEQ®

CHRONIC LYMPHOCYTIC LEUKEMIA

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia.¹ When diagnosed, patients are assessed for risk status to determine whether/when to initiate treatment and to inform treatment selection.^{1,2} An important aspect of response assessment in CLL is evaluating minimal residual disease, also known as measurable residual disease (MRD).

According to clinical practice guidelines, undetectable MRD (U-MRD or MRD negativity) in the peripheral blood following treatment is an important predictor of treatment efficacy.²

ABILITY TO PREDICT CLINICAL OUTCOMES

clonoSEQ predicts outcomes three months after the completion of therapy

clonoSEQ® predicts progression free survival

The CLL14 study assessed the combination of obinatumab plus venetoclax versus obinatumab plus chlorambucil in previously untreated CLL patients with coexisting conditions. MRD was assessed in peripheral blood samples from 337 patients three months after completion of therapy using an MRD threshold of 10^{-5} . U-MRD by clonoSEQ significantly predicted progression-free survival (PFS; $P = 3.075 \times 10^{-19}$; Figure 1) with a 6.64-fold higher event risk in MRD-positive patients compared to U-MRD patients (95% CI: 3.65-12.1).³

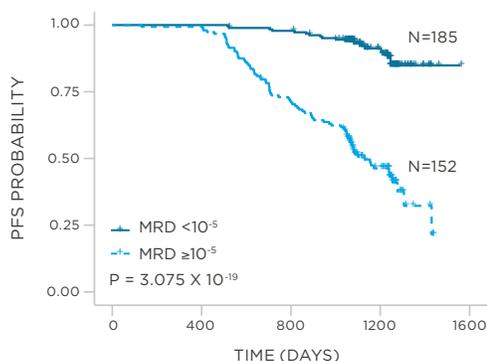


Figure 1: Kaplan-Meier survival curves for PFS given clonoSEQ MRD detection at a threshold of 10^{-5} three months after completion of therapy

THE CLONOSEQ ASSAY HAS DEMONSTRATED SEVERAL CLINICAL BENEFITS

- U-MRD by clonoSEQ significantly predicted PFS with a 6.64-fold higher event risk in MRD-positive patients compared to U-MRD patients.³
- Patients with U-MRD or very low levels of MRD ($<10^{-5}$) have better outcomes than patients with higher levels of MRD ($\geq 10^{-5}$).³
- clonoSEQ identifies MRD in cases where disease is not detected by flow cytometry.³
- Both bone marrow and peripheral blood can be used for MRD assessment by clonoSEQ as both sample types have been shown to predict clinical outcomes.³

clonoSEQ® is available as an FDA-cleared *in vitro* diagnostic (IVD) test service provided by Adaptive Biotechnologies to detect measurable residual disease (MRD) in bone marrow from patients with multiple myeloma or B-cell acute lymphoblastic leukemia (B-ALL) and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL). clonoSEQ is also available for use in other lymphoid cancers as a CLIA-validated laboratory developed test (LDT) service. For important information about the FDA-cleared uses of clonoSEQ including test limitations, please visit clonoSEQ.com/technical-summary.

Lower levels of MRD are correlated with better outcomes

When assessing the correlation between clinical outcomes and continuous clonoSEQ MRD levels (vs. at a pre-specified sensitivity threshold), a patient's risk of an event was shown to increase by 2.15-fold for every 10-fold increase in MRD (95% CI: 1.86-2.48).

Patients with U-MRD or very low levels of MRD detected by clonoSEQ have the best outcomes. Patients with clonoSEQ MRD $< 10^{-6}$ or between 10^{-6} and 10^{-5} have the longest PFS, followed by patients with MRD between 10^{-5} and 10^{-4} and patients with MRD $\geq 10^{-4}$ ($P = 4.902 \times 10^{-31}$). These data demonstrate that patients with MRD $< 10^{-5}$ have better outcomes than patients with MRD $\geq 10^{-5}$, and that increasing MRD levels above 10^{-5} are associated with an increased risk of disease progression within the follow-up time of this study.³

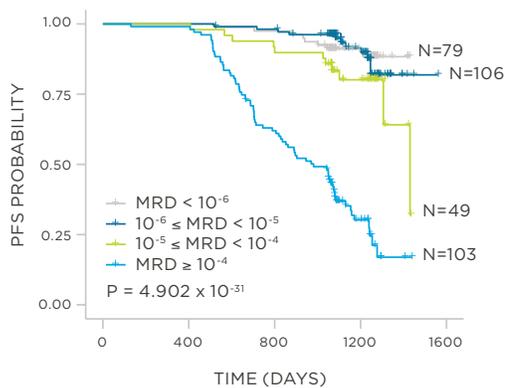


Figure 2: Kaplan-Meier Survival Curve for PFS using clonoSEQ at four MRD levels in CLL: $<10^{-6}$, $10^{-6} - 10^{-5}$, $10^{-5} - 10^{-4}$, $\geq 10^{-4}$.

clonoSEQ is more predictive than other factors

The results also show that the MRD level is a stronger predictor of PFS than other prognostic variables identified as clinically relevant covariates, or the treatment arm of the clinical trial.³

clonoSEQ identified additional patients with disease compared to flow cytometry

In this correlation study, 299 samples were assessed by flow cytometry and clonoSEQ. In a comparison of qualitative calls between flow cytometry and clonoSEQ, MRD negativity was defined as MRD $< 10^{-4}$ for flow and MRD below the limit of detection for clonoSEQ. There was high concordance of positive calls between flow cytometry and clonoSEQ, while more than half of samples that were MRD-negative by flow cytometry (107 of 204 samples) were identified as MRD-positive by clonoSEQ (Table 1).³

Table 1: Summary of flow cytometry and clonoSEQ MRD results

	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		

CONCORDANCE BETWEEN BLOOD AND BONE MARROW

clonoSEQ predicts outcomes in both bone marrow and peripheral blood samples

In bone marrow samples, clonoSEQ is predictive of PFS

Thompson et al assessed 62 previously untreated CLL patients who received therapy with fludarabine, cyclophosphamide, and rituximab (FCR). These patients were initially determined to be U-MRD by 4-color flow cytometry at an MRD threshold of 10^{-4} . From these patients, 57 bone marrow (BM)

samples were evaluated at the end of treatment by clonoSEQ. Patients who achieved the lowest levels of MRD in the bone marrow (10^{-5} – 10^{-6}) or who had a U-MRD status ($<10^{-6}$) experienced the best outcomes. Patients who had a U-MRD status at a threshold of $<10^{-5}$ had superior PFS compared to patients with $MRD \geq 10^{-5}$ ($P = 0.01$; Figure 3).⁴

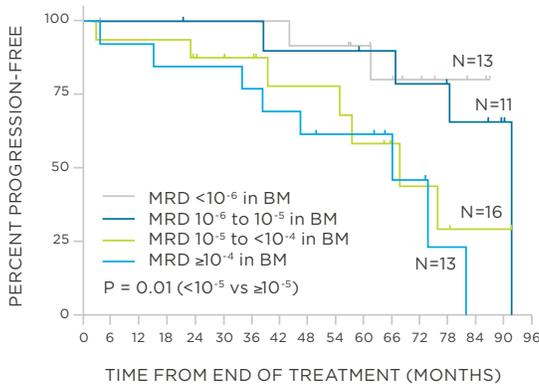


Figure 3: Patients who had a U-MRD status at a threshold of $<10^{-5}$ had longer PFS compared to MRD-positive patients ($\geq 10^{-5}$)

clonoSEQ predicts outcomes in both blood and bone marrow

An expanded analysis of the Thompson et al data set supported the clinical validation of clonoSEQ. This data shows a significant association between PFS and continuous clonoSEQ MRD measurement (vs. at a pre-specified sensitivity threshold) in both blood and bone marrow, after end of treatment ($P = 9.6 \times 10^{-4}$ for blood; $P = 2.13 \times 10^{-4}$ for bone marrow). Additionally, patients who had U-MRD status at a threshold $<10^{-6}$ had superior PFS compared to patients with $MRD \geq 10^{-6}$ ($P = 7.74 \times 10^{-2}$ for blood, Figure 4A; and $P = 8.10 \times 10^{-2}$ for bone marrow, Figure 4B).³

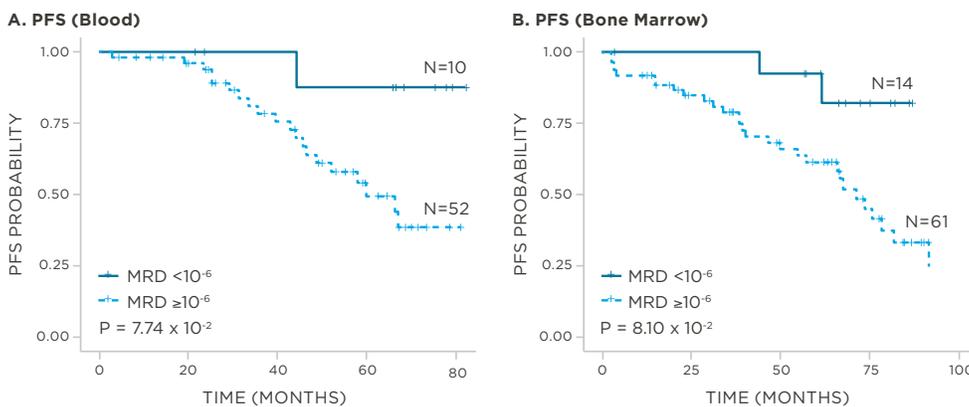


Figure 4A and B: Patients with U-MRD ($<10^{-6}$) had longer PFS compared to patients with detectable MRD whether assessed in blood or bone marrow.

There was high correlation between MRD results in blood and bone marrow

Analysis of 26 CLL patients comparing disease burden between blood and bone marrow demonstrated 85% concordance in positive/negative MRD calls. The median MRD level was 27% lower in blood than bone marrow which is consistent with previously published analysis.³

Table 2: Correlation of MRD results in blood and bone marrow in CLL

	Bone Marrow +	Bone Marrow -
Blood+	19	1
Blood -	3	3

Conclusions

- U-MRD by clonoSEQ significantly predicted PFS with a 6.64-fold higher event risk in MRD-positive patients compared to U-MRD patients.³
- Patients with U-MRD or very low levels of MRD ($<10^{-5}$) have better outcomes than patients with higher levels of MRD ($\geq 10^{-5}$).³
- clonoSEQ identifies MRD in cases where disease is not detected by flow cytometry.³
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REFERENCES

1. Turtle CJ, et al. *J Clin Oncol*. 2017;35(26):3010-20.
2. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Chronic Lymphocytic Leukemia/Small Lymphocytic Leukemia V.4.2020. © National Comprehensive Cancer Network, Inc. 2020. All rights reserved. Accessed June 9th, 2020. To view the most recent and complete version of the guideline, go to NCCN.org.*
3. clonoSEQ®. [technical summary]. Seattle, WA: Adaptive Biotechnologies Corporation; 2020.
4. Thompson P, et al. *Blood*. 2019;134(22):1951-1959

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Test Limitations

ALL, MM and CLL: MRD values obtained with different assay methods may not be interchangeable due to differences in assay methods and reagent specificity. The results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings. The clonoSEQ Assay is for use with specimens collected in EDTA tubes. Results may vary according to sample time within the course of disease or by sampling site location. The assay may overestimate MRD frequencies near the limit of detection (LoD). The MRD frequency LoD varies based on the amount of gDNA that is tested and using lower gDNA input may prevent MRD detection at low frequencies. Sample processing and cell enrichment strategies may affect the measured MRD frequency. The volume and cellularity of sampled input material may affect the ability to detect low levels of disease. False positive or false negative results may occur for reasons including, but not limited to: contamination; technical and/or biological factors such as the type of rearrangement or the size of the junction region. The assay has been validated with the Illumina NextSeq500 and 550.

For CLL: 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 play an independent role in patient risk status and its impact on PFS/OS.