clonoSEQ®

ACUTE LYMPHOBLASTIC LEUKEMIA

The NCCN Guidelines for ALL recommend measurable residual disease (MRD) testing at a sensitivity of 10^{-4} or better because of its clinical utility. Next-generation sequencing (NGS) is listed as one of the recommended methods for MRD assessment in these Guidelines.[1]

ADDITIONAL PATIENTS CAPTURED WITH CLONOSEQ MRD DETECTION

The sensitivity and specificity of clonoSEQ MRD enables robust detection of disease

Additional 55 MRD-positive patients captured

A study evaluated MRD in the bone marrow of 579 pediatric ALL patients. Next-generation sequencing MRD detection identified an additional 55 patients who were MRD-positive by the clonoSEQ Assay and MRD-negative* by flow cytometry at a sensitivity level of 10^{-4}. 17 patients were identified as MRD-positive by flow cytometry and MRD-negative by clonoSEQ at a sensitivity of 10^{-4} (Table 1). When assessing MRD at a sensitivity level of 10^{-5}, clonoSEQ identified an additional 87 patients with disease who were MRD-negative by flow cytometry.[2]

clonoSEQ MRD is able to predict event-free survival (EFS) in pediatric ALL

This study demonstrated that 55 patients who had no detectable MRD by flow cytometry and who were MRD-positive by NGS had a worse EFS than those who were MRD-negative by NGS and had no detectable MRD by flow cytometry (sensitivity of 10^{-4}). Using an MRD cutoff level of 10^{-4}, flow cytometry identified 17 patients as MRD-positive that NGS identified as MRD-negative. When those 17 patients were assessed at an MRD cutoff level of 10^{-5}, NGS identified residual disease in 11 of these patients. Additionally, NGS MRD (sensitivity of 10^{-4}) was able to predict EFS in the standard risk subgroup. Patients who were NGS MRD-negative had longer EFS compared to the NGS MRD-positive patients (P=0.0226).[2]

THE CLONOSEQ ASSAY HAS DEMONSTRATED SEVERAL CLINICAL BENEFITS

- *The clonoSEQ Assay detected additional patients with residual disease who were initially classified as MRD-negative by flow cytometry.[2]*
- *The clonoSEQ Assay demonstrated concordance with traditional methods for MRD detection and offers increased sensitivity.[3]*
- *The clonoSEQ Assay has been shown to be predictive of relapse and survival.[4]*
- *The clonoSEQ Assay has been shown to have prognostic value in the post-transplant setting.[4]*

*** Per ALL clinical practice guidelines, MRD negativity is defined as the absence of detectable cancer cells using a method with a minimum sensitivity of 10^{-4} nucleated cells or higher.[1] MRD status should be evaluated in the context of clinical clinopathological features and is not a determination of the absence of disease.**

Table 1: *Comparison between the clonoSEQ Assay and flow cytometry (both assessed at 1/10,000)* [2,5]

Flow Cytometry MRD Status	clonoSEQ MRD Status	Number of Patients	P-value
Negative	Positive	55	0.036
Negative	Negative	409	
Positive	Positive	87	0.61
Positive	Negative	17	

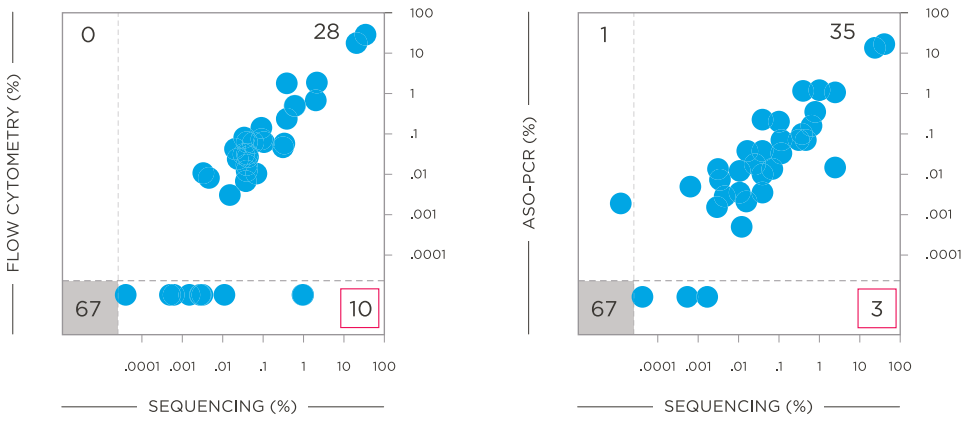
CONCORDANCE

The clonoSEQ Assay is highly concordant with traditional MRD detection methods in ALL

In a study of more than 100 pediatric ALL patients, the clonoSEQ Assay showed quantitative concordance with both flow cytometry and allele-specific oligonucleotide PCR (ASO-PCR; Figure 2).[3]

Increased sensitivity

The clonoSEQ Assay was able to detect additional patients with disease present below the detection limits of flow cytometry (N=10) and ASO-PCR (N=3), respectively (Figure 2, red boxes).[3] ASO-PCR identified one patient with residual disease that was MRD-negative by clonoSEQ.



The number of concordant measurements are shown in the lower left and upper right. The number of discordant measurements are shown in the upper left and lower right.

Boxed numbers highlight increased sensitivity provided by NGS MRD detection over other methods.

Figure 2: *Comparison between sequencing and flow cytometry and ASO-PCR*

PREDICTIVE POWER OF CLONOSEQ MRD

clonoSEQ MRD assessment pre-transplant may predict relapse and overall survival

Analysis of pre-transplant bone marrow samples from 41 pediatric patients with ALL found that clonoSEQ MRD detection predicted relapse and overall survival post-allogeneic transplant significantly better than 6-color flow cytometry (Table 2).[4]

Table 2:

	2-Year Relapse Probability
clonoSEQ MRD-Negative	0%
Flow MRD-Negative	16%
P-value	0.02

	2-Year Overall Survival Probability
clonoSEQ MRD-Negative	96%
Flow MRD-Negative	77%
P-value	0.003

PROGNOSTIC VALUE

clonoSEQ MRD assessment has demonstrated prognostic value post-transplant in the pediatric and adult ALL settings

Analysis of bone marrow samples from 53 pediatric patients analyzed post-allogeneic transplant showed that clonoSEQ can be used to predict relapse. In the case of discordant MRD determinations, there were 11 patients identified as clonoSEQ MRD-positive and flow cytometry MRD-negative and 3 patients identified as clonoSEQ MRD-negative and flow cytometry MRD-positive.[4]

Strong predictive power

One month after transplant, flow cytometry was unable to distinguish between patients who ultimately relapsed and those who did not (p=0.91; Figure 3). NGS MRD showed an estimated relapse probability of 67% in MRD-positive patients vs. 25% in MRD-negative patients (p=0.01).[4]

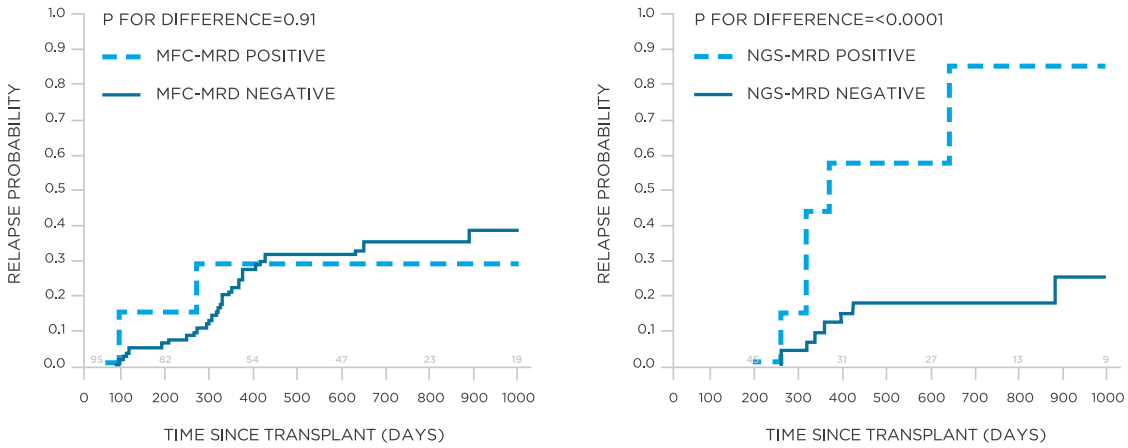


Figure 3: *Flow cytometry (MFC) vs. next-generation sequencing (NGS) MRD +30 days post-transplant*

Long range predictive power

Better predictive power of post-transplant NGS MRD detection vs. flow cytometry continued at day 100 and 8 months post-transplant.[4]

Long range predictive power

Better predictive power of post-transplant NGS MRD detection vs. flow cytometry continued at day 100 and 8 months post-transplant.[4]

Conclusions

- The clonoSEQ Assay detected additional patients with residual disease who were initially classified as MRD-negative by flow cytometry.[2]
- The clonoSEQ Assay demonstrated concordance with traditional methods for MRD detection and offers increased sensitivity.[3]
- The clonoSEQ Assay has been shown to be predictive of relapse and survival.[4]
- The clonoSEQ Assay has been shown to have prognostic value in the post-transplant setting.[4]

REFERENCES

1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Pediatric Acute Lymphoblastic Leukemia V.2.2020. © National Comprehensive Cancer Network, Inc. 2020. All rights reserved. Accessed March 11th, 2020. To view the most recent and complete version of the guideline, go to NCCN.org.

Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Acute Lymphoblastic Leukemia V.1.2020. © National Comprehensive Cancer Network, Inc. 2020. All rights reserved. Accessed March 11th, 2020. To view the most recent and complete version of the guideline, go to NCCN.org.*

2. Kirsch L, et al. *SIOP.* 2016; O-031.**

3. Faham M, et al. *Blood.* 2012;120(26):5173-5180.**

4. Pulsipher M, et al. *Blood.* 2015;125(22):3501-8.***

5. Wood B, et al. *Blood.* 2018;131(12):1350-1359.

***NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

***Study author was an employee of Adaptive at time of publishing.

***Study author's research was supported, in part, by product grants from Adaptive.

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.

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.

PM-US-cSEQ-0063-4