The NCCN Guidelines for ALL recommend 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

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* 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. MRD status should be evaluated in the context of clinical clinopathological features and is not a determination of the absence of disease.

The clonoSEQ Assay is sold as a CLIA-certified laboratory service and is not currently cleared by the FDA. clonoSEQ should not be used as the sole determinant of patient care.
Table 1: Comparison between the clonoSEQ Assay and flow cytometry (both assessed at 1/10,000)\textsuperscript{2,5}

<table>
<thead>
<tr>
<th>Flow Cytometry MRD Status</th>
<th>clonoSEQ MRD Status</th>
<th>Number of Patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>55</td>
<td>0.036</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>409</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>87</td>
<td>0.61</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

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).\textsuperscript{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).\textsuperscript{3} ASO-PCR identified one patient with residual disease that was MRD-negative by clonoSEQ.

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

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.

PREDICTIVE POWER OF CLONOSEQ MRD

clonoSEQ MRD assessment pre-transplant predicts 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).\textsuperscript{4}
**Table 2:**

<table>
<thead>
<tr>
<th></th>
<th>2-Year Relapse Probability</th>
<th>2-Year Overall Survival Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>clonoSEQ MRD-Negative</td>
<td>0%</td>
<td>96%</td>
</tr>
<tr>
<td>Flow MRD-Negative</td>
<td>16%</td>
<td>77%</td>
</tr>
<tr>
<td>P-value</td>
<td>0.02</td>
<td>0.003</td>
</tr>
</tbody>
</table>

**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

**Superior 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

**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

**Relapse prediction in the first 100 days**

A study of peripheral blood samples from 29 adult patients who had undergone allogeneic hematopoietic stem cell transplantation found that MRD positivity (>10^-6) in the first 100 days post-transplant using clonoSEQ, was highly predictive of relapse (Figure 4).6
Figure 4: MRD positivity (≥10^-6) at any time through day +100 post-transplant predicted subsequent relapse

3 month clinical relapse lead time
clonoSEQ MRD detection in peripheral blood was shown to provide 3 months lead-time before clinical relapse (range 0-207 days), which could offer an opportunity to apply additional therapeutic maneuvers while disease burden is low (Figure 5).6

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 measurable residual disease (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 Acute Lymphoblastic Leukemia V.1.2017. © National Comprehensive Cancer Network, Inc. 2017. All rights reserved. Accessed June 13, 2017. To view the most recent and complete version of the guideline, go online to NCCN.org.*

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


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