Monitoring Minimal Residual Disease in AML

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Monitoring for residual disease in AML

- **When to monitor**
  - After induction
  - After consolidation

- **How to monitor**
  - Bone marrow biopsy with <5% blasts
  - Cytogenetics
  - PCR for rearrangements
  - Flow cytometry
MRD predicts poor outcome after AML induction

MRD predicts poor outcome after stem cell transplant

Disease-free survival after allogeneic stem cell transplant

Patients with no “Minimal Residual Disease” may still have billions of AML cells

AML surface markers are different in each patient, and frequently change between diagnosis and relapse

<table>
<thead>
<tr>
<th>Antigen</th>
<th>N*</th>
<th>Expressed at diagnosis</th>
<th>Lost at relapse†</th>
<th>Gained at relapse‡</th>
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</thead>
<tbody>
<tr>
<td>CD2</td>
<td>126</td>
<td>14 (11)</td>
<td>2 (14)</td>
<td>16 (14)</td>
</tr>
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<td>3 (100)</td>
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<td>3 (3)</td>
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<td>132</td>
<td>100 (76)</td>
<td>6 (6)</td>
<td>5 (16)</td>
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</table>

Baer et al. Blood (2001)
Flow cytometry for AML MRD is technically challenging ➔ sensitivity is limited

- AML surface markers vary in each patient, and can be gained and lost during treatment

- Result: sensitivity is variable
  - At UW, most cases can get 0.1% sensitivity
  - ~30% of cases can get better sensitivity (e.g. 0.01%)
  - Counting more cells will **not** necessarily improve sensitivity.

- Sensitivity is **very** lab-dependent
  - UW looks at a huge number of markers simultaneously
  - Expert interpretation is required
Additional challenge: AML consists of multiple clones.

We do not have a good way to simultaneously monitor several clones.

Could we track mutations to monitor MRD? Example: NPM1 mutations as marker of residual disease

**Problems:**
- Only 30% of AML cases have NPM1 mutation
- Approximately 50 different types of NPM1 mutations have been described; challenging to cover all of them with one assay
- NPM1 mutations could potentially be lost at relapse

AML cells have hundreds of mutations. Most mutations persist at relapse. 

Could we monitor clearance of mutations as a general strategy to track MRD?

Question:
Can we track AML mutations to monitor residual disease?

- Mutations are stable markers → may give more reliable results than flow cytometry
- Mutations can allow tracking multiple clones → may allow determining whether all clones are cleared
Study Design

- 50 newly diagnosed AML cases
- All patients achieved CR (<5% blasts) after “7+3”

**Step 1.** Sequence AML sample at diagnosis to identify mutations.
  - Exome sequencing of peripheral blood

**Step 2.** Sequence paired sample at day 30, determine if the mutations seen at diagnosis persist.
  - 25 cases: exome sequencing of peripheral blood
  - 25 additional cases: targeted PCR-based sequencing of formalin-fixed bone marrow
**Results:** mutations can be tracked over time

Figure 2. Clearance Patterns of Acute Myeloid Leukemia–Associated Mutations Detected by Exome Sequencing

Klco et al. JAMA (2015)
NPM1 and FLT3 mutations were always cleared, even in patients who relapse.

→ NPM1/FLT3 may not be good mutations to track.

Cleared mutations

Persistent mutations

Klco et al. JAMA (2015)
failure to clear mutations $\rightarrow$ poor outcomes

If mutations are not cleared $\rightarrow$
100% of patients relapse by 3 years, 90% die by 5 years

Klco et al. JAMA (2015)
Conclusions

• Mutation tracking can detect residual disease in AML
• Some mutations might be better for tracking than others
  – NPM1, FLT3 mutations frequently lost → not a good choice for disease monitoring?
• Failure to clear mutations results in extremely poor outcomes (0% 3-yr EFS, 10% 5-yr OS)
Questions

• How does mutation tracking compare to flow cytometry for MRD monitoring?
  – Head-to-head comparison needed

• Can the sensitivity be improved beyond 2.5%?
  – Track more mutations?
  – Use higher accuracy sequencing methods?

• Can this approach be scaled to a clinical assay?
  – Need rapid turn-around, need to consistently identify mutations for monitoring

• Can this approach be applied to other cancers?
Mutation tracking predicts relapse in localized breast cancer


Sequence tumor biopsy at diagnosis.

After tumor resection, look for the tumor-specific mutations in blood.

Standard follow-up:
- Blood samples every 6 months.

![Graph showing disease-free survival after surgery.](https://example.com/graph)

- Red line: ctDNA detected; n = 30
- Black line: ctDNA not detected; n = 13

**Statistical Analysis:**
- P < 0.0001
- Hazard Ratio (HR), 12.0 (95% CI, 3.36-43.07)
Thanks

Pam Becker

Brent Wood

Jerry Radich