MRD and impact on Center Performance Measures

Bart Scott           Selina Luger,
Stella Davies        Wael Saber,
Chris Hourigan       Daniel Weisdorf
Primary Questions

Should we incorporate MRD measures into Center performance?
1 year survival post Allogeneic HCT

How should data collection questions be modified to improve precision and prepare for future analyses?

Which diseases? Acute Leukemia
but recognize ALL and AML differently
• How are centers collecting data currently? Wael Saber

• MRD and technique sensitivity. ALL and AML. Stella Davies, Bart Scott

• Differing techniques for molecular testing & CHIP Chris Hourigan

• How should we use it in Center Performance Score

• Recommendation on:
  How should we revise the data collection forms &
  How should we use the data in the Center Specific Analysis of Outcome
What do we collect now and on which form in ALL/AML

• Molecular data/cytogenetic data at 3 time points: dx, between dx and HCT, and at HCT
• Single time point: flow cytometry to test for MRD at HCT only (if CR is achieved).
• No sensitivity threshold is asked
• In AML, molecular panel asked now includes: FLT3-ITD, FLT3-TKD, IDH1/2, CEBPA, KIT, NPM1, Others
• In ALL, molecular panel includes: BCR/ABL, TEL-AML/AML1, Others
• Disease classification form (f2402)
<table>
<thead>
<tr>
<th>MRD testing by center volume, AML in CR1/CR2</th>
<th>High volume</th>
<th>Low volume</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>6107</td>
<td>1666</td>
<td>0.03a</td>
</tr>
<tr>
<td>MRD testing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>407 (7)</td>
<td>86 (5)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5700 (93)</td>
<td>1580 (95)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MRD testing by center volume, ALL in CR1/CR2</th>
<th>High volume</th>
<th>Low volume</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>2734</td>
<td>828</td>
<td>0.39a</td>
</tr>
<tr>
<td>MRD testing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>110 (4)</td>
<td>39 (5)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2624 (96)</td>
<td>789 (95)</td>
<td></td>
</tr>
</tbody>
</table>

Hypothesis testing: * Pearson chi-square test
TED; first all for all indications; US only; 2017-2019
### MRD testing according to center volume

#### AML in CR1/CR2

<table>
<thead>
<tr>
<th></th>
<th>High volume</th>
<th>Low volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>5700</td>
<td>1580</td>
</tr>
<tr>
<td>MRD testing method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow only</td>
<td>2547 (45)</td>
<td>788 (50)</td>
</tr>
<tr>
<td>NGS/PCR only</td>
<td>358 (6)</td>
<td>28 (2)</td>
</tr>
<tr>
<td>Both</td>
<td>2795 (49)</td>
<td>764 (48)</td>
</tr>
</tbody>
</table>

#### ALL in CR1/CR2

<table>
<thead>
<tr>
<th></th>
<th>High volume</th>
<th>Low volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>2624</td>
<td>789</td>
</tr>
<tr>
<td>MRD testing method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow only</td>
<td>1430 (54)</td>
<td>474 (60)</td>
</tr>
<tr>
<td>NGS/PCR only</td>
<td>78 (3)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Both</td>
<td>1116 (43)</td>
<td>298 (38)</td>
</tr>
</tbody>
</table>
A systematic review of outcomes after stem cell transplantation in acute lymphoblastic leukemia with or without measurable residual disease

Shweta Shah\textsuperscript{a}, Amber Martin\textsuperscript{b}, Monica Turner\textsuperscript{b}, Ze Cong\textsuperscript{a}, Faraz Zaman\textsuperscript{a}, and Anthony Stein\textsuperscript{c}

\textsuperscript{a} Amgen Inc., Thousand Oaks, CA, USA; \textsuperscript{b} EVI DERA, Evidence, Synthesis, Modeling, and Communications, Waltham, MA, USA; \textsuperscript{c} City of Hope National Medical Center, Duarte, CA, USA
Hazard Ratios for OS in Adults with ALL With and Without MRD

**Study** | **HR for OS (95% CI)**
--- | ---
Zhou, 2014: With MRD vs. Without MRD | 1.51 (0.86–2.70); p=0.14*
Kanakry, 2014: With MRD vs. Without MRD | 2.24 (0.79–6.38); p=0.13
Appelbaum, 2013: With MRD vs. Without MRD | 2.39; p=0.0005
Zhang, 2016: High MRD (SCT) vs. Low MRD | 2.65 (1.23–5.69); p=0.013
Cai, 2017: With MRD vs. Without MRD | 3.86 (1.32–11.29); p=0.014
Kebriaei, 2017: With MRD vs. Without MRD | 2.54; p=0.01

CI = confidence interval; HR = hazard ratio
* Values based on author calculations

**Figure 2.** Available hazard ratios for overall survival. CI: confidence interval; HR: hazard ratio; MRD: measurable residual disease; OS: overall survival; SCT: stem cell transplantation.
# Outcome Of Allo HSCT for Adults with ALL in CR1

<table>
<thead>
<tr>
<th>Time point</th>
<th>Measure</th>
<th>No. of studies</th>
<th>Range in patients with MRD</th>
<th>Range in patients without MRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>Median OS</td>
<td>One [15]</td>
<td>1.98 months</td>
<td>Not reached</td>
</tr>
<tr>
<td></td>
<td>Median RFS</td>
<td>One [23]</td>
<td>6.5 months</td>
<td>Not reached</td>
</tr>
<tr>
<td></td>
<td>Median DFS</td>
<td>One [15]</td>
<td>1.16 months</td>
<td>Not reached</td>
</tr>
<tr>
<td>2-year results</td>
<td>OS rate</td>
<td>Three [10,17,19]</td>
<td>27–37.7%</td>
<td>66–41.3%</td>
</tr>
<tr>
<td></td>
<td>RFS rate</td>
<td>Two [16,19]</td>
<td>40.2–57%</td>
<td>61–70.3%</td>
</tr>
<tr>
<td></td>
<td>DFS rate</td>
<td>Two [24,25]</td>
<td>54%</td>
<td>52–66%</td>
</tr>
<tr>
<td>3-year results</td>
<td>OS rate</td>
<td>Two [14,21]</td>
<td>27–64%</td>
<td>68–92%</td>
</tr>
<tr>
<td></td>
<td>DFS rate</td>
<td>One [21]</td>
<td>72%</td>
<td>73%</td>
</tr>
<tr>
<td>5-year results</td>
<td>OS rate</td>
<td>Three [15,18,20]</td>
<td>33–53%</td>
<td>58–75%</td>
</tr>
<tr>
<td></td>
<td>DFS rate</td>
<td>Three [15,20,25]</td>
<td>10–41%</td>
<td>47–72%</td>
</tr>
<tr>
<td>10-year results</td>
<td>DFS rate</td>
<td>One [26]</td>
<td>30%</td>
<td>35%</td>
</tr>
</tbody>
</table>

CR1: first complete remission; DFS: disease-free survival; MRD: measurable residual disease; OS: overall survival; RFS: relapse-free survival.
## Outcome of Allo HSCT for Adults with ALL in CR2

<table>
<thead>
<tr>
<th>Time point</th>
<th>Measure</th>
<th>No. of studies</th>
<th>Range in patients with MRD</th>
<th>Range in patients without MRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean survival</td>
<td>DFS</td>
<td>One [28]</td>
<td>36–52 months</td>
<td>35–62 months</td>
</tr>
<tr>
<td>Median OS</td>
<td>Four</td>
<td>[29–31,34]</td>
<td>8–17 months</td>
<td>7 months to not reached</td>
</tr>
<tr>
<td>Median RFS</td>
<td>One</td>
<td>[34]</td>
<td>10.5 months</td>
<td>51 months</td>
</tr>
<tr>
<td>Median EFS</td>
<td>Two</td>
<td>[29,31]</td>
<td>6–7 months</td>
<td>5–18 months</td>
</tr>
<tr>
<td>2-year results</td>
<td>DFS rate</td>
<td>One [37]</td>
<td>51.4%</td>
<td>51.5%</td>
</tr>
<tr>
<td></td>
<td>EFS rate</td>
<td>Two [29,31]</td>
<td>61.2%</td>
<td>74.4%</td>
</tr>
<tr>
<td></td>
<td>PFS rate</td>
<td>One [32]</td>
<td>0–19%</td>
<td>7–46%</td>
</tr>
<tr>
<td>3-year results</td>
<td>DFS rate</td>
<td>One [27]</td>
<td>29.6%</td>
<td>29.8%</td>
</tr>
<tr>
<td></td>
<td>PFS rate</td>
<td>One [27]</td>
<td>29.6%</td>
<td>29.8%</td>
</tr>
<tr>
<td></td>
<td>DFS rate</td>
<td>Three [28,36,38]</td>
<td>27–50%</td>
<td>40–73.9%</td>
</tr>
<tr>
<td>6-year results</td>
<td>DFS rate</td>
<td>One [30]</td>
<td>24%</td>
<td>74%</td>
</tr>
<tr>
<td>10-year results</td>
<td>DFS rate</td>
<td>One [26]</td>
<td>23%</td>
<td>32%</td>
</tr>
</tbody>
</table>

Shah et al, 2020
### Table 2. Studies supporting the prognostic significance of MRD prior to HSCT.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Study Type</th>
<th>Technique</th>
<th>Sensitivity</th>
<th>N</th>
<th>Age, Years, Median (Range)</th>
<th>Remission</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knechtli [32]</td>
<td>1998</td>
<td>R</td>
<td>PCR</td>
<td>$&lt;$10^{-3} - 10^{-5}</td>
<td>64</td>
<td>18</td>
<td>CR1, CR2</td>
<td>2-year EFS 73% MRD− vs. 0% MRD+ $p &lt; 0.001</td>
</tr>
<tr>
<td>Van der Velden [33]</td>
<td>2001</td>
<td>R</td>
<td>PCR</td>
<td>$&lt;$10^{-4}</td>
<td>17</td>
<td>15</td>
<td>CR1, CR2</td>
<td>5-year EFS 80% MRD− vs. 33% MRD+ $p = 0.03</td>
</tr>
<tr>
<td>Sanchez [34]</td>
<td>2002</td>
<td>P</td>
<td>MCF</td>
<td>$&lt;$10^{-4}</td>
<td>24</td>
<td>18 (3–49)</td>
<td>≥CR1</td>
<td>2-year EFS 73% MRD− vs. 33% MRD+ $p = 0.03</td>
</tr>
<tr>
<td>Bader [35]</td>
<td>2002</td>
<td>R</td>
<td>PCR</td>
<td>$&lt;$10^{-4}</td>
<td>41</td>
<td>9.8 (1.5–17.8)</td>
<td>≥CR1</td>
<td>5-year EFS 78% MRD− vs. 32% MRD+ $p = 0.011</td>
</tr>
<tr>
<td>Krejci [36]</td>
<td>2003</td>
<td>R</td>
<td>PCR</td>
<td>$&lt;$10^{-4}</td>
<td>140</td>
<td>19</td>
<td>≥CR1</td>
<td>5-year EFS 75.2% MRD− vs. 29.8% MRD+</td>
</tr>
<tr>
<td>Imashuku [37]</td>
<td>2003</td>
<td>P</td>
<td>PCR</td>
<td>$&lt;$10^{-4}</td>
<td>95</td>
<td>9 (0.3–20)</td>
<td>Not remission, ≥CR1</td>
<td>Available data in 19 relapses, all 19 were MRD+</td>
</tr>
<tr>
<td>Goulden [38]</td>
<td>2003</td>
<td>R</td>
<td>PCR</td>
<td>$&lt;$10^{-4}</td>
<td>64</td>
<td>Pediatric</td>
<td>≥CR1</td>
<td>3-year EFS 73% MRD− vs. 17% MRD+ $p &lt; 0.001</td>
</tr>
<tr>
<td>Sramkova [39]</td>
<td>2007</td>
<td>P</td>
<td>PCR</td>
<td>$&lt;$10^{-4}</td>
<td>25</td>
<td>1.1–19</td>
<td>Partial remission, CR1, CR2</td>
<td>EFS 94% MRD− vs. 13% MRD+ $p &lt; 0.001</td>
</tr>
<tr>
<td>Paganin [40]</td>
<td>2008</td>
<td>P</td>
<td>PCR</td>
<td>$&lt;$10^{-4}</td>
<td>60</td>
<td>5 (0.6–17)</td>
<td>CR2</td>
<td>3-year EFS 73% MRD− vs. 19% MRD+ $p &lt; 0.05</td>
</tr>
<tr>
<td>Bader [41]</td>
<td>2009</td>
<td>P</td>
<td>PCR</td>
<td>10^{-3}</td>
<td>91</td>
<td>11.1 (3–22.6)</td>
<td>CR2, CR3</td>
<td>3-year EFS 60% MRD− vs. 27% MRD+ $p &lt; 0.05</td>
</tr>
<tr>
<td>Elorza [42]</td>
<td>2010</td>
<td>P</td>
<td>MCF</td>
<td>$&lt;$10^{-4}</td>
<td>31</td>
<td>7 (&lt;1–16)</td>
<td>≥CR1</td>
<td>2-year EFS 74% MRD− vs. 20% MRD+</td>
</tr>
<tr>
<td>Leung [43]</td>
<td>2012</td>
<td>R</td>
<td>MFC</td>
<td>10^{-3}</td>
<td>64</td>
<td>11.3 (0.6–25.1)</td>
<td>≥CR1</td>
<td>5-year OS 87.5% MRD− vs. 48.5% MRD+</td>
</tr>
<tr>
<td>Ruggeri [44]</td>
<td>2012</td>
<td>R</td>
<td>PCR/MFC</td>
<td>10^{-3}–5</td>
<td>170</td>
<td>6.5 (&lt;1–17)</td>
<td>CR1, CR2, CR3</td>
<td>4-year CIR 24% MRD− vs. 39% MRD+</td>
</tr>
<tr>
<td>Bachanova [45]</td>
<td>2012</td>
<td>P</td>
<td>MFC</td>
<td>10^{-3}</td>
<td>86</td>
<td>20 (6–63)</td>
<td>CR1, CR2, CR3</td>
<td>2-year RR 26% MRD− vs. 30% MRD+</td>
</tr>
<tr>
<td>Shah [46]</td>
<td>2014</td>
<td>R</td>
<td>MFC</td>
<td>10^{-4}</td>
<td>34</td>
<td>&lt;21</td>
<td>CR2</td>
<td>RR 35% MRD− vs. 64% MRD+</td>
</tr>
<tr>
<td>Balduzzi [47]</td>
<td>2014</td>
<td>P</td>
<td>PCR</td>
<td>$&lt;$10^{-4}</td>
<td>82</td>
<td>8 (&lt;1–20)</td>
<td>CR1, CR2, CR3</td>
<td>5-year EFS 77.7% MRD− vs. 30.8% MRD+ $p &lt; 0.001</td>
</tr>
<tr>
<td>Bar [48]</td>
<td>2014</td>
<td>R</td>
<td>MCF</td>
<td>10^{-3}–10^{-4}</td>
<td>153</td>
<td>24.6 (0.6–61.8)</td>
<td>≥CR1</td>
<td>3-year EOR 17% MRD− vs. 38% MRD+</td>
</tr>
</tbody>
</table>
Pre-HCT MRD in AML

AML CR1 MRD- n=147
AML CR2 MRD- n=52
AML CR1 MRD+ =36
AML CR2 MRD+n=18

MAC: Bu4, H-TBI, Treo, RAB

Walter et al. *Blood* 2013;122:1813-1821
Pre-HCT MRD in AML

**Overall Survival**
AML 1st CR n=152
8 color MFC 0.01% sensitivity

- 66.9%
- 48.8% p=0.008

**Relapse**

<table>
<thead>
<tr>
<th></th>
<th>MRD-negative</th>
<th>MRD-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>104</td>
<td>48</td>
</tr>
<tr>
<td>event</td>
<td>13</td>
<td>16</td>
</tr>
</tbody>
</table>

- 32.6%
- 14.4% p=0.002

Oran et al. Haematologica 2017;102
Pre-HCT MRD in AML

359 AML MAC HCT 2006-2014
Bu4, H-TBI, Treo, RAB

MRD Modifies Effect of Conditioning Intensity

190 of 218 AML patients
51kB multiplex PCR targeting 13 genes
VAF as low as 0.1% (1/1000), or
0.02% (1/5000) for insertions in mutated NPM1 and FLT3-ITD.

Does the Method of Detection of MRD Matter?

286 AML in CR
62% MAC
38% RIC
C=standard karyotype and AML
FISH probe
F=10 color MFC

Fang et al. Cancer 2012;118:2411-1419
Araki et. al., JCO 2016
Regardless of test used:

AML MRD in CR before Allo-HCT = worse survival after transplant.

Buckley et. al., Haematologica, 2017
qPCR

Uses:
- CBF (Inv16, t8,21)
- NPM1mut (A, B and D)
- BCR-ABL1

Advantages:
- Ubiquitous presence in most clinical labs
- Fast turnaround time
- High sample throughput
- Broad dynamic range.

Disadvantages:
- Limited number of suitable targets/assays
- Relative lack of multiplexing ability
- Need to validate each target/assay individually
- Limited ability to quantify at v.low MRD

Hourigan and Freeman, ASH Educational, 2019
Real-time qPCR ...high sensitivity ... therefore currently considered the gold standard....limited to ... ~40% of AML patients

- 100ng cDNA/rxt (10K ABL1 copy)
- Run Triplicates
- EAC assays/criteria
- Ref. standards, pos. and no template control

<table>
<thead>
<tr>
<th>Target</th>
<th>Classification</th>
<th>Target</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPM1</td>
<td>Insertion mutation</td>
<td>NPM1</td>
<td>Insertion mutation</td>
</tr>
<tr>
<td>PML-RARA</td>
<td>Fusion transcript</td>
<td>PML-RARA</td>
<td>Fusion transcript</td>
</tr>
<tr>
<td>CBFB-MYH11</td>
<td>Fusion transcript</td>
<td>CBFB-MYH11</td>
<td>Fusion transcript</td>
</tr>
<tr>
<td>RUNX1-RUNX1T1</td>
<td>Fusion transcript</td>
<td>RUNX1-RUNX1T1</td>
<td>Fusion transcript</td>
</tr>
<tr>
<td>BCR-ABL1</td>
<td>Fusion transcript</td>
<td>BCR-ABL1</td>
<td>Fusion transcript</td>
</tr>
</tbody>
</table>

Bone Marrow (5-10ml, first pull, EDTA or Heparin okay) AND Blood

Complete molecular remission: Must be in morphological CR. Two successive MRD negative samples obtained within interval of ≥ 4 weeks at a sensitivity level of at least 1 in 1000.

Molecular Relapse: ↑MRD level of 1 log\(^{10}\) between 2 positive samples (4wk) in a patient who previously tested negative.

Molecular Persistence: <100-200 copies/\(^{10^4}\) ABL copies corresponding to <1% to 2% of target to reference gene or allele burden. Progression: ↑MRD level of 1 log\(^{10}\) any 2 positive samples.
Digital PCR

Uses:
As qPCR:
- CBF (Inv16, t8,21)
- NPM1mut (A, B and D)
- BCR-ABL1

Advantages:
- Absolute quantification – good for low MRD
- Doesn’t need standard curve

Disadvantages:
- Technology not in common clinical use
- Assays not clinically validated (unlike qPCR)
- Cost > qPCR

Hourigan and Freeman, ASH Educational, 2019
NGS – diagnostic
aka: “myeloid panel”

**Uses:**
Genetic profiling of AML when blasts >5%
**Not** for measurable residual disease
Typical gDNA input 20-200ng

**Advantages:**
Broad panel = lots of targets

**Disadvantages:**
Very high false **positive** rate for variants <5%
Very high false **negative** rate for variants <5%

Hourigan and Freeman, ASH Educational, 2019
Ivey et al.
NEJM. 2016
NGS – MRD Depth

**externally validated test not yet available clinically**

**Uses:**
Research (clinical soon hopefully)
200ng to 2ug gDNA input

**Advantages:**
Broad panels – can track lots of variants
Detection down to to 0.001 or below
Potential for patient personalization

**Disadvantages:**
Cost
Clinical utility of detected variants unknown
Clinical utility of VAF thresholds unknown

Hourigan and Freeman, ASH Educational, 2019
Hourigan and Karp, Nature Reviews Clinical Oncology, 2013

Detectability limit (fraction of leukemia cells)
Take-home messages on molecular MRD

- ELN recommendation is currently only for qPCR (CBF, NPM1, BCR-ABL)

- Cytos and FISH low sensitivity (not MRD) but may be helpful if positive in cytomorphological remission

- ~80% of flow+ cases post induction will be deep NGS+. Also many flow- cases.

- Diagnostic NGS “myeloid panels” insufficient to test for MRD negativity

- “Late” mutations (FLT3, RAS, KIT) often lost at relapse = helpful if positive

- “Early” mutations (DTA) often persist in cured patients = ?helpful if negative
Not all mutations are cancer — example: “DTA”
Questions?

hourigan@nih.gov

@DrChrisHourigan
Does MRD always matter?

Yes for AML and ALL  
CR1  MRD + or –
CR2  MRD + or –
CR3+  OK to ask but we do not know if matters for CR3+

MRD+ is as risky as morphologic disease pre transplant

Centers without high sensitivity MRD testing (and thus MRD unknown) are including patients with higher risk of relapse.
Recomendations: **Revise the questions** to ask the following:
For ALL, AML and MDS (consider the same questions for CLL, myeloma)

**Pre-transplant**

1. In Morphologic CR, was MRD assessed?  y/n.

2. If Flow was tested
   - Was an original leukemia immunophenotype used for detection?  y/n
   - Was an aberrant phenotype used for detection?  y/n
   - What is the lower limit of detection?

3. Was molecular assay (PCR or NGS) used for MRD detection?  y/n
   - Was MRD detected?  y/n

4. Were cytogenetic assays (Metaphase or FISH) used for MRD detection?  y/n
   - Was MRD detected?  y/n
Recommendations

For the Outcomes Analysis of 1 year survival.

Include these changes **only for ALL, AML**

Use modified pre-transplant disease status definitions:
- CR1 (or CR2 or later CR) without MRD
- CR with MRD+
- and
- CR with no high sensitivity testing for MRD
How complete is molecular data is (AML as example)?

• Selection: first alloHCT for AML since F2402R2 (July 2017, when time point of between dx and HCT are added)

• Select molecular/cytogenetic abnormalities (7- by FISH, CEBPA, FLT3-TKD, FLT3-ITD, NPM1)

• Data complete across all 3 time points in only 10%

• Data complete across two time points (dx and at HCT):
  - CEBPA 12%
  - FLT-TKD 17%
  - FLT-ITD 23%
  - NPM1 19%
  - 7 by FISH 12%