MINIMAL/MEASURABLE RESIDUAL DISEASE (MRD) IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Disclosure Statement

• I have no conflict of interest
Outline & Objectives

• Discuss the significance of MRD in ALL

• Review the common laboratory tests used in MRD assessment.

• List the advantages and disadvantages of various techniques

Acute Lymphoblastic Leukemia

• ~6000 new cases in the USA/year

• 75% occur in children aged < 6 years

• > 20% lymphoblasts in bone marrow or peripheral blood

• Diagnosis: Morphology, flow cytometry analysis and cytogenetic and molecular analysis

• Major Subtypes: B-cell ALL (85%), T-cell ALL and NK cell ALL
2016 WHO classification of ALL

Precursor lymphoid neoplasms

B-lymphoblastic leukaemia/lymphoma, not otherwise specified
B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13.2;q22.1); ETV6-RUNX1
B-lymphoblastic leukaemia/lymphoma with t(11;19)(q23;p13.3); TCF3-PBX1
B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34.1;q11.2); BCR-ABL1
B-lymphoblastic leukaemia/lymphoma with t(4;11)(q32;q22.3); KVMT24-rearranged
B-lymphoblastic leukaemia/lymphoma with t(1;14)(q32;q32.1); IGF1R/IL3
B-lymphoblastic leukaemia/lymphoma with t(4;14)(q34.1;q32.1); E2A/FOXN1
B-lymphoblastic leukaemia/lymphoma with t(1;19)(q13;p13.3); TCF3-PBX1
B-lymphoblastic leukaemia/lymphoma with BCR-ABL1-like
B-lymphoblastic leukaemia/lymphoma with IAMP21

T-lymphoblastic leukaemia/lymphoma
NK lymphoblastic leukaemia/lymphoma

Lineage Based B/T/NK
Cytogenetic Based Ph+/Ph-/Ph-like

Ph +

Poor Prognosis
- Hypodiploid
- Most commonly lost chromosomes include 3, 4, 7, 9, 13, 17, and 20
- Worse prognostic score hz,(25-25 chromosomes) and low hypodiploid (33-39 chromosomes)

Ph like

T Lymphoblastic Leukemia/Lymphoma (T-ALL)
- Translocations involving T-cell receptor (TCR): error during TCR gene rearrangements
- t(9;22)(q34;q34); NUP214-ABL1 strictly associated with T-ALL <6% cases
- MYC rearrangements (6% cases)
- NOTCH1 (70% cases), CDKN1a, IGHV1-2 (cryptic deletions >70% cases) mutations

Early T Precursor Acute Lymphoblastic Leukemia (ETP-ALL)
- FLII, NRAS/KRAS, DNMT3A, IDH1/2

www.amp.org/AMPEducation
Diagnostic Algorithm (Ph+ ALL) helpful in MRD monitoring

Suspected ALL
Order FISH
4(9;22) BCR-ABL1 (Ph)

Ph+ by FISH
Order Qualitative BCR-ABL 1 Assay
p210 isoform detected
Order Quantitative assay For isoform p210
p190 isoform detected
Order Quantitative assay For isoform p190

BCR-ABL1 FISH cytogenetic testing detects the presence BCR-ABL1 fusion but does not differentiate between the different isoforms (p190, p210)

ABL1 test detects the presence of the p190, p210 isoforms but does not measure the levels of the transcripts.

Qualitative BCR-ABL1 testing is indicated for monitoring of disease for p210 or p190 BCR-ABL1 fusion gene by qualitative assay.

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MRD DEFINITION: The amount of residual leukemic cells present in bone marrow and/or circulating in the peripheral blood after treatment

https://www.knowmrd.com/hcp/

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Importance of MRD in ALL

- MRD is accepted as the strongest independent prognostic factor in ALL.
- MRD positivity after induction or consolidation therapy may point to an impending relapse and thus enable salvage treatment to be initiated earlier, which could possibly improve treatment outcomes.
- The prognostic significance of pre-transplantation MRD is shown by several studies and MRD high-risk patients are shown to benefit from stem cell transplantation (SCT).
- Achieving MRD less than $10^{-4}$ ($0.01\%$; MRD-negative status) is a critical milestone.

MRD-negative status has better clinical outcome

- Meta-analysis of 39 studies with 13,737 patients
- Trials across the globe including different types of chemotherapy, protocols, MRD time points and methods of detection
- 10 year EFS within the first 3 cycles of chemotherapy was 64% for adults achieving MRD-negative status vs. 21% in those with MRD-positive disease.

Berry et al, JAMA Oncol. 2017;3(7)
Goal Of MRD Based Risk Directed Approach

- Establish standardized protocols as to the timing of assessment and methodology used, to limit inter laboratory variability
- Identify patients with suboptimal initial response to therapy who may benefit from more intensive or novel therapies
- Identify patients with an excellent response to initial therapy who may be candidates for therapeutic reduction to limit toxicity
- Identify MRD high-risk patients who may benefit from stem cell transplantation (SCT)

NCCN Guidelines Recommend:
- Baseline characterization of leukemic clone as a part of the diagnostic work-up to facilitate subsequent MRD analysis
- MRD testing at completion of induction (+ additional time points guided by the regimen used)
- MRD testing to identify patients who are in Complete Response and MRD-positive >> should receive targeted therapy
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For patients with suspected or confirmed acute leukemia, the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of minimal residual disease (MRD).

14. For adult patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t(12;21)(p13.2;q22) TEL-AML1, t(9;22)(q34.1;q11.2) BCR-ABL1, and t(15;17)(q22;q12) PML-RARA translocations (< 1% in bone marrow cells) is performed.

15. For patients with suspected or confirmed ALL, the pathologist or treating clinician may order appropriate mutational analysis for selected genes that influence diagnostic, prognostic, and/or therapeutic management, which includes, but is not limited to, FAS, JAK1, JAK2, and/or NRAS and KRAS mutations and/or FISH for t(4;11)(q21.2;q23) and BCAP31. Testing for overexpression of CREB2 may also be performed for B-ALL.
Assays for MRD Assessment

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Reproducibility and applicability</th>
</tr>
</thead>
<tbody>
<tr>
<td>The ability to discriminate between malignant and normal cells</td>
<td>The ability to detect one malignant cell in at least 1000 to 10000 normal cells</td>
<td>Standardized and reproducible technique and results must be available in a timely manner</td>
</tr>
</tbody>
</table>

**Multicolor Flow Cytometry (MFC)** - Discrimination of the leukemia-associated immunophenotype (LAIP)

**Quantitative PCR (RT-qPCR)** - Detection of fusion gene transcripts & rearrangements of immunoglobulin and T-cell receptor (IG/TR) genes by real time quantitative polymerase chain reaction

**Next Generation Sequencing (NGS)** - High-throughput sequencing of leukemia-specific rearrangements of immunoglobulin and T-cell receptor (IG/TR) genes

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**Multicolor Flow Cytometry (MFC)**

**Basics of Flow Cytometry**

- **Fluidics**
- **Optics**
- **Electronics**

Cells in suspension flow in a single-file through an illuminated flow cell...

...filtered & detected...

...and converted to digital values that are analyzed and stored on a computer.

**Cellular Antigens**

- CD19
- CD20
- CD25
- CD79a
- CD79b
- CD22
- CD56
- CD49
- CD10
- Cytokines
- Metabolic surface proteins
- Enzymes
- Adhesion molecules
**Multicolor Flow Cytometry Assay for MRD**

**PRINCIPLE:** Leukemic blasts show altered patterns of antigen expression secondary to underlying genetic mutation, thus differing from normal hematopoietic progenitors of the same lineage through all stages of differentiation.

2 methodological approaches:

- The observation of a combination of antigens expressed on the leukemic blasts that are absent in normal progenitors, referred to as **“leukemia-associated immunophenotypes” (LAIPs)”**
- An alternative strategy, the **“difference from normal”** approach detects leukemic blasts by recognizing immunophenotypic deviation from the patterns of antigen expression on normal hematopoietic progenitors of similar lineage and maturational stage.
Multicolor Flow Cytometry Assay for MRD

STANDARD (adapted from COG Protocol)

Goal = MRD to at least 0.01%
- First draw of bone marrow must be used
- Ideally begin with a million cells for acquisition of up to 500,000 events (cluster of 50 events considered POI - population of interest; <50 events with abnormal phenotype acceptable)
- Most labs with 8 color flow cytometers are able to acquire between 200,000 to 500,000 events per tube, and sensitive to 10-40 events in POI

Higher the Quality & Quantity of Input Sample = Higher the Sensitivity of Assay

BM: B-cell Precursor Differentiation
In precursor B-ALL:

- CD38 is generally dimmer and shows more variability in expression levels
- CD10 is generally brighter
CAR-T Cell Treated ALL

Reverse transcriptase PCR (RT-PCR) used to detect fusion transcripts
- BCR/ABL in t(9;22)
- E2A/PBX1 in t(1;19)
- MLL/AF4 in t(4;11)
- TEL/AML1 in t(12;21)

Advantages
- Sensitive
- Rapid
- Not patient specific

Limitations
- Used in limited cases
- RNA degradation and inefficient conversion of mRNA to cDNA
- Method variations by center
- Risk of cross-contamination, requiring strict quality control

PCR Assays for MRD – RT-PCR

Reverse transcriptase polymerase chain reaction (RT-PCR)

Reverse transcriptase:
- RNA isolation from a tissue or cells
- RNA is converted to cDNA

PCR:
- PCR products are visualized on an ethidium bromide/agarose gel after electrophoresis
PCR Assays for MRD – Real Time quantitative PCR

Real-time quantitative PCR — Accumulation of PCR products over time is measured directly by fluorescence and quantitation is estimated from the cycle number required for the reaction to generate sufficient fluorescence to cross a set threshold. Targets are Immunoglobulin (Ig) or T cell receptor (TCR) gene rearrangements OR fusion gene transcripts.

Advantages
- High sensitivity $10^{-4}$ to $10^{-6}$.
- Wide applicability
- Sample stability
- Minimal tissue requirements.
- Standardized method.

Limitations:
- Need for diagnostic sample for appropriate primers
- Contamination of the reaction product, strict quality control
- Poor reproducibility when small numbers of transcripts
- Expensive & time consuming to determine the unique clonal rearrangement
- False-negative and false-positive results due to clonal evolution/subclone formation/presence of oligoclonal populations
PCR Assays for MRD – Allele Specific Oligonucleotide Assay

Allele specific oligonucleotide (ASO) hybridization and dot-blot analysis

Limitation for MRD assessment:
- The specific immune gene rearrangements must be sequenced at diagnosis and allele-specific oligonucleotide (ASO) primers designed complimentary to the unique patient or leukemic-specific junctional region sequences. The resulting primers are then applied to follow-up samples post therapy to quantify MRD.
- Need for diagnostic sample.

Next Generation Sequencing for MRD

Amplicon based high-throughput multiplex PCR assay designed with primers that simultaneously amplify all possible combinations of the rearranged IG or TCR loci in B- and T-ALL.

Advantages:
- This eliminates the requirement for patient or leukemic-specific primers and enable the creation of a general purpose MRD assay for ~95% of patient.
- In addition, multiple clones and subclones can be identified simultaneously, potentially reducing false negative results seen in qPCR-based assays caused by clonal evolution or emergence of new clones.

Limitations:
- Increased bioinformatics data for analysis
- Need for diagnostic sample
Flow Cytometry • Widely available
• Rapid turnaround time
• Simultaneously evaluates multiple antigens
• Sensitivity of detection of 0.01%.

qPCR • Better reproducibility
• One log more sensitive (0.01-0.001%) than flow cytometry.

NGS • No need for patient-specific primers, as consensus primers are used by multiplex PCR to detect all potential rearrangements, and used as a single standardized assay.

Concordance between flow cytometry & qPCR is >90% in ALL in most studies, if a cut-off of 0.01% is used

High level MRD (>0.01%): Concordant
Low level MRD (<0.01%): Discordant (Flow-/PCR +)

Advantages and Disadvantages of MRD Assays

<table>
<thead>
<tr>
<th>Feature</th>
<th>Flow Cytometry</th>
<th>qPCR</th>
<th>NGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiparametric Flow Cytometry</td>
<td>3-4 colors: 0.1-0.001%</td>
<td>0.01-0.001%</td>
<td>0.001%</td>
</tr>
<tr>
<td>Target</td>
<td>“difference from normal” or leukemia-associated immunophenotypes</td>
<td>IG/TCR gene rearrangements</td>
<td>Leukemic fusion transcripts</td>
</tr>
<tr>
<td>Specimen</td>
<td>Fresh viable cells</td>
<td>DNA</td>
<td>RNA Day 1-3</td>
</tr>
<tr>
<td>Turn-around time</td>
<td>1-2 days</td>
<td>-4 weeks to generate primers</td>
<td>Majority of ALL</td>
</tr>
<tr>
<td>Applicability</td>
<td>ALL</td>
<td>More expensive</td>
<td>Most expensive</td>
</tr>
<tr>
<td>Cost</td>
<td>Moderate expense</td>
<td>Widely available</td>
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</tr>
<tr>
<td>Advantages</td>
<td>Rapid</td>
<td>Direct quantification</td>
<td>High sensitivity</td>
</tr>
<tr>
<td></td>
<td>Potential to detect cells with leukemia</td>
<td>Highly standardized procedure and analysis</td>
<td>Generally applicable</td>
</tr>
<tr>
<td></td>
<td>Identification and monitoring treatment targets</td>
<td>with consensus guidelines</td>
<td>Can detect subclones and clonal evolution</td>
</tr>
</tbody>
</table>

Disadvantages

- Inadequate interlaboratory standardization
- Requires high-level of expertise to interpret data
- False negativity resulting from immunophenotypic shifts or confounding regenerating blasts

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<td>Reverse Transcriptase</td>
<td>Time consuming and labor-intensive for initial characterization of targets and construction of patient-specific primers</td>
<td>Only applicable to a subset of ALL with detectable abnormalities</td>
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<tr>
<td>Quantitative PCR</td>
<td>Requires prior knowledge of patient-specific IG/TCR gene rearrangements</td>
<td>Requires complex bioinformatics</td>
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<tr>
<td>Next Generation Sequencing</td>
<td>False negativity resulting from clonal evolution</td>
<td>Uncertain quantification of leukemic blasts</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; MRD, minimal residual disease; PCR, polymerase chain reaction.

Chen, Wood, 2017
Practical Issues

- Baseline characterization of leukemic clone as a part of the diagnostic work-up to facilitate subsequent MRD analysis

- Higher the quality of input sample = Higher the assay sensitivity

- For T-lineage ALL, peripheral blood is same as bone marrow for yield in MRD (Blood MRD levels were comparable to or up to 1 log lower than bone marrow MRD levels)

- For B-lineage ALL bone marrow is better than peripheral blood for yield in MRD (Blood MRD levels were 1 to 3 logs lower than bone marrow MRD levels)

- Sensitivity of flow cytometry is about 1 log lower than that of the molecular methods; however is less laborious and faster than molecular methods allowing quicker TAT which is useful in making therapeutic decisions.

Practical Issues

FALSE NEGATIVE/FALSE POSITIVE MRD:

- Phenotypic shifts frequently occur in MRD cells as well as in normal cells during therapy

- Antibody-based therapies (e.g. targeting CD19, CD20 or CD22) influence the detectability of residual leukemic cells

- Clonal evolution of IG/TR gene rearrangements in immature leukemic blasts might lead to the loss of leukemia-specific IG/TR sequence and false negative MRD result by PCR and NGS

- Massive bone marrow regeneration after treatment can cause unspecific primer annealing and false positive MRD results.
Summary of MRD Testing in Acute Lymphoblastic Leukemia

- B-ALL Ph+ (25%)
- B-ALL Ph- (75%)
- T-ALL

- Quantitative PCR (BCR-ABL Fusion)
- Flow Cytometry

- Flow Cytometry
- PCR Ig/TR
- NGS

Thank You!
Question?

UNMC Nebraska Medicine