Advances in B Lymphoblastic Leukemia MRD

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Measures of Response

• Clinical outcome
  – OS, EFS, RFS, etc.

• Blast count
  – Remission = < 5% by morphology

• Minimal Residual Disease
  – Cytogenetics, Flow, Molecular
Pediatric AML

- Blast count does not correlate with MRD

Pediatric AML

• Morphologic blast count is not predictive

AML Pre-Transplant

- Pre-Tx Blasts > 5% = MRD+ Blasts < 5%

ALL Morphology vs. Flow

0.8% M1 but Flow > 5%

6.5% M1 but Flow > 5%

ALL Morphology vs. Flow

Need a new definition of remission

• Morphologic blast counts correlate poorly with response in AML

• Shouldn’t use in clinical trial design

• Assess residual disease by other methods
Reproducibility
Sources of Variability

• Identification (false positive or negative)
  – Insufficiently informative reagents
  – Improper assay validation
  – Immunophenotypic shift
  – Inexperienced interpreters

• Quantitation
  – Too few events acquired
  – Denominator effects (2 fold)
  – Sample degeneration
  – Hemodilution
Figure S4: MRD detection in samples exchanged between the Eastern and Western reference laboratories.

The single point at <.01% for both labs represents 10 samples mutually interpreted as negative.

Flow MRD on AALL03B1

% of cases MRD >.01%

- JHU (n=2282)
- UW (n=1947)

* day 8 M1 patients excluded

Unpublished data, courtesy Mike Borowitz
Day 29 Flow MRD on AALL0232

Correlation between labs

Correlation between labs

AALL0232

0.1% <= Day 29 MRD < 1.0%

Eastern Region (n = 121)
Western Region (n = 114)

P = 0.5612

Interpretive Variability

First 3 rounds

Last 2 rounds

Experience with MRD Testing in B- ALL By Flow Cytometry Does Not Prevent Interpretative Discordance

Keeney, Wood et al. (2017) Cytometry B
Reproducibility

Flow cytometry is capable of reproducible MRD detection and enumeration

Lack of standardization in implementation is the source of variability in current practice
Opportunities
Anti-CD19 Immunotherapy

• Targets primary gating reagent
  – Identifying B cells difficult

• Eliminates expression of CD19
  – Rapidly after administration
  – Selects for CD19 (-) subset of leukemia
  – CD19 (-) MRD and relapse

• Need alternate strategy
  – CD22, CD24 + CD66b, cCD79a
  – Now routine assay
Anti-CD19 Immunotherapy

CD19-negative MRD with background hematogones

MRD by Flow Cytometry

• Advantages
  – Fast
  – Relatively inexpensive
  – Large instrument base
  – Reproducible

• Disadvantages
  – Subjective interpretation
  – Immunophenotypic drift after therapy
  – Moderate sensitivity
  – Poorly standardized
IgH and TCR Diversity
MRD by NGS

Day 0 Cancer clone Frequency

Day 29 Cancer clone Frequency

93 / 98 (95%) with IgH rearrangement
Remainder: 2 with D-J, 1 with clonal D-J + many VH, 2 none

MRD AND TRANSPLANT
Flow vs NGS

Better definition of low risk through higher sensitivity

Pulsipher et al Blood 2014;123:2017  
Pulsipher et al Blood 2015;125:3501
B-LL End of Induction MRD

Flow and HTS are equivalent at 0.01%
Use of cutoff < 0.01% does not improve risk stratification
B-LL End of Induction MRD

Lower MRD more informative for high risk
Absence of detectable MRD by NGS = Excellent outcome
Standard risk patients
B-LL End of Induction MRD

Absence of detectable MRD by NGS for High risk patients ≠ Standard risk patients
Cut Point Analysis

Optimal cut point for combined patients is ~0.01%

Hazard Ratios

All patients
Standard risk
High risk

MRD

Optimal cut point for combined patients is ~0.01%
Discordant Flow and HTS has intermediate outcome
Similar result for Flow+/HTS- (N=17)
Discordant = Concordant MRD- at lower HTS cutoff of 0.001%
SmMIPS

- Minimal error rate for via single molecule tagging
- Targets multiple genomic regions of interest
- Simple, scalable protocol
- Modular and cost-effective target enrichment
- Low sample input requirements
AML MRD – NPM1 by NGS

Abnormal myeloid blasts (%) vs. NPM1 mutant VAF (%)

N = 353

AML MRD – NPM1 by NGS

Non-transplant N = 37


#### Mutation Type
- Exclusive across categories
- Co-occurring across categories
- Co-occurring within category
- 2-Hit mutation

#### Cytogenetic Risk
- Unfavorable
- Intermediate
- Favorable
- Unknown

#### Dendrix++ Group
- A
- B
- C

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200 AML Samples
### AML MRD NGS

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32 gene panel of AML mutations  
Coverage for ~85% of AML  
Sensitivity of 0.011%  

Good correlation between multigene assay and standard NPM1

NGS Conclusions

• NGS is capable of MRD detection
  – Can reproduce risk groups
• Sensitivity = NGS \((10^{-6})\) > ASO-PCR > Flow
• Uses
  – Standardize testing
  – Simplify sample requirements
  – Define MRD(-) low-risk group early
  – Define MRD(+) high-risk group late (EOC)
  – ? better for targeted immunotherapy
• TAT of > 1 week currently
Subclonal CRLF2

Harvey et al. (2016) submitted

CRLF2 deletion

P2RY8-CRLF2

Harvey et al. (2016) submitted
Molecular Cytometry

The ability to measure multiple molecular features at the single cell level for entire populations of cells
Tumor Heterogeneity

Conclusions

• Diagnosis is immunophenotypic, classification is genotypic

• MRD detection provides important unique prognostic information in acute leukemia
  – Is standard of care for B-ALL

• MRD by flow cytometry can be done reproducibly

• MRD by NGS is feasible for B-ALL, T-ALL and AML

• Single cell molecular methods are the next frontier
Acknowledgements

• Hematopathology Laboratory at UWMC
• Michael Borowitz MD PhD
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  – Steven Hunger, Bill Carroll, Mignon Loh
• Adaptive Biotechnologies
  – Harlan Robins (FHCRC)