Molecular Monitoring in AML Can Inform Prognosis

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ACCME/Disclosure

Dr. Klco has nothing to disclose

Talk Overview

• Traditional approach to risk stratification
• Comprehensive genomics of AML and revised risk stratification
• The utility of molecular tests after therapy
• Future studies and issues for molecular testing
• Overall recommendations for clinical testing
AML Outcome and Treatment

• In adults, the 5 year overall survival is ~30%
• Patients are typically treated with cytarabine and anthracycline-based induction chemotherapy
• Therapy for AML has changed very little in the last 30 years
• Goal of AML therapy is the achievement of remission
  – ~20% of patients never reach remission (primary refractory)
  – ~40% of patients relapse

Current approach to risk stratification in AML

At the time of presentation

• Age
• Cytogenetics
• White blood cell count
• Mutational profiling:
  – FLT3
  – NPM1
  – CEBPA

Increasing age at diagnosis is associated with inferior outcome

Pre-treatment cytogenetics are associated with outcome

Smith et al, Blood Reviews, 2011
Minimal Residual Disease by Multi-color Flow Cytometry

- Current standard of care at SJCRH
- Requires the detection of immunophenotypically abnormal cells
  - Leukemia-associated immunophenotype
    - “Different than normal”
- SJCRH approach
  - Establish a leukemia-associated immunophenotype at diagnosis using a panel of 18 markers: CD45, CD33, CD34, CD117, CD13, CD15, CD36, CD44, CD11b, CD15, HLA-DR, CD38, CD4, CD235A.
  - 0.1% is our current threshold
- Has clearly shown improvement over morphologic evaluation
- Although not standard for adult AML, it is becoming more common

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What are the limitations to risk stratification using genomics at presentation?

1. Many of the associations with outcome require large cohorts and lack prognostic value for individual patients.

2. AML is a heterogeneous disease both in terms of mutations and subclones.
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**AML 31**
- 55 year old man with NK-AML, FAB M5 (BM: >90% leukemia)
- Relapse at day 505 (BM: ~40% leukemia)
- WGS was performed at both diagnosis and relapse and all positions were validated by targeted deep sequencing

**Mutations**
- DNMT3A p.R882H
- NPM1 p.W288fs
- IDH1 p.R132H
- FLT3-ITD
- FLT3 p.D835H
- IDH2 p.R140Q

**AML Subclonal Heterogeneity**
- The monocyte and blast populations both have mutations in DNMT3A and NPM1
- The blast population corresponds to a subclone enriched with the IDH2 mutation
- This subclone also expanded at disease relapse

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Genomics in AML Risk Stratification

• Can we use genomics to follow Minimal Residual Disease?
• Design:
  – Identified 50 adult AML patients that achieved complete remission after induction chemotherapy
  – Exome sequencing of skin biopsy material, day 0 leukemia and day 30 (all in morphologic remission), and a later timepoint (remission or relapse, if available)
    • NimbleGen V3 exome with spiked-in IDT probes covering all exons of 264 genes recurrently mutated in adult AML (AML-RMG)
      – Coverage (mean): Exome, 199x; AML-RMG, 383x
    • Ampliseq at day 30 on a subset of patients

All leukemia associated variants are cleared and patient remains in remission

All leukemia-associated variants (both founding clone and subclones) persist and patient experiences disease relapse

Founding clone variants persist and subclonal populations are cleared
The continued detection of leukemia-associated variants is an independent predictor of poor outcome.
Molecular Monitoring in AML

- Other studies have demonstrated the persistence of DNMT3A mutations in remission (Ploen et al, BJH, 2014)
- IDH1 or IDH2 mutations are a better MRD target than DNMT3A (Debarri et al, Oncotarget, 2015)
- Deep sequencing for the detection of RUNX1 mutations in remission can identify resistant disease (Kohlman et al, Leukemia, 2014)
- Quantitative detection of NPMc transcripts is associated with risk of relapse (Ivey et al, NEJM 2016).

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Are we ready to move to molecular approaches for AML MRD?

Open questions:
1. How does molecular detection of variants correlate with MRD detection by flow cytometry?
2. How do we interpret molecular MRD findings in the context of clonal hematopoiesis?
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SJCRH AML MRD Study

• Goal: How does sequencing compare to MRD by multicolor flow cytometry?

• Cohort: 36 pediatric AML samples with material available at diagnosis and the first timepoint after induction therapy (~day 22)
  — Intermediate/high risk AML
  — Patients with MLL-rearranged AMLs, APL, CBF-AMLs were excluded
  — MRD results
    • Positive: 18
    • Negative: 14
    • Unavailable/unable to follow: 4

• Sequencing approach: Nextera Rapid Capture Expanded Exome (Illumina) at day 0 and day 22
  — Coverage: 100x at day 0, ~250x at day 22.

- Patient relapsed at day 343
- BRAF, SETD2 and NRAS mutations were present at relapse
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Molecular MRD for AML

• Tumor/normal comparison is critical
• What is the optimal source of “normal” comparator gDNA?
  • Avoid remission samples
  • Flow sorted T cells
  • Skin biopsy if peripheral counts are <25 k/cumm
    — Monocytic leukemias can be especially tricky
• What is the optimal sequencing platform?
  — Small focused panels may not reveal the full complexity of AML
  — Exome sequencing in combination with spiked-in probes offers a great combination of depth and discovery
Molecular MRD for AML

- RNA-based approaches are important additions
  - Fusion detection and mutant NPM1 transcripts
  - Only effective in a limited number of cases

- What is the optimal sensitivity for calling persistent variants after chemotherapy?
  - This will likely vary depending on the timepoint during therapy
  - Changes over time will be most important

Conclusions: Molecular Monitoring in AML

- Molecular approaches to detect residual disease in AML can provide important prognostic information

- Additional large studies are needed to determine:
  - If these approaches will supplant or compliment current MRD approaches, such as multicolor flow cytometry.
  - The significance of clonal hematopoiesis after chemotherapy
  - Optimal sequencing approaches

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