Molecular Diagnosis of Acute Myeloid Leukemia: Present and Future Challenges

This presentation will begin momentarily!
Molecular Diagnosis of Acute Myeloid Leukemia: Present and Future Challenges

David R. Czuchlewski, MD, FCAP and Mohammad A. Vasef, MD, FCAP

May 31, 2012
David Czuchlewski, MD, FCAP

- Assistant Professor of Pathology at the University of New Mexico
- Associate Director of the Molecular Diagnostic Laboratory at TriCore Reference Laboratories
- He has written widely on molecular genetic testing and hematopathology including in the recent editions of Foucar’s Bone Marrow Pathology, Diagnostic Pathology: Blood and Bone Marrow, and Henry’s Clinical Diagnosis and Management by Laboratory Methods
Mohammad Vasef, MD, FCAP

- Professor of Pathology at the University of New Mexico
- Director of the Molecular Diagnostic Laboratory at TriCore Reference Laboratories
- He has published on hematopathology and molecular genetic pathology-related topics including *The Pathology of the Spleen* in ‘The Complete Spleen’ and Foucar’s *Diagnostic Pathology: Blood and Bone Marrow*
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Disclosure

• We have no disclosures.
Acute Myeloid Leukemia (AML) Classifications

• Pre-French American British (FAB) proposal era
• Original FAB classification proposal (1976)
• Revised FAB classification proposal (1985)
• WHO classification (2001)
• WHO classification (2008)
Acute Myeloid Leukemia Classifications

Pre-French American British (FAB) proposal

- Morphologic and exclusion-based:
  - Acute non-lymphoid leukemia (ANLL)

Proposals for the classification of the acute leukemias. French-American-British (FAB) co-operative group*

Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C.

Br J Haematol (1976), 33:451-458*
FAB proposal for AML classifications

• An attempt for a uniform classification system
• Defined subgroups of acute leukemias
• Provided diagnostic guidelines based on:
  o Conventional morphology
  o Few cytochemical stains (MPO & NSE)
• AML subtypes grouped as M1-M6 (M0 and M7)
  o Line (s) of differentiation & extent of maturation

Br J Haematol (1976), 33:451-458*
FAB proposal for AML classification

- FAB system used in clinical trials by many groups
- FAB proposal was revised in 1985*
  - The FAB nomenclature retained but modified to:
    - further clarify different M categories
    - define M6 and its distinction from RAEB
- FAB classification of AML used for almost 3 decades
- M3 (APL) and M4Eo morphologic subtypes predicted underlying genetic abnormalities

*Ann Intern Med (1985), 103:626-629*
Discovery of chromosomal translocations in leukemias

- Chromosomal studies in leukemias continued since 1960s despite technical limitations
- Many chromosomal translocations discovered since introduction of G-banding in 1970
- t(8;21)-1972, 1st described recurrent chromosomal translocation

Discovery of chromosomal translocations in leukemias

Nowell P & Hungerford DA. *Science*, 1960;132:1497
Importance of Diagnostic Cytogenetics on Outcome in AML

The Importance of Diagnostic Cytogenetics on Outcome in AML: Analysis of 1,612 Patients Entered Into the MRC AML 10 Trial

By David Grimwade, Helen Walker, Fiona Oliver, Keith Wheatley, Christine Harrison, Georgina Harrison, John Rees, Ian Hann, Richard Stevens, Alan Burnett, and Anthony Goldstone on behalf of the Medical Research Council Adult and Children's Leukaemia Working Parties

- Trial began 1988, closed in 1995
- Median follow up: 61 months
- Accrued 1,966 pts including 364 children
- Three prognostic groups by cytogenetics:
  1. Favorable
  2. Intermediate
  3. Unfavorable
Correlation of Chromosome Abnormalities with Survival in AML

OS for AML patients classified according to cytogenetic risk group

614 patients with *de novo* AML

- favorable n = 94 (censored 69)
- intermediate n = 280 (censored 120)
- unfavorable n = 79 (censored 19)

OS in AML patients classified according to FAB criteria

614 patients with de novo AML

A

B

years from start of therapy

J Clin Oncol 2003;21:256-265
Importance of chromosomal aberrations in AML

- **Diagnostic importance:**
  - $t(8;21)$ - a more precise classification than FAB M2
  - $t(15;17)$ - restricted to APL
  - $inv(16)$ - a few more cases in addition to M4EO

- **Prognostic importance:**
  - Achievement of complete remission
  - Risk of relapse
  - Overall survival
  - Response to therapy (high with CBF leukemias)
Discoveries of critical genes due to study of translocations

- Examples of genes involved in AML identified by cloning translocation breakpoints:
  - \textit{RUNX1} (\textit{AML1/CBFA})
  - \textit{RUNX1T1(ETO)}
  - \textit{PML}
  - \textit{CBFB}
  - \textit{ETV6 (TEL)}
  - \textit{MLL}
Study of chromosomal translocations

Chromosomal translocations

Diagnostic/Prognostic/Predictive

Discovery of Critical Genes

Microarrays/Epigenetics

microRNAs/Gene mutations
WHO classification of AML (2001)

- AML with recurrent genetic abnormalities:
  1. APL with t(15;17)(q22;q12)
  2. AML with t(8;21)(q22;q22)
  3. AML with inv(16)(p13q22)/t(16;16)
  4. AML with 11q23 abnormalities

- AML with multi-lineage dysplasia

- AML and MDS, therapy related

- AML, not otherwise categorized
Acute promyelocytic leukemia AML-M3 (FAB)

• Rapid and accurate diagnosis is crucial due to:
  o Potential life-threatening coagulopathy
  o Unique response to all-trans-retinoic acid alpha

• All-trans-retionnic-acid alpha (ATRA):
  o Allows differentiation of promyelocytes
  o Can induce complete response in APL with PML-RARA fusion
Acute promyelocytic leukemia “continued’

- 2 morphologic subtypes:
  - Hypergranular variant with abn promyelocytes
  - Hypogranular variant

- Morphology in conjunction with MPO cytochemical stain highly predictive of correct diagnosis
  - Characteristic blasts with bi-lobed nuclei
  - Strong MPO reactivity in both variants
Myeloperoxidase
Core binding factor AML

Transcription of genes necessary for hematopoiesis

inv(16)/t(16;16) t(8;21)

KIT mutation is seen in up to 40% of CBF AML
Core binding factor AML

Repression of genes necessary for hematopoiesis

$\text{inv}(16)/t(16;16)$

$\text{t}(8;21)$

$\text{KIT}$ mutation is seen in up to 40% of CBF AML

$\text{KIT}$ mutation is seen in up to 40% of CBF AML
Core binding factors leukemias

- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)
  - 8% of adult AML cases (~10% of pediatric AML)
  - Increased myeloblasts and immature monocytes
  - Abnormal eosinophils with dark purple granules
  - inv(16) may be overlooked on cytogenetics
  - FISH or RT-PCR at diagnosis if indicated
  - Additional ch abnormalities, common
Core binding factors leukemias

- AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
  - 7% of adult AML cases (a third of FAB M2 cases)
  - Blasts may accounts for <20% of nucleated cells
  - Expression of CD19, PAX-5 and cCD79A
  - Increased mast cells may be seen
  - Additional ch abnormalities, common
  - KIT mutations may have adverse effect
AML1 (RUNX1)/ETO (RUNX1T1) positive FISH
\( t(8;21) \) probe set
AML with recurrent genetic abnormalities (2008 WHO)

- APL with t(15;17)(q22;q12)
- AML with t(8;21)(q22;q22)
- AML with inv(16)(p13;q22)/t(16;16)
- AML with t(9;11)(p22;q23)
- AML with t(6;9)(p23;q34)
- AML with inv(3)(q21q26)/t(3;3)
- AML with t(1;22)(p13;q13)
- AML with mutated NPM1
- AML with mutated CEBPA
WHO 2008 recognizes gene mutations, especially:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency (all de novo AML)</th>
<th>Utility</th>
<th>Prognostic impact</th>
<th>Associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIT</td>
<td>~5%</td>
<td>Prognostic factor</td>
<td>Poor</td>
<td>t(8;21), inv(16)/t(16;16)</td>
</tr>
<tr>
<td>FLT3</td>
<td>20-40%</td>
<td>Prognostic factor</td>
<td>Poor</td>
<td>--</td>
</tr>
<tr>
<td>NPM1</td>
<td>~30%</td>
<td>Defines provisional entity</td>
<td>Good (if no FLT3 mutation)</td>
<td>M4, M5, CD34(-)</td>
</tr>
<tr>
<td>CEBPA</td>
<td>~10%</td>
<td>Defines provisional entity</td>
<td>Good</td>
<td>M1, M2, CD7(+)</td>
</tr>
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</table>
KIT mutations in AML

- **Exon 17**: point mutations, codons 816, 822 (~2/3)
- **Exon 8**: especially affecting codon 419 (~1/3)
KIT codon 816 mutation by pyrosequencing
Adverse impact of \textit{KIT} mutation in \textit{t}(8;21) AML

Relapse more frequent and inferior survival

Boissel et al. Leukemia 2006; 20: 965-70
**KIT mutation can change treatment**

<table>
<thead>
<tr>
<th>RISK STATUS</th>
<th>CYTOGENETICS</th>
<th>MOLECULAR ABNORMALITIES</th>
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<tr>
<td>Better-risk</td>
<td><strong>inv(16)(^1) or t(16;16)(^1)</strong> t(8;21)(^1) t(15;17)**</td>
<td>Normal cytogenetics: with NPM1 mutation or isolated CEBPA(^3) mutation in the absence of FLT3-ITD</td>
</tr>
<tr>
<td>Intermediate-risk</td>
<td>Normal cytogenetics +8 t(9;11) Other non defined</td>
<td>t(8;21), inv(16), t(16;16): with c-KIT(^4) mutation</td>
</tr>
<tr>
<td>Poor-risk</td>
<td>Complex (≥3 clonal chromosomal abnormalities) -5, 5q-, -7, 7q-11q23 - non t(9;11) inv(3), t(3;3) t(6;9) t(9;22)(^2)</td>
<td>Normal cytogenetics: with FLT3-ITD mutation(^5)</td>
</tr>
</tbody>
</table>
Targeted therapy in CBF-AML

• **Hypothesis:** Dasatinib, a potent inhibitor of KIT, would be effective in CBF-AML

Cancer and Leukemia Group B (CALGB), NCT01238211

German-Austrian AML Study Group, NCT00850382

• **KIT** mutation is NOT an eligibility criterion for these trials!
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Physiologic roles of FLT3, NPM1, CEBPA

**NPM1:**
Cytosol/nuclear shuttle

**FLT3:**
Cell surface tyrosine kinase

**CEBPA:**
Hematopoietic transcription factor
Gene mutations in cytogenetically normal AML.
**FLT3**

- Internal tandem duplication
- Tyrosine kinase domain mutation

**NPM1**

- Insertion
- NLS
FLT3 and NPM1 mutation detection

Normal NPM1

Normal FLT3

NPM1 mutation

FLT3 mutation
The two types of CEBPA mutations

- **N-terminal (frame shift)**
  - Truncated protein lacks transactivating domain

- **C-terminal (in frame)**
  - Defective DNA binding domain

CEBPA mutation detection requires more comprehensive sequencing and/or screening methods
CEBPA: Double is the trouble

Gene mutations can change treatment

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<td>Normal cytogenetics: with NPM1 mutation or isolated CEBPA(^3) mutation in the absence of FLT3-ITD</td>
</tr>
<tr>
<td></td>
<td>t(6;12)(^1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(8;21)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>inv(16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other non defined</td>
<td></td>
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<tr>
<td>Intermediate-risk</td>
<td>Normal cytogenetics</td>
<td>t(8;21), inv(16), t(16;16): with c-KIT(^4) mutation</td>
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<tr>
<td>Poor-risk</td>
<td>Complex (≥3 clonal chromosomal abnormalities)</td>
<td>Normal cytogenetics: with FLT3-ITD mutation(^5)</td>
</tr>
<tr>
<td></td>
<td>-5, -10, -7, 7q-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13p-3 non t(9;11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>inv(16), t(3;3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(6;12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(8;21)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(9;22)</td>
<td></td>
</tr>
</tbody>
</table>
Gene mutations can change AML post-remission therapy

<table>
<thead>
<tr>
<th>Risk</th>
<th>HiDAC without transplant</th>
<th>Autologous transplant</th>
<th>Allogeneic transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Better risk</td>
<td>✓</td>
<td>✓</td>
<td>x</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Poor risk</td>
<td>x</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
**FLT3: Additional clinical relevance**

High *FLT3* allele burden confers worse prognosis

- Mutant *FLT3* / total *FLT3*

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**FLT3 inhibitors**

- Multiple open clinical trials
- Quizartinib: very successful phase I trial
- Some trials permit *FLT3*-TKD mutation!
### NPM1 and CEBPA in special situations

<table>
<thead>
<tr>
<th>NPM1 or CEBPA mutation with:</th>
<th>Prognostic significance unclear</th>
<th>More recent prognostic data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal cytogenetics</td>
<td></td>
<td>NPM1: Favorable even with abnormal karyotype (?)</td>
</tr>
<tr>
<td>Myelodysplasia-related changes</td>
<td>Prognostic significance unclear</td>
<td>NPM1: Favorable even with MDS changes</td>
</tr>
</tbody>
</table>
Stratification of Pediatric AML is different

- Current COG AAML1031 Protocol:

<table>
<thead>
<tr>
<th>“Low risk”</th>
<th>“High risk”</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPM1 or CEBPA mutation regardless of monosomy 7, monosomy 5, or del5q</td>
<td>Any FLT3-ITD+ with high allelic ratio &gt; 0.4</td>
</tr>
</tbody>
</table>
TET2

- Mutated in 10-15% of de novo AML, but even more common in elderly
- Common in MDS/MPN, especially CMML
- Prognostic impact controversial
- Encodes demethylase, so mutation induces hypermethylation (?)
**IDH1 and IDH2**

- Mutated in ~18% of de novo AML
- Associated with normal cytogenetics and NPM1 mutation
- Apparently poor prognosis, though controversial
- Encode citric acid cycle enzymes (cytosolic and mitochondrial)
- Mutation induces “neomorphic” enzyme activity
Other mutations include . . .

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency (all de novo AML)</th>
<th>Comment</th>
<th>Prognostic impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT3A</td>
<td>~20%</td>
<td>Results in hypomethylation</td>
<td>Poor</td>
</tr>
<tr>
<td>RUNX1</td>
<td>~10%</td>
<td>Independent of t(8;21)</td>
<td>Poor</td>
</tr>
<tr>
<td>WT1</td>
<td>~10%</td>
<td>WT1 overexpression also observed</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>RAS</td>
<td>~10-15%</td>
<td>Signal transducer (KIT, FLT3, etc.)</td>
<td>None?</td>
</tr>
<tr>
<td>MLL-PTD</td>
<td>7%</td>
<td>Independent of MLL translocation</td>
<td>Poor; OK with intensification</td>
</tr>
</tbody>
</table>
Many mutations are not mutually exclusive

The interpretive challenge will increase with next generation sequencing. . .
AML whole genomes

57-year-old female with cytogenetically normal AML
Ley TJ et al. Nature 2008; 456: 66-72

38-year-old male with cytogenetically normal AML
Mardis ER et al. NEJM 2009; 361: 1058-66

Genetic alterations include:

Genetic changes in normal AML:
- NPM1
- FLT3
- PTPRT
- CDH24
- PCLKC
- SLC15A1
- KNDC1
- GPR123
- EBI2
- GRINL1B

Genetic changes in non-AML:
- NPM1
- NRAS
- IDH1
- IMPG2
- ANKRD26
- LTA4H
- FREM2
- C19orf62
- SRRM1
- PCDHA6
- CEP170
AML survival remains poor: a challenge and an opportunity

Age 45-54

Molecular Genetics of Pancreatic Neoplasms
Wednesday, June 13th, 9:00-10:00 am Central
- Ralph Hruban, MD

The recent whole exome sequencing of pancreatic cancer and the other common tumors of the pancreas has provided unparalleled insight into pancreatic neoplasia. An integration of the results of this sequencing with histopathology allows for the creation of a new classification of pancreatic neoplasia; one that incorporates molecular changes. This webinar will review the results of the whole exome sequencing of the six most common tumor types of the pancreas with an emphasis on how this sequencing will impact practicing pathologists.
<table>
<thead>
<tr>
<th>Date</th>
<th>Title</th>
<th>Presenter(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 13</td>
<td>Molecular Genetics of Pancreatic Neoplasms</td>
<td>Ralph Hruban, MD</td>
</tr>
<tr>
<td>July 24</td>
<td>Biospecimens and Biorepositories for the Community Pathologist</td>
<td>Raj C. Dash, MD, FCAP, David L. Booker, MD, FCAP, and James Robb, MD, FCAP</td>
</tr>
<tr>
<td>Sept 27</td>
<td>Biomarkers in HPV-associated Lower Anogenital Squamous Lesions from the CAP-ASCCP Lower Anogenital Squamous Terminology Project</td>
<td>Mark Stoler, MD, FCAP</td>
</tr>
<tr>
<td>Nov 14</td>
<td>Molecular Markers for the Evaluation of Colorectal Cancer- joint webinar with AMP (hosted by AMP)</td>
<td>Stanley Hamilton, MD, FCAP, Federico Monzon, MD, FCAP, and Wayne Grody, MD, FCAP</td>
</tr>
</tbody>
</table>
Don’t Forget to Check Out Past Webinars!

• Past Webinars Available Now Online at www.cap.org/institute
  o Clinical Next-Generation Sequencing: Just Another Lab Test
  o Molecular Testing Selection Guidelines for Selecting Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors
  o Clinical Use of Whole Genome and Whole Exome Sequencing Today
  o Validating Whole Slide Imaging Systems for Diagnostic Use in Pathology
  o The Why, What and How of Identifying Patients at Risk
  o Molecular Diagnosis for Lung Cancer Patients
  o Whole Genome Analysis as a Universal Diagnostic: A Pathologist’s Perspective
  o Next-Generation Sequencing for the Clinical Laboratory

• Go to www.cap.org/institute For All Upcoming Webinars!
• **CAP/ASH Algorithm for Initial Work-up of Acute Leukemia**
  - To provide recommended testing for the initial workup for proper diagnosis, determination of prognostic factors and for possible future monitoring of acute leukemias, including AML, ALL and mixed phenotype acute leukemia in children and adults. This project is a joint collaboration with the American Society of Hematology (ASH).

• **CAP Bone Marrow Synoptic Reporting for Hematologic Neoplasms**
  - To address the highly variable reporting methodologies to provide clear, appropriate reporting for patient safety, treatment, and prognosis.
## CAP Learning – Leukemia CME/CE

<table>
<thead>
<tr>
<th>Course</th>
<th>Learning Objectives</th>
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</table>
| Molecular Testing for AML Cases                                         | -Recognize molecular oncology knowledge and skills required of pathologists that can mitigate problems and enhance patient care with respect to specimen handling  
-Realize the effects that appropriate specimen handling and communication throughout all stages of diagnosis have in enhancing patient care  
-Reflect on your own knowledge and skills in specimen handling and patient care, and identify what can help you and your practice be more effective in these areas of molecular oncology |
| Archives Applied - Acute Erythroid Leukemia (SAM eligible)              | -Identify the current criteria for the diagnosis of AEL.  
-Identify the clinical features of AEL.  
-Identify typical morphologic and cytochemical findings of AEL.  
-Identify Immunophenotypic findings of AEL.  
-Distinguish the features that indicate a diagnosis of AEL versus other types of AML.                                                                 |
-Recognize the characteristics of additional B cell lymphomas included in the morphological category of small B cell lymphomas.  
-Formulate a differential diagnosis of small B cell lymphomas based on morphological, immunophenotypical and cytogenetic parameters.  
-Understand the clinical and prognostic significance in bone marrow evaluation in patients with small B cell lymphomas. |
The CAP Learning Portal landing page on the cap.org website replaces the previous Education Programs page design. A user must log into cap.org in order to access further information.

The CAP Learning Portal includes new tools to support the learning needs of pathologists such as:

- Learning Options search/catalog
- Competency Model for Pathologists
- Personal Progress Check (member only tool)
- My Learning Plan (member only tool)
- Help Center

Benefits:
- Increase effectiveness to plan and manage learning
- Increase efficiency to target learning needs and identify premium learning solutions
- Increase satisfaction with learning solutions that meet specific learner needs
- Increase capability to maintain professional certifications
To learn more…

- For more details and to register for/access Leukemia educational offerings:
  1. Log in to the cap.org website
  2. Click on Launch Portal
  3. Click on the Learning Options tab
  4. Type Leukemia in the Search box

A list of available learning options displays
Thank you for attending our webinar “Molecular Diagnosis of Acute Myeloid Leukemia: Present and Future Challenges” by David Czuchlewski, MD, FCAP and Mohammad Vasef, MD, FCAP.

For comments about this webinar or suggestions for upcoming webinars, please contact Jill Kaufman, PhD, Director of Personalized Health Care at jkaufma@cap.org

NOTE: There is no CME/CE credit available for today’s free webinar.