Genetic Testing in Diagnosis of Acute Leukemias: Summary

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The Diagnosis of Acute Leukemia

• Genetics play an increasingly important role in the diagnosis of acute leukemia

• But,

• An accurate diagnosis using the WHO classification requires more than just genetics:
  – Clinical information
  – Morphology
  – Immunophenotype
  – Cytogenetics
  – Molecular genetics
# AML with Recurrent Genetic Abnormalities

<table>
<thead>
<tr>
<th>Case #</th>
<th>Contributor</th>
<th>Panel Diagnosis</th>
<th>Other</th>
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<tbody>
<tr>
<td>SH2017-0150</td>
<td>M. Vasef</td>
<td>APL with <em>PML-RARA</em></td>
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<td>University of New Mexico</td>
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<td>SH2017-0180</td>
<td>C.C. Yin</td>
<td>APL with variant <em>RARA</em> rearrangement (<em>IRF2BP2-RARA</em>)</td>
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<td>MD Anderson Cancer Center</td>
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<td>SH2017-0118</td>
<td>R. Juskevicius</td>
<td>APL with variant <em>RARA</em> rearrangement (<em>ZBTB16-RARA</em>)</td>
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<td>Vanderbilt University Medical Center</td>
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<td>SH2017-0262</td>
<td>V. Reddy</td>
<td>1. AML with t(8;21) 2. CLL</td>
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<td>University of Alabama at Birmingham</td>
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<td>SH2017-0290</td>
<td>H. Yu</td>
<td>AML with inv(3)</td>
<td>Myeloid T markers</td>
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<tr>
<td>SH2017-0291</td>
<td>M. Menon</td>
<td>AML with <em>BCR-ABL1</em></td>
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<td>A. Vogel</td>
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<td>SH2017-0335</td>
<td>S. Garces</td>
<td>AML with <em>BCR-ABL1</em></td>
<td>Acquired</td>
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<td>SH2017-0053</td>
<td>H. Kurt</td>
<td>AML with mutated <em>RUNX1</em></td>
<td>Aberrant CD79A and TdT (subset)</td>
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<td>MD Anderson Cancer Center</td>
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<td>SH2017-0164</td>
<td>G. Mikita</td>
<td>AML with mutated <em>RUNX1</em></td>
<td>Ambiguous immunophenotype and myeloid genetic mutations.</td>
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<td>Weill Cornell Medicine</td>
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<td>SH2017-0281</td>
<td>D. Yang</td>
<td>AML with mutated <em>RUNX1</em></td>
<td>Other co-mutations</td>
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<td>University of Wisconsin</td>
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<tr>
<td>SH2017-0313</td>
<td>A. Quesada</td>
<td>AML with mutated <em>RUNX1</em></td>
<td>Salmon pink granules</td>
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<td>MD Anderson Cancer Center</td>
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<tr>
<td>SH2017-0370</td>
<td>S. Jain</td>
<td>AML with mutated <em>RUNX1</em></td>
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<td>University of Pittsburgh</td>
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</table>
AML with *BCR-ABL1*

- Difficult to distinguish from myeloid blast crisis of chronic myeloid leukemia
- Deletion of antigen receptors (*IGH, TCR, IKZF1*, and/or *CDKN2A*) may support a diagnosis of de novo disease
- Patients may benefit from targeted (TKI) therapy
- New provisional entity

AML with Acquired **BCR-ABL1**

Initial Marrow

- **NRAS** mutation
- **BCR-ABL1** negative

Case 335  
S. Garces

Refractory AML

- **NRAS** mutation
- **BCR-ABL1** positive
AML with mutated \textit{RUNX1}

- Gene located at 21q22
- Encodes the alpha subunit of the core binding factor
- Mutation in 12.5-13.2\% of AML
- More frequent in older male patients
- Frequent prior history of MDS, or prior exposure to radiation
- Frequent among FAB M0 cases, but wide morphologic spectrum
- Frequently associated \textit{KMT2A}-PTD, \textit{IDH1}, \textit{IDH2} or \textit{ASXL1} mutations
- Rare \textit{CEBPA} or \textit{NPM1} mutations
- Poor response to therapy with shortened survival
- Germline mutations should be evaluated

Mendler et al. J Clin Oncol 30:3109, 201
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Mutations in *RUNX1*

- Germline
- Post radiation
  - Therapy-related myeloid neoplasm
- History of MDS or MDS/MPN
  - AML with myelodysplasia-related changes
- de novo
  - AML with mutated *RUNX1*
### Other AML Types

<table>
<thead>
<tr>
<th>Case #</th>
<th>Contributor</th>
<th>Panel Diagnosis</th>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>SH2017-0024</td>
<td>N. Baste</td>
<td>AML-MRC</td>
<td>Normal karyotype; ASXL1 mutation; history of aCML</td>
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<tr>
<td>SH2017-0054</td>
<td>B. Chen</td>
<td>AML-MRC</td>
<td>i(17q); FLT3-ITD, IDH2, NRAS and BCOR mutations</td>
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<tr>
<td>SH2017-0107</td>
<td>C. Liu</td>
<td>AML-MRC</td>
<td>Diagnosis limited by biopsy only; CD34 negative; complex karyotype</td>
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<tr>
<td>SH2017-0201</td>
<td>M. Jan</td>
<td>AML-MRC</td>
<td>Complex karyotype that included t(4;17); RARA translocation excluded</td>
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<tr>
<td>SH2017-0325</td>
<td>M. Foshat</td>
<td>AML-MRC</td>
<td>High WBC, MLD, splenomegaly, marrow fibrosis, CALR mutation. AML vs BC of MPN</td>
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<tr>
<td>SH2017-0128</td>
<td>M. Yabe</td>
<td>AML-MRC</td>
<td>10% total blasts reported; KMT2A translocation detected in myeloid component only</td>
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<tr>
<td>SH2017-0288</td>
<td>R. Crotty</td>
<td>Therapy-related myeloid neoplasm (AML)</td>
<td>PEL with ZYMA-REL rearrangement</td>
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<tr>
<td>SH2017-0323</td>
<td>C. Cotta</td>
<td>AML with t(8;21); favor therapy-related</td>
<td>3 years post lenalidomide therapy for myeloma</td>
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<tr>
<td>SH2017-0328</td>
<td>K. Holder</td>
<td>Therapy-related myeloid neoplasm (AML) with t(9;11)</td>
<td>Post therapy for APL</td>
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<tr>
<td>SH2017-0276</td>
<td>N. Patel</td>
<td>AML-NOS</td>
<td>Morphologic features of megakaryocytic lineage</td>
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</tbody>
</table>
Other AML Types

AML with myelodysplasia-related changes

- May arise from MDS or MDS/MPN
- Recurring cytogenetic abnormalities not included in AML RGA are rare, but do occur
- Revised 2016 WHO criteria have de novo cases with $NPM1$ or biallelic $CEBPA$ mutations trumping this category

Case 54
B. Chen
i(17q)

Case 325
M. Foshat
$CALR$ mutation
Other AML Types

Therapy-related myeloid neoplasms

- May have recurring cytogenetic abnormalities that impact prognosis and should be noted (cases 128, 323 and 328)
- May occur after therapy for another AML type (case 328)

Case 328
K. Holder
t-MN (AML) with t(9;11)
Other AML Types

AML, not otherwise specified

- Morphology is often enough to subclassify
- Megakaryocyte lineage, however, must be confirmed by immunophenotyping

Case 276
N. Patel
AML Genetic Testing Recommendations

- Karyotype
- Molecular testing
  - For all or most cases
    - FLT3-ITD, NPM1, CEBPA, RUNX1
    - Others: IDH1, IDH2, TET2, WT1, DNMT3A, TP53, (FLT3-TKD), (ASXL1)
  - For select cases
    - KIT for core binding factor leukemias
    - PML-RARA if APL suspected

Based on CAP/ASH Guidelines Arch Pathol Lab Med. 2017 Feb 22. [Epub ahead of print].
Genes in parentheses are not part of the guideline, but are included due to newly available drug targeting the abnormality (FLT3-TKD) or required for full evaluation of ELN risk group (Blood. 2017 Jan 26;129(4):424-447).
### Immature Ambiguous and T-cell Neoplasms

<table>
<thead>
<tr>
<th>Case #</th>
<th>Contributor</th>
<th>Panel Diagnosis</th>
<th>Sub-diagnosis and Additional Information</th>
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<tbody>
<tr>
<td>SH2017-0113</td>
<td>E. Castro-Echeverry University of Pittsburgh</td>
<td>MPAL, B/myeloid, not otherwise specified</td>
<td>Cryptic NUP98/NSD1 t(5;11)(q35;p15) by SNP microArray analysis</td>
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<tr>
<td>SH2017-0163</td>
<td>G. Griffin Boston Children's Hospital</td>
<td>MPAL, B/T</td>
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<tr>
<td>SH2017-0188</td>
<td>J. Aster Brigham &amp; Women's Hospital</td>
<td>MPAL, T/myeloid, not otherwise specified favored</td>
<td>NOTCH1, DNMT3A, ETV6, and IKZF1 mutations ETP-ALL vs. MPAL</td>
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<tr>
<td>SH2017-0221</td>
<td>Y. Xie University of California San Francisco</td>
<td>MPAL, T/myeloid, not otherwise specified</td>
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<tr>
<td>SH2017-0319</td>
<td>Z. Hu MD Anderson Cancer Center</td>
<td>MPAL, T/myeloid, not otherwise specified</td>
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<td>SH2017-0026</td>
<td>M. Xu Yale University</td>
<td>AUL</td>
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<tr>
<td>SH2017-0103</td>
<td>S. Williams University of Minnesota</td>
<td>Acute leukemia, not definitively classifiable</td>
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<tr>
<td>SH2017-0189</td>
<td>J. Cortazar Brigham &amp; Women's Hospital</td>
<td>T-ALL</td>
<td>IL7R exon 6 insertion mutation</td>
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<tr>
<td>SH2017-0259</td>
<td>G. Wertheim Children's Hospital of Philadelphia</td>
<td>T-ALL favored</td>
<td>NGS support for ETP lymphoma?</td>
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</tbody>
</table>
Early T-Precursor Acute Lymphoblastic Leukemia (ETP-ALL)

- Early T-Precursor (ETP) ALL comprises 10-15% of T-ALL
- Defined immunophenotypically by expression of CD7, CD3 (surface or rarely cytoplasmic) but not CD1a or CD8
  - Express one or more of the following CD34, CD117, HLA-DR, CD11b, CD65, CD33, or CD13, but not MPO
  - Usually express CD2; CD5 negative or absent in 25% or more of cells
- Molecular genetics
  - Increase in AML-associated mutations (FLT3, NRAS/KRAS, DNMT3A, IDH1, IDH2)
  - Infrequent NOTCH pathway (T-ALL-associated) mutations
- Initially considered high risk due to higher rate of induction failure
- Recent COG study suggests no outcome difference with current T-ALL therapy

Immature Ambiguous and T-cell Neoplasms

- Both ETP-ALL and MPAL are diagnosed by immunophenotype
  - MPAL is subclassified based on detection of \( KMT2A \) translocations or \( BCR-ABL1 \)
- Other more specific disease categories trump MPAL
- As currently defined, ETP-ALL must be MPO negative
MPAL and T-ALL Genetic Testing Recommendations

• MPAL
  – Karyotype
  – *BCR-ABL1*
  – *KMT2A* translocations

• T-ALL
  – Karyotype
  – *NOTCH1, FBXW7* mutations may be performed

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<th>Contributor</th>
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<tr>
<td>SH2017-0078</td>
<td>K. D. Li University of Utah</td>
<td>B-ALL, BCR-ABL1-like IGH-CRLF2 rearrangement</td>
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<tr>
<td>SH2017-0110</td>
<td>C. S. Wilson University of New Mexico</td>
<td>B-ALL, BCR-ABL1-like Ph-like gene expression signature and JAK2 mutation</td>
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<td>SH2017-0120</td>
<td>A. Wu UCLA</td>
<td>B-ALL, BCR-ABL1-like Xp22 translocation, presumably involving CRLF2</td>
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<td>SH2017-0123</td>
<td>Y. Hui University of Pennsylvania</td>
<td>B-ALL, BCR-ABL1-like novel GOLGA5-JAK2 fusion and sensitivity Ruxolitinib</td>
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<td>SH2017-0131</td>
<td>K. Ganapathi University of California San Francis</td>
<td>B-ALL, BCR-ABL1-like</td>
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<tr>
<td>SH2017-0142</td>
<td>S. Li MD Anderson Cancer Center</td>
<td>B-ALL, BCR-ABL1-like with CRLF2 rearrangement and JAK2 Mutation</td>
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<td>SH2017-0233</td>
<td>V. Leventaki St. Jude Children’s Research Hospital</td>
<td>B-ALL, BCR-ABL1-like with IGH-CRLF2 rearrangement</td>
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<tr>
<td>SH2017-0242</td>
<td>M.-L. Zhu Cleveland Medical Center</td>
<td>B-ALL, BCR-ABL1-like with CRLF2 positivity by flow cytometry, cryptic CRLF2 translocation t(Yp11.32;?), as well as t(X;20)(p22;q13.3) and deletion of IKZF1</td>
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<td>C. Ryder Cleveland Medical Center</td>
<td>B-ALL, BCR-ABL1-like P2YR8-CRLF2 translocation, JAK2 and IL7R Mutations, and somatic trisomy 21</td>
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<td>T. Zhou Baylor College of Medicine</td>
<td>B-ALL, BCR-ABL1-like with CRLF2 expression and rearrangement</td>
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<tr>
<td>SH2017-0322</td>
<td>M. Alikhan University of Chicago</td>
<td>B-ALL, BCR-ABL1-like JAK2 mutation with CRLF2 alteration in two siblings: a possible inherited cancer predisposition</td>
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<tr>
<td>SH2017-0343</td>
<td>S. Ondrejka Cleveland Clinic</td>
<td>B-ALL, BCR-ABL1-like PDGFRB rearrangement</td>
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<tr>
<td>SH2017-0362</td>
<td>R. Pillai City of Hope</td>
<td>B-ALL, BCR-ABL1-like with P2RY8-CRLF2 fusion, JAK2 and JAK1 mutations</td>
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BCR-ABL1-like B-ALL

- **BCR-ABL1**-like B-ALL is a high risk ALL with a gene expression profile similar to that of BCR-ABL1+ ALL
- Accounts for 10% of pediatric and 25% of adult ALL; associated with Hispanic ethnicity; poor clinical outcomes; some cases respond to TKI therapy
- Need to establish clear diagnostic criteria
  - **CRLF2** translocations
    - Usually show increased expression of CRLF2 by flow cytometry analysis
  - Some have activating mutations or translocations of genes, such as ABL1, ABL2, JAK2, PDGFRB, NTRK3, TYK2, CSF1R, and/or EPOR
  - The full spectrum of genetic changes is still being investigated

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Case 322 M. Alikhan

BCR-ABL1-like B-ALL

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- CRLF2 translocations
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  - Some have activating mutations or translocations of genes, such as ABL1, ABL2, JAK2, PDGFRB, NTRK3, TYK2, CSF1R, and/or EPOR
- The full spectrum of genetic changes is still being investigated

B-ALL, *BCR-ABL1*-like

- 12 of 14 cases had some evidence of *CRLF2* abnormalities (expression, FISH, karyotype, sequencing)
- 6 of 6 cases with clear data of testing were *JAK2* mutated or translocated
- 7 of 14 cases had a *BCR-ABL1*-like signature on a send out panel
- 1 case (343) had a *PDGFRB* translocation
- We are probably still missing a significant percentage of cases with current testing approaches
# Other ALL Types

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<tr>
<td>SH2017-0235</td>
<td>M. Koo</td>
<td>B-ALL with iAMP 21</td>
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<td>UCLA</td>
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<td>SH2017-0366</td>
<td>M. Harris</td>
<td>B-ALL with iAMP 21</td>
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<td>SH2017-0045</td>
<td>C. Roth</td>
<td>B-ALL hypodiploid with TP53 mutation</td>
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<td>Baylor College of Medicine</td>
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<td>SH2017-0108</td>
<td>E. Mason</td>
<td>LPL in setting of B-ALL KMT2A rearrangement</td>
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<td>Vanderbilt University Medical Center</td>
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<td>SH2017-0183</td>
<td>J. Cheng</td>
<td>B-ALL, possibly therapy-related</td>
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<td>University of Chicago</td>
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<td>SH2017-0347</td>
<td>J. Gomez-Gelvez</td>
<td>B-ALL, BCR-ABL1 positive</td>
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B-ALL with iAMP21

• Intrachromosomal amplification of chromosome 21 (iAMP21) accounts for about 2% of pediatric B-ALL
• Uncommon in adults
• Adverse outcomes when treated with standard risk therapy; but improved when treated as high risk ALL
• Presence of 5 or more copies of RUNX1 on a single cell or 3 or more extra copies on a single abnormal chromosome 21 in metaphase FISH
• Reliably detected by FISH for RUNX1 used to evaluate for B-ALL with ETV6-RUNX1

Harrison et al. Leukemia 28:1015, 2014
B-ALL Genetic Testing Recommendations

- Karyotype
- BCR-ABL1
- KMT2A translocations
- PAX5, JAK1, JAK2, IKZF1 mutations may be performed*
- Pediatrics
  - t(12;21)(p13.2;q22.1); ETV6-RUNX1
  - iAMP21
  - Trisomy 4 and 10

*commercial assays for BCR-ABL1-like ALL not readily available

Diagnostic Hierarchy of Acute Myeloid (and Mixed Phenotype) Leukemias

Clinical information
- Prior cytotoxic therapy
- Down syndrome
- Prior MDS or MDS/MPN
  (Germline mutations)

Genetics
- Specific cytogenetic and molecular
  genetic abnormalities

Morphology
- Blast count
  - <20% MDS
  - >20% AML

Genetics
- Multilineage dysplasia

Immunophenotype
- MPAL, NOS

Genetics
- AML or MPAL with recurrent
  genetic abnormalities

Prognosis
- Therapy-related myeloid neoplasm
- Myeloid proliferations related to
  Down syndrome
- AML with myelodysplasia-related changes
- AML, NOS
- AML with myelodysplasia-related changes
- AML, NOS
Thank you for your attention