The Revised 2016 WHO Classification of Acute Leukemias

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Acute leukemias

• Aggressive hematopoietic neoplasms with maturation arrest of the myeloid or lymphoid lineage leading to the accumulation of blasts

• Heterogeneous clinical behavior and treatment
  – Due to the varied genetic abnormalities that cooperate to cause hyperproliferation and impaired maturation of the malignant clone
  – Treatment plan based on type of leukemia (AML versus ALL), disease subcategorization, and patient factors
Overall 2016 WHO acute leukemia classification

- Acute leukemia
  - AML and related neoplasms
  - Acute leukemias of ambiguous lineage
  - B-ALL
  - T-ALL

- Blastic plasmacytoid dendritic cell neoplasm
Getting to a diagnosis of acute leukemia

• Are they blasts?
• Are the blasts at least 20% of all nucleated marrow cells or blood leukocytes?

• If blasts are <20% in blood or marrow, is one of these ‘loopholes’ fulfilled?
  — AML-defining translocation is present
    • PML-RARA, t(8;21), inv(16)/t(16;16)
  — Blasts + blast equivalents are ≥20%
Pitfalls in the diagnosis of acute leukemias

Proliferations of non-neoplastic blasts, erythroids, or monocytes

Growth factor effect, infections

Megaloblastic anemia

Hematogone proliferations

Lymphomas

Other neoplasms with excess blasts

Blast mimics

AML

ALL

MDS

MPN

MDS/MPN
Blast: AML

Not blast: CLL prolymphocyte

Not blast: Mantle cell lymphoma

Not blast: Burkitt lymphoma
Exceptions: ‘Blast equivalents’

- **AML with** \textit{PML-RARA}
  - Promyelocytes are counted along with myeloblasts

- **AML with monocytic differentiation**
  - Promonocytes (not monocytes) are counted along with monoblasts

- **Pure erythroleukemia**
  - Pronormoblasts are blast equivalents
  - $>80\%$ immature erythroids with $\geq 30\%$ pronormoblasts
APML in bone marrow aspirate
Promonocytes are blast equivalents

Monoblasts

Promonocytes

Monocytes

BLAST EQUIVALENTS
Pure erythroid leukemia
Acute myeloid leukemia: WHO 2008

• AML with recurrent genetic abnormalities
  – Cytogenetically-defined entities
  – AML with mutated NPM1
  – AML with mutated CEBPA
• AML with myelodysplasia-related changes
• Therapy-related myeloid neoplasms
• AML, not otherwise specified
• Myeloid sarcoma
• Myeloid proliferations related to Down syndrome
• Blastic plasmacytoid dendritic cell neoplasm
2016 WHO AML Classification

AML

“De novo”

“Secondary”

Genetics

AML with recurrent genetic abnormalities

AML, not otherwise specified

Morphology

Therapy-related AML

AML with MDS-related changes

History

Morphology

Myeloid proliferations related to Down syndrome

History

Genetics

Myeloid neoplasms with germline predisposition

History
AML with recurrent genetic abnormalities

- with t(15;17)(q22;q12); PML-RARA
- with inv(16)(p13.1q22)/t(16;16)(p13.3;q22); CBFB-MYH11
- with t(8;21)(q22;q22.1); RUNX1-RUNX1T1

- Tend to develop rapidly, with few other genetic lesions
- Define AML even if <20% blasts
- Favorable prognosis

Importance of cytogenetic abnormalities in risk-stratifying AML

Byrd JC et al. Blood 2002;100:4325
Important to recognize AML with t(15;17)!

• Abundant abnormal promyelocytes
  – Classical: heavily granulated with frequent Auer rods
  – Microgranular: granules are indistinct, prominently bilobed nucleus

• Flow cytometry clues
  – HLA-DR negative, CD11 negative, CD34 negative, strong MPO

• Clinical clues
  – Disseminated intravascular coagulation (DIC), fulminant or incipient
APML in bone marrow trephine biopsy
Refinement of APML definition in 2016

• Name changed from APML with t(15;17) to AML with \textit{PML-RARA}
  
  – Some \textit{PML-RARA} rearrangements may be cryptic and missed on routine karyotype
  
  – Complex cytogenetic rearrangement may produce same \textit{PML-RARA} fusion but with different karyotype abnormality (e.g. 3-way translocation)

• APML variants with alternative \textit{RARA} fusions are also recognized
  
  – \textit{NUMA-RARA, BCOR-RARA, PRKAR1A-RARA, NPM1-RARA}: some respond to ATRA therapy

AML with t(8;21)

AML with inv(16)
Poorer prognosis AML with recurrent genetic abnormalities

- with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A*
- with t(6;9)(p23;q34.1);*DEK-NUP214*
  - Younger individuals, basophilia
- with inv(3)(q21.3;q26.2)/t(3;3)(q21.3;q26.2)*GATA2,MECOM (EVI1)*
  - Small megakaryocytes, often increased platelets
- with t(1;22)(p13.3;q13.3);*RB1M15-MKL1*
  - Infants, megakaryoblastic subtype
New entity: AML with $BCR-ABL1$

- Very rare (0.5%) AML, poor prognosis
- Challenge to distinguish from CML blast crisis
  - Recent data suggest frequent loss of $IGH$, $IKZF$, and/or $CDKN2A$ loci in these leukemias
  - Splenomegaly and basophilia are usually absent in AML with $BCR-ABL1$, unlike CML blast crisis)
- Important to recognize, because these patients may benefit with TKI treatment

Mutations in AML ca. 2008

*NPM1* mutation

*CEBPA* mutation

*FLT3* ITD

Arrested myeloid maturation + Hyperproliferation

**Acute leukemia**
**NPM1** and **CEPBPA**-mutated AML: 2016 WHO mutation-defined entities

- **NPM1**-mutated AML
  - Favorable prognosis if not accompanied by **FLT3 ITD**
  - Often monocytic phenotype, CD34 negative

- **CEBPA**-mutated AML
  - Favorable prognosis
  - Mutation must be bi-allelic (usually different mutations on different gene alleles)
AML with *NPM1* and *FLT3* ITD mutations
Bone marrow biopsy in AML with *NPM1* mutation
NPM1 immunohistochemistry
Other gene mutations in AML

- Epigenetic modifiers
- Cohesin complex
- RNA splicing
- Tyrosine kinase and RAS signalling
- Transcription factors
- DNA Repair

The Cancer Genome Atlas Research Network. NEJM 2013;368:2059
Other mutations influence outcome of genetically-defined AML entities

Papaemmanuil E et al. NEJM 2016;374: 2209
New entity: AML with mutated \textit{RUNX1}

- Encodes the alpha subunit of the core binding factor
- Mutation in \(~13\%\) of AML mostly older male patients
  - Cases occurring after therapy or following MDS are classified separately
- Poor response to therapy with short survival

Courtesy of Drs Dan Arber and James Vardiman

Secondary AML

• Therapy-related AML
  – Any AML arising in a patient with history of cytotoxic chemotherapy and/or radiotherapy

• AML with myelodysplasia-related changes
  - Tend to be genetically complex
  - Often occur after antecedent MDS
  - Poor prognosis
Therapy-related AML (t-AML)

- AML occurring after exposure to cytotoxic chemotherapy or radiotherapy
  - Alkylating agents, topoisomerase II inhibitors, anthracyclines, therapeutic radiation exposing hematopoietic bone marrow
- Frequent high-risk genetics (90% abnormal)
- Prognosis very poor compared with de novo AML
- Prognosis not dependent on blast count: therapy-related MDS is equally aggressive
Complex karyotype in therapy-related AML
• Frequent TP53 mutations in t-AML
  • 40% of cases versus 10% in de novo AML
  • Correlate with complex karyotype

Cleven AJ et al. Mod Pathol 2015;28:552
Therapy-related AML: the precise mechanism remains unclear

• Cytotoxic therapy induces mutations in key oncogenes in a stem cell, leading to transformation to leukemia?

• Cytotoxic therapy alters the marrow environment and allows expansion of a mutated clone already present?

• Germline mutations may confer patient vulnerability to cytotoxic chemotherapy/XRT
  – Potential to pre-identify patients at higher risk
  – Importance of family history in providing clues to risk

AML with myelodysplasia-related changes (AML-MRC)

SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR, STAG2 mutations are strongly associated with AML post-MDS

Three ways to diagnose AML-MRC

- Any prior diagnosis of MDS or MDS/MPN
- MDS-associated cytogenetic abnormality
- Severe morphologic dysplasia
  - >50% of cells from at least 2 lineages are dysplastic
Cytogenetic abnormalities that define AML-MRC

- Can be used to diagnose AML-MRC even in patients with no history of MDS or MDS/MPN
- Supercede *NPM1* or double-*CEPBA* mutation status

<table>
<thead>
<tr>
<th>Complex karyotype (3 or more abnormalities)</th>
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<tbody>
<tr>
<td>Unbalanced abnormalities</td>
</tr>
<tr>
<td>- -7/del(7q)</td>
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<tr>
<td>- del(5q)/t(5q)</td>
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<tr>
<td>- i(17q)/t(17p)</td>
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<tr>
<td>- -13/del(13q)</td>
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<tr>
<td>- del(11q)</td>
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<tr>
<td>- del(12p)/t(12p)</td>
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<tr>
<td>- idic(X)(q13)</td>
</tr>
<tr>
<td>-- del(9q)</td>
</tr>
<tr>
<td>Balanced abnormalities</td>
</tr>
<tr>
<td>- t(11;16)(q23.3;p13.3)</td>
</tr>
<tr>
<td>- t(3;21)(q26.2;q22.1)</td>
</tr>
<tr>
<td>- t(1;3)(p36.3;q21.2)</td>
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<tr>
<td>- t(2;11)(p21;q23.3)</td>
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<tr>
<td>- t(5;12)(q32;p13.2)</td>
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<tr>
<td>- t(5;17)(q32;p13.2)</td>
</tr>
<tr>
<td>- t(5;10)(q32;q21.2)</td>
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<tr>
<td>- t(3;5)(q25.3;q35.1)</td>
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</table>
AML with myelodysplasia-related changes
Significance of dysplastic changes in AML in the absence of history or cytogenetic features of AML-MRC?

• Some studies suggest that morphologic dysplasia alone may not be independently significant
  – May merely reflect underlying genetic abnormalities
• Further study is needed to more specifically examine dysplastic changes and their associations with mutations and outcome
  – Dysplasia in myeloid and megakaryocytic lineages may be more significant than in erythroid lineage

AML, not otherwise specified

- De novo AML case that fails to place in any other WHO group
  - No defining genetic abnormality (karyotype, $RUNX1$, $NPM1$, biallelic $CEBPA$)
  - Not therapy-related or MDS-related
- Most cases have normal karyotype
- Classified according to morphology (FAB)
  - Myeloid, myelomonocytic, monocytic, pure erythroid leukemia, acute megakaryoblastic leukemia
  - Pure erythroid leukemia appears to be the only prognostically relevant entity within AML-NOS
Pure erythroid leukemia (AML M6B)

- Very rare, highly aggressive leukemia
- Only myeloid neoplasm where erythroblasts can be considered as blast equivalents
  - >80% of erythroid cells with >30% pronormoblasts
  - E-cadherin+, CD71+/-, glycophorin+/-, CD117+, CD45-, CD34-
- Almost all cases have an abnormal, highly complex karyotype
- Must be distinguished from florid reactive erythroid proliferations

Liu W et al. Mod Pathol 2011;24:375
Pure erythroid leukemia
Myeloid neoplasms with germline predisposition: new WHO category

- Encompasses myeloid neoplasms (MDS, AML, MDS/MPN) arising in the background of a predisposing mutation or congenital syndrome
- Can include cases with or without a known syndrome, platelet disorder, or family history
  - Examples: *CEBPA, RUNX1, ETV6, ANKRD26, DDX41, GATA2*
- Germline predisposition should be appended to diagnosis, e.g.
  - Refractory cytopenia of childhood with germline *GATA* mutation
  - MDS with excess blasts associated with Fanconi anemia

AML with germline GATA2 mutation

Courtesy of L Peterson and J Vardiman
Challenges in genetic predisposition syndromes

• Identifying the germline mutation
  – Need to sequence non-hematopoietic tissue to know for certain that the mutation is germline
  – Need to be alert to clues: detailed personal & family history and use of experienced genetic counselors

• Some entities present in adulthood
  – MDS/AML with $DDX41$ mutation

• Distinguishing platelet disorders and bone marrow failure conditions from MDS

• Implications for family members (including potential bone marrow donors)
Acute myeloid leukemia: WHO 2016

- AML with recurrent genetic abnormalities
  - Cytogenetically-defined entities
    - AML with \textit{BCR-ABL1}
    - AML with mutated \textit{NPM1}
    - AML with \textit{biallelic} mutations of \textit{CEBPA}
    - AML with mutated \textit{RUNX1}
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- AML, not otherwise specified
  - Acute erythroid leukemia, erythroid/myeloid-subtype
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
WHO 2016 B-ALL Classification

B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities

- with t(9;22)(q34.1;q11.2); **BCR-ABL1**
- with t(v;11q23.3); **KMT2A** rearranged
- with t(12;21)(p13.2;q22.1); **ETV6-RUNX1**
- with t(5;14)(q31.1;q32.3); **IL3-IGH**
- with t(1;19)(q23;p13.3); **TCF3-PBX1**
- with hyperdiploidy
- with hypodiploidy

**BCR-ABL1**-like (provisional)

- with iAMP21 (provisional)

**B-lymphoblastic leukemia/lymphoma, NOS**

*Blood* 127:2391, 2016
<table>
<thead>
<tr>
<th>Subtype name</th>
<th>Genes involved</th>
<th>Frequency</th>
</tr>
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<tbody>
<tr>
<td>t(12;21)(p13.2;q22.1)</td>
<td><em>ETV6-RUNX1</em></td>
<td>Children&gt;adults</td>
</tr>
<tr>
<td>Hyperdiploidy</td>
<td>&gt;50 chromosomes</td>
<td>Children&gt;adults</td>
</tr>
<tr>
<td>t(9;22)(q34.1;q11.2)</td>
<td><em>BCR-ABL1</em></td>
<td>Adults&gt;children</td>
</tr>
<tr>
<td>Hypodiploidy*</td>
<td>&lt;46 chromosomes</td>
<td>4-5%</td>
</tr>
<tr>
<td>t(v;11q23.2)</td>
<td><em>KMT2A (MLL)</em></td>
<td>3-6%</td>
</tr>
<tr>
<td>t(1;19)(q23;p13.3)</td>
<td><em>TCF3-PBX1</em></td>
<td>Rare, mostly children</td>
</tr>
<tr>
<td>t(5;14)(q31.1;q32.3)</td>
<td><em>IL3-IGH</em></td>
<td>&lt;1%; eosinophilia</td>
</tr>
</tbody>
</table>
New entity:  
**BCR-ABL1-like B-ALL**

- Gene expression profiling identifies a subset of B-ALL cases with similar expression profile to **BCR-ABL1+ B-ALL**
  - Share **IKZF** deletion with **BCR-ABL1+ B-ALL**
- 10% of pediatric, up to 25% of adult B-ALL
- Altered ABL/JAK pathway signalling
- Often have rearrangements of tyrosine kinase or cytokine genes
  - Can respond to appropriate targeted therapies

Roberts KG et al. NEJM 2014;371:1005
BCR-ABL1-like B-ALL has poor prognosis

**BCR-ABL1-like B-ALL: How to identify?**

- 50% of cases overexpress *CRLF2*, detectable by flow cytometry
- Specific translocations or mutations of *ABL1*, *JAK2*, *FLT3*
- Limited-panel (“low-density”) gene expression microarray?

<table>
<thead>
<tr>
<th>Kinase Gene</th>
<th>Tyrosine Kinase Inhibitor</th>
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<tbody>
<tr>
<td><em>ABL1</em></td>
<td>Dasatinib</td>
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<tr>
<td><em>ABL2</em></td>
<td>Dasatinib</td>
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<tr>
<td><em>CSF1R</em></td>
<td>Dasatinib</td>
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<tr>
<td><em>PDGFRB</em></td>
<td>Dasatinib</td>
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<tr>
<td><em>CRLF2</em></td>
<td>JAK2 inhibitor</td>
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<tr>
<td><em>JAK2</em></td>
<td>JAK2 inhibitor</td>
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<tr>
<td><em>EPOR</em></td>
<td>JAK2 inhibitor</td>
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<tr>
<td><em>DGKH</em></td>
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<tr>
<td><em>IL2RB</em></td>
<td>JAK1 inhibitor, JAK3 inhibitor, or both</td>
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<tr>
<td><em>NTRK3</em></td>
<td>Crizotinib</td>
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<tr>
<td><em>PTK2B</em></td>
<td>FAK inhibitor</td>
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<tr>
<td><em>TSLP</em></td>
<td>JAK2 inhibitor</td>
</tr>
<tr>
<td><em>TYK2</em></td>
<td>TYK2 inhibitor</td>
</tr>
</tbody>
</table>

B-ALL with iAMP21

• Intrachromosomal amplification of *RUNX1* locus on chromosome 21
• Adverse outcome treated with standard ALL therapy, but patients benefit when treated as high-risk ALL
• Easily recognizable by interphase FISH using *RUNX1* probe
  – At least 5 *RUNX1* signals/nucleus
• Will be added as a new genetically defined B-ALL subgroup

Identifying B-ALL with iAMP21

ETV6-RUNX1 probe

No fusion, but multiple RUNX1 signals

Courtesy of Mike Borowitz and Nyla Heerema
B-ALL with hypodiploidy

• Currently defined as any B-ALL with <46 chromosomes

• Update identified 3 distinct subgroups, each having different gene expression and mutational profiles
  – Near haploid (24-31 chromosomes)
  – Low hypodiploid (23-39 chromosomes)
  – Near diploid (40-45 chromosomes)

• Near haploids may undergo chromosome doubling and be mistaken for near diploid or hyperdiploid cases

Poorer prognosis
GEP identifies distinct subsets of hypodiploidy ALL

RAS signalling

IKZF alterations

TP53 mutations

WHO 2016: Leukemias of ambiguous lineage and T-ALL/LBL

Acute undifferentiated leukemia

Mixed phenotype acute leukemias with recurrent genetic abnormalities

with t(9;22)(q34.1;q11.2); BCR-ABL1
with t(v;11q23.3); KMT2A rearranged

Mixed phenotype acute leukemia, B/myeloid, not otherwise specified

Mixed phenotype acute leukemia, T/myeloid, not otherwise specified

T-lymphoblastic leukemia/lymphoma

Early T-cell precursor lymphoblastic leukemia

Blood 127:2391, 2016
Lineage-associated markers in acute leukemia

<table>
<thead>
<tr>
<th>B-ALL</th>
<th>T-ALL</th>
<th>AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19, cCD22, cCD79a</td>
<td>cCD3</td>
<td>MPO</td>
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<tr>
<td>CD10, sCD22, PAX5, CD24</td>
<td>CD7</td>
<td>CD13, CD33, CD117</td>
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<tr>
<td>TdT</td>
<td>TdT</td>
<td>NSE, CD11c, CD14, CD64, lysozyme</td>
</tr>
<tr>
<td>CD34, HLA-DR</td>
<td>CD2, sCD3, CD5, CD4, CD8, CD1a, CD10</td>
<td>CD34, HLA-DR</td>
</tr>
<tr>
<td>CD20</td>
<td>CD34</td>
<td></td>
</tr>
</tbody>
</table>
Different types of blast phenotype ambiguity...

Mixed phenotype: Bilineal

Mixed phenotype: Biphenotypic

2 distinct populations, each with lineage-associated markers

Acute undifferentiated leukemia

<2 lineage-associated markers for any lineage

Lineage-specific markers from 2 lineages co-expressed on the same cells
Early T-precursor acute lymphoblastic leukemia (ETP-ALL)

- 10-15% of T-ALL
- Defined by expression of cytoplasmic CD3 + CD7 + low (<75%) CD5 and no CD1a, CD4 or CD8
- Expresses CD34 and one or more myeloid-related antigens (CD117, CD33, or CD13) but not MPO
- Thought to arise from an early progenitor cell with lineage plasticity
- Molecular genetics
  - More frequent AML-associated mutations
  - NOTCH pathway (T-ALL-associated) mutations are rare
- Considered high risk

Conclusions

- The current leukemia classification integrates clinical, morphologic, and genetic data to identify subtypes with distinctive behavior.

- Recently data have allowed refinement of leukemia classification, with new entities associated with distinct prognosis or therapies.

- Translating new genetic data into improved patient outcome will require ongoing collaborations between pathologists and treating clinicians: there’s still a lot of work to be done!