Updated WHO Classification of Hematological Neoplasms: Acute Leukemias and Myelodysplastic Syndromes

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Overview

- Acute lymphoblastic leukemia
- Acute myeloid leukemia
- Myelodysplastic syndromes
- Myeloid neoplasms with germline predisposition

Acute Lymphoblastic Leukemia

• B-cell lymphoblastic leukemia/lymphoma (B-ALL)
  – B-ALL with intrachromosomal amplification of chromosome 21 (iAMP21)
  – B-ALL with translocations involving tyrosine kinases or cytokine receptors (*BCR-ABL1*-like ALL)
  – Association between low hypodiploid ALL and often constitutional mutations in *TP53*

• T-cell lymphoblastic leukemia/lymphoma (T-ALL)
  – Indolent T-lymphoblastic proliferations
  – Early T-precursor (ETP) ALL
B-ALL with iAMP21

- Intrachromosomal amplification of chromosome 21 (iAMP21) accounts for about 2% of pediatric B-ALL
- Generally in older children (median age 9 years) with lower WBC count
- 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
**BCR-ABL1-like B-ALL**

(B-ALL with Translocations Involving Tyrosine Kinases or Cytokine Receptors)

- *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; 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*
    - Diagnostic significance of deletions/mutations of *IKZF1*, *CDKN2A/B*, *JAK1* less clear
  - The full spectrum of genetic changes is still being investigated

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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, 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 and cCD3; CD5 negative or absent in 25% or more of cells
- Thought to arise from an early progenitor cell with lineage plasticity that may be more closely related to human stem cells than to early T-cell precursors
- 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

2016 WHO Classification of Lymphoblastic Leukemia/Lymphoma

B lymphoblastic leukemia/lymphoma

B lymphoblastic leukemia/lymphoma, NOS
B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
  B lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);BCR-ABL1
  B lymphoblastic leukemia/lymphoma with t(v;11q23.3);KMT2A rearranged
  B lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); ETV6-RUNX1
  B lymphoblastic leukemia/lymphoma with hyperdiploidy
  B lymphoblastic leukemia/lymphoma with hypodiploidy
  B lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3) IL3-IGH
  B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);TCF3-PBX1

Provisional entity: B lymphoblastic leukemia/lymphoma, BCR-ABL1-like
Provisional entity: B lymphoblastic leukemia/lymphoma with iAMP21

T lymphoblastic leukemia/lymphoma

Provisional entity: Early T-cell precursor lymphoblastic leukemia
Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma

Acute Myeloid Leukemia

- AML with recurrent genetic abnormalities
- 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
AML with Recurrent Genetic Abnormalities

Cytogenetic groups

• Minor changes
  – APL with PML-RARA
  – AML with t(9;11)(p21.3;q23.3); KMT2A-MLLT3
  – AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM

• New provisional entity
  – AML with BCR-ABL1
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 Recurrent Genetic Abnormalities

Mutations

• Revised categories
  – AML with mutated \textit{NPM1}
  – AML with biallelic mutations of \textit{CEBPA}

• New provisional entity
  – AML with mutated \textit{RUNX1}
AML with mutated CEBPA

- 7-20% of AMLs have mutations of CEBPA
  - More frequent with normal or intermediate karyotype
- 12.5-47% are single/monoallelic
- Double mutant/biallelic cases (CEBPA\textsuperscript{dm}) predict a favorable prognosis
  - Low frequency of other mutations or other cytogenetic abnormalities

**NPM1 and CEBPA Mutations and Multilineage Dyplasia**

- Significance of multilineage dysplasia in the presence of *NPM1* mutation, a normal karyotype and no history of MDS
  - MLD found in 74/318 (23%) de novo *NPM1* mutated AML
  - No prognostic significance for MLD

- **CEBPA** mutations
  - MLD found in 28/108 (25.9%) *CEBPA* mutated AML patients
  - No significant survival difference in MLD+ and MLD- groups

Survival curves of patients up to 60 years with intermediate-risk cytogenetics AML depending on \textit{NPM1} status and presence of multilineage dysplastic features (MLD)

\begin{itemize}
\item[A] Mutated \textit{NPM1}
\item[B] Wild-type \textit{NPM1}
\end{itemize}

\begin{align*}
P &= .97 \\
P &= .012
\end{align*}

AML with Recurrent Genetic Abnormalities

Revisions

- AML with mutated $NPM1$ and AML with biallelic mutations of $CEBPA$ no longer provisional entities
- Biallelic mutations required for $CEBPA$ category
- Multilineage dysplasia alone no longer trumps these mutations
AML with mutated 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 *KMT2A*-PTD, *IDH1/2* or *ASXL1* mutations
- Rare *CEBPA* or *NPM1* mutations
- Poor response to therapy with shortened survival
- Germline mutations should be evaluated

AML with mutated *RUNX1*

• Provisional entity
  – Only includes de novo cases without MDS-related cytogenetic abnormalities
  – Does not include therapy-related cases
2016 WHO Classification of AML with Recurrent Genetic Abnormalities

AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
APL with *PML-RARA*
AML with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*
AML with t(6;9)(p23;q34.1); *DEK-NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); *RBM15-MKL1*

*Provisional entity:  AML with BCR-ABL1*
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*

*Provisional entity:  AML with mutated RUNX1*

AML with Myelodysplasia-Related Changes

• Multilineage dysplasia alone no longer trumps a diagnosis of AML with mutated *NPM1* or AML with biallelic mutations of *CEBPA*

• Changes to the MDS-related cytogenetic abnormality table
AML with mutated \textit{NPM1} or \textit{CEBPA} and an abnormal karyotype

- Abnormal karyotype identified in 14.7\% of \textit{NPM1} mutated AML cases
- +8, +4, -Y, del(9q) and +21 most frequent
- del(9q) is currently considered an MDS-related cytogenetic abnormality, but it appears to be unusually common in \textit{NPM1} and \textit{CEBPA} mutated cases
- Not clear if del(9q) has prognostic significance in this setting
- MDS-related cytogenetic abnormality list to diagnose AML with myelodysplasia-related changes is now changed to not include del(9q)

MDS-related cytogenetic abnormalities

• **Complex karyotype***

• **Unbalanced abnormalities**
  – -7/del(7q)
  – -5/del(5q)/t(5q)
  – i(17q)/t(17p)
  – -13/del(13q)
  – del(11q)
  – del(12p)/t(12p)
  – del(9q)
  – idic(X)(q13)

• **Balanced abnormalities**
  – t(11;16)(q23.3;p13.3)
  – t(3;21)(q26.2;q22.1)
  – t(1;3)(p36.3;q21.1.2)
  – t(2;11)(p21;q23.3)
  – t(5;12)(q32;p13.2)
  – t(5;7)(q32;q11.2)
  – t(5;17)(q32;p13.2)
  – t(5;10)(q32;q21)
  – t(3;5)(q25.3;q35.1)

* >3 abnormalities
AML with Myelodysplasia-Related Changes

- Detection of multilineage dysplasia
  - Two non-blast cell lines must show dysplasia in at least 50% of cells
- MDS-related cytogenetic abnormalities, or
- History of MDS or MDS/MPN
- Absence of the specific cytogenetic abnormalities of AML with recurrent genetic abnormalities
- Absence of prior history of therapy
AML, Not Otherwise Specified

• Elimination of the category of erythroid/myeloid type of acute erythroid leukemia

• Eliminates the use of non-erythroid cell blast counts for classification
AML, Not Otherwise Specified

AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis

Myelodysplastic Syndrome Changes

- **Nomenclature**
- **Morphology**
  - MDS, unclassifiable
- **Genetics and molecular genetics**
  - MDS with ring sideroblasts
  - MDS with isolated del(5q)


Nomenclature

- WHO scheme classifies based on dysplasia and blast counts, not cytopenia
  - Cytopenias are captured in IPSS-R system
- Type of dysplasia often does not agree with the cytopenic lineage in refractory cytopenia with unilineage dysplasia (RCUD)
  - Cannot predict peripheral counts from dysplasia
  - Subgroups of refractory anemia, neutropenia, and thrombocytopenia will be eliminated

Myelodysplastic Syndromes – Revised Terminology

- Refractory cytopenia with unilineage dysplasia
  - Refractory anemia
  - Refractory neutropenia
  - Refractory thrombocytopenia
- Refractory anemia with ring sideroblasts
- Refractory cytopenia with multilineage dysplasia
- Refractory anemia with excess blasts
  - RAEB-1
  - RAEB-2
- Myelodysplastic syndrome with isolated del(5q)
- Myelodysplastic syndrome, unclassifiable
- Childhood myelodysplastic syndrome
  - Refractory cytopenia of childhood

- MDS with single lineage dysplasia
- MDS with ring sideroblasts and single lineage dysplasia
- MDS with ring sideroblasts and multilineage dysplasia
- MDS with multilineage dysplasia
- MDS with excess blasts
  - With excess blasts-1
  - With excess blasts-2
- MDS with isolated del(5q)
- MDS, unclassifiable
- Refractory cytopenia of childhood
MDS, Unclassifiable

• Findings of MDS with single lineage dysplasia or multilineage dysplasia (<5% marrow blasts), but 1% peripheral blood blasts
  – 1% PB blasts must be measured on at least two separate occasions

• Findings of MDS with single lineage dysplasia, but pancytopenia
  – Cytopenias must be below IPSS levels: ANC<1.8 x 10^9/L, HGB<10 g/dL, PLT<100 x 10^9/L

• MDS-associated cytogenetic abnormality in association with cytopenias, less than 1% blood and less than 5% marrow blasts, but less than 10% dysplasia in any cell line
Genetics in MDS

• Somatic mutations in MDS
  – Prognostic significance of mutations of TP53, EZH2, ETV6, RUNX1, ASXL1 and others (Bejar R et al. NEJM 2011;364:2496)
• SF3B1 mutations in MDS with ring sideroblasts
• MDS with isolated del(5q)

Ribosomal proteins: RPS14
Epigenetic regulators: TET2, ASXL1
RNA splicing: SF3B1, SRSF2, U2AF1
Transcription factors: RUNX1, ETV6
Tyrosine kinase signaling: RAS
Tumor suppressor genes: TP53

MDS with ring sideroblasts
(with single lineage or multilineage dysplasia)

• Frequent association with mutations of SF3B1 and a favorable prognosis with low risk of transformation to acute leukemia

• WHO revision:
  – >15% ring sideroblasts (among erythroid precursors), or
  – >5% in the presence of an SF3B1 mutation
  – Blast cell increases exclude this diagnosis
    • If multilineage dysplasia without a blast cell increase is present, case is classified as MDS with ring sideroblasts and multilineage dysplasia

MDS with Isolated del(5q) (5q-minus Syndrome)

- 2008 WHO restricted category to allow del(5q) as the only abnormality
- Revision now allows a second (except monosomy 7) cytogenetic abnormality
- Cases with >2 abnormalities will not qualify for this category
- Recommend TP53 mutation assessment or p53 staining

Germing Leukemia 26:1286, 2012;
MDS with Isolated del(5q) (5q-minus Syndrome)

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Germing Leukemia 26:1286, 2012;
Mallo Leukemia 24:110, 2011; Jadersten JCO 29:1971; 20
Acute erythroid leukemia (erythroid/myeloid type) is now MDS based on total blast count

- 2008 definition of acute erythroleukemia (erythroid/myeloid type) in AML, NOS required ≥50% marrow erythroid precursors and ≥20% myeloblasts among non-erythroid cells
- These cases will now be classified as MDS based on the total blast cell count

Wang et al. Mod Pathol 2015 abstract
## Diagnostic approach to myeloid neoplasms when erythroid precursors comprise ≥50% of bone marrow (BM) nucleated cells

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<td>≥50%</td>
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<td>MDS**</td>
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<td>&gt;80% immature erythroid precursors with &gt;30% proerythroblasts</td>
<td>&lt;20%</td>
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<td>AML, NOS, acute erythroid leukemia (pure erythroid type)</td>
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* Cases of AML t(8;21)(q22;q22.1); RUNX1-RUNX1T1, AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11 or acute promyelocytic leukemia with PML-RARA, may rarely occur in this setting with less than 20% blasts and those diagnoses would take precedence over a diagnosis of AML, NOS or MDS.

** Classify based on myeloblast percentage of all BM cells and of peripheral blood leukocytes and other MDS criteria

Abbreviations: BM, bone marrow; PB, peripheral blood; AML, acute myeloid leukemia; AML-MRC, acute myeloid leukemia with myelodysplasia-related changes; AML-NOS, acute myeloid leukemia, not otherwise specified; NA, not applicable; MDS, myelodysplastic syndrome

Myelodysplastic Syndromes

MDS with single lineage dysplasia
MDS with ring sideroblasts
  MDS with ring sideroblasts and single lineage dysplasia
  MDS with ring sideroblasts and multilineage dysplasia
MDS with multilineage dysplasia
MDS with excess blasts
MDS with isolated del(5q)
MDS, unclassifiable

Provisional entity: Refractory cytopenia of childhood

Myeloid Neoplasms with Germline Predisposition

Myeloid neoplasms with germline predisposition are likely more prevalent than realized.

**Myeloid neoplasms with germline predisposition without a pre-existing disorder or organ dysfunction**

- AML with germline *CEBPA* mutation
- Myeloid neoplasms with germline *DDX41* mutation*

**Myeloid neoplasms with germline predisposition and pre-existing platelet disorders**

- Myeloid neoplasms with germline *RUNX1* mutation*
- Myeloid neoplasms with germline *ANKRD26* mutation*
- Myeloid neoplasms with germline *ETV6* mutation*

**Myeloid neoplasms with germline predisposition and other organ dysfunction**

- Myeloid neoplasms with germline *GATA2* mutation
- Myeloid neoplasms associated with bone marrow failure syndromes
- Myeloid neoplasms associated with telomere biology disorders
- Juvenile myelomonocytic leukemia associated with neurofibromatosis, Noonan syndrome or Noonan syndrome-like disorders
- Myeloid neoplasms associated with Down syndrome*

* Lymphoid neoplasms also reported.

Summary

• The 2016 revision of the WHO classification clarifies the diagnostic criteria for some existing neoplasms and introduces some new categories as provisional entities.

• The classification recognizes the prognostic significance of many genetic changes, especially mutations, even if they do not directly impact diagnostic categories.
Acknowledgements

• Jim Vardiman
• Jürgen Thiele
• Attilio Orazi
• Robert Hasserjian
• Kathy Foucar
• LoAnn Peterson
• Dick Brunning
• Michelle LeBeau
• Mike Borowitz
• Myeloid CAC
  – Clara Bloomfield
  – Mario Cazzola