WHO Classification

Classifying Hematopoietic Disorders

French-American-British (FAB) World Health Organization (WHO)

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Myeloproliferative Disorders (MPDs)
Myeloproliferative Neoplasms

FAB Classification Begun in 1976

• Based on Wright-stain peripheral blood (PB) and bone marrow (BM) morphology
• Cytochemistry: special dyes
  – Myeloperoxidase & Sudan black B
  – Esterase: specific and non-specific
• Cytogenetics: Ph’ chromosome
  – Balanced reciprocal translocation
  t(9;22)(q34;q11.2)


Developments of the 1990s

• FAB classification becomes confounded as technology develops
  – Phenotyping by flow cytometry: lymphomas, acute and chronic lymphocytic leukemia
  – Karyotyping extended: for example, t(15,17), 5q-
  – Fluorescent in situ hybridization (FISH)

WHO initiates 1995 meeting of…
  – The European Association of Pathologists
  – The International Society for Hematopathology


Philadelphia Chromosome

• Juxtaposition of c-abl proto-oncogene (chrom 9) with portion of bcr gene (chrom 22) yields BCR/ABL fusion gene
• Produces 210-kD bcr/abl fusion protein with abnormal tyrosine kinase activity
  – Activates signal transduction pathways
  – Raises proliferation, suppresses apoptosis
• Occasional patients lack Ph’ but show BCR/ABL rearrangement by PCR
• Occasionally found months before CML Dx
WHO Classification

2001: morphology, cytochemistry, immunophenotype, genetic and clinical features
2008 updates: molecular diagnosis
- MPDs renamed myeloproliferative neoplasms (MPNs)
- Identified genetic abnormalities in pathogenesis of BCR/ABL-negative MPNs
- Clonal abnormalities of genes that encode cytoplasmic or membrane receptor tyrosine kinases that activate signal transduction pathways to cause proliferation
- Histologic features: megakaryocytic localization, marrow stromal changes, multiple cell lineages involved in proliferation used as criteria to identify subtypes
- Correlation with clinical features


MPN: Molecular Characteristics Beyond BCR/ABL
- JAK2: Janus 2 kinase
  - Most common mutation at 9p24: JAK2 V617F
- Platelet derived growth factor receptor-α (PDGFRα)
- Platelet derived growth factor receptor-β (PDGFRβ)
- FGF1: Fibroblast growth factor 1

WHO Classification of MPNs
- CML, BCR-ABL positive
- Chronic neutrophilic leukemia (CNL)
  - BCR-ABL negative
- PV: 90% JAK2 V617F positive
- Primary myelofibrosis (MMM, PMF); 50% JAK2 Pos
- ET; PLT count threshold now ≥450,000/μL; 50% JAK2 Pos
- Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB and FGFR1
- MPN, unclassifiable (MPN,U)
- Mastocytosis

Polycythemia Vera
- HGB
  - Males >18.5 g/dL
  - Females >16.5 g/dL
  - JAK2 V617F
- Progenitor cells hypersensitive to cytokines
  - Erythropoietin
  - SCF, GM-CSF, IL-3, TPO, IGF-1
- In PMF and ET, also some cytokine hypersensitivity

Chronic Neutrophilic Leukemia
- No Ph' chromosome
- WBC > 25,000/μL
  - Mature neutrophils predominate
  - No evidence of infection
- Leukocyte alkaline phosphatase ↑, not ↓
- Slowly progressive
Primary Myelofibrosis (PMF)
- MOD/MKD reactive marrow fibrosis
- Myeloid metaplasia: hepatosplenomegaly
- Leukoerythroblastic: Immature PB RBCs and neutrophils
- WBC <30,000/mL
- N/N anemia: teardrop RBCs
- FAB names: Agnogenic myelofibrosis with myeloid metaplasia (AMMM, MMM)
- WHO 2001 name: Chronic idiopathic myelofibrosis (IMF)

PMF Diagnosis: WHO 2008
Major Criteria: Must meet all three
1. Megakaryocyte (MK) atypical proliferation
   - With either reticulin or collagen fibrosis
   - In the absence of fibrosis, MK changes must be accompanied by ↑ BM cellularity with myelocytic proliferation and ↓ erythropoiesis
2. JAK2 (50% of patients) or other clonal marker such as MPL W515K/L
   - If no clonal marker, BM fibrosis cannot be secondary to infection, autoimmune disorder or other chronic inflammatory condition, lymphoid neoplasm or metastatic malignancy
3. Exclude WHO criteria for PV, BCR-ABL CML, MDS or other myeloid neoplasms

Minor Criteria: Must meet two
- Leukoerythroblastosis
- Raised serum lactic dehydrogenase
- Anemia
- Splenomegaly

ET: WHO 2008 Criteria
1. Sustained PLT count ≥450,000/uL
   - Reduced from ≥600,000/uL in 2001
2. BM biopsy: massive megakaryocytic proliferation
   - No left-shift or significant increase of myelopoiesis or erythropoiesis
3. JAK2 50%, or other clonal marker
   - In absence of JAK2, must show no evidence for reactive thrombocytosis
4. Exclude WHO criteria for PV, BCR-ABL CML, MDS or other myeloid neoplasms

Myelogenous or Lymphoid Neoplasms with Eosinophilia & Abnormalities of PDGFRA, PDGFRB or FGFR1
- Three rare specific disease groups
- All result from a fusion gene that encodes aberrant tyrosine kinase activity
- Eosinophilia characteristic, not invariable
- Important to recognize since tyrosine kinase inhibitors are targeted therapy

Mastocytosis
- Clonal proliferation of mast cells that accumulate in one or more organ systems
- Multifocal compact clusters or cohesive aggregates of abnormal mast cells
- Same as WHO 2001, except now included in MPN
MPN-Unclassifiable
- Definite clinical, and laboratory features of MPN, but fail to meet criteria for any of the specific MPN entities
- MPN that presents with features overlapping or more of the MPN
- May be early stages of PV, PMF or ET in which features are not yet developed
- May be advanced MPN
  - Multiple, combined, or co-existing neoplastic or inflammatory disorder that obscures MPN Dx features

Myelodysplastic/Myeloproliferative Neoplasms
- Clonal myeloid disorders that possess both dysplastic and proliferative features that are not classified as either MDS or MPN
- Atypical (aCML): BCR/ABL negative
- Chronic myelomonocytic leukemia (CMML)
- Juvenile myelomonocytic leukemia (JMML)
- Myelodysplastic/myeloproliferative neoplasm, unclassifiable

Myelodysplastic Syndromes (MDS)
- Abnormal myeloblastic clones mixed with normal maturation
  - Altered, unbalanced gene functions
- Variable anemia and cytopenia, may smolder for years
  - Some are transfusion dependent
- 20-40% transform to acute myeloblastic leukemia

MDS FAB Classification
- Refractory anemia (RA)
- Refractory anemia with ringed sideroblasts (RARS)
- Refractory anemia with excess blasts (RAEB)
- Refractory anemia with excess blasts in transformation (RAEB-t)

MDS: WHO 2001
- Refractory anemia with or without ringed sideroblasts
- Refractory pancytopenia with multilineage dysplasia
- Refractory anemia with excess blasts
- Unclassified myelodysplasia
- 5q- syndrome

Significant MDS Differences
- RAEB-t eliminated
- RA and RARS have unilineage dysplasia: only dyserythropoiesis
- Refractory cytophenia with multilineage dysplasia (RCMD) added
- MDS with del (5q) as sole genetic abnormality added as 5q- syndrome
- Therapy-related MDS/AML grouped as a single syndrome
- CMML moved to MDS/MPN category
MDS: 2008 WHO Updates

- Refractory cytopenia with unilineage dysplasia (RCUD, 2008)
- Refractory anemia with or without ringed sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia (RCMD)
- RAEB-1 (<5% blasts, no Auer rods)
- RAEB-2 (5-19% blasts, Auer rods)
- MDS-U
- MDS with isolated del (5q), (5q-)
- Childhood MDS (2008)

RCUD

- Refractory anemia (RA)
- Refractory neutropenia (RN)
- Refractory thrombocytopenia (RT)
- Peripheral blood
  - Unicytopenia
  - No or <1% blasts
- Bone marrow
  - Unilineage dysplasia
  - >10% in one myeloid lineage
  - <5% blasts
  - <15% ring sideroblasts

RCMD

- Bicytopenia or pancytopenia with dysplasia in 10% of two or more cell lines
  - <1% blasts in PB
  - <5% blasts in BM
  - No Auer rods
- 50% have karyotypic abnormalities
  - Monosomy 5
  - Del (5q)
  - Monosomy 7
  - Del (7q)
  - Trisomy 8
  - Del (20q)

5q- Syndrome

- Deletion 5q (5q-32-33.3) is sole abnormality
- Macrocytic anemia with otherwise normal blood counts
- Blasts in marrow <5%
- Hypolobulated megakaryocytes
- Normal or high platelets
- Male: female ratio 1:3
- Favorable: low progression to AML

Acute Myeloblastic Leukemia (AML)

- Neoplastic cells escape apoptosis resulting in expansion of leukemic clone
- Failure to differentiate into functional hematopoietic cells
- Proliferate and accumulate in BM

FAB Acute Myeloblastic Leukemia

<table>
<thead>
<tr>
<th>FAB</th>
<th>Name</th>
<th>Phenotype</th>
<th>Image</th>
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<tbody>
<tr>
<td>M0</td>
<td>AML, minimally differentiated</td>
<td>CD13, 33, 34, 117</td>
<td></td>
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<tr>
<td>M1</td>
<td>AML without differentiation</td>
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<tr>
<td>M2</td>
<td>AML with differentiation</td>
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<tr>
<td>M3</td>
<td>Acute promyelocytic (APL)</td>
<td>Genotype: t(15;17)</td>
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<td>M4</td>
<td>Acute myelomonocytic (AMML)</td>
<td>CD4, 11c, 13, 14, 33, 36, 64</td>
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<tr>
<td>M5</td>
<td>Acute monoblastic (AMoL)</td>
<td>CD4, 11b, 13, 14, 36, 64</td>
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<tr>
<td>M6</td>
<td>Acute erythroid leukemia</td>
<td>CD11b, 13, 15, 33, 34, 45, 71</td>
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<tr>
<td>M7</td>
<td>Acute megakaryocytic</td>
<td>CD 41, 42, 61</td>
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AML With Recurrent Translocations

- AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
- AML with inv(16)(p13.1q22); CBFB-MYH11
- APL with t(15;17)(q22;q12); PML/RARA
- AML with t(9;11)(q22;q22); MLLT3-MLL
- AML with t(6;9)(p23;q34); DEK-NUP214
- AML with inv(3)(q21;q26.2); RPN1-EVI1
- AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1

The last 3 are defined in 2008.

AML With MDS-related Changes*

- Category has ≥ 20% PB or BM blasts
- And any of following:
  - Prior history of MDS
  - MDS-related cytogenetic abnormality
  - Multilineage dysplasia
- And absence of...
  - Prior cytotoxic therapy for an unrelated diagnosis.
  - Recurring genetic abnormality.
- For prognosis, monitor for FLT3, NPM1, and CEBPA

*This category replaces 2001 “AML with multilineage dysplasia.”

AML Not Otherwise Specified*

- AML without maturation
- AML with minimal differentiation
- AML with maturation
- Acute myelomonocytic leukemia (AMML)
- Acute monoblastic/monocytic leukemia (AMoL)
- Acute erythroid leukemia (AEL)
  - Erythroleukemia, erythro/myeloid
  - Pure erythroid leukemia
- Acute megakaryoblastic leukemia (AMkL)
- Acute basophilic leukemia (ABL)
- Acute panmyelosis with myelofibrosis

*Do not fill criteria for any other AML categories, follow FAB classification.

Two 2008 Additions

- Myeloid proliferations related to Down syndrome
  - MDS and AML are identical in Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm
  - Formerly blastic NK-cell lymphoma/leukemia, CD4, 56
Acute Leukemias of Ambiguous Lineage

- Acute undifferentiated leukemia: no lineage-specific markers
- Mixed phenotype AL with t(9;22)(q34;q11.2); BCR-ABL
- Mixed phenotype AL with t(v;11q23); MLL rearranged
  - B/myeloid, not otherwise specified
  - T/myeloid, not otherwise specified

Lymphoblastic Leukemia

- B-lymphoblastic leukemia/lymphoma, not otherwise specified
- B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
  - t(9;22)(q34;q11.2) BCR-ABL
  - t(v;11q23); MLL rearranged
  - t(12;21)(p13;q22); TEL-AML1 (ETV6-RUNX1)
  - Hyperdiploidy, hypodiploidy
  - t(5;14)(q31;q32) IL3-IGH
  - t(1;19)(q23;p13.3); TCF3-PBX1
- T-lymphoblastic lymphoma