Burning issues regarding AML & MDS classification: origin (de novo & secondary), genetics, grey zones

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Data Management Education sessions
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Education session on AML & MDS: outline

- AML & MDS: definition, evolution
- Classification (WHO, FAB)
- Relevance of genetics in AML & MDS
- Grey zones & confounding concepts
- Capturing complexity in MED-A & MED-B forms
- Q & As
Acute myeloid leukemia (AML): WHO 2008 definition

• Clonal expansion of myeloid blasts in bone marrow (BM), peripheral blood (PB) or other tissue

• Minimum threshold of blast cells for AML diagnosis (BM):
  - 20% blasts (WHO, 2008)
  - Any blast count in cases with RUNX1/RUNX1T1, CBFb/MYH11 or PML/RARA rearrangement

Proliferation + differentiation block/maturation arrest
Normal hematopoiesis
Hematopoiesis in AML patients

HSC

Leukemia stem cell

AML BLASTS

B cell

T cell

NK cell

DC

Erythrocyte
Myelodysplastic syndromes (MDS): concept

- Heterogeneous disorder
- Clonal neoplasm
- Abnormal (dysplastic) hematopoietic precursors
- Ineffective hematopoiesis – cytopenia & malfunctioning
- <20% of blasts but…
- Risk of transformation to AML
## AML vs. MDS: differential characteristics

<table>
<thead>
<tr>
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<th>Blood counts</th>
<th>BM blasts</th>
<th>BM cellularity</th>
<th>Morphology</th>
<th>Genetics</th>
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<tbody>
<tr>
<td><strong>MDS</strong></td>
<td>Cytopenias</td>
<td>&lt;20%</td>
<td>Increased, with maturation</td>
<td>Dysplasia in 1 or more lineages</td>
<td>Characteristic genetic lesions</td>
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<tr>
<td><strong>AML</strong></td>
<td>↑ WBC (blasts) or leucopenia</td>
<td>≥20%</td>
<td>Increased, w/o maturation</td>
<td>Absent or present (AML with MRC)</td>
<td>Characteristic genetic lesions, although in some pts with MDS</td>
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I. AML with recurrent cytogenetic abnormalities
- AML with t(8;21)(q22;q22)/RUNX1-RUNXT1
- AML with inv(16) or t(16;16)(p13;q22)/CBFβ-MYH11
- Acute promyelocytic leukemia [t(15;17) & PML-RAR-α]
- AML with t(9;11)(p22;q23)/AF9(MLLT3)-MLL
- AML with t(6;9)(p23;q34)/DEK-CAN(NUP214)
- AML with inv(3) or t(3;3)(q21;q26)/RPN1-EVI1
- Megakaryoblastic AML with t(1;22)(p13;q13)/RBM15-MKL1
- AML with mutated NPM
- AML with normal karyotype and CEBPA mutation

II. AML with myelodysplasia-related changes

III. Therapy-related AML/MDS

IV. AML, not otherwise specified
- AML without differentiation
- AML minimally differentiated
- AML with differentiation
- AML myelomonocytic
- AML monoblastic and monocytic
- Acute erythroid leukemia
- Acute megakaryoblastic leukemia

V. Myeloid sarcoma

VI. Myeloid proliferations related to Down syndrome

VII. Blastic plasmacytoid dendritic cell neoplasms
Impact of cytogenetic entities recognized in 2008 WHO classification on survival

MRC/NCRI AML Trials: Overall Survival
Ages 16–59

% alive

Years from entry

AML classification before 2001 (FAB)

- **M0** Myeloid without differentiation
- **M1** Myeloid minimally differentiated
- **M2** Myeloid with differentiation
  - $t(8;21)$, RUNX1-RUNX1T1
- **M3** Promyelocytic
  - $t(15;17)$, PML/RARA
- **M4** Myelomonocytic
  - Eosinophilia, Inv(16) or $t(16;16)$, CBFB/MYH11
- **M5** Monoblastic/monocytic
- **M6** Erythroid
- **M7** Megakaryoblastic
WHO 2008
Classification for Myelodysplastic syndromes

- Refractory cytopenia with unilineage dysplasia (RCUD)
  - Refractory anaemia (RA)
  - Refractory neutropenia (RN)
  - Refractory thrombocytopenia (RT)
- Refractory anaemia with ring sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia (RCMD)
- Refractory anaemia with excess blasts (RAEB I & II)
  - RAEB-1: 5-9% bone marrow blasts
  - RAEB-2: 10-19% BM blasts
- Myelodysplastic syndrome with isolated del (5q)
- Myelodysplastic syndrome unclassifiable (MDS,U)
- Childhood myelodysplastic syndrome
WHO Classification

Survival

Cumulative proportion surviving

Survival time (months)

RA/RARS
RCMD/RCMD-RS
RAEB-1
RAEB-2
AML

Most-Frequent Chromosome Abnormalities in Patients with MDS

Patients (%)

- del 5q
- -7
- +8
- del 20q
- -5
- -Y
- -18
- +Mar
- +21
- del 7q
- complex

n=2072

Good prognosis (OS ≥24 months)
Intermediate prognosis (OS 12–24 months)
Poor prognosis (OS ≤12 months)
Intermediate to poor prognosis

OS = overall survival.

When classifications capture reality: SF3B1 mutation was the underlying mutation in Refractory Anemia with Ring Sideroblasts (RARS)
De novo AML: arising in a patient w/o previous antecedent of hematologic neoplasia or exposition to leukemogenic agents (tAML)

De novo AML

Myelodysplastic syndrome (MDS)

Myeloproliferative neoplasm (MPN)

Secondary AML
## Therapy-related AML (tAML) & other myeloid neoplasms: cytotoxic agents

### Alkylating agents
- Melphalan, cyclophosphamide, nitrogen mustard, chlorambucil, busulfan, carboplatin, cisplatin, dacarbazine, procarbazine, carmustine, mitomycin C, thiotepa, lomustine, etc.

### Ionizing radiation therapy
- Large fields including active bone marrow

### Topoisomerase II inhibitors
- Etoposide, teniposide, doxorubicin, daunorubicin, mitoxantrone, amsacrine, actinomycin
- *Topoisomerase II inhibitors may also be associated with therapy-related lymphoblastic leukaemia

### Others
- Antimetabolites: thiopurines, mycophenolate, fludarabine
- Antitubulin agents (usually in combination with other agents): vincristine, vinblastine, vindesine, paclitaxel, docetaxel
AML with myelodysplasia-related changes: diagnostic criteria

≥20% blasts (BM or PB) +

1. Prior history of MDS or MDS/MPN (secondary AML) and/or

2. MDS-related cytogenetic abnormalities (de novo or secondary AML) and/or

3. Multilineage dysplasia (≥50% of the cells in at least 2 BM myeloid lineage) (de novo or secondary)
Diagnosis of AML/MDS: integration of multiple tools

- Clinical history
- **Cytology**: PB slide, BM (BM aspirate ± BM biopsy)
- **Cytochemistry**: MPO & Sudan Black B (myeloid); \( \alpha \)-naphthyl acetate & \( \alpha \)-naphthyl butyrate (monocytic)
- **Immunophenotype**: multiparameter flow cytometry – based
- **Cytogenetics**
  - Conventional cytogenetics
  - FISH; other (CGH, SNParray,...)
- **Molecular biology**
  - Fusion transcripts (RT-PCR): PML/RAR-\( \alpha \), AML1/ETO, CBF-\( \beta \)/MYH11, MLL/..., BCR/ABL, DEK/CAN,...
  - Gene mutations: FLT3-ITD, CEBPalpha, NPM1, ...
Diagnosis of AML with myelodysplasia-related changes: dysplasia assessment

• **Dysgranulopoiesis**: 25-100 neutrophils
  – hypogranular cytoplasm, hyposegmented nuclei or bizarrely segmented nuclei, cytoplasmic vacuoles
  – MPO deficiency (50%, 20 cells)

• **Dyserythropoiesis**: at least 25 mature erythroblasts
  – megaloblastosis, karyorhexitis & nuclear irregularity, fragmentation or multinucleation
  – ring sideroblasts, PAS positivity

• **Dysmegakaryopoiesis**: at least 6 megakaryocytes
  – micromegakaryocytes, normal sized or large megakaryocytes with non-lobulated or multiple nuclei

Evaluation of dysplasia
CD34+ (partial), CD56-
B-cell markers (CD19, CD10, CD20): negative
T-cell markers (CD2, CD3) negative CD7++ and CD5 (partial)
HLA-DR+
Myeloid markers: CD117+ MPO+ (weak) CD13+(het) CD33++(hom)
Monocytic markers: CD4+ CD36 (partial) CD64+ lysozyme+ CD11+ (weak)
CD66- CD65- NG2- CD14- CD87-
Conventional cytogenetics

46, XX, del (5)(q22q33)
FISH in MDS

del(5)(q31)
RT-PCR of Exon 12 of Nucleophosmin (NPM1)

**Heterozygous mutation (4-bp insertion)**

PCR of exons 11-12 of FLT3 gene (screening of internal tandem duplication, ITD)

Presence of a FLT3-ITD mutation of 90 bp and a ratio mutant allele/wt 0.45
CEBPA mutation

FORWARD SEQUENCE

beginning of sequence deletion (13pb)

REVERSE SEQUENCE
AML mutations: distribution into categories of related genes

The Cancer Genome Atlas Research Network, NEJM 2013
Acute promyelocytic leukemia (APL): an example of integrated diagnosis

FISH: PML/RARA fusion signal

bcr1

bcr3

t(15;17)(q22;q12)

RT-PCR PML/RAR-alpha
• AML & MDS: clearly differentiated entities (clinical presentation, hematological picture, genetics)
• … although AML represents an evolutive phase of MDS in some patients
• AML & MDS are heterogenous diseases defined by complex, distinct genetic background: moving towards molecularly-defined classification
• Importance/difficulties of translating reality/biological complexity to pre-defined forms (MED-A & MED-B)
Questions?