Acute erythroid leukemias have a distinct molecular hierarchy from non-erythroid acute myeloid leukemias

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Supplementary material and methods

The 8 antibodies used for sorting CD34+ subpopulations were purchased from BD Biosciences: CD45-V500 (HI-30), CD34-PE-Cy7 (8G12), CD38-BV421 (HIT2), CD90-FITC (5E10), CD123-PE (9F5), CD45RA-APC-H7 (HI100), CD10-APC (HI 10a), and 7-Amino-Actinomycin D-PerCPCy5.5 (68981E) as a marker of cell viability. The antigens have been shown to be characteristic of the major types of hematopoietic stem and progenitor cells including CD34+CD38-CD90+CD45RA- hematopoietic stem cells (HSC), CD34+CD38-CD90-CD45RA- multipotent progenitors (MPP), CD34+CD38+CD10+CD45RA+ common lymphoid progenitor (CLP), CD34+CD38+CD123+CD45RA- common myeloid progenitors (CMP), CD34+CD38+CD123+CD45RA+ granulocyte-monocyte progenitors (GMP), CD34+CD38+CD123-CD45RA- megakaryocyte-erythrocyte progenitors (MEP). We tried to discriminate leukemic stem cell (LSC) from HSC based on an hypothetical leukemic phenotype CD34+CD38-CD90+CD45+RA expression. CD45RA is a specific marker for leukemia stem cell sub-populations in AML and aberrant marker expression is a possibility to differentiate LSC from HSC (Kersten et al Bjh 2016).

Seven antibodies were used to sort CD34- subpopulations: CD45-V450 (2D1), CD3-PE (UCHT1), CD19-APC (SJ25C1), CD16-APC-H7 (3G8), CD33-PerCP-Cy5.5 (P67.6), CD235-FITC (11E4B-7-8), and the marker of viability Live/Dead fixable Aqua Stain-Amcyan, and this allowed the recovery of T-lymphocytes, B-lymphocytes, neutrophils/NK, immature granulocytes and erythroblasts, respectively.
**Supplementary Table S1.** Clinical, biological and molecular data of the 12 patients studied.

**Supplementary Table S2.**
The 227 genes studied by targeted next generation sequencing on Illumina Miseq using a custom made Hemato v14 panel (HaloPlex Design ID: 27066-1485800404, Agilent Technologies).

**Supplementary Figure S1.** FACS analysis.

A. Example of FACS analysis in bone marrow cells from an *NPM1*-mutated M6-AML patient.

1. In the CD34+ fraction, HSC, LSC, MPP, LMPP, GMP, CMP, MEP, and CLP were sorted.
2. In the CD34- fraction, erythrocytes, granulocytes, PNN, T-lymphocytes and B-lymphocytes were sorted.

B. Example of FACS analysis in bone marrow cells from a *TP53*-mutated M6-AML patient. 1 and 2 same as in A.

**Supplementary Figure S2.** Comparison of the number of cells in subpopulations of the cellular hierarchy in CD34+ (A) and CD34- (B) fractions. The percentage of living cells is depicted in four groups of patients: 3 *NPM1*-mutated M6-AMLs, 3 *TP53* mutated M6-AMLs, 3 *NPM1*-mutated non-M6-AMLs and 2 *TP53*-mutated non-M6-AMLs.
Supplementary Figure S3. aCGH profiles showing losses at regions of chromosome 17 (including TP53 and NF1) and of chromosome 7 (including EZH2) in sorted subpopulations of M6 AMLs (A), and losses of chromosome 17 and chromosome 7 (left) or chromosome arm 7q (right) in sorted subpopulations but not in CD235+ cells of non-M6 AMLs (B). Dotted vertical line is the reference for absence of either loss or gain.

Supplementary Figure S4. Schematic structural organization of EPOR (A) and TRIM10 (B). The localization, type and variant allele frequency (VAF) of the mutations are indicated. In A, the left part shows the exonic organization of the EPOR gene and the right part the dimerization of the EPOR receptor bound to its EPO ligand and its JAK2 signal transducer. In B, domains of TRIM10 are indicated; the ring finger, B-box and coiled-coil regions are also present in the PML and RFP proteins, and are conserved in the truncated PML and RFP moieties found in the PML-RARA and RFP-RET oncogenic fusions.
### Supplementary Table S1: Clinical and biological data of the 12 patients studied by FACS.

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<th>NEUTROPHILS (G/l)</th>
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<th>ERYTHRO-BLASTS (%)</th>
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**Notes:**
- **M4**: Myeloid leukemia
- **AML**: Acute myeloid leukemia
- **IPSS**: International Prognostic Scoring System
- **R-IPSS**: Revised International Prognostic Scoring System
- **2017**: Year of publication

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**Legend:**
- **WBC (G/l)**: White blood cells
- **HEMOGLOBIN (g/dl)**: Hemoglobin concentration
- **NEUTROPHILS (G/l)**: Neutrophils per liter
- **BLASTS (%)**: Percentage of blasts
- **ERYTHRO-BLASTS (%)**: Percentage of erythroblasts
- **AUTOGRAPH**: Autologous transplantation
- **GRAFT**: Allogenic transplantation
- **IPSS or AML**: International Prognostic Scoring System or acute myeloid leukemia
- **PROGNOSIS (R-IPSS)**: Revised International Prognostic Scoring System
- **2017**: Year of publication
Supplementary Table S2: Genes studied by sequencing

HEMATO V14 used on Illumina
Miseq_ HaloPlex Design ID: 27066-1485800404

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TRIM33
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USP9X
WDR5
WHSC1
WT1
ZMYM3
ZNF717
ZRSR2
Supplementary Figure S1: Sorting analysis

A. HD-2295: NPM1-mutated M6-AML

1. CD34+ fraction

2. CD34- fraction
B. HD-2170: TP53-mutated M6-AML

1. CD34+ fraction

2. CD34- fraction

ALIVE
Supplementary Figure S2: Percentage of cells in each hematopoietic compartment

A. CD34+ fraction

1. HSC

2. LSC

3. MPP

4. LMPP

5. CMP

6. MEP

7. GMP

8. CLP
B. CD34- fraction

1. ERYTHROBLASTS

2. GRANULOCYTES

3. POLYNUCLEAR CELLS

4. B- LYMPHOCYTES

5. T- LYMPHOCYTES
Supplementary Figure S3: aCGH profiles of chromosome 7 and chromosome 17 in sorted subpopulations of M6-AMLs and non-M6 AMLs.

A. M6-AMLs

B. non-M6 AMLs
Supplementary Figure S4

A

Cases
- HD-2702
- HD-0177

Variants
- S407X (VAF: 6.92)
- L436fs (VAF: 14)

Exons
- 1
- 8

Amino-acids
- 306
- 508

Regions
- Extra-cytoplasmic
- TM
- Intra-cytoplasmic

EPOR

B

Case HD-2180

Amino-acids
- 1
- 309
- 489

Regions
- Ring
- Coiled-coil
- SPRY domain

Variant
- G377fs (VAF: 24)

TRIM10