GENETICS OF HEMATOLOGICAL MALIGNANCIES

INTERUNIVERSITY CERTIFICATE IN HUMAN GENETICS

Université catholique de Louvain
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Center for Human Genetics
Genetics of hematological malignancies

I - Introduction

II - Diagnostic

III- Prognosis

IV – Minimal residual disease

V - Targeted treatment

VI – Genetic predisposition
Normal hematopoiesis

Hematopoietic precursor neoplasms

Mature hematopoietic cell neoplasms
Hematopoiesis: transcription factors
I - INTRODUCTION

Hematopoiesis: growth factors

Multipotent hematopoietic stem cell (Hemocytoblast)

Common lymphoid progenitor
- FLT-3 ligand
- TNF-α
- TGF-β1

Common myeloid progenitor

Small lymphocyte
- IL-1
- IL-2
- IL-4
- IL-6
- IL-7

Erythrocyte
- SCF
- TPO
- IL-3
- GM-CSF

Myeloblast
- SCF
- Epo
- IL-3
- GM-CSF

B lymphocyte
- IL-3
- IL-5
- GM-CSF

Megakaryocyte
- SCF
- G-CSF
- GM-CSF
- IL-3
- IL-6

Thrombocytes
- Basophil
- Neutrophil
- Eosinophil
- Monocyte

I - INTRODUCTION

Neoplasms of hematopoietic precursors

Clonal proliferation of bone marrow hematopoietic precursors

With a block of differentiation

= acute

Myeloid

Acute Myeloblastic Leukemia (AML)

Blast cells

Lymphoid

Acute Lymphoblastic Leukemia (ALL)

Without a block of differentiation

= chronic

Quantitative Anomaly

One or more myeloid lineage

Myeloproliferative Neoplasm (MPN)

Qualitative Anomaly

Myelodysplastic Neoplasm (MDS)

Acute transformation
I - INTRODUCTION

Neoplasms of mature lymphoid cells

Clonal proliferation developed from a mature lymphoid cell

Classification according to the cell of origin:
- B-cell or T-cell lineage
- differentiation and/or activation status (B & T-cell immune response)
II - DIAGNOSIS

Clonality = marker of autonomous proliferation

• All cells derive from the same ancestral cell where occurred the acquired initiating mutation

• Thrombocytosis, polycythemia, myelofibrosis : Myeloproliferative neoplasm or reactive proliferation ?
  
  – JAK2 V617F mutation = clonal myeloproliferative neoplasm
    polycythemia vera / Vaquez (95%)
    essential thrombocytosis (50-60%)
    myelofibrosis (50-60%)
  – CalR (ex9) = clonal myeloproliferative neoplasm
    essential thrombocytosis (25%)
    myelofibrosis (35%)
## II - DIAGNOSIS

### Myeloproliferative neoplasms

<table>
<thead>
<tr>
<th>Mast cell</th>
<th>Systemic mastocytosis</th>
<th>Activating mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>Polycythaemia vera</td>
<td>JAK2V617F, JAK2 Exon 12</td>
</tr>
<tr>
<td>Platelets</td>
<td>Essential thrombocythaemia</td>
<td>JAK2V617F, MPLW515L/K</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Chronic eosinophilic leukemia</td>
<td>FIP1L1–PDGFRA</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Chronic myeloid leukaemia, Chronic myelomonocytic leukaemia, Primary myelofibrosis</td>
<td>BCR-ABL, TEL–PDGFRB, BCR–PDGFR, TEL–JAK2, other fusion TKs, JAK2V617F, MPLW515L/K</td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Nat Rev Cancer © 2007 Nature Publishing Group
II - DIAGNOSIS

Classification of acute myeloid leukemias (AML)

FAB Classification (1976)

*Morphology (+ immunophenotyping 1991)*

- (AML minimally differentiated) M0
- AML without maturation M1
- AML with maturation M2
- Promyelocytic acute leukemia M3
- Myelomonocytic leukemia M4
- AML-M4 with abn eosinophils M4Eo
- Monoblastic/monocytic AL M5a/b
- Erythroid AL M6
- Megacaryoblastic AL M7
Uses all available information to define entities:
- Clinics
- Morphology
- Immunophenotype
- Genetics

No real "gold standard" criteria

Aims:
- To define "real disease" entities
- Reproducibility
- Easy to use in the daily practice
2008 WHO Classification

Genetics + Morphology + Immunophenotyping

AML with recurrent genetic abnormalities

AML with balanced translocations / inversions

- \(t(8;21)(q22;q22)\) \(RUNX1-RUNX1T1\)
- \(t(15;17)(q22;q12)\) \(PML-RARa\) promyelocytic (M3)
- \(t(9;11)(p22;q23)\) \(MLLT3-MLL\)
- \(t(1;22)(p13;q13)\) \(RBM15-MKL1\) megacaryoblastic (M7)
- \(t(6;9)(p23;q34)\) \(DEK-NUP214\) basophilia
- \(inv(t(3;3)(q21;q26.2)\) \(RPN1-EVI1\)

AML with gene mutations

- Normal karyotype
  - \(FLT3-ITD\), \(FLT3-TKD\)
  - \(MLL-PTD\)
  - \(NPM1\) mutations (ex12)
  - \(CEBP\alpha\) mutations

- \(t(8;21), inv/t(16;16)\) \(KIT\) mutations
AML with myelodysplasia-related changes
   following a myelodysplastic syndrome (MDS) or myeloproliferative disorder
   without prior MDS

AML & myelodysplastic syndromes, therapy related
   Alkylants agents
   Ionizing radiation therapy
   Topoisomerase II inhibitors
   Others

AML not otherwise specified
   FAB
   AML with minimally differentiation          M0
   AML without maturation                      M1
   AML with maturation                         M2
   Acute myelomonocytic leukemia               M4
   Acute monoblastic & monocytic leukemia      M5a/b
   Acute erythroid leukemia                    M6
   Acute megacaryoblastic leukemia             M7
   Acute basophilic leukemia                   M7
   Acute panmyelosis with myelofibrosis

Myeloid sarcoma
Chromosomal translocations targeting the Core Bing Factor: RUNX1/21q22 & CBFβ/16q22

AML-M2: t(8;21)(q22;q22) RUNX1-RUNX1T1

AML-M4Eo: t/inv(16)(p13q22) CBFβ-MYH11

GM-CSF, IL-3, M-CSF-R, TCRα, TCRβ, p21/WAF1, MPO, NE, granzyme B, NP-3,….

Dominant negative effect of the fusion protein → inhibition of the target genes involved in myeloid differentiation
II - DIAGNOSIS

Classification of acute myeloid leukemias

Acute promyelocytic leukemia: Chromosomal translocation targeting the Retinoic Acid Receptor-α

RARα

PML-RARα

t(15;17)(q22;q21)
II - DIAGNOSIS

Classification of acute myeloid leukemias

Chromosomal translocations targeting \textit{MLL}/11q23

- Wild-type MLL

- MLL Fusion Proteins
  - \textit{ENL}: t(11;19)
  - \textit{AF4}: t(4;11)
  - \textit{AF9}: t(9;11)
  - \textit{AF10}: t(6;11)

- MLL Partial Tandem Duplication (MLL-PTD)
  - AML (8%)

- ALL (7%)
  - Infant ALL (80%)

- AML (8%)

- MLL and partners - 58 recurrent translocations and 150 partner genes. Edits [29/08/17; last update 22/04/17]

- Normal stem cell development
  - precursor to mature cell

- Leukemia development
  - MLL fusion
  - rapid self renewal

- Hox/Meis pathway
II - DIAGNOSIS

Classification of acute myeloid leukemias

Molecular heterogeneity of AML with normal karyotype
II - DIAGNOSIS

Classification of acute myeloid leukemias

NGS : 5 classes of cooperating mutations
### Classification of acute lymphoid leukemias (ALL)


**Morphology** + Immunophenotype + **Genetics**

#### ALL of B-cell precursors

<table>
<thead>
<tr>
<th>Type</th>
<th>Pro-B-ALL</th>
<th>Common ALL</th>
<th>Pre-B-ALL</th>
<th>Mature B-ALL</th>
<th>(Child / Ad%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL-L1</td>
<td></td>
<td>t(9;22)(q34;q11.2)</td>
<td></td>
<td></td>
<td>2-4% / 25% BCR-ABL</td>
</tr>
<tr>
<td>ALL-L2</td>
<td>t(v;11q23)</td>
<td>2-3%</td>
<td></td>
<td></td>
<td>2-3% MLL Rgt</td>
</tr>
<tr>
<td>ALL-L3</td>
<td>t(12;21)(p13;q22)</td>
<td>25%</td>
<td>t(12;21)(p13;q22)</td>
<td>0% ETV6-RUNX1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hyperdiploidy &gt; 50</td>
<td>~25% / &lt;5% Hypodiploidy 1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t(5;14)(q31;q32)</td>
<td>IL3-IGH</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t(1;19)(q23;p13.3)</td>
<td>TCF3-PBX1</td>
<td></td>
</tr>
</tbody>
</table>

#### ALL of T-cell precursors

<table>
<thead>
<tr>
<th>Type</th>
<th>(Child / Ad%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-T-ALL</td>
<td>25%</td>
</tr>
<tr>
<td>Pre-T-ALL</td>
<td>30% p16</td>
</tr>
<tr>
<td>Cortical T-ALL</td>
<td>7-31% TLI1</td>
</tr>
<tr>
<td>Medullary T-ALL</td>
<td>13-20% TLX3</td>
</tr>
<tr>
<td></td>
<td>9-30% CALM-AF10</td>
</tr>
<tr>
<td></td>
<td>8% MLL-v</td>
</tr>
<tr>
<td></td>
<td>6% NUP214-ABL</td>
</tr>
<tr>
<td></td>
<td>5% TCRβ-HOXA</td>
</tr>
</tbody>
</table>
II - DIAGNOSIS

Classification: B-cell lymphomas

Diagram showing the processes of somatic hypermutation, class switch recombination, and apoptosis in the context of lymphoma development.
II - DIAGNOSIS

Classification: Diffuse Large B-Cell Lymphoma

• Clinical heterogeneity of DLBCL
• Non reproducibility of morphology and immunohistochemistry
• Gene expression profiling: 2 main molecular subtypes of DLBCL:
  – derived from the lymph node germinal center (GCB)
  – derived from an activated B-cell (ABC)

![Gene expression profiling diagram](image)

NFkB activation
REL amplification
BCL2 translocation

Ash A. Alizadeh, Nature 2000
III - Prognosis

Childhood ALL

Impact of cytogenetics on survival

(Moorman AV Blood 2007)
Impact of cytogenetics on survival

Cytogenetics: 1366 (90%) of 1522 patients

Successful: 1003 cases (73%)

Abnormal clone: 796 cases (79%)

(Moorman AV Blood 2007)
III - Prognosis

Adult AML

Impact of cytogenetic entities on survival

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

Grimwade D et al. Blood 2010
### Adult AML

#### Prognostic subgroups according to genetic results (ex: Hovon)

<table>
<thead>
<tr>
<th>Prognostic subgroup</th>
<th>Cytogenetic/molecular results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Favorable</strong></td>
<td>- t(15;17)/PML-RARA&lt;br&gt;- t(8;21)/AML-ETO&lt;br&gt;- inv(16)/CBFB-MYH11&lt;br&gt;- isolated <em>NPM1</em>-mutations (normal karyotype)&lt;br&gt;- isolated <em>CEPBA</em>-mutations (normal karyotype)</td>
</tr>
<tr>
<td><strong>Intermediate</strong></td>
<td>- Normal karyotype&lt;br&gt;- trisomy 8</td>
</tr>
<tr>
<td><strong>Unfavorable</strong></td>
<td>- complex aberrations (≥3 chrom. anomalies)&lt;br&gt;- monosomy 7&lt;br&gt;- anomalies of chromosome 3&lt;br&gt;- <em>FLT3</em> length mutations (<em>FLT3</em>-LM/<em>FLT3</em>-ITD)&lt;br&gt;- <em>MLL</em>-PTD</td>
</tr>
</tbody>
</table>
Minimal Residual Disease

- Real-time PCR
- Multi-color Flow cytometry
- NGS (high coverage)
- Acute lymphoblastic leukemia
- Chronic myelocytic leukemia
- ...
V– Targeted treatment

Promyelocytic acute leukemia (AML-M3/APL)

“faggot cells” = bundles of Auer rods
Promyelocytic acute leukemia (AML-M3/APL)

t(15;17)(q22;q21)
**V— Targeted treatment**

**Promyelocytic acute leukemia (AML-M3/APL)**

- **LMA M3**

  - t(15;17)(q22;q21) = fusion between
    - **PML** (15q22) and
    - **RARA**, retinoic acid receptor alpha (17q21)

  - Reduced sensitivity of the nuclear receptor to the ligand, retinoic acid

  - **All Trans Retinoic Acid – ATRA (Vesanoid®) – (Δ vitamin A)**
1μM ATRA: differentiation induction with growth inhibition and apoptosis
RARα

PML-RARα

NPM-RARα

NuMA-RARα

ATRA sensitivity

45 mg/m²/jr

PLZF-RARα

STAT5b-RARα

Poor ATRA sensitivity

45 mg/m²/jr
Chronic myeloid leukemia (CML) fusion between BCR (22q11) and ABL1 (9q34). Constitutively activated chimeric tyrosine kinase.

Tyrosine kinase inhibitor (STI571 / Imatinib / Glivec®)
Inhibitors of tyrosine kinase activity

competitive antagonists of the ATP-binding site of bcr-abl

Chronic Myelocytic leukemia (CML)

V– Targeted treatment

Druker, B. J. Blood 2008;112:4808-4817
**V– Targeted treatment**

**Chronic Myelocytic leukemia (CML)**

Glivec resistance

**BCR-ABL amplification**

**ABL tyrosine kinase domain mutations**

and overexpression of drug-efflux proteins (ABCB1 and ABCG2)

Development of new tyrosine kinase inhibitors (*Dasatinib, Nilotinib, …*)
**ABL KD Mutations**

Sensitivity of *BCR/ABL* Kinase Domain Mutations to TKIs (values are given as IC\(_{50}\))

![Table showing IC\(_{50}\) fold increase for different mutations and drugs](image)

• < 1% underestimated, esp. in adults ↑ with NGS

• Mainly myeloid disorders :
  – Bone marrow failure
  – Myelodysplasia / AML

• Genes involved in :
  – DNA repair : Fanconi complementation group
  – Telomere maintenance : TERC, TERT
  – Ribosome : RPS19, SDBS
  – Hematopoïèsis : CEPBA, RUNX1, GATA2, PAX5, ETV6

• Other disorders : low penetrance susceptibility
VI – GENETIC PREDISPOSITION

Hematological malignancies

• Crucial to identify for MDS/AML:
  – Selection of an unaffected familial donor in case of hematopoïetic stem cell transplantation
  – Fanconi Anemia, Telomeropathies are associated with an increased sensitivity to chemotherapy/radiotherapy
    → dose adaptation to limit toxicity

• Genetic test:
  – on a non invaded sample (hair follicle, skin, buccal swap)
  – before allo-graft