Basic mechanisms of leukemogenesis

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Basic molecular mechanisms of leukemogenesis

Part I

What is leukemia?

Genetic aberrations leading to leukemia
a) Alterations in tyrosine kinases (BCR/ABL)
b) Alterations in transcriptional regulators (CBF, RARα)

Cooperative model - therapeutic consequences

Part II

Leukemic stem cells
Blood formation: hematopoietic hierarchy

- Stem cells
- LT-HSC
- ST-HSC
- Self renewal
- MPP
- Myeloid
  - CMP
  - MEP
  - Erythrocytes
  - Platelets
  - Granulocytes
- Lymphoid
  - CLP
  - Pro-DC
  - Pro-T
  - Pro-NK
  - Pro-B
  - Macrophages
  - Dendritic-cells
  - T-cells
  - NK-cells
  - B-cells
Normal cells as detected in smear of peripheral blood.
Leukemia: cancer of the hematopoietic system

Too many hematopoietic cells with normal or blocked differentiation occupy the hematopoietic organs such as peripheral blood, bone marrow, and spleen.

The main consequence is functional insufficiency of the blood cell forming system resulting in anemia, infection, and bleeding episodes.
LEUKEMIA = LEUKOS = “White blood”
Leukemia: cancer from blood-forming system often associated with an increase in white blood cells

- **Chronic leukemia**
  - Clinic: gradual
  - Normal differentiation

- **Acute leukemia**
  - Clinic: rapid
  - Block in normal cell differentiation
“Leukemia” summarizes a large number of different diseases

**Chronic myeloproliferative disorders**
- Chronic myeloid leukemia (CML)
- Polycythemia vera (PV)
- Essential thrombocythemia (ET)
- Hypereosinophilic syndrome (HES)
- Chronic idiopathic myelofibrosis (OMF)

Myelodysplastic/Myeloproliferative disorders (e.g. CMML)
Myelodysplastic syndromes (MDS)

**Chronic lymphoproliferative disorders (CLL)**

**Acute myeloid leukemia (AML)**

B-cell acute lymphoblastic leukemia (ALL)

T-cell/NK-cell acute lymphoblastic leukemia
Leukemia: cancer of the hematopoietic system

Chronic myeloid leukemia: affects all myeloid lineages: *differentiation maintained*

CML: chronic myeloid leukemia; CLL: chronic lymphocytic leukemia;
Leukemia: cancer of the hematopoietic system

Acute leukemia: *differentiation block at progenitor stage*

**ALL**

- Lymphoid Stem Cell
- Trilineage Myeloid Stem Cell

**AML**

- Pluripotent Stem Cell

- AML: acute myeloid leukemia
- ALL: acute lymphoblastic leukemia;
AML-FAB-M0

- MPO +/- CD13/CD33+, ev. CD22/CD79α (B), CD3 (T)
- 5q-, 7q-, +13, rearr. 2p, 12p

AML-FAB-M2

- CD34+, CD13+, ev. CD19+, ev. CD56+
- t(8;21)(q22;q22): AML1/ETO

AML-FAB-M3

- HLA-DR-, CD33/13+ CD65/15 +/-, ev CD2+
- t(15;17)(q22;q11): PML/RARα

AML-FAB-M4

- CD34+, CD13+, ev. CD2+
- In(16)(p13;q22) t(16;16)(p13q22) CBFβ/SMMHC (MYH11)

AML-FAB-M6

- HLA-DR+/-, CD34+/-, CD14-GlycoA+, CD36+, CD41-
- -5, -7, -12p, +8

AML-FAB-M7

- CD41/CD61+, CD13/CD33+/- CD7-
- +21; t(1;22)(p13;q13): OTT/MAL1

Morphology | Immunophenotype | Karyotype & Molecular genetics
Somatic *mutations* in leukemia

- **chromosomal translocations**
  - fusion protein
  - abnormal gene expression

- **numerical chromosomal aberrations**
  - deletions, aneuploidy

- **Mutations** (point-, ITD..) in known oncogenes or tumor suppressor genes.
Chromosome studies on normal and leukemic human leukocytes


FIG. 13

Metaphases from Leukemia case 4 (male); 46 chromosomes.

Note: minute chromosome (arrow), x 2700.
1983-86: Identification of the **BCR/ABL fusion** product that is present in close to 100% of CML cases.
Clonal chromosomal abnormalities in human cancer

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Cases (n)</th>
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<tbody>
<tr>
<td>AML</td>
<td>12,124</td>
</tr>
<tr>
<td>MDS</td>
<td>4,008</td>
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<tr>
<td>CML</td>
<td>2,999</td>
</tr>
<tr>
<td>CMD</td>
<td>1,180</td>
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<tr>
<td>ALL</td>
<td>7,194</td>
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<tr>
<td>B-ML</td>
<td>7,504</td>
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<tr>
<td>T-ML</td>
<td>1,094</td>
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<tr>
<td>HD</td>
<td>231</td>
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<td>Respiratory system</td>
<td>759</td>
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<td>Digestive system</td>
<td>1,334</td>
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<tr>
<td>Breast</td>
<td>799</td>
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<tr>
<td>Female genital organs</td>
<td>790</td>
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<tr>
<td>Male genital organs</td>
<td>297</td>
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<td>Urinary tract</td>
<td>1,590</td>
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<td>Endocrine system</td>
<td>150</td>
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<td>Nervous system</td>
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<tr>
<td>Skin</td>
<td>264</td>
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<td>Bone</td>
<td>570</td>
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<tr>
<td>Soft tissue</td>
<td>1,169</td>
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</table>

[Mittelman et al., Nat. Rev. Cancer 2007]
Chromosomal translocations: principle

Promoter exchange:
Deregulated expression

Oncogenic fusion genes
Genetic aberrations in leukemogenesis

Functional classification

Affecting cellular *growth & survival*


Protein tyrosine kinases and related signaling mediators


X-ABL, X-PDGFR
FLT3
JAK2
<table>
<thead>
<tr>
<th>Protein</th>
<th>Alteration</th>
<th>Disease/Condition</th>
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</thead>
<tbody>
<tr>
<td><strong>ABL</strong></td>
<td>(9q22)</td>
<td><strong>BCR-ABL</strong> (p210/p190/p230)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>CML (&gt;90%)</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>B-ALL (&gt;15%)</strong></td>
</tr>
<tr>
<td><strong>PDGFβR</strong></td>
<td>(5q31-33)</td>
<td><strong>TEL-PDGFβR</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>HIP-PDGFβR</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>H4-PDGFβR</strong></td>
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<tr>
<td></td>
<td></td>
<td><strong>Rap5-PDGFβR</strong></td>
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<tr>
<td></td>
<td></td>
<td><strong>CEV14-PDGFβR</strong></td>
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<tr>
<td></td>
<td></td>
<td><strong>CMML, EoCMPD</strong></td>
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<tr>
<td></td>
<td></td>
<td><strong>EoL, MDS+Eo</strong></td>
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<tr>
<td><strong>FLT3</strong></td>
<td>(13q12)</td>
<td><strong>FLT3-ITD</strong></td>
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<td><strong>FLT3-TKD</strong></td>
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<td></td>
<td></td>
<td><strong>X-FLT3</strong></td>
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<tr>
<td></td>
<td></td>
<td><strong>AML (&gt;20%)</strong></td>
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<tr>
<td></td>
<td></td>
<td><strong>AML/ALL</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>MPD/AML (rare)</strong></td>
</tr>
</tbody>
</table>
Chronic myeloid leukemia (CML)

- >90% Ph+ = t(9;22) = BCR/ABL

- expansion of the myeloid lineage with “normal” maturation

- inevitable transformation from a chronic phase into a blast crisis resembling AML/ALL.
Chronic myeloid leukemia (CML) is induced by t(9;22) ["Philadelphia chromosome"] in hematopoietic stem cells.
Experimental approaches to assess the transforming properties of a PTK fusion

A) *in vitro*

- cytokines (days)

Number of viable cells

[Growth-factor dependent cell lines]

B) *in vivo*

Retroviral infection

Transgenic animals

“Leukemia”

BMT
Malignant transformation by protein tyrosine kinase fusions through *multiple* signal transduction pathways

5’ partners: *BCR, TEL, ...*

Di-/ oligomerization

PTKs: *ABL, PDGFβR, JAK2...*

Auto-/ trans tyrosine-phosphorylation

RAS/MAPK  PI3K/PKB(AKT)  JAK/STAT  NF-κB

Maligant phenotype
Imatinib mesylate - STI-571 - Gleevec™

[Schindler et al., Science, 289:1938, 2000]
CML Therapy: the impact of imatinib

TREATMENT OF CHRONIC MYELOID LEUKEMIA

P. A. Stryckmans, M.D.

Table 1 Results of selected therapies for chronic myeloid leukemia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of patients</th>
<th>All have Phֳ ‰</th>
<th>Median survival (months)</th>
<th>Mode of treatment</th>
<th>Survival</th>
<th>Ref.</th>
<th>Year</th>
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<tr>
<td>Supportive</td>
<td>52</td>
<td>no</td>
<td>36c</td>
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<td>S</td>
<td>14</td>
<td>1924</td>
</tr>
<tr>
<td>Busulfan daily</td>
<td>30</td>
<td>no</td>
<td>42</td>
<td>D</td>
<td>S</td>
<td>7</td>
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<tr>
<td>Busulfan single doses</td>
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<td>D</td>
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<td>48</td>
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<td>40</td>
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<td>31</td>
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<td>1968</td>
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<td>Dibromomannitol</td>
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<td>Hydroxyurea</td>
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<td>39.6</td>
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<td>1973</td>
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<td>Busulfanf</td>
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<td>D</td>
<td>D</td>
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<td>Dibromomannitolf</td>
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<td>D</td>
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<td>Melphalan</td>
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<td>T</td>
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<td>L-S protocole</td>
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<td>50</td>
<td>D</td>
<td>D</td>
<td>37</td>
<td>1979</td>
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</tbody>
</table>

a: discontinuous, C: continuous.
b: from first symptoms, D: from diagnosis, T: from beginning of treatment.
c: Mean survival.
d: Except one Ph− case.
e: See text.
f: Randomized trial.

d7350

Five-Year Follow-up of Patients Receiving Imatinib for Chronic Myeloid Leukemia

Druker B, et al., 2006
Imatinib mesylate [STI-571/Gleevec™]: Resistance

Multifunctional resistance mechanisms (variable latency)

- Apoptosis
- Gene Amplification
- Targeted Mutations (E255V/K, T315I)
- MDR expression
- mdr
- AGP binding (?)

STI
X-ABL  
X-PDGFβR

Small molecule PTK inhibitors

Primary and secondary drug resistance

Signaling pathways

“where to interfere? “...
[Van Etten RA, 2007]
Protein tyrosine kinase fusion genes in human myeloproliferative disorders
**Functional classification of genetic alterations in leukemia**

<table>
<thead>
<tr>
<th>Tyrosine kinase fusions</th>
<th>Gain of function point mutations</th>
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</thead>
<tbody>
<tr>
<td>- ABL</td>
<td>- FLT3</td>
</tr>
<tr>
<td>- JAK2</td>
<td>- RAS</td>
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<tr>
<td></td>
<td>- KIT</td>
</tr>
<tr>
<td></td>
<td>- PTPN11</td>
</tr>
</tbody>
</table>

Conferring proliferative and/or survival advantage but do not affect differentiation

“Class I mutations”

Transforming activity *in vivo*:
- BCR-ABL
- TEL-PDGFβR
- TEL-JAK2
- FLT3-ITD

Myeloproliferative disease (MPD, “CML-like”)  
[short latency / polyclonal]

“Sufficient”
Genetic aberrations in leukemogenesis

Functional classification

**Affecting cellular growth & survival**
- Protein tyrosine kinases and related signaling mediators
  - X-ABL, X-PDGFR
  - FLT3
  - JAK2...

**Affecting self renewal & cellular differentiation**
- Transcriptional regulators
  - CBF
  - RARα
  - MLL...
Transcriptional regulators of hematopoiesis

- Molecular regulators that induce a gene expression program essential for differentiation of hematopoietic cells.
Master transcriptional regulators of hematopoiesis*

[not a complete list*]
Genetic alterations in transcriptional master regulators

- Co-Factor (TF)

Gene expression program for normal differentiation

- Mutations in key transcription factors and/or co-regulators

Block in differentiation ("blasts")
Genetic alterations target transcriptional regulators

Block in cellular differentiation and/or induction of aberrant self-renewal

**Fusion genes involving:**
- Core binding factor (CBF)
- Retinoic acid receptor α (RARα)
- Homeobox factors (HOX)
- Co-activators: CBP/p300

**Point mutations involving:**
- Core binding factor (CBF)
- CCAAT/enhancer binding protein (CEBP/α)
- PU.1

“Class II mutations”
### Most frequent genetic alterations in acute myeloid leukemia targeting transcriptional regulators

**A) Loss of function mutations**

- **AML1** (21q22) truncation/missense mutations (DN) AML-M0, FPD/AML, MDS
- **C/EBP α** (19q13) truncation/missense mutations (DN) AML-M2
- **PU.1** (11p11) truncation/missense mutations (DN) AML (rare)

**B) Fusion genes**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Translocation/Partner</th>
<th>Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RAR α</strong> (17q21)</td>
<td>t(15;17)(q22;q21) PML/RARα</td>
<td>APL / AML-M3</td>
</tr>
<tr>
<td></td>
<td>t(11;17)(q23;q21) PLZF/RARα</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(5;17)(q32;q21) NPM/RARα</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(11;17)(q13;q21) NuMA/RARα</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(17;17)(q11;q21) STAT5B/RARα</td>
<td></td>
</tr>
<tr>
<td><strong>MLL/ALL1</strong> (11q23)</td>
<td>&gt;40 translocations &gt;40 fusion partners</td>
<td>AML/ALL</td>
</tr>
<tr>
<td><strong>AML1</strong> (21q22)</td>
<td>t(3;21)(q26;q22) MDS/EVI1/AML1</td>
<td>t-AML/MDS</td>
</tr>
<tr>
<td></td>
<td>t(8;21)(q22;q22) AML1/ETO</td>
<td>AML-M2</td>
</tr>
<tr>
<td></td>
<td>t(16;21)(q24;q22) MTG16/AML1</td>
<td>AML</td>
</tr>
<tr>
<td><strong>CBF β</strong> (16p13)</td>
<td>Inv(16)(p13;q22) SMMHC/CBFβ</td>
<td>AML-M4</td>
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<td></td>
<td>t(11;16)(q23;p13) MLL/CBP</td>
<td>AML-M5</td>
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<tr>
<td></td>
<td>t(8;16)(p11;p13) MOZ/CBP</td>
<td>AML-M5</td>
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<td>t(10;16)(q22;p13) MORF/CBP</td>
<td>AML-M5</td>
</tr>
<tr>
<td><strong>p300</strong> (22q13)</td>
<td>t(11;22)(q23;q13) MLL/p300</td>
<td>AML</td>
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<tr>
<td></td>
<td>t(8;22)(p11;q13) MOZ/p300</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>Inv(8)(p11q13) MOZ/TIF2</td>
<td>AML</td>
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<tr>
<td><strong>HOX gene cluster</strong></td>
<td>t(7;11)(p15;p15) NUP98/HOXA9</td>
<td>AML</td>
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<td></td>
<td>t(7;11)(p15;p15) NUP98/HOXA11</td>
<td>AML</td>
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<tr>
<td></td>
<td>t(7;11)(p15;p15) NUP98/HOXA13</td>
<td>AML</td>
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<tr>
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<td>t(11;12)(p15;q13) NUP98/HOXC13</td>
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<td>t(11;12)(p15;q13) NUP98/HOXC11</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>t(2;11)(q31;p15) NUP98/HOXD13</td>
<td>AML</td>
</tr>
<tr>
<td><strong>PMX</strong> (1q23)</td>
<td>t(1;11)(q23;p15) NUP98/PMX1</td>
<td>AML</td>
</tr>
<tr>
<td><strong>CDX2</strong> (13q12)</td>
<td>t(12;13)(p13;q12) TEL/CDX2</td>
<td>AML</td>
</tr>
<tr>
<td><strong>GSH2</strong> (4q12)</td>
<td>t(4;12)(q12;p13) CHIC2/TEL</td>
<td>AML</td>
</tr>
<tr>
<td><strong>HLXB9</strong> (7q36)</td>
<td>t(7;12)(q36;p13) HLXB9/TEL</td>
<td>AML</td>
</tr>
</tbody>
</table>
The core binding factor (CBF)
AML1 [RUNX1] is essential for definitive hematopoiesis

fetal liver E11.5-12.5

liver touch preparation

AML1 -/- mice

[Okuda et al., Cell, 1996]
Major alterations targeting the core-binding factor (CBF)

*Fusion genes

- AML1/ETO
- CBF\(\beta\)/MYH11
- TEL/AML1

*“Loss of function” mutations

- AML1

>20% of human acute myeloid leukemias have CBF aberrations!
Core binding factor (CBF) and leukemia

$\text{AML1/ETO} \quad >15\% \text{ of AML cases}$
CBF-containing fusion proteins are *dominant-negative* to CBF-mediated transcription & differentiation.
PU.1

JunB

Jun

AML1/ETO [t(8;21)]

PML/RARα [t(15;17)]

Mutations?

Normal myeloid differentiation

Acute myeloid leukemia

[p16INK4A, Cyclin D1, Bcl2, BclXL]

Monocytic lineage differentiation block

[according to Somervaille & Cleary, Cancer Cell, 2006]
## Most frequent genetic alterations in acute myeloid leukemia targeting transcriptional regulators

### A) Loss of function mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration</th>
<th>Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AML1 (21q22)</strong></td>
<td>truncation/missense mutations (DN)</td>
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<td><strong>PU.1 (11p11)</strong></td>
<td>truncation/missense mutations (DN)</td>
<td>AML (rare)</td>
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</tbody>
</table>

### B) Fusion genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Breakpoints</th>
<th>Partner Gene</th>
<th>Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RAR α (17q21)</strong></td>
<td>17q21</td>
<td>(15;17)(q22;q21)</td>
<td>PML/RARα</td>
<td>APL / AML-M3</td>
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<tr>
<td></td>
<td>17q21</td>
<td>(11;17)(q23;q21)</td>
<td>PLZF/RARα</td>
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<td></td>
<td>17q21</td>
<td>(5;17)(q32;q21)</td>
<td>NPM/RARα</td>
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<tr>
<td></td>
<td>17q21</td>
<td>(11;17)(q13;q21)</td>
<td>NuMA/RARα</td>
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</tr>
<tr>
<td></td>
<td>17q21</td>
<td>(17;17)(q11;q21)</td>
<td>STAT5B/RARα</td>
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</tr>
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</table>

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<td></td>
<td>17q21</td>
<td>(17;17)(q11;q21)</td>
<td>STAT5B/RARα</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Breakpoints</th>
<th>Partner Gene</th>
<th>Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MLL/ALL1 (11q23)</strong></td>
<td>11q23</td>
<td>&gt;40 translocations</td>
<td>&gt;40 fusion partners</td>
<td>AML/ALL</td>
</tr>
<tr>
<td><strong>AML1 (21q22)</strong></td>
<td>21q22</td>
<td>(3;21)(q26;q22)</td>
<td>MDS/EVI1/AML1</td>
<td>t-AML/MDS</td>
</tr>
<tr>
<td></td>
<td>21q22</td>
<td>(8;21)(q22;q22)</td>
<td>AML1/ETO</td>
<td>AML-M2</td>
</tr>
<tr>
<td></td>
<td>11q23</td>
<td>(16;21)(q24;q22)</td>
<td>MTG16/AML1</td>
<td>AML</td>
</tr>
<tr>
<td><strong>CBF β (16p13)</strong></td>
<td>16p13</td>
<td>Inv(16)(p13q22)</td>
<td>SMMHC/CBFβ</td>
<td>AML-M4</td>
</tr>
<tr>
<td></td>
<td>16p13</td>
<td>(16;16)(p13;q22)</td>
<td>SMMHC/CBFβ</td>
<td>AML-M4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Breakpoints</th>
<th>Partner Gene</th>
<th>Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CBP (16p13)</strong></td>
<td>16p13</td>
<td>(11;16)(q23;p13)</td>
<td>MLL/CBP</td>
<td>AML-M5</td>
</tr>
<tr>
<td></td>
<td>16p13</td>
<td>(8;16)(p11;p13)</td>
<td>MOZ/CBP</td>
<td>AML-M5</td>
</tr>
<tr>
<td></td>
<td>16p13</td>
<td>(10;16)(q22;p13)</td>
<td>MORF/CBP</td>
<td>AML-M5</td>
</tr>
<tr>
<td><strong>p300 (22q13)</strong></td>
<td>22q13</td>
<td>(11;22)(q23;q13)</td>
<td>MLL/p300</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>22q13</td>
<td>(8;22)(p11;q13)</td>
<td>MOZ/p300</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>22q13</td>
<td>Inv(8)(p11q13)</td>
<td>MOZ/TIF2</td>
<td>AML</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Breakpoints</th>
<th>Partner Gene</th>
<th>Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HOX gene cluster</strong></td>
<td>11p15</td>
<td>(7;11)(p15;q15)</td>
<td>NUP98/HOXA9</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>11p15</td>
<td>(7;11)(p15;q15)</td>
<td>NUP98/HOXA11</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>11p15</td>
<td>(7;11)(p15;q15)</td>
<td>NUP98/HOXA13</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>11p15</td>
<td>(11;12)(p15;q13)</td>
<td>NUP98/HOXC13</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>11p15</td>
<td>(11;12)(p15;q13)</td>
<td>NUP98/HOXC11</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>11p15</td>
<td>(2;11)(q31;p15)</td>
<td>NUP98/HOXD13</td>
<td>AML</td>
</tr>
<tr>
<td><strong>PMX (1q23)</strong></td>
<td>1q23</td>
<td>(1;11)(q23;p15)</td>
<td>NUP98/PMX1</td>
<td>AML</td>
</tr>
<tr>
<td><strong>CDX2 (13q12)</strong></td>
<td>13q12</td>
<td>(12;13)(p13;q12)</td>
<td>TEL/CDX2</td>
<td>AML</td>
</tr>
<tr>
<td><strong>GSH2 (4q12)</strong></td>
<td>4q12</td>
<td>(4;12)(q12;p13)</td>
<td>CHIC2/TEL</td>
<td>AML</td>
</tr>
<tr>
<td><strong>HLXB9 (7q36)</strong></td>
<td>7q36</td>
<td>(7;12)(q36;p13)</td>
<td>HLXB9/TEL</td>
<td>AML</td>
</tr>
</tbody>
</table>
Class II alterations: translocations targeting RARα

Characterized by an accumulation of abnormal promyelocytes: AML-M3 (FAB); or APL

Molecularly characterized by distinct cytogenetic aberrations targeting the RARα gene on 17q

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Frequency</th>
<th>Fusion Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(15;17)(q22;q21)</td>
<td>&gt;95%</td>
<td>PML/RARα</td>
</tr>
<tr>
<td>t(11;17)(q23;q21)</td>
<td>1%</td>
<td>PLZF/RARα</td>
</tr>
<tr>
<td>t(11;17)(q13;q21)</td>
<td>rare</td>
<td>NuMA-RARα</td>
</tr>
<tr>
<td>t(5;17)(q35;q21)</td>
<td>&lt;1%</td>
<td>STAT5b-RARα</td>
</tr>
</tbody>
</table>
Pharmacological doses of RA [ATRA] relieves repression

ATRA is not sufficient to relieve the repressive state
## Therapeutic targeting the molecular defect: APL

Table 1. Outcomes with simultaneous administration of all-trans retinoic acid (ATRA) plus chemotherapy-based regimens in patients with newly diagnosed acute promyelocytic leukemia (APL).

<table>
<thead>
<tr>
<th>Cooperative group, Reference</th>
<th>Induction Regimen</th>
<th>n</th>
<th>Non-Eligible n (%)</th>
<th>Median age (Range)</th>
<th>CR n (%)</th>
<th>DFX %</th>
<th>CIR %</th>
<th>OS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIMEMA Mandelli et al (1997)</td>
<td>ATRA + Idarubicin</td>
<td>253</td>
<td>21 (8)</td>
<td>38 (2-74)</td>
<td>229 (95)</td>
<td>79 (2y)</td>
<td>NA</td>
<td>87 (2y)</td>
</tr>
<tr>
<td>European APL Fenaux et al (1999)</td>
<td>ATRA + Daunorubicin + Cytarabine</td>
<td>99*</td>
<td>NA</td>
<td>43 (7-63)</td>
<td>NA (94)</td>
<td>NA</td>
<td>11 (2y)</td>
<td>84 (2y)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>163**</td>
<td>NA</td>
<td>42 (1-73)</td>
<td>NA (90)</td>
<td>NA</td>
<td>29 (2y)</td>
<td>77 (2y)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42 †</td>
<td>NA</td>
<td>69 (65-77)</td>
<td>NA (90)</td>
<td>NA</td>
<td>7 (2y)</td>
<td>69 (2y)</td>
</tr>
<tr>
<td>MRC Burnett et al (2000)</td>
<td>ATRA + Anthracycline + Cytarabine +/- Etoposide</td>
<td>120 ‡</td>
<td>NA</td>
<td>NA</td>
<td>NA (67)</td>
<td>72 (4y)</td>
<td>20 (4y)</td>
<td>71 (4y)</td>
</tr>
<tr>
<td>GAMLCG Længfeldor et al (2000)</td>
<td>ATRA + Daunorubicin + Cytarabine + Thioguanine</td>
<td>51</td>
<td>11 (18)</td>
<td>43 (16-60)</td>
<td>47 (92)</td>
<td>96 (2y)</td>
<td>NA</td>
<td>88 (2y)</td>
</tr>
<tr>
<td>PETHEMA Sanz et al (1999)</td>
<td>ATRA + Idarubicin</td>
<td>123</td>
<td>13 (10)</td>
<td>42 (1-74)</td>
<td>109 (89)</td>
<td>92 (2y)</td>
<td>NA</td>
<td>82 (2y)</td>
</tr>
<tr>
<td>PETHEMA Sanz et al (2004)</td>
<td>ATRA + Idarubicin$</td>
<td>251</td>
<td>28 (10)</td>
<td>39 (2-81)</td>
<td>227 (90)</td>
<td>90 (3y)</td>
<td>7.5 (3y)</td>
<td>85 (3y)</td>
</tr>
</tbody>
</table>

* Patients aged < 65 years with WBC < 5 ×10⁹/L
** Patients with WBC > 5 ×10⁹/L
† Patients aged > 65 years with WBC < 5 ×10⁹/L
‡ extended ATRA arm;
§ Risk-adapted consolidation with ATRA for intermediate and high-risk patients.
Abbreviations: CR, complete remission; DFX, desferrioxamine; CIR, cumulative incidence of relapse; OS, overall survival

[Sanz, Hematology, 2006]
A very simplified general model of action of transcription factor fusions associated with acute leukemia.

DEREGULATED TARGET GENE EXPRESSION

[e.g. CBF, X-RARα]

[e.g. MOZ, CBP]
Genetics of leukemia: working model

activated kinase $\rightarrow$ proliferation/apoptosis $\rightarrow$ Chronic leukemia

altered transcription $\rightarrow$ block in differentiation $\rightarrow$ Acute leukemia?
**In vivo** transforming activity of mutations targeting transcriptional regulators of hematopoiesis

- **Retroviral infection**
- **BMT**
- **Transgenic animals**

- **AML1-ETO**
- **CBFβ/MYH11**
- **PML/RARα**
- **NUP-HOXA9**
- **MLL/CBP**
- **MLL/ENL**
- **MLL/GAS6**
- **MOZ/TIF2**

- [long latency (weeks-months) / monoclonal]

- “Not sufficient, needs additional hits “

**MDS/AML-like disease**
Clinical evidence for *functional cooperation* of genetic alterations in acute leukemia

<table>
<thead>
<tr>
<th>TK (class I)</th>
<th>TF (class II)</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT3-ITD</td>
<td>AML1/ETO</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>PML/RARα</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBFβ/MYH11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DEK/CAN</td>
<td></td>
</tr>
<tr>
<td>FLT3-KD</td>
<td>MLL/AF4</td>
<td>AML/ALL</td>
</tr>
<tr>
<td></td>
<td>MLL/SEPT6</td>
<td></td>
</tr>
<tr>
<td>KIT-mutation</td>
<td>CBFβ/MYH11</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>AML1/ETO</td>
<td></td>
</tr>
<tr>
<td>N-RAS, K-RAS mutation</td>
<td>CBFβ/MYH11</td>
<td>AML</td>
</tr>
<tr>
<td>TEL/PDGFβR</td>
<td>AML1/ETO</td>
<td>CMML (BC)</td>
</tr>
<tr>
<td>TRK-A up-regulation</td>
<td>AML1/ETO</td>
<td>AML</td>
</tr>
<tr>
<td>JAK2V617F</td>
<td>AML1/ETO</td>
<td>AML</td>
</tr>
<tr>
<td>BCR/ABL</td>
<td>AML1/EVI1/MDS1</td>
<td>CML (BC)</td>
</tr>
<tr>
<td></td>
<td>NUP98/SHOXA9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NUP98/DDX10</td>
<td></td>
</tr>
</tbody>
</table>
**In vivo cooperation of genetic alterations demonstrated in mouse models of leukemia**

**CLASS I mutations**
- BCR/ABL
- TEL/PDGFβR
- FLT3-ITD
- ....

**CLASS II mutations**
- AML1-ETO
- NUP-HOXA9
- MLL-X
- ....

**Myeloproliferative disease** (MPD, “CML-like”)
- [short latency / polyclonal]
- “Sufficient”

**MDS-like disease**
- [long latency / monoclonal]
- “Not sufficient, needs additional hits “

**ACUTE LEUKEMIA**
- [short latency, clonal]

- BCR/ABL + NUP98/HOXA9
- BCR/ABL + AML1/EVI1
- TEL/PDGFβR + AML1/ETO
- FLT3-ITD + PML-RARα
- FLT3-ITD + AML1/ETO
- FLT3-ITD + MLL-X
- KIT-TKD + AML1/ETO
activated kinase

proliferation

apoptosis

Chronic leukemia

Acute leukemia

altered transcription

block in differentiation

Myelodysplasia
Optimized targeted therapy for acute leukemia

1. Determination of class I & class II alterations.
2. Select compound(s) to target distinct alteration(s).
3. Adapt upon development of resistance.

Class I mutation + Class II mutation = Acute leukemia

- siRNA, AS-ODN, ribozymes
- Small molecules or peptides inhibiting kinase activity or/and oligomerization.
- Small molecules (peptides) inhibiting aberrant histone modification, processing proteases, co-repressor recruitment.
ACUTE LEUKEMIA

Leukemic Stem Cell (LSC)

[“gain of function”]

CLASS I

PROLIFERATION & SURVIVAL

- FLT3-ITD
- X-ABL
- KIT
- RAS
- PTPN11 mutations

PTK-inhibitors
FT-inhibitors
PI3K-inhibitors

[“loss of function”]

CLASS II

BLOCK IN DIFFERENTIATION

- CBF (AML1, CBFβ)
- X-RARα
- MLL-X
- CBP, TIF2
- C/EBPα
- GATA1

ATRA (APL)
HDAC inhibitors ?
Basic molecular mechanisms of leukemogenesis

Part I

What is leukemia?

Genetic aberrations leading to leukemia
a) Alterations in tyrosine kinases (BCR/ABL, FLT3-ITD)
b) Alterations in transcriptional regulators (CBF, RARα)

Cooperative model - therapeutic consequences

Part II

Leukemic stem cells
The cancer stem cell hypothesis is not new!!

1937: Furth & Kahn: transmission of leukemia by transplantation into mice

1960’s: Bruce & Van der Gaag: only a small subsets of primary tissue is able to proliferated in vivo.

Not all cells of a tumor can proliferate and form a colony in vitro or a tumor when transplanted!

Why is tumor/leukemia cell transplantation so inefficient?
Models of leukemia initiation and maintenance

A) “Stochastic”
- Leukemia induction [Tpl]

B) “Leukemic stem cell”
- Leukemia [Tpl]
- No leukemia [transplant]

[acc. Hope & Dick, 2005]
Self-renewal Assay in NOD/SCID Mice
[Non-obese diabetic/severe combined immunodeficiency]
The leukemic stem cell (LSC) hierarchy

- **HSC [SRC]**
  - [CD34+, CD38-, Thy-1+, c-kit+, IL3Rα-]

- **LSC [SL-IC]**
  - [CD34+, CD38-, Thy-1-, c-kit-, IL3Rα+]
  - [0-2 out of 10^6 tumor cells]

  - Leukemic [LT-IC]
  - Leukemic [CFU]

Transforming genetic alterations

AML-M1 (FAB)  AML-M2 (FAB)  AML-M3 (FAB)  AML-M4 (FAB)  AML-M5 (FAB)  AML-M6 (FAB)  AML-M7 (FAB)

-Leukemic blast cells-
The Leukemic Stem Cell paradigm (LSC)

- Despite differentiation arrest, AML clones are organized in a hierarchical manner similar to normal HSCs.

- AML-LSC [0-2 : 10^6] : CD34+, CD38-, CD71-, HLA-DR-, CD90-, CD117-, CD123+ [different from normal HSCs]: a high percentage is negative for the Ki-67 proliferation marker [most cells in G_0/G_1 of the cycle].

- Experimental systems: AML-repopulating capacity in vivo [e.g. in NOD-SCID mice] and long-term in vitro cultivation systems.

- Leukemic stem cells (LSC) are AML-initiating and maintaining
Cancer Stem Cells (CSCs) - Leukemic Stem Cells (LSCs)

**Stem Cells** = undifferentiated cells that are capable of *self-renewing* and *differentiating* into diverse mature progeny.

*[Embryonic Stem (ES) cells : totipotent; Adult Stem cells: pluripotent]*

Hematopoietic Stem Cells (HSC): generation of all cell types in the blood

Cancer stem cells / *leukemic stem cells* (like normal stem cells) have the ability to *self-renew* and give rise to heterogeneous differentiated cells.

Cancer stem cells (CSCs) are being identified in solid cancers as well such as breast, colon, prostate, pancreas...

*Is the model true for all malignancies??*
### Putative cancer stem cells in human malignancies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Marker</th>
<th>% in tumors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>CD34+ CD38- IL3RA+ CD123+ CD90-CD150+?</td>
<td>0.2-1%</td>
<td>Bonnet et al., Nat Med 1997</td>
</tr>
<tr>
<td>APL</td>
<td>CD34- CD38+</td>
<td></td>
<td>Turhan et al., Blood 1995</td>
</tr>
<tr>
<td>B-ALL</td>
<td>CD34+ CD38- CD19+/- (CD10+)</td>
<td>1%</td>
<td>Castor et al., Nat Med 2005</td>
</tr>
<tr>
<td>CML-CP</td>
<td>CD34+ CD38-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CML-BC</td>
<td>CD34+ CD38- IL3RA+ CD45RA+</td>
<td></td>
<td>Jamieson et al., NEJM 2004</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>CD133+</td>
<td>6-20%</td>
<td>Singh et al., Nature 2004</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>CD133+</td>
<td>19-29%</td>
<td>Singh et al., Nature 2004</td>
</tr>
<tr>
<td>Ependymomas</td>
<td>CD133+ Nestin+ RC2 BLBP+</td>
<td>0.001-1.5%</td>
<td>Taylor et al., Cancer Cell 2005</td>
</tr>
<tr>
<td>Breast CA</td>
<td>ESA+ CD44+ CD24-/low, Lin-</td>
<td>0.5-5%</td>
<td>Al-Haji et al. PNAS 2003</td>
</tr>
<tr>
<td>Melanoma</td>
<td>CD20+ MCAM+</td>
<td>20%</td>
<td>Fang et al., Cancer Res 2005</td>
</tr>
<tr>
<td>Lung adeno CA</td>
<td>SP-C+, CCA+</td>
<td></td>
<td>Kim et al., Cell 2005</td>
</tr>
<tr>
<td>Prostate</td>
<td>CD44+, a2b1 Int-high, CD133+</td>
<td>0.10%</td>
<td>Collins et al., Cancer Res 2005</td>
</tr>
<tr>
<td>Liver CA</td>
<td>CD133+</td>
<td></td>
<td>Suetsugu et al. BBRC, 2006</td>
</tr>
<tr>
<td>Colon CA</td>
<td>CD133+</td>
<td>0.001-2%</td>
<td>O'Brien et al., Ricci-Vitiani et al., Nature 2006</td>
</tr>
<tr>
<td>HNSCC</td>
<td>CD44+ CD166+, EpCAMhigh</td>
<td>&lt;10%</td>
<td>Prince et al., PNAS, 2007</td>
</tr>
<tr>
<td>Pancreas CA</td>
<td>CD44+, CD24+, ESA+</td>
<td>0.2-0.8%</td>
<td>Li et al., Cancer Res 2007</td>
</tr>
</tbody>
</table>

[adapted according to Guo et al. Pediatric Res 2006]
Only less than 50% of patients with acute myeloid leukemia (AML) are cured using current therapies. RELAPSE is the major reason for therapy failure in acute leukemia!

[Hiddemann et al., Crit Rev Hem Onc, 2005]
Relapse: the "achilles heel" of leukemia therapy

- Leukemic bulk population
- Minimal residual disease (MRD)
- Relapse

Treatment: CHT, Imatinib

Relapse originating from quiescent "stem cell"

"LSC" (Leukemic Stem Cell)

Quiescent not touched.
Leukemia stem cells: evading cancer therapy?

- *Quiescent phenotype* - protection from agents that target actively cycling cells

- ↑ ABC and other transporters – prevent buildup of therapeutic drug dosage

- HSC-”niche” may be a “haven”

- Differential requirement for oncogene(s) between stem cells and less primitive tumor cells
Targeting leukemic stem cells in therapy of acute leukemia

Current Chemotherapy → Tumor debulking → Targeting LSCs + Conventional Chemotherapy → Tumor involution → Disease remission

Current Chemotherapy → Tumor debulking → Disease relapse
Therapeutic targeting of leukemic stem cells?

- Effectively target leukemia stem cells whilst selectively sparing normal HSC function

- Target potential biological differences between LSC and normal HSC:
  - Surface phenotype?
  - Apoptosis mechanisms?
  - Aberrant self-renewal?
LSC: targeting the surface phenotype

HSC:
- CD34+/38-
- CD90+/123lo/117+
- CD71+/HLA-DR
- CD44+

LSC:
- CD34+/38-
- CD90+/123+/CLL1+/117+
- CD71+/HLA-DR
- CD44++

NORMAL

LEUKEMIA

Δ CD123/IL-3Rα
Targeting the AML LSC surface phenotype

Imunoconjugate
e.g. IL-3-Diphteria toxin

NORMAL

LEUKEMIA

CD123/IL-3Rα
Targeting LSCs by diphteria-toxin-IL3 conjugates

DT388IL3 showed limited toxicity in monkeys:
a phase I/II study in human AML patients is currently ongoing

Therapeutic targeting of leukemic stem cells?

• Target potential biological differences between LSC and normal HSC:
  - Surface phenotype
  - Self-renewal?
  - Apoptosis?
  - Differentiation?

Need to understand the molecular pathways of LSCs !!
Cell fate decisions for leukemic stem cells

- Quiescence
- Differentiation
- Self Renewal
- Apoptosis
Leukemic stem cells: molecular pathways

Proliferation/Survival/Self-renewal - PI3K/PKB

Proliferation/Survival - JUN/AP1
[Passegue et al., Cell 2004; Steidel et al., Nat Genet 2006]

Differentiation/Self renewal - Homeobox/HOX
[Kroon et al., 2001; Scholl et al., JCI, 2007]

Self renewal - βCat/WNT
[Jaimieson et al., NEJM, 2004]

Survival - NFκB
[Guzman et al., PNAS, 2002; Blood, 2001, 2005]

Homing & Differentiation - CD44
[Jin et al., Nat Med 2006; Krause et al., Nat Med 2006]

Homing & Migration - CXCR4
Self renewal of leukemic stem cells

- Unlimited self-renewal = hallmark of cancer

- Molecular pathways regulating self-renewal in normal stem cells: *HOX, WNT, NOTCH, PI3K/PTEN, Hedgehog, Polycomb(BMI1),..*

- Certain oncogenic fusion genes alter committed murine progenitor cells and confer self-renewal: *HSCs are not the only target for malignant transformation in acute leukemia!*

- Determination of a *self-renewal transcriptional program* in LSCs?
Molecular regulators of stem cell self-renewal

PROLIFERATION + DEVELOPMENTAL POTENTIAL = SELF-RENEWAL

Involved in leukemogenesis?

[adapted Molofsky et al., Current Opinion in Cell Biology]
Signaling pathways regulating self-renewal in normal and cancer stem cells

WNT

- Fzd
- LRP
- GSK3β
- β-catenin
- LEF
- β-catenin
- Cyclin D1

Stem/progenitor cell self-renewal

Haematopoetic
Epidermal
Gut

Tumorigenesis

- Colon carcinoma
- Epidermal tumours

SHH

- Ptc
- Glial
- Glial
- Ptc

Stem/progenitor cell self-renewal

Haematopoetic
Neural
Germ line

Tumorigenesis

- Medulloblastoma
- Basal cell carcinoma

NOTCH

- Notch1
- CBF1
- Hes-1

Stem/progenitor cell self-renewal

Haematopoetic
Neural
Germ line

Tumorigenesis

- Leukaemia
- Mammary tumours

[Reya et al, 2001]
Blood formation: hematopoietic hierarchy

Self renewal

LT-HSC → Self renewal

ST-HSC → Self renewal

MPP

Myeloid

CMP → CMP

MEP

GMP

Erythrocytes
Platelets
Granulocytes
Macrophages
Dendritic-cells
T-cells
NK-cells
B-cells

Lymphoid

CLP

Pro-DC

Pro-T

Pro-NK

Pro-B
Acute leukemia: product of cooperating genetic alterations
Collaborating Class I mutation? [MOZ/TIF2, MLL/ENL..]

[Huntley et al., Cancer Cell, 2004]
Hunting for genetic profile of self-renewal of LSCs

Identification of a putative “self-renewal” genetic footprint in leukemic stem cells [L-GMP-MLL/AF9]

Leukemic stem cells: molecular pathways

**Proliferation/Survival/Self-renewal - PI3K/PKB**  

**Proliferation/Survival - JUN/AP1**  
[Passegue et al., Cell 2004; Steidel et al., Nat Genet 2006]

**Differentiation/Self renewal - Homeobox/HOX**  
[Kroon et al., 2001; Scholl et al., JCI, 2007]

**Self renewal - βCat/WNT**  
[Jaimieson et al., NEJM, 2004]

**Survival - NFⱽB**  
[Guzman et al., PNAS, 2002; Blood, 2001, 2005]

**Homing & Differentiation - CD44**  
[Jin et al., Nat Med 2006; Krause et al., Nat Med 2006]

**Homing & Migration - CXCR4**  
- Increased entry into cycle of HSCs followed by marked reduction of in HSC number over time.

- Initial myeloproliferation (feedback?) followed by transplantable acute leukemia (myeloid/lymphoid)

[Yilmaz et al, Zhang et al., Nature 2006; Trumpp et al.]
[Yilmaz et al., Nature, 2006]
Activation of the PKB/AKT kinase in AML cells

In AML patients by Rapamycin (mTOR-inhibitor) [clinical phase I/II trials are ongoing]

Leukemic stem cells: molecular pathways

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Homing & Differentiation - CD44
[Jin et al., Nat Med 2006; Krause et al., Nat Med 2006]

Homing & Migration - CXCR4
Aberrant NF-κB activation in many cancers is linked to increased cellular survival/proliferation & self-renewal

[Jost et al., Blood 2007]
[Basseres & Baldwin, Oncogene, 2006]
Constitutive activation of NF-κB in LSCs
Parthenolide (NF-κB inhibitor) induces apoptosis in CD34+ CD38- AML cells but not in normal cells.

The NF-κB (IKK)-inhibitor AS602868 enhances cytotoxic effects of DOX & AraC on leukemic blasts but not on normal CD34+ hematopoietic cells.
Leukemic stem cells: molecular pathways

Proliferation/Survival/Self-renewal - PI3K/PKB

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Homing & Migration - CXCR4
Directing differentiation of leukemic stem cells?

Restoration of blocked AML differentiation *in vitro* by ligation of CD44: glycoprotein involved in cell adhesion through interaction with matrix (e.g. hyaluronic acid) (*Charrad et al., Nat Med, 1999*)

CD44 ligation by a monoclonal antibody (H90) induces differentiation in human AML cells

[Charrad RS, Nat Med, 5:669-676, 1999]
[Jin et al., Nat Medicine, 2006]
LSC-NICHE: key molecular pathways

Stem Cell Niche

SDF1

ILK

Integrins

CXCR4

CD44

Leukemic Stem Cell [LSC]
SDF1α[CXCL12]-CXCR4 signaling for homing of normal and leukemic hematopoietic stem cells

[adapted from Burger & Bürkle, BJH, 2007]
Leukemic cell homing/engraftment assessed by *in vivo* confocal imaging

[Sipkins et al, Nature 2005]
a-f) Co-localization in the scull of Nalm-6 leukemic cells with SDF-1α 30 min after injection

g-i) Inhibition of Nalm-6 cell homing by CXCR4 blockade (AMD3100); Reduction of homing in absence of E-selectin.

[Sipkins et al., Nature, 2005]
Therapeutic targeting of LSCs

- Homing/Migration: CD44/CXCR4/ITK
- Self-renewal: WNT/HOX/PcG
- Proliferation/Survival: PI3K/AKT; JAK/STAT, NF-κB
- Differentiation: HOX/RARα/NOTCH/CD44
### Potential therapeutic leukemic stem cell targeting

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Target(s)</th>
<th>Approach(es)</th>
</tr>
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<tbody>
<tr>
<td>IL3-signaling?</td>
<td>IL-3Rα [CD123]</td>
<td>Immunoconjugate: <strong>IL-3-Diphteria toxin</strong> (DS388-IL-3)</td>
</tr>
<tr>
<td>AP1</td>
<td>JunB, c-Jun</td>
<td>??</td>
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<tr>
<td>PI3K/AKT</td>
<td>PI3K</td>
<td>NG, PI107, ?</td>
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<tr>
<td></td>
<td>mTOR</td>
<td><strong>Rapamycin/RAD001</strong></td>
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<tr>
<td>NF-κB</td>
<td>IKK</td>
<td><strong>IKK inhibitors</strong></td>
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<tr>
<td></td>
<td>IκB</td>
<td>Parthenoloide</td>
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<td>Proteosome inhibitors</td>
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<tr>
<td>CD44</td>
<td>CD44</td>
<td><strong>Activating Antibody (H90)</strong> to induce cellular differentiation?</td>
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<tr>
<td>SDF1α/CXCR4</td>
<td>CXCR4</td>
<td>AMD3000</td>
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<td>Blocking Ab</td>
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<td></td>
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<td>Blocking peptide (RCP168)</td>
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Leukemic stem cells (LSCs): Summary

Leukemia (Cancer) is hierarchically organized with a small proportion of cells with stem cell properties: *aberrant self-renewal and ability to differentiate.*

LSCs are leukemia *essential for leukemia initiation and maintenance* [key experiment: transplantation of human AML cells in NOD-SCID mice]

LSCs may escape current chemotherapies: *origin of relapse* (?)

A better understanding of the biology of LSCs (molecular pathways) could offer new *therapies targeting LSCs:*

- targeting LSC surface antigens (e.g. CD123-toxin complexes)
- targeting proliferation/survival (e.g. NF-κB, PI3K/AKT,..)
- targeting interaction of LSCs with the niche (e.g. block of CXCR4, CD44)