Novel treatment approaches in (pediatric) BCR-ABL1-like acute lymphoblastic leukemia

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For children, against cancer

Mission:
to cure every child with cancer, and to provide them optimum quality of life

Aims:
• Identify molecular markers and drugable targets in childhood B-cell precursor ALL
• Prioritize genes and proteins to study the underlying biology
Leukemia

Acute Lymphoblastic Leukemia
~200 new patients/year in the Netherlands (~2/3 pediatric)
Pathobiology of ALL in the genome area

**Initiating lesions**
- ETV6-RUNX1, BCR-ABL1, MLL
- TAL1, LMO1,2

**Stem cell / lymphoid progenitor**

**Arrested B or T cell**

**Secondary lesions**
- Aberrant RAG activity, developmental arrest
- IKZF1, BTG1, EBF1, PAX5 / NOTCH1, PTEN

**Developmental arrest**

**Mature B / T cell**

**Cooperating events**
- CDKN2A, TP53, RB
- Ras pathway
- CRLF2, JAK, CREBBP

**Diagnosis**

**Therapy selected**
- Selection of clones or genetic events that confer resistance
- TP53, IKZF1, NR3C1
- CREBBP, NT5C2

**Relapse**

*Adapted from Inaba, Greaves and Mullighan, Lancet, 2013*
Outcome of pediatric ALL in the Netherlands

Data Dutch Childhood Oncology Group 1988-present

Slide: Frank van Leeuwen, RadboudUMC
Genetic classification of childhood ALL

- BCR-ABL1-like
- MLL
- ETV6-RUNX1
- TCF3-PBX1
- High hyperdiploid (51-65 chr)
- B-other

• cytogenetics
• gene expression profiling
• copy number profiling
• next generation sequencing

⇒ average 10 events/cell

BCP-ALL subtypes across age groups

- **1-15 yrs**: 88 (BCR-ABL1), 24 (BCR-ABL1-like), 441 (other B-ALL)
- **16-20 yrs**: 6 (BCR-ABL1), 5 (BCR-ABL1-like), 13 (other B-ALL)
- **21-39 yrs**: 18 (BCR-ABL1), 9 (BCR-ABL1-like), 21 (other B-ALL)
- **40-71 yrs**: 19 (BCR-ABL1), 6 (BCR-ABL1-like), 30 (other B-ALL)
Gene expression profiling identifies BCR-ABL1-like subgroup

COALL-treated patients (discovery cohort)

Den Boer et al. Lancet Oncology 2009
Validation of *BCR-ABL1*-like in 4 new childhood ALL cohorts

**BCR-ABL1*-like in children:**
- 15% of BCP-ALL
- 50% of B-other group

Van der Veer et al, Blood 2013
**BCR-ABL1-like adult BCP-ALL**

- 17% of adult BCP-ALL (21/127)
- 29% non-response rate vs 6% in remaining BCP-ALL
- 67% 5-yr CIR vs 45% in remaining BCP-ALL

Boer et al, Haematologica 2015
Prognostic markers in DCOG ALL-10

35 out of 48 relapsed cases were MRD-intermediate

<table>
<thead>
<tr>
<th></th>
<th>BCR-ABL1-like</th>
<th>IKZF1-deleted</th>
<th>CRLF2-high</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRD low</td>
<td>14.3%</td>
<td>16.7%</td>
<td>33.3%</td>
</tr>
<tr>
<td>MRD intermediate</td>
<td>67.9%</td>
<td>76.7%</td>
<td>61.1%</td>
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<tr>
<td>MRD high</td>
<td>17.8%</td>
<td>6.7%</td>
<td>5.6%</td>
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</tbody>
</table>

Van der Veer et al., Blood 2013

COX univariate model

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL1-like</td>
<td>3.7</td>
<td>1.2-11.5</td>
<td>0.026</td>
</tr>
<tr>
<td>IKZF1-deleted</td>
<td>2.7</td>
<td>1.0-7.0</td>
<td>0.043</td>
</tr>
<tr>
<td>CRLF2-high</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

Van der Veer et al., Blood 2013
**BCR-ABL1**-like and Philadelphia-like expression signature comparison

**BCR-ABL1**-like
- HC signature
- DCOG/Erasmus MC
- dic(9;20)
- Kinase-activating fusions
- JAK2 mutations
- IKZF1
- CRLF2
- VPREB1

**Philadelphia-like**
- Hispanic
- iAMP21
- Kinase-activating fusions
- JAK2 mutations
- IKZF1
- CRLF2
- VPREB1

**HC + PAM signature**
- Hispanic

Boer et al. Haematology 2015
Tyrosine kinase-activating lesions

- ABL class tyrosine kinase fusion genes
  - ABL1, ABL2, PDGFRB, CSF1R
- JAK2 fusion genes and truncated EPO receptor
  - JAK2, EPOR

- Overexpression of TSLP-receptor CRLF2
  - JAK2 mutation (50%) or other activating JAK pathway lesions

- RAS pathway mutations
  - NRAS, KRAS, FLT3, PTPN11
## Tyrosine kinase fusion genes

<table>
<thead>
<tr>
<th>Inhibitor type</th>
<th>TK gene</th>
<th>Protein function</th>
<th>Partner gene(s)</th>
<th>Type of fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABL class</strong></td>
<td><strong>ABL1</strong></td>
<td>Tyrosine kinase</td>
<td>ETV6, NUP214, ZMIZ1, RCSD1, NUP153, SFPQ, RANBP2, SNX1, SNX2, SPTAN1, FOXP1</td>
<td>Chimeric protein</td>
</tr>
<tr>
<td></td>
<td><strong>ABL2</strong></td>
<td>Tyrosine kinase</td>
<td>RCSD1, PAG1, ZC3HAV1</td>
<td>Chimeric protein</td>
</tr>
<tr>
<td></td>
<td><strong>PDGFRB</strong></td>
<td>Cytokine receptor tyrosine kinase</td>
<td>EBF1, ATF7IP, SSBP2, TNIP1, ZEB2, SNX29</td>
<td>Chimeric protein</td>
</tr>
<tr>
<td></td>
<td><strong>CSF1R</strong></td>
<td>Cytokine receptor tyrosine kinase</td>
<td>SSBP2, MEF2D</td>
<td>Chimeric protein</td>
</tr>
<tr>
<td></td>
<td><strong>PDGFRA</strong></td>
<td>Cytokine receptor tyrosine kinase</td>
<td>FIP1L1</td>
<td>Chimeric protein</td>
</tr>
<tr>
<td><strong>JAK2 class</strong></td>
<td><strong>JAK2</strong></td>
<td>Tyrosine kinase</td>
<td>PAX5, BCR, ATF7IP, EBF1, PPFIBP1, SSBP2, STRN3, TPR, TERF2, ETV6, OFD1, SMU1, ZNF340</td>
<td>Chimeric protein</td>
</tr>
<tr>
<td></td>
<td><strong>EPOR</strong></td>
<td>Cytokine receptor</td>
<td>IGH, IGK, LAIR1, THADA</td>
<td>Overexpression truncated protein</td>
</tr>
<tr>
<td></td>
<td><strong>CRLF2</strong></td>
<td>Cytokine receptor</td>
<td>P2RY8, IGH, CSF2RA</td>
<td>Overexpression</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td><strong>TYK2</strong></td>
<td>Tyrosine kinase</td>
<td>MYB, SMARCA4, ZNF340</td>
<td>Chimeric protein</td>
</tr>
<tr>
<td></td>
<td><strong>NTRK3</strong></td>
<td>Cytokine receptor tyrosine kinase</td>
<td>ETV6</td>
<td>Chimeric protein</td>
</tr>
<tr>
<td></td>
<td><strong>FLT3</strong></td>
<td>Cytokine receptor tyrosine kinase</td>
<td>ZMYM2</td>
<td>Chimeric protein</td>
</tr>
<tr>
<td></td>
<td><strong>PTK2B</strong></td>
<td>Tyrosine kinase</td>
<td>TMEM2</td>
<td>Chimeric protein</td>
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<tr>
<td></td>
<td><strong>IL2RB</strong></td>
<td>Cytokine receptor</td>
<td>MYH9</td>
<td>Overexpression</td>
</tr>
</tbody>
</table>
These fusion genes facilitate leukemic transformation by inducing constitutive kinase activation and signaling through the activation of ABL1 and/or JAK-STAT pathways.
Co-occurring aberrations

- **IKZF1** deletions are frequent in tyrosine kinase fusion cases.
- JAK2 mutations are frequent in **CRLF2**-rearranged cases.
**BCR-ABL1-like: heterogeneous outcome**

- **US pediatric and AYA**
  - Roberts et al. NEJM 2014
- **Netherlands pediatric**
  - Boer et al. Oncotarget 2016
- **Japan pediatric**
  - Imamura et al. BCJ 2016

**5-year event-free survival**

- **CLRF2-rearranged JAK2mutant**
- **CRLF2-rearranged JAK2wildtype**
- **JAK2/EPOR**
- **Other JAK/STAT**
- **ABL class**

Japan pediatric
Imamura et al.
BCJ 2016

Netherlands pediatric
Boer et al.
Oncotarget 2016
Question

How would you treat an adult patient with *ETV6-ABL1* identified at diagnosis?

1. the current treatment protocol plus ABL inhibitor

2. the current treatment protocol and monitor response before deciding on ABL inhibitor
What do we need for introduction of tyrosine kinase inhibitors in treatment?

- Preclinical evidence for tyrosine kinase inhibitor sensitivity
  - In vitro, patient cells ex vivo and in mice (xenograft)

- Early clinical trials and case studies showing safety and efficacy

- Fast and reliable detection of tyrosine kinase aberrations at diagnosis
Preclinical and early clinical evidence JAK class

<table>
<thead>
<tr>
<th>Sensitive to ruxolitinib</th>
<th>In vitro (transduced Arf-/- or BA/F3 cells)</th>
<th>Ex vivo (patients' blast cells)</th>
<th>Mouse (patient-derived xenograft)</th>
<th>Patients (early clinical trials/case reports)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAX5-JAK2</td>
<td><img src="#" alt="Green" /></td>
<td><img src="#" alt="Green" /></td>
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<td></td>
</tr>
<tr>
<td>BCR-JAK2</td>
<td><img src="#" alt="Green" /></td>
<td><img src="#" alt="Green" /></td>
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<td></td>
</tr>
<tr>
<td>ATF7IP-JAK2</td>
<td><img src="#" alt="Green" /></td>
<td><img src="#" alt="Green" /></td>
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</tr>
<tr>
<td>SSBP2-JAK2</td>
<td><img src="#" alt="Green" /></td>
<td><img src="#" alt="Green" /></td>
<td><img src="#" alt="Green" /></td>
<td><img src="#" alt="Orange" /> no response?</td>
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<tr>
<td>STRN3-JAK2</td>
<td><img src="#" alt="Green" /></td>
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<tr>
<td>TERF2-JAK2</td>
<td><img src="#" alt="Green" /></td>
<td><img src="#" alt="Green" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGH-EPOR</td>
<td><img src="#" alt="Green" /></td>
<td></td>
<td><img src="#" alt="Green" /></td>
<td><img src="#" alt="Green" /> transient, partial</td>
</tr>
<tr>
<td>IL7Rmut/SH2B3del</td>
<td><img src="#" alt="Green" /></td>
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</tr>
<tr>
<td>CRLF2r/JAK2mut</td>
<td><img src="#" alt="Green" /></td>
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<td><img src="#" alt="Green" /></td>
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</tr>
</tbody>
</table>

JAK2 translocations and mutations are sensitive to ruxolitinib

Steeghs et al. Oncotarget, accepted

Ex vivo patients’ cells
JAK2 inhibition by ruxolitinib results in accumulation of phosphorylated JAK2

Accumulation of pJAK2\(^{Y1007}\) results in a rebound effect when the drug is washed out

\[ \text{reactivation of downstream STAT signaling} \]
### Preclinical and early clinical evidence ABL class

<table>
<thead>
<tr>
<th>Sensitive to imatinib/dasatinib</th>
<th>In vitro (transduced cells)</th>
<th>Ex vivo (patients’ cells)</th>
<th>Mouse (patient-derived xenograft)</th>
<th>Patients (early clinical trials/case reports)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETV6-ABL1</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>NUP214-ABL1</td>
<td></td>
<td></td>
<td></td>
<td>transient response</td>
</tr>
<tr>
<td>RANBP2-ABL1</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>ZMIZ1-ABL1</td>
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<tr>
<td>RCSD1-ABL1</td>
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<td></td>
</tr>
<tr>
<td>RCSD1-ABL2</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>EBF1-PDGFRB</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ATF7IP-PDGFRB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSBP2-CSF1R</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>SNX2-ABL1</strong></td>
<td>Sensitive to nilotinib</td>
<td></td>
<td></td>
<td>transient/partial responses</td>
</tr>
</tbody>
</table>

Different breakpoints in ABL genes

ABL1

SH3

SH2

PTK

F-actin binding

ABL2

SH3

SH2

PTK

F-actin binding

pecan.stjude.org
RAS pathway mutations

- 50% of *BCR-ABL1*-like have RAS pathway mutations
- 30% clonal, 20% subclonal

Jerchel et al. Leukemia 2017
RAS mutant cases sensitive to MEK inhibition

Ex vivo patients’ cells

Jerchel et al. Leukemia 2017
Kerstjens et al. Oncotarget 2017 (infants)
Trametinib sensitizes to prednisolone

Ex vivo patients’ cells

Jerchel et al. Leukemia 2017
Summary: Tyrosine kinase-activated cases

- often respond poorly to initial treatment
  - poor prednison response, lower complete remission rate, detectable minimal residual disease

- do better on MRD-guided protocols
  - Roberts et al. JCO 2014

- are sensitive to tyrosine kinase inhibitors in preclinical and early clinical studies
  - ABL class: imatinib/dasatinib
  - JAK class: ruxolitinib
  - RAS pathway: trametinib
Lessons from *BCR-ABL1*-positive ALL

- Tyrosine kinase inhibitors used to induce remission followed by stem cell transplantation
- Important in context of standard cytotoxic drugs
- Intrinsic resistance: alternative pathways activated
  - microenvironment activates IL7 signaling in ALL cells
- Acquired resistance: mutations in the drug binding site
  - 2\textsuperscript{nd} and 3\textsuperscript{rd} generation ABL inhibitors
- Future: multiple inhibitors to block alternative pathways rather than sequential inhibitors
What do we need for introduction of tyrosine kinase inhibitors in treatment?

- Preclinical evidence for tyrosine kinase inhibitor sensitivity
  - In vitro, patient cells ex vivo and in mice (xenograft)

- Early clinical trials and case studies showing safety and efficacy

- Fast and reliable detection of tyrosine kinase aberrations at diagnosis
<table>
<thead>
<tr>
<th>Detection method</th>
<th>Target</th>
<th>Variant type</th>
<th>Number of screened fusions</th>
<th>TK gene</th>
<th>Partner gene</th>
<th>Distance from breakpoint location</th>
<th>DNA breakpoint detected</th>
<th>Diagnostic test</th>
<th>Turnaround time</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescent in situ hybridization</td>
<td>DNA</td>
<td>Structural variant</td>
<td>Single</td>
<td>Fixed</td>
<td>Not detected</td>
<td>Flexible</td>
<td>No</td>
<td>Yes</td>
<td>1 week</td>
<td>Low</td>
</tr>
<tr>
<td>Reverse transcriptase-PCR plus Sanger sequencing</td>
<td>RNA</td>
<td>Chimeric transcript</td>
<td>Single or multiple</td>
<td>Fixed</td>
<td>Fixed</td>
<td>Restricted</td>
<td>No</td>
<td>Yes</td>
<td>1 week</td>
<td>Low</td>
</tr>
<tr>
<td>Capture or amplicon next-generation sequencing</td>
<td>DNA/RNA</td>
<td>Chimeric gene; mutation</td>
<td>Multiple</td>
<td>Fixed</td>
<td>All</td>
<td>Restricted</td>
<td>Yes</td>
<td>In progress</td>
<td>1-2 weeks</td>
<td>Medium</td>
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<tr>
<td>Targeted locus amplification</td>
<td>DNA</td>
<td>Structural variant; mutation</td>
<td>Multiple</td>
<td>Fixed</td>
<td>All</td>
<td>Flexible</td>
<td>Yes</td>
<td>No</td>
<td>2 weeks</td>
<td>Medium</td>
</tr>
<tr>
<td>Whole transcriptome sequencing</td>
<td>RNA</td>
<td>Chimeric gene; mutation</td>
<td>All</td>
<td>All</td>
<td>All</td>
<td>All</td>
<td>No</td>
<td>No</td>
<td>3-6 weeks</td>
<td>Medium</td>
</tr>
<tr>
<td>Whole genome sequencing</td>
<td>DNA</td>
<td>Structural variant; copy number; mutation</td>
<td>All</td>
<td>All</td>
<td>All</td>
<td>All</td>
<td>Yes</td>
<td>No</td>
<td>3-6 weeks</td>
<td>High</td>
</tr>
</tbody>
</table>
Question

How would you treat an adult patient with *ETV6-ABL1* identified at diagnosis?

1. the current treatment protocol plus ABL inhibitor

2. the current treatment protocol and monitor response before deciding on ABL inhibitor
Use of genetic markers in ALL treatment

- Patients
- Molecular diagnostics/sequencing
- Genetic markers
- Risk assignment
- Targeted therapy
Use of genetic markers in ALL treatment

- Better risk stratification
  - Genetic risk factors, independent of minimal residual disease
    - *IKZF1* deletion in pediatric DCOG ALL11 protocol: MR-treated patients receive additional year of maintenance
    - *iAMP21* in pediatric UKALL 2003: considered genetic high risk, receive more intensive treatment

- Sensitivity to new agents
  - Improve outcome and/or reduce toxic side effects
    - Tyrosine kinase inhibition for *BCR-ABL1*-like fusion cases
  - Sensitize to cytotoxic drugs
    - MEK inhibitors for RAS-mutated cases
Future perspectives

- Tyrosine kinase inhibitors: Type II JAK inhibitors
- RAS mutated/activated ALL: MEK inhibitors
- Combination treatment of tyrosine kinase inhibitor with alternative pathway inhibitor
  - eg. PI3K/MTOR inhibitors
- Combination of targeted treatment with cytotoxic drugs
  - Synergy, sensitization

- New clinical trial design: basket trials
  - Prediction of sensitivity based on aberrations, not tumor type
  - ITHER, INFORM, MAPPYACTS, e-SMART, LEAP
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- William Evans

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