TARGET-All: targeted NGS tool for ALL genetic diagnostics

Sarra Ryan
Leukaemia Research Cytogenetics Group
Chromosomal abnormalities are important in stratifying patients for treatment in B-ALL

Specific chromosomal changes are linked to a good or poor outlook.
Risk of relapse by chromosomal abnormalities

Moorman et al., Lancet Oncology 2010
Risk of relapse by chromosomal abnormalities

- Further patient stratification and new therapies are required

Moorman et al, Lancet Oncology 2010
Subgroups of B-other ALL harbour cryptic genetic lesions

Data taken from Mullighan, *J Clin Invest* 2012

Clappier *et al* 2014 *Leukemia*

Tyrosine kinase and cytokine signalling activating lesions

Den Boer *et al* 2009 *Lancet Oncology*
High-risk Ph-like ALL patients may respond to novel targeted therapies

### Table 1. Kinase Fusions Identified in Ph-like Acute Lymphoblastic Leukemia.

<table>
<thead>
<tr>
<th>Kinase Gene</th>
<th>Tyrosine Kinase Inhibitor</th>
<th>Fusion Partners</th>
<th>Patients</th>
<th>5' Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL1</td>
<td>Dasatinib</td>
<td>6</td>
<td>14</td>
<td>ETV6,11 NUP214,11 RCSD1,11 RANBP2,11 SNX2,10 ZMIZ1,20</td>
</tr>
<tr>
<td>ABL2</td>
<td>Dasatinib</td>
<td>3</td>
<td>7</td>
<td>PAG1,* RCSD1,* ZC3HAV1*</td>
</tr>
<tr>
<td>CSF1R</td>
<td>Dasatinib</td>
<td>1</td>
<td>4</td>
<td>SSBP2*</td>
</tr>
<tr>
<td>PDGFRB</td>
<td>Dasatinib</td>
<td>4</td>
<td>11</td>
<td>EBF1,15-17 SSBP2,* TNIP1,* ZEB2a</td>
</tr>
<tr>
<td>CRLF2</td>
<td>JAK2 inhibitor</td>
<td>2</td>
<td>30</td>
<td>IGH,21 P2RY822</td>
</tr>
<tr>
<td>JAK2</td>
<td>JAK2 inhibitor</td>
<td>10</td>
<td>19</td>
<td>ATF71P,* BCR,11 EBF1,* ETV6,21 PAX5,11 PPF1BP1,* SSBP2,24 STRN3,11 TERF2,25 TPR*</td>
</tr>
<tr>
<td>EPOR</td>
<td>JAK2 inhibitor</td>
<td>2</td>
<td>9</td>
<td>IGH,11 IGK*</td>
</tr>
<tr>
<td>DGKH</td>
<td>Unknown</td>
<td>1</td>
<td>1</td>
<td>ZFAND3*</td>
</tr>
<tr>
<td>IL2RB</td>
<td>JAK1 inhibitor, JAK3 inhibitor, or both</td>
<td>1</td>
<td>1</td>
<td>MYH9*</td>
</tr>
<tr>
<td>NTRK3</td>
<td>Crizotinib</td>
<td>1</td>
<td>1</td>
<td>ETV625-27</td>
</tr>
<tr>
<td>PTK2B</td>
<td>FAK inhibitor</td>
<td>2</td>
<td>1</td>
<td>KDM6A,* STAG2*</td>
</tr>
<tr>
<td>TSLP</td>
<td>JAK2 inhibitor</td>
<td>1</td>
<td>1</td>
<td>IQGAP2*</td>
</tr>
<tr>
<td>TYK2</td>
<td>TYK2 inhibitor</td>
<td>1</td>
<td>1</td>
<td>MYB*</td>
</tr>
</tbody>
</table>

* The gene is a previously unreported fusion partner.
†ETV6-NTRK3 has been reported in multiple cancers, including congenital fibrosarcoma25,26 and secretory breast carcinoma,27 but it has not previously been described in acute lymphoblastic leukemia.28,29

Roberts et al, NEJM, 2014
How do we identify B-other ALL patients using emerging genomic data (i.e. Ph-like, ERG\textsubscript{abn})?

<table>
<thead>
<tr>
<th>Method detection</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-banding</td>
<td>Established, cost-effective</td>
<td>No detection of cryptic or focal SV and CNA or mutations</td>
</tr>
</tbody>
</table>
| FISH RT-PCR      | Established, cost-effective | Prior knowledge of fusion partner  
No detection of CNA or mutations |
| Copy number array | Established | No detection of cryptic or focal SV and CNA or mutations |
| Gene expression  | Established and accurate for some groups | Define groups not individual patients  
(Boer JM et al, Blood, 2013)  
No detection of SV, CNA or mutations |
| RNA-seq          | Can detect most SV, CNA and mutations | Expensive, bioinformatician required |
| WGS              | Can detect most SV, CNA and mutations | Expensive, bioinformatician required |
The detection of genetic aberrations by 100bp paired-end sequencing
The detection of genetic aberrations by 100bp paired-end sequencing

Translocation

Chr5

1000bp

100bp

Chr12

100bp
The detection of genetic aberrations by 100bp paired-end sequencing.

- **Normal**: Human reference genome, chr21
- **Mutation**: Human reference genome, chr21
- **Deletion**: Human reference genome, chr21
- **Translocation**: Human reference genome, chr21 and chr12
Plan of action: B-other ALL

391 UKALL2003 patient samples
- HPA: other/normal/failed
- Diagnostic DNA available

WGS

TARGET-All

164 patient samples
Identify genetic subgroups

Published genomic data

Design TARGET-All to capture ALL regions

227 patient samples
Identify genetic subgroups

A: Incidence and genomic landscape of novel and established subgroups
B: Clinical significance of individual or combined genetic abnormalities
C: Functional relevance of novel and established genetic abnormalities
Plan of action: B-other ALL

- 391 UKALL2003 patient samples
  - HPA: other/normal/failed
  - Diagnostic DNA available

  WGS

  TARGET-All

  164 patient samples
  Identify genetic subgroups

  Published genomic data

  Design TARGET-All to capture ALL regions

  227 patient samples
  Identify genetic subgroups

  A: Incidence and genomic landscape of novel and established subgroups

  B: Clinical significance of individual or combined genetic abnormalities

  C: Functional relevance of novel and established genetic abnormalities
TARGET-All: test cohort

- 29 B-other patient samples
  - 8 x Ph-like SV
  - 17 x PAX5 abnormal
  - 4 x ZNF384 rearrangement

- Whole genome amplified (WGA) DNA

- Detection of SV and fusion partner gene

- Identification of known/unknown CNA and mutations

- All known genetic aberrations detected by TARGET-All
Dicentric (9;12)
Karyotype: 45,XX,dic(9;12)(p11;p11)
Unbalanced translocation
PAX5 rearrangement
Karyotype: 46,XY,t(7;9)(q11;p13)
Balanced translocation
PAX5 complex rearrangement
Karyotype: 46-50,XX,del(3)(p?21),t(6;13)(p11.2;q22),der(9)t(3;9)(p14;p12),del(9)(p13),+11,add(14)(q?32)[cp9]

PAX5-FBRSL1
t(9;12)(p13;q24)

PAX5-KDMC4
t(9;9)(p13;p24)
Rearrangements in test cohort

TK rearrangements

ETV6
ATF7IP
EBF1

PDGFRB
NM_002609
- Ig1_PDGF-alpha - First immunoglobulin (Ig) like domain of platelet-derived growth factor... 
- Ig4_PDGF-alpha - Fourth immunoglobulin (Ig) like domain of platelet-derived growth factor...
- Ig - Immunoglobulin domain...
- Pkc_like - Protein Kinases, catalytic domain...

CSF1R
NM_0012511
- Ig - Immunoglobulin domain...
- Ig4_CSFR_liko - Fourth immunoglobulin (Ig) like domain of stem cell factor receptor (5... 
- PTK1c_CSFR1R - Catalytic domain of the Protein Tyrosine Kinase, Colony Stimulating Factor Receptor...

Other rearrangements

TAF15
TCF3
EWSR1
ARID1B

ZNF384
NM_00107920
- zfHDC2_2 - Zinc-finger double domain...

ETV6
ARHGAP22
C20orf112
KIF3B

ETV6
P2RY8
JAK2
GREB1L

ETV6
P2RY8
JAK2
GREB1L

DACH2
ESRRB
C20orf112

PAX5
NM_014734
- PAX - Paired Box domain...
- Pax2_C - Paired box protein 2 C terminal...

FOXP1
KDM4C
FBRSL1
AUTS2

ZMYND8
TARGET-All: validation cohort

- 61 more cases
- Coverage: mean 301x, median 273x
TARGET-All: discovery cohort

- 5% (3/61) cases failed
- DNA extracted from fixed cells in all failed cases
TARGET-All: resolution of genetic defects

- 96% (74/77) of known (FISH and MLPA tested) SVs detected
- 14 abnormalities detected that were undetected or unresolvable by FISH or MLPA (subclonal, focal, cryptic), i.e.
  - t(9;20)
  - t(7;9)
  - Subclonal ERG(del) x2
  - ETV6-PDGFRB t(5;12)
  - IKZF1 duplication
  - CNA (IKZF1 Ex4-7 and CDKN2A/B deletion)
Mutational spectrum

- Mutations also observed in *CSF1R, KRAS, NCOR1, SETD2* and *SH2B3*
TARGET-All Summary

• Reliable detection of a range of genetic abnormalities in B-other ALL, including ERG, PAX5 and kinase genes

• Development of CNA bioinformatic pipelines

• The assay design is dynamic

• Test a larger cohort to assess the incidence, genomic profile and clinical significance of genetic defects

• Potential for the assay to be used for routine diagnostic subgrouping
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