Where we are with *BCR-ABL1*-like ALL!
Gene expression profiling identifies cytogenetic subgroups

Diagnostic ALL BM samples (n=327)

Yeoh et al 2002 Cancer Cell 1: 133-43
**BCR-ABL1-like/Ph-like ALL**

- BCR-ABL1-negative cases which show a gene expression profile similar to that of BCR-ABL1-positive ALL.
- Account for ~15% of BCP-ALL.
- Mutually exclusive of other established cytogenetic subgroups.
- They share the same high-risk of relapse and poor outcome.

den Boer *et al* 2009 Lancet Oncology 10: 125-34
Mullighan *et al* 2009 NEJM 360: 470-80
BCR-ABL1-like ALL shares the same high-risk of relapse and poor outcome as BCR-ABL1 positive ALL

Den Boer et al 2009 Lancet Oncology 10: 125-34
Loh et al 2013 Blood 121: 485-8
Problems with using the gene expression signature as a diagnostic test for *BCR-ABL1*-like ALL

- Facility not available for all clinical trials
- Signatures define groups not individuals
- Some overlap but classification of different patients when different groups examine the same cohort using different predictive algorithms!!
- Thus for diagnosis of this poor risk group, we need to make use of emerging genomic data…..
80% B-cell development genes/CDKN2A
40% IKZF1 deletions

15-20% Ph-like

50% CRLF2 rearrangement
8% EBF1-PDGFRB

Other (~20%)
- ABL1, JAK2, PDGFRB and additional kinase rearrangements and sequence mutations

- 25% JAK mutations
- 25% unknown lesions
- 12% IL7R mutations
- 10% FLT3 mutations

These genes facilitate leukemic transformation by inducing constitutive kinase activation and signaling through the activation of ABL1 and/or JAK-STAT pathways.
Fusion of *NUP214* to *ABL1* on episomes in T-ALL

Graux *et al* 2004 Nature Genetics 36: 1084-1089
FISH detection of *NUP214-ABL1* fusion in T-ALL

Barber *et al* 2004 Leukemia 18: 1153-6
Gain of one copy of DNA sequence between \textit{NUP214} and \textit{ABL1}

NUP214-ABL1 positive BCP-ALL

Eyre, Schwab et al 2012 Blood 120: 4441-3
Novel treatment based on genetics

**BCR-ABL1** positive ALL

- Treatment with tyrosine kinase inhibitors plus conventional chemotherapy has significantly improved event free survival
- Promising results of EsPhALL trial reported

Schultz *et al* 2009 J Clin Oncol 27: 5175-81
Biondi *et al* 2012 Lancet Oncology 13: 936-45
Four cases of NUP214-ABL1 positive BCP-ALL

Eyre et al 2012 Blood 120: 4441-3
What about other $ABL1$ partners?

t(9;10)(q34;q22.2) $ZMIZ1$-$ABL1$ fusion (n=6)
Zinc finger MIZ-type 1
regulates the activity of various transcription factors

Soler et al 2008 Leukemia 22: 1278-80
Moorman 2012 Blood Reviews 26: 123-35
What about other ABL1 partners?

t(5;9)(q22;q34)/SNX2-ABL1 fusion (n=2)
Sortin nexin family gene

Poor outcome but transient response to Imatinib

Kiyokawa et al 2013 European J Haematol (in press)
Ernst et al 2011 Br J Haematol 153: 33-6
These genes facilitate leukemic transformation by inducing constitutive kinase activation and signaling through the activation of ABL1 and/or JAK-STAT pathways.

Original identification of EBF1-PDGFRB

RNA-Seq
Cytogenetics

Predicted domain structure
RT-PCR
Sanger Sequencing

PDGFRB 5q32
EBF1 5q33

FISH detection of *PDGFRB* rearrangements

Design probes specific for different partner genes for example *EBF1*
**EBF1-PDGFRB**

by SNP arrays and MLPA

<table>
<thead>
<tr>
<th>MLPA Probe</th>
<th>Probe Ratio</th>
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<tbody>
<tr>
<td>EBF1_Ex01</td>
<td>0.972</td>
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<td>EBF1_Ex10</td>
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<td>EBF1_Ex14</td>
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<tr>
<td>EBF1_Ex16</td>
<td>0.589</td>
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</table>

**PDGFRB**

149.5 Mb

**EBF1**

158.1 Mb

Exon 16

**EBF1**

Exon 1
Response to Imatinib Mesylate in Patients with Chronic Myeloproliferative Diseases with Rearrangements of the Platelet-Derived Growth Factor Receptor Beta

Jane F. Apperley, M.D., Martine Gardembas, M.D., Junia V. Melo, M.D., Robin Russell-Jones, M.D., Barbara J. Bain, M.D., E. Joanna Baxter, Ph.D., Andrew Chase, Ph.D., Judith M. Chessells, M.D., Marie Colombat, Ph.D., Claire E. Dearden, M.D., Sasa Dimitrijevic, Ph.D., François-X. Mahon, M.D., David Marin, M.D., Zariana Nikolova, M.D., Eduardo Olavarria, M.D., Sandra Silberman, M.D., Beate Schultheis, M.D., Nicholas C.P. Cross, Ph.D., and John M. Goldman, D.M.

Kinase-activating fusions induce growth factor-independence and show response to tyrosine kinase inhibitors

Response of \textit{EBF1-PDGFRB} ALL to imatinib in two patients

- Two males of 10 years and 16 years with refractory B-ALL
- \textit{EBF1-PDGFRB} positive
- Started imatinib with immediate clinical improvement
- Morphologic remission
- Negative MRD

- Remains in remission at 1 year
- Bone marrow transplant

\textit{Weston et al} 2013 J Clinical Oncology 31: 413-6 \hspace{1cm} \textit{Lengline et al} 2013 Haematologica 98: 146-8
EBF1-PDGFRB fusion in ALL2003

• Estimated overall incidence ~0.5% BCP-ALL

• **EBF1-PDGFRB** patients
  – More likely NCI high risk
  – All MRD positive at day 28

  – Outcome:
    • More likely refractory or late remitters
    • Relapse unless treated on intensive treatment arm
Signalling pathways associated with CRLF2 rearrangements and JAK mutations in which TSLP induces phosphorylation of STAT5 and PI3K.

Tasian et al 2012 Blood 120: 833-42
Maude et al 2012 Blood 120: 3510-8
<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Genes</th>
<th>Drug</th>
<th>Mode of Action</th>
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<tr>
<td>t(9;22)(q34;q11)</td>
<td>BCR-ABL1</td>
<td>Imatinib or derivatives</td>
<td>Tyrosine kinase inhibitor</td>
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<td>BCR-ABL1-like</td>
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<tr>
<td>ABL1 partners</td>
<td>NUP214-ABL1, other ABL1 partners</td>
<td>Imatinib or derivatives</td>
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<td>CRLF2 rearrangements</td>
<td>CRLF2, JAK2</td>
<td>Ruxolitinib</td>
<td>JAK inhibitor</td>
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<td>IGH@-EPOR</td>
<td>EPOR</td>
<td>Ruxolitinib</td>
<td>JAK inhibitor</td>
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<td>Others</td>
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<td>CREBBP</td>
<td>CREBBP</td>
<td></td>
<td>Histone deacetylase inhibitors</td>
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<tr>
<td>Mutations in Ras/RTK pathway genes and PI3K pathway</td>
<td>Including FLT3, NF1, NRAS, KRAS, MAPK1, PTPN11</td>
<td>Rapamycin</td>
<td>PI3K/mTOR inhibitors</td>
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<tr>
<td>MLL rearrangements</td>
<td>DOTL1, FLT3</td>
<td>EPZ004777, lesautanib (CEP-70), PKC412</td>
<td>Inhibitor of histone methyltransferase: DOTL1, FLT3 inhibitors</td>
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<td>Hypodiploidy</td>
<td>TP53, RAS/RTK/PI3K pathways</td>
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<td>MEK inhibitors, PI3K inhibitors</td>
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<td>Hyperdiploidy</td>
<td>RAS pathway</td>
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<td>MEK inhibitors</td>
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</table>
Strategy for ALL2011 (and non trial patients)

• Whilst we are awaiting consensus from international collaboration:

  – Test **VERY HIGH RISK** patients for *EBF1-PDGFRB* fusion

  – Who are they?

  – How to test?
Very high risk patients

- Patients who fail to achieve complete remission by Day 28 or
- Remain MRD positive at week 14

- Account for 10-20 patients per year
- Notified to LRCG who will contact the local cytogenetics lab
How to test for \textit{EBF1-PDGFRB} fusion

- Two stage FISH approach

1. Commercially available dual colour break apart probe for \textit{PDGFRB}: result in loss of telomeric signal if \textit{EBF1} is partner, or split signal if another partner or \textit{t(5:5)(q35;q35)}

2. Home grown \textit{EBF1} breakapart probe to confirm \textit{EBF1}: by LRCG or locally
How to test for *EBF1-PDGFRB* fusion

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2. Home grown *EBF1* breakapart probe to confirm *EBF1*: by LRCG or locally
How to test for *EBF1-PDGFRB* fusion

- **RT-PCR**
  - Primers published
    - (Roberts *et al* 2012 Cancer Cell 22: 153-66)

- **SNP arrays**

- **MLPA with P335 kit** indicates the *EBF1* exon 16 deletion
Cautionary note

CSF1R fusions encode constitutively active tyrosine kinases also sensitive to imatinib

Lilljebjorn et al 2013 Leukemia epub
Roberts et al ASH Abstract
For now we test for *EBF1-PDGFRB* fusion

- *ABL1* fusions

- JAK-STAT pathway activating abnormalities

- Await further evidence unless requested specifically