Chromosomal translocations in childhood leukaemia

- Translocations arise in haematopoietic stem cells
- Translocations initiated by double-strand DNA breaks
- Products of translocations are fusion genes generating a chimaeric fusion protein with novel properties
- In childhood leukaemia, translocations can arise mainly before birth during foetal haematopoiesis
- Chromosomal translocations can initiate leukaemogenesis but likely require further genetic changes (‘hits’) for leukaemia to arise
Hypothetical model of stem cell origins of chromosome translocations in infant and childhood leukaemia

Greaves and Wiemels, 2003
Infant Leukaemia and the *MLL* Gene

- Rearrangements of the *MLL* gene occur in majority of infants with leukaemia

- *MLL* disruption appears central to the development of leukaemia by the mechanism of gene-fusion

- Extremely bad prognosis
Chromosomal translocation

Before translocation

Chromosome 11

Chromosome 4

After translocation

Derivative Chromosome 11

Derivative Chromosome 4

Fused gene (MLL-AF4)
Cytogenetic Abnormalities Involving MLL from recent patient cohort at GOSH

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>ALL</th>
<th>AML</th>
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<tbody>
<tr>
<td>Total</td>
<td>66</td>
<td>20</td>
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<tr>
<td>t(4;11)</td>
<td>MLL-AF4</td>
<td>36 (55%)</td>
</tr>
<tr>
<td>t(9;11)</td>
<td>MLL-AF9</td>
<td>8 (12%)</td>
</tr>
<tr>
<td>t(11;19)</td>
<td>MLL-ENL</td>
<td>12 (18%)</td>
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<tr>
<td>t(10;11)</td>
<td></td>
<td>5 (7.5%)</td>
</tr>
<tr>
<td>Other 11q23</td>
<td></td>
<td>5 (7.5%)</td>
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</table>
EFS in infants with ALL according to category of 11q23 rearrangement
Genes that are specifically expressed in MLL, ALL or AML

Armstrong et al, Nature genetics, 2002
How do MLL fusion genes transform cells?

A

Transfect cells with fusion gene under inducible control

B

Induce expression of fusion gene

C

Analyse gene expression profiles of B and C to identify targets of the gene of interest
Inducible expression of MLL fusion proteins

- **Myc Tag**
  - MLL ~1400 aa’s
  - Fusion partner

- **HA Tag**
  - MLL-AF9 170kDa
  - MLL-ENL 220 kDa
  - MLL-AF4 240 kDa

**Constructs**

<table>
<thead>
<tr>
<th>Dox</th>
<th>Anti-Myc</th>
<th>Anti-HA</th>
<th>Construct</th>
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<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>MLL-AF9</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>MLL-ENL</td>
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<td></td>
<td>+</td>
<td>+</td>
<td>MLL-AF4</td>
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<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>M.W. (kDa)</td>
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</tbody>
</table>

- 212
- 158
- 116
MLL-AF9 protects TONBAF cells from AraC

AraC for 48 hrs. Dox for 24 hrs before AraC
Identify **target** genes regulated by the MLL fusion gene

Determine the contribution of the **target** gene to the leukaemia phenotype i.e. proliferation or senescence or apoptosis

Use **targetted** therapy e.g. retroviral mediated siRNA or dominant negative mutant protein or drug to block contribution of this **target** to leukaemia *in vitro* then animal models
Summary of gene chip analysis from BaF3 MLL fusion inducible clones

- **MLL-AF9**: 225 genes
  - 42% up
  - 58% down

- **MLL-AF4**: 237 genes
  - 50% up
  - 50% down

- **MLL-ENL**: 164 genes
  - 79% down
  - 21% up

- Overlapping genes:
  - 13 genes
  - 10 genes
  - 6 genes
Experimental outline.

E12 Foetal Liver

\[\rightarrow\]

Purify c-Kit\(^+\) Ter119\(^-\) HSCs

\[\rightarrow\]

2 rounds of retroviral infection (LinXE packaging cells)

\[\rightarrow\]

Serial rounds of methylcellulose colony forming assays

In vitro experiments
The Tet-Off system.

pMSCV-tTA-IRES-EGFP

| 5’LTR | ψ⁺ | tTA | IRES-EGFP | 3’LTR | Amp⁺ |

pMSCV-TRE-MLL-ENL

| 5’LTR | ψ⁺ | Neo⁺ | TRE | MLL-ENL | 3’LTR | Amp⁺ | Dox | tTA |
Generation of an inducible MLL-ENL cell line from bone marrow.
Candidate target genes of MLL fusions

- Identification of candidate target genes up-regulated following inducible expression of MLL-AF4, MLL-ENL and MLL-AF9 in B cell and myeloid cell lines
- Identification of candidate target genes down-regulated following switching off the expression of MLL-ENL and MLL-AF9 in transformed primary haematopoietic cells
- Overlapping candidates from MLL-ENL and MLL-AF9 are being assessed for contribution to leukaemic phenotype
25% of paediatric pre-B cell ALL

Additional mutations are required for leukaemia: Twin studies, LOH @ TEL locus

Mouse models: suggest that TEL-AML1 does not directly induce leukaemia
Establishment of a model of the t(12;21) translocation using retroviral gene transduction

E12 mouse foetal liver (Ly5.2+)

FACS

MSCV Retroviruses

c-kit⁺ Ter119⁻ cells

Infected c-kit⁺ cells-GFP+
48 hours post-infection

Serial re-plating
(every 6-10 days)

Irradiated recipient Ly5.1+

Methylcellulose assay: Growth factors

Myeloid: SCF, IL3 IL6, GM-CSF

Pre-B: SCF, IL7, FLT3L

Liquid culture: derive cell line
Expression of TEL-AML1 in c-kit+ haematopoietic progenitor cells

Diagram showing the expression of TEL-AML1 and EGFP in TEL-AML1 and Vector constructs. The graphs compare the expression levels of EGFP in TEL-AML1 and Vector-transfected cells, with 83.6% and 87.6% expression respectively.

HA and Tubulin Western blots are also shown, indicating the expression levels of these proteins.
TEL-AML1 promotes B lineage development in vitro

**Total cell number**

![Graph showing total cell number over rounds of plating for TEL-AML1 and Vector control groups.](image)

**Colony number**

![Graph showing colony number over rounds of plating for TEL-AML1 and Vector control groups.](image)

**Pre-B cell numbers**

![Graph showing B220+ cell numbers harvested per plate over rounds of plating for TEL-AML1 and Vector control groups.](image)
Infection of HPC with low titres of retrovirus

- **0.07%** uninfected
- **16.6%** vector
- **16.2%** TEL-AML1

EGFP
TEL-AML1 promotes the development of B and myeloid lineage, but not T cells

Spleen 6 weeks post-transplantation

B cells

Myeloid cells

T cells

% B220+ Ly5.2+ cells

% Mac1+ Ly5.2+ cells

% Thy1.2+ Ly5.2+ cells

UN Vector T/A-1

EGFP negative

EGFP positive
Expression of TEL-AML1 enhances B cell, but not myeloid cell, development and the self-renewal of B cell precursors in vitro.

TEL-AML1 induces the growth of immortalized growth factor dependent pre-B cell lines which are not leukaemogenic in vivo.

TEL-AML1 enhances haematopoietic reconstitution of both B and myeloid lineages in vivo.

Expression of TEL-AML1 in progenitor cells does not cause a block B cell development
E2A-HLF

• E2A-HLF is a chimaeric protein formed as a result of t(17;19) chromosomal translocation found in about 1% of childhood ALL

• t(17;19) is associated with a poor outcome
Ch 17 - transcription factor HLF

- Member of the PAR (proline and acidic amino acid rich) subfamily of the bZIP (basic leucine zipper) transcription factors
- Expressed in liver, lung and kidney but not in haematopoietic cells
- Homology with the bZIP protein E4BP4

Ch 19 - transcription factor E2A

- Encodes for E12 and E47- contains 2 transactivation domains and a basic helix-loop-helix (bHLH) domain
- Ubiquitous expression
- Interacts with tissue-specific HLH proteins
- Essential for B cell development
E2A-HLF

- **E2A**
  - Transactivation domain (TAD)
  - Nuclear localization domain (NLS)

- **E2A-HLF**
  - Transactivation domain (TAD)
  - Proline & acidic acid rich domain (PAR)
  - Basic leucine zipper domain (bZIP)
  - Basic helix-loop-helix domain (bHLH)

**HLF** (Hepatic Leukaemia Factor)

- Transactivation domain (TAD)
- Proline & acidic acid rich domain (PAR)
- Basic leucine zipper domain (bZIP)
E2A

Encodes for transcription factors E12, E47 and E2-5

Is essential for B-cell development

HLF (Hepatic Leukaemic Factor)

Encodes a homolog of CES-2 a a pro-survival C. Elegans gene

Hypothesis: E2A-HLF contributes to leukaemia by promoting survival of B cell progenitors.
Detection of the t(17;19) translocation in a 6-year-old female patient with B cell ALL by fluorescence *in situ* hybridisation.

Chromosome 17 – Red
Chromosome 19 – Green

Derivative chromosome 19 is indicated by the white arrow.
Detection of E2A-HLF by RT-PCR in patient samples

Diagnosis
Relapse
Jurkat
HAL-01
No RNA

Diagnosis
Relapse
Jurkat
HAL-01
No RNA

Neg control (H₂O)

YCUB2
HAL-01

RT

bp
650
500
400
300
200
100

E2A-HLF
Inducible expression of E2A-HLF in mixed populations of BaF3 cells
Survival of E2A-HLF-inducible Baf-3 cells after IL-3 withdrawal.
Overview of experimental approach to identify transcriptional targets of E2A-HLF

- Ton-E2A-HLF clones

Triplicate experiments

- Dox (12 h)

RNA

Generation of Probes

Hybridisation to Chips

Scan Chips

Data Analysis
Identification of E2AHLF targets by comparison of data from all chips
### E2A-HLF targets genes identified by gene chip experiments

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<th>Gene</th>
<th>Fold change</th>
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<td>1.6</td>
<td>Activated in T-ALL</td>
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<td><strong>IL-15</strong></td>
<td><strong>2.0</strong></td>
<td><strong>T cell growth factor</strong></td>
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<tr>
<td>N-myc</td>
<td>1.5</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>1.3</td>
<td>Regulation of growth and differentiation</td>
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<td>Bcl2</td>
<td>1.4</td>
<td>Inhibitor of apoptosis</td>
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<td>E2F1</td>
<td>1.7</td>
<td>Transcription factor</td>
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<td>Enigma</td>
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<td>Adaptor molecule</td>
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<td>Sox4</td>
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E2A-HLF induces the expression of IL-15

<table>
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<tr>
<th>Time (h)</th>
<th>Empty Vector</th>
<th>E2A-HLF #6</th>
<th>E2A-HLF #7</th>
<th>E2A-HLF #9</th>
<th>Dox</th>
<th>LMO2</th>
<th>GAPDH</th>
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<th>GAPDH</th>
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<td>- - - - + + +</td>
<td>- - - - + + +</td>
<td>- - - - + + +</td>
</tr>
</tbody>
</table>

[Images of gel electrophoresis showing expression levels over time for different conditions.]
E2A-HLF trans-activates LMO2 and IL-15 promoter constructs

![Graph showing fold change for -3190LMOLUC and mL15/Luc constructs with different treatments](image-url)
E2A-HLF expands myeloid progenitor cells in methycellulose culture

![Graph showing colony numbers and cell numbers per plate over different rounds of replating for Empty Vector and E2A-HLF conditions.](image)
E2A-HLF alone and in combination with Bcl-2 immortalizes haematopoietic progenitor cells derived from E13 murine foetal liver
How E2A-HLF promotes survival of B cells?
IKAROS IS A TRANSCRIPTION FACTOR INVOLVED IN THE DIFFERENTIATION OF BOTH THE MYELOID AND LYMPHOID LINEAGES

Expression

Knockout mice

HSC

++

+++
IKAROS CAN BE ALTERNATIVE SPLICED INTO MULTIPLE ISOFORMS
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<tr>
<th>Case number</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Sex</th>
<th>WBC count 10^9/l</th>
<th>Blast count %</th>
<th>Main karyotypic abnormality</th>
<th>MLL rearrangement by FISH</th>
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<td>1</td>
<td>PreB-ALL</td>
<td>1yr11m</td>
<td>F</td>
<td>117.7</td>
<td>&gt;95</td>
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<tr>
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<td>M</td>
<td>4.88</td>
<td>1</td>
<td>t(8;14)</td>
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<td>4</td>
<td>B-ALL</td>
<td>10yrs6m</td>
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<tr>
<td>5</td>
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<td>t(7;9;11)</td>
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<td>9</td>
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<tr>
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<td>AML M5</td>
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<td>t(3;9;11)</td>
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<td>1yr1m</td>
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<td>13.6</td>
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**N/D, not done**
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</table>

- Expression pattern between leukemic samples and bone marrow is similar
- Only 3 of 49 samples have a clear shift to the expression of the ikaros 6 isoform
- These three samples harbour t(4;11) translocations
The expression of the ikaros 6 isoform by Western analysis correlates with the one seen by RT-PCR in samples 29 and 38.
Ikaros6 DELAYS CELL DEATH IN BaF3 CELLS AFTER IL3 WITHDRAWAL

Clone 10
Clone 5
Clone 13
Clone 19
Clone 10
Clone 5
Clone 13
Clone 19

Dox
- - - + + + +

Actin
Ik6

Clone 10

Clone 5

Clone 19

Clone 13
Current questions about Ik6

• What is the genomic structure of the *ikaros* gene in our Ik6-expressing patients, are any changes present *in utero* concurrent with presence of t(4;11) ?

• How does Ik6 act on blood cell development ? Infection of HSCs with Ik6 retrovirus, to study action of Ik6 on haematopoiesis both *in vitro* and *in vivo*
How does mapping the molecular pathways downstream of chromosomal translocations help us treat leukaemia in children?

- Tells us which additional genetic changes are required for full transformation to leukaemic cell
- Provides targets for therapeutic intervention either as drug target or target for genetic therapy
- Enhances epidemiological studies examining causality - even if initial translocation happens in utero, post-natal exposure and subsequent genetic changes are likely to be crucial to development of childhood leukaemia
Institute of Child Health, University College London and Gt Ormond St Hospital for Children

Hugh Brady

Owen Williams

Jenny Yeung
Elaine O’Sullivan
Julianne Ellu

Anna Ruiz
Tanzina Chowdhury
Dale Moulding
Inusha DeSilva

Sarah Horton
Michelle Morrow

Ian Hann
Helena Kempski
Mike Hubank

Funding:

Children with Leukaemia

MRC NIMR, London
Dimitris Kioussis

Leukaemia Research Fund
Welcome to the

International Scientific Conference

for

Childhood Leukaemia

incidence
causal mechanisms
prevention