35th UKCCCG Meeting
Joint UKCCCG and CHO Annual Conference

9th – 10th April 2013
Newcastle upon Tyne
Welcome!

35th UKCCCG Meeting

Joint UK Cancer Cytogenetics Group and Centre Haemato-Oncology Annual Conference
Thank you very much to all our sponsors!
Dinner at Prima

Registered delegates

Tonight at 7pm

The LRCG and sponsors would like to invite you to dinner at Prima Pasta, Newcastle Quayside.

Address: 40-46 The Side, Quayside, Newcastle upon Tyne, NE1 3JA
Centre for Haemato-Oncology
Guest Lecture

Next-Generation Sequencing Technologies for Characterization and Prognostic Stratification of Haematological Malignancies

Dr Alexander Kohlmann, Munich Leukemia Laboratory, Germany
The Molecular Genetic Makeup of Acute Lymphoblastic Leukemia

*Dr Charles Mullighan, Associate Member, St. Jude Faculty, St Jude’s Children’s Research Hospital, Memphis, USA*
IKZF1, CRLF2 and all that in Childhood Acute Lymphoblastic Leukemia trials in France

Dr Hélène Cavé, Hôpital Robert Debré Paris, France
Other external speakers

To MRD and Beyond
*Dr Bella Patel*, Department of Haematology, Royal Free Hospital, London

A Novel Subgroup in ALL with Amplification of *PAX5*
*Jorieke Weiden*, Radboud University Nijmegen Medical Centre, The Netherlands

*RUNX1* Disrupted by a Complex Rearrangement of Chromosome 21 Causes Familial Predisposition to Haematological Malignancies
*Simone Snijder*, Department of Clinical Genetics, Academic Medical Centre, Amsterdam

How Genetics Determines Treatment for Patients with Neuroblastoma Now and in the Future
*Professor Debbie Tweddle*, Northern Institute for Cancer Research, Newcastle University
IGH@ Translocations, CRLF2 Deregulation, and Microdeletions in Adolescents and Adults With Acute Lymphoblastic Leukemia.

Genes commonly deleted in childhood B-cell precursor acute lymphoblastic leukemia: association with cytogenetics and clinical features.
Schwab CJ et al Haematologica 2013

Abnormalities of the der(12)t(12;21) in ETV6-RUNX1 acute lymphoblastic leukemia.
Al-Shehhi H et al Genes Chromosomes Cancer 2013 52(2): 202-213

The clinical characteristics, therapy and outcome of 85 adults with acute lymphoblastic leukemia and t(4;11)(q21;q23)/MLL-AFF1 prospectively treated on UKALLXII/ECOG2993.
Marks DI et al Haematologica 2013

Episomal amplification of NUP214-ABL1 fusion gene in B-cell acute lymphoblastic leukemia.
Eyre T et al Blood 2012 120(22):4441-4443

Treatment outcome of CRLF2 -rearranged childhood acute lymphoblastic leukaemia: a comparative analysis of the AIEOP-BFM and UK NCRI-CCLG study groups.
Other publications on UK trials

Outcomes in older adults with acute lymphoblastic leukaemia (ALL): results from the international MRC UKALL XII/ECOG2993 trial.


Impact of NOTCH1/FBXW7 mutations on outcome in pediatric T-cell acute lymphoblastic leukemia patients treated on the MRC UKALL 2003 trial.

*Jenkinson S et al* Leukemia 2012 26:1-7
European collaborative studies

*BTG1* deletions do not predict outcome in Down syndrome acute lymphoblastic leukemia.

*Buitenkamp TD et al* *Leukemia* 2012 26(8):1-2

Outcome in children with Down syndrome and Acute Lymphoblastic Leukemia: role of *IKZF1* deletions and *CRLF2* aberrations.

*Buitenkamp TD et al* *Leukemia* 2012 26(3):2204-2211
Small sizes and indolent evolutionary dynamics challenge the potential role of P2RY8-CRLF2-harboring clones as main relapse-driving force in childhood ALL. *Morak M et al* Blood 2012

Distinct patterns of gained chromosomes in high hyperdiploid acute lymphoblastic leukemia with t(1;19)(q23;p13), t(9;22)(q34;q22) or MLL rearrangements. *Paulsson K et al* Leukemia 2012 26(10):139-140


Genomic analysis drives tailored therapy in poor risk childhood leukemia.

*Harrison CJ* Cancer Cell 2012 22(2):139-140

The clinical relevance of chromosomal and genomic abnormalities in B-cell precursor acute lymphoblastic leukaemia.


Burkitt's lymphoma.

Update of current studies
Deletions of B-cell differentiation genes and cell cycle progression in BCP-ALL by SNP arrays

Mullighan et al 2007 Nature
Kuiper et al 2007 Leukemia
Strefford et al 2007 Oncogene
Multiplex Ligation-dependent Probe Amplification (SALSA MLPA kit P335-A1 ALL IKZF1)

- Probes for genes most frequently deleted in BCP-ALL
  - **IKZF1 (8)** — Early B-cell differentiation gene (deleted in 29% high risk ALL)
  - **CDKN2A/B (3)** — Cell cycle G1 control - CDK inhibitor and p53 stabilizer (deleted in 20% BCP-ALL)
  - **PAX5 (6)** — B-cell differentiation gene (deleted in 32% of BCP-ALL)
  - **EBF1 (4)** — Early B-cell differentiation gene activating PAX5
  - **ETV6 (6)** — Transcription factor required for haematopoiesis (involved in translocations in ALL and deleted in 5%)
  - **BTG1 (4)** — Negative regulator of cell cycle
  - **RB1 (5)** — Negative regulator of cell cycle (deleted in 5-11% B-ALL)
  - **CRLF2/CSF2RA/IL3RA (1 each)** — Deregulated in 6% BCP-ALL

Claire Schwab
MLPA analysis of 1427 childhood patients from ALL97 and ALL2003

Incidence and type of abnormalities

- **Whole gene**: 30%
- **Exons 4-7**: 31%
- **Exons 4-8**: 7%
- **Exons 2-3**: 8%
- **Exons 2-7**: 10%
- **Miscellaneous**: 14%

**RB1**
- Majority: exons 18-26

**IKZF1** and **PAX5** heterogeneous
Schwab et al Haematologica (in press)
Prospective collection of viable cells for:

- MLPA studies
- Other genetic tests as appropriate
Prognostic subgroups within high hyperdiploidy & *ETV6-RUNX1* – fact or artefact?

**Panel: Definition of cytogenetic risk groups**

**Good risk**
- High hyperdiploidy (51–65 chromosomes)
- *ETV6-RUNX1*

**Intermediate risk**
- `t(1;19)(q23;p13)`
- `IGH-CEBP`
- `IGH-ID4`
- `del(6q)`
- Abnormal 9p
- Abnormal 11q
- `dup(1q)`
- `-7`
- `dic(9;20)(p13;q11)`
- `dic(9;12)(p11-21;p11-13)`
- Any other abnormality
- Normal karyotype

**Poor risk**
- `t(9;22)(q34;q11.2)`
- `iAMP21`
- *MLL* translocations
- Near haploidy (<30 chromosomes)
- Low hypodiploidy (30–39 chromosomes)
- `t(17;19)(q23;p13)`
- Abnormal 17p
- Loss of 13q

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**Risk Group** | **%** | **EFS @ 5y** | **OS @ 5y**
--- | --- | --- | ---
**Good** | 59% | 86% | 94% |
**Intermediate** | 30% | 76% | 85% |
**Poor** | 11% | 45% | 61% |

Prognostic subgroups within high hyperdiploidy & ETV6-RUNX1 – fact or artefact?

- High Hyperdiploidy
  - +6 | +4,+10 | +10,+17 | +18 | +4,+10,+17 | +5
  - Modal chromosome number

- ETV6-RUNX1
  - deletion of native ETV6 allele
  - +der(21)t(12;21)
  - +21
  - NCI Risk Status

- New factors
  - IKZF1 deletions
  - CRLF2 rearrangements

- Aim: To examine risk factors in a single cohort (ALL97) with long follow-up (>8 years)
Outcome of *ETV6-RUNX1* patients by NCI Risk Status

Univariate Cox Regression
- Risk of event = 2.45 (1.41-4.23), p=0.001
- Risk of death = 5.40 (2.21-13.2), p<0.001
No prognostic impact of ETV6 deletions among ETV6-RUNX1 patients

Univariate Cox Regression
Risk of event = 1.21 (0.62-2.37), p=0.570
Risk of death = 0.82 (0.30-2.25), p=0.699
No prognostic impact of +21/+der(21) among ETV6-RUNX1 patients

![Graph showing event-free survival for different groups with varying numbers: Normal (n=166), +der(21) (n=21), +21 (n=40), +21,+der(21) (n=17).]
Outcome of high hyperdiploid patients by NCI Risk Status

Overall Survival
Hazard Ratio
2.42 (1.34-4.34)
p=0.003

Relapse Risk
Hazard Ratio
1.73 (1.07-2.78)
p=0.025

NCI SR - 93%
NCI HR - 84%
NCI High Risk, n=439 (79%)
NCI Standard Risk, n=114 (21%)
Modal number is NOT linked to outcome

Overall Survival

Relapse Risk

Years from diagnosis

Probability

0.00 0.25 0.50 0.75 1.00

Modal Number
58-65
54-57
51-53

Modal Number
51-53
58-65
54-57
Triple trisomy (+4, +10, +17) is NOT linked to outcome

Overall Survival
Hazard ratio
0.82 (0.43-1.56)
p=0.544

Relapse Risk
Hazard ratio
0.74 (0.45-1.12)
p=0.233
Trisomy 18 is associated with a good outcome

Effect was independent of NCI risk, trial (97 v 99) and treatment (standard v high risk)

Overall Survival
Hazard ratio 0.43 (0.23-0.81)
p=0.009

Relapse Risk
Hazard ratio 0.47 (0.29-0.76)
p=0.002
Spectrum of micro-deletions differs between $ETV6$-$RUNX1$ and high hyperdiploid patients.
EFS of patients with good risk cytogenetics by *IKZF1* deletion status

Event Free Survival

Normal (n=244, 95%) 86% (81-90%)

IKZF1 deleted (n=12, 5%) 58% (27-80%)

5 events: 4 patients relapsed, 1 patient died in remission, 4 were SERs

Years from diagnosis
Summary & Conclusions

- NCI risk status was prognostic in ETV6-RUNX1 and high hyperdiploid patients.

- **High hyperdiploidy**: Trisomy 18 was associated with outcome.

- **ETV6-RUNX1**: No evidence for outcome heterogeneity according to secondary abnormalities affecting ETV6 or RUNX1

- Spectrum and number of micro-deletions varies between ETV6-RUNX1 and high hyperdiploidy.

- High rate of relapse among the small number of patients with an IKZF1 deletion.
IGH@ translocations in lymphoid malignancies

Identified in BCP-ALL

- 19q13 - CEBPG
- 19q13 - CEBPA
- 17q21 – IGF2BP1
- 14q11 – CEBPE
- 11q24 – miR-125b
- 11q13 - UK
- 10q24 - NFKB2
- 9p13 - PAX5
- 8q11 - CEBPD
- 7q21 – TRG@
- 6q22 – ID4
- 5q31 – IL3
- 3p14 – LAPT5
- 1p34 – LAPTM5
- 1p22 - BCL10
- 1q21 – ITRA1
- 1q21 - BCL9
- 1q21- FCGR2B
- 1q24 - LHX4
- 2p13 - BCL11A
- 3p14 - FOXP1
- 3q27 - BCL6
- 4p16 - FGFR3
- 4p16 - WHSC1
- 4p13 - RHOH
- 7q21 - CDK6
- 7q21 - ERVWE1
- 8q24 – MYC

Mature leukaemia/lymphoma

- 20q13 - CEBPB
- 20q11 - MAFB
- 19q13 - BCL3
- 1q21 - mir-125b
- 1p34 – LAPT5
- 1p22 - BCL10
- 1q21 – ITRA1
- 1q21 - BCL9
- 1q21- FCGR2B
- 1q24 - LHX4
- 2p13 - BCL11A
- 3p14 - FOXP1
- 3q27 - BCL6
- 4p16 - FGFR3
- 4p16 - WHSC1
- 4p13 - RHOH
- 7q21 - CDK6
- 7q21 - ERVWE1

Lisa Russell
IGH@ translocations in childhood and adult ALL

- Overall incidence is 5% (160/3274) in B- and T-ALL
**IGH@ translocations in childhood and adult ALL**

- Median age 16 years
- Peak incidence in 20-24 year olds

Other = All other known *IGH@* partner genes
Outcome of *IGH@* patients

(a) **Childhood ALL**

- Overall Survival/Relapse Rate (%)
- Follow-up time (years)
- IGH@ -ve
- IGH@ +ve

- p = 0.003
- No difference

(b) **Adult ALL**

- Overall Survival (%)
- Follow-up time (years)
- IGH@ -ve
- IGH@ +ve

- p = 0.002
- No difference

(c) **Outcome of IGH@ patients**

- Relapse Rate (%)
- Follow-up times (years)
- IGH@ -ve
- IGH@ +ve

- No difference
Intrachromosomal amplification of chromosome 21

iAMP21

Normal signal pattern

Abnormal signal pattern

Incidence ~2%
Older children median age 9 years

ETV6-RUNX1 extra signal

Harewood et al 2003 Leukemia
Soulier et al 2003 Leukemia
Robinson et al, 2003; 2007
Complexity of chromosome 21
(185K Agilent arrays)
Paired end sequencing of chromosome 21 from iAMP21 patient

Claire Schwab
Sarra Ryan

Yilong Li
Peter Campbell
Wellcome Trust
Sanger Centre
International study of 525 patients: iAMP21 associated cytogenetic changes
iAMP21 associated copy number changes
ALL97: Risk of relapse for iAMP21

Survival vs Years from diagnosis

- ETV6-RUNX1
- HeH
- Other
- 13 Loss
- MLL
- 17p
- t(9;22)
- iAMP21

Moorman et al, 2010 Lancet Oncology
Outcome of iAMP21 patients on ALL97

Overall Survival - 5yr 69% (SE 8.6)

Event Free Survival - 5yr 26% (SE 8.3)

N=29

22 relapses (15 BM, 5 CNS, 2 BM&CNS)

11 deaths (1 in 1st CCR)

October 2007 Update

Leukaemia Research Cytogenetics Group
EFS of iAMP21 patients treated on ALL2003 and ALL97

10 events: 4 remissions deaths, 6 relapses

P<0.0001

EFS @ 5yrs
ALL2003 = 78%
ALL97 = 28%

February 2012 Update
Relapse rate for iAMP21 patients

No risk factors could be established – age, sex, white cell count, genetics.
Survival of iAMP21 patients

- **ALL2003 (N=52)**: 89% (76-95%)
- **ALL97 (N=28)**: 67% (47-82%)

P<0.01

Years from diagnosis
Recommendation

• Similar results shown by Children’s Oncology Group

• iAMP21 patients should be treated as high risk

• Detection requires FISH with probes directed to the **RUNX1** gene

• 3 or more extra copies (≥5) of the **RUNX1** gene on a single abnormal chromosome 21
Probes for genes frequently abnormal in T-ALL

- **STIL-TAL 1p32** (5 probe) — deletion leading to STIL-TAL1 fusion gene (10-30% T-ALL)
- **LEF1 4q25** (5 probes) — deletions in 11% T-ALL. Good Prognosis
- **CASP8AP2 6q15** (6 probes) — deleted in 12% correlates with poor treatment response
- **MYB (5 probes) 6q23** — Tandem duplication seen in 8-15% of T-ALL. Therapeutic target
- **CDKN2A/B and MTAP 9p21** (1 probe each) — Cell cycle G1 control -CDK inhibitor and p53 stabilizer (deleted in ~70% T-ALL)
- **NUP214-ABL1 (4) 9q34** — amplified in patients with TLX3 rearrangement. Treatment with imatinib.
- **PTEN 10q23** (4 probes) - deleted in ~9% of T-ALL patients. Associated with early treatment failure
- **LMO2 11p12-13** — deletion of exon 1 leads to activation fusing to exon 1 of RAG2
- **NF1 17q12** (3 probes) — deleted in ~10% of T-ALL patients. May correlate with poor response to induction
- **PTPN2 18p12** (5 probes) — 7% of T-ALL show deletion. Negative regulator of NUP214-ABL1 fusion.
- **PHF6 Xq26** (6 probes)- inactivated in 16-38% of T-ALL. Poor survival in adults.
43 GENES

- **TCRB** (7q34)
- **TCRAD** (14q11)
- **TAL1** (1p32)
- **LEF1** (4q25)
- **MEF2C** (5q14)
- **NKG2-5** (5q35)
- **TLX3** (5q35)
- **CASP8AP2** (6q15)
- **GRK2** (6q16)
- **C-MYB** (6q23)
- **IKZF1** (7p11)
- **CDKN2A** (9p21)
- **JAK2** (9p24)
- **ABL1** (9q34)
- **NUP214** (9q34)
- **NOTCH1** (9q34)
- **PTEN** (10q23)
- **WT1** (11p13)
- **LMO2** (11p13)
- **LMO1** (11p15)
- **NUP98** (11p15)
- **CALM** (11q14)
- **MLL** (11q23)
- **ETV6** (12p13)
- **NF1** (17q12)
- **PTPN2** (18p12)
- **LCK** (1p34)
- **TAL2** (9q32)
- **AF10** (10p13)
- **TLX1** (10q24)
- **HOXA** (7p15)
- **C-MYC** (8q24)
- **CCND2** (12p13)
- **NKG2-1** (14q13)
- **TCL1** (14q32)
- **BCL11B** (14q32)
- **LYL1** (19p13)
- **NKG2-2** (20q11)
- **OLIG2** (21q22)
- **RUNX1** (21q22)
- **IRS4** (Xq22)
- **PHF6** (Xq26)
- **MTCP1** (Xq28)
Mutations in T-ALL
Jan Cools, Leuven

Mutations:

– NOTCH1, FBXW7, PTEN, PHF6, BCL11B, JAK1, JAK3, IL7R, NRAS, KRAS

– RPL10, CNOT3, PTPRC (CD45)
Integrated FISH and MLPA results of 90 T-ALL patients

137 abnormalities detected

CDKN2A/B (79%)

MYB dup (3.5%)

CASP8AP2 (11%)

LEF1 (11%)

MYB (7%)

NF1 (1%)

PTEN (13.5%)

PTPN2 (5.5%)

PHF6 (3.5%)

NUP214-ABL1 amp (4.5%)

STIL-TAL Fusion (22.5%)
Distribution by major genetic group

TAL/LMO (n=30)

HOXA (n=12)

TLX3 (n=18)

Not Classified (n=23)

TLX1 (n=2)

NKX2-1 (n=3)
Ongoing studies

- MLPA on ALL2003
- Expand the FISH cohort
- Prospective MLPA screening in ALL2011 with FISH for major subgroups: TLX1 and TLX3
Mutations in the **RAS Signaling, B-Cell Development, TP53/RB1, and JAK** Signaling Pathways Are Common in High Risk BCP-ALL.


Coding region and UTR of 125 genes sequenced in 187 high risk childhood BCP-ALL in COG P9906
**BCR-ABL1-like/Ph-like ALL**

Ph-negative cases show a gene expression profile similar to that of Ph-positive ALL and share the same high-risk of relapse and poor outcome

Charles Mullighan *et al* 2009 Nature Genetics
Monique den Boer *et al* 2009 Lancet Oncology
Can we identify *BCR-ABL1*-like from their genomic profile?
Similar distribution of copy number abnormalities in *BCR-ABL1* and “other” group points to presence of *BCR-ABL1*-like ALL.
Rearrangements, mutations and deletions affecting kinase and cytokine signaling in BCR-ABL1-like ALL

Roberts et al 2012 Cancer Cell
FISH detection of *NUP214-ABL1* fusion

Eyre T *et al* Blood 2012
Roberts *et al* 2012 Cancer Cell
FISH detection of *PDGFRB* rearrangements

Found among non-remitters

Known response to Imatinib
Thanks
Leukaemia Research Cytogenetics Group past and present

UK Cancer Cytogenetics Group
Scientific and Clinical Collaborators