PROFESSIONAL GUIDELINES FOR CLINICAL CYTOGENETICS

ACUTE LYMPHOBLASTIC LEUKAEMIA (2011) v1.00

July 2011

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1   INTRODUCTION

Professional guidelines for Cytogenetics laboratories incorporate the standards imposed by regulatory bodies (Clinical Pathology Accreditation (CPA) [1]) and by statute (Clinical Governance), while taking into account current practice in the UK. Elements of the service not subject to statute may be varied according to local constraints and agreements. It must be noted that these guidelines are minimum requirements and that professional judgment is of paramount importance for many circumstances.

It is important that laboratories are aware of, and comply with, any additional requirements relating to cytogenetic analyses which may be set by national clinical trials.

Cytogenetic laboratories should provide onward referral to specialist laboratories when necessary, or advice on where appropriate further testing may be performed.

The use of 'must' in this document indicates a requirement and the use of 'should' indicates a recommendation. Where there appears to be contradiction between available guidelines, the most recently published should be taken to apply to all.

All cytogenetic laboratories must be accredited to nationally or internationally accepted standards. Laboratories must participate in an External Quality Assessment Scheme in all aspects of their service for which a scheme is available [2].

These guidelines relate to Acute Lymphoblastic Leukaemia and make up part of the Haemato-Oncology Best Practice Guidelines. They should be used in conjunction with the following documents; Professional Guidelines for Clinical Cytogenetics: General Best Practice Guidelines (2007) [3], Professional Guidelines for Clinical Cytogenetics: Haemato-oncology (2007) [4].
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2   ACUTE LYMPHOBLASTIC LEUKAEMIA

2.1   Introduction

Acute lymphoblastic leukaemia (ALL) comprises neoplastic precursor cells committed to the B or T cell lineages. B-lineage ALL is more frequent, accounting for 85% of childhood ALL and 75% of adult ALL [5].

ALL is primarily a disease of childhood, with 75% of cases occurring under six years of age. Cytogenetic abnormalities are seen in the leukaemic clone in the majority of cases of B-ALL and often define specific entities with unique haematological and prognostic features [5]. Some of the non-random abnormalities are listed in the appendix: this list is not intended to be exhaustive, but can be used as a basis for cytogenetic testing strategies.

In childhood B-cell ALL, the ploidy of the leukaemic cells is a well-established prognostic factor. High hyperdiploidy with 51-65 chromosomes per cell (usually including gains of 4, 6, 10, 14, 17, 18, 21 and X) accounts for about 25% of cases of B-ALL and is associated with a favourable prognosis.   By contrast, low hypodiploidy (30-39 chromosomes) and near-haploidy (23-29 chromosomes) confer a poor prognosis. In childhood ALL, near-haploidy is more common than low hypodiploidy, whereas the converse is true for adult ALL.

As reviewed by Harrison et al. [6], the most significant genetic prognostic factors in paediatric ALL are;
- t(9;22)(q34;q11) Philadelphia translocation
- *MLL* rearrangements - particularly translocation t(4;11)(q21;q23)
- *ETV6-RUNX1* fusion
- iAMP21 (intrachromosomal amplification of chromosome 21)
- t(17;19)(q22;p13)
- Ploidy

Since these abnormalities may alter treatment approaches, it is essential that strategies for karyotyping and FISH (or RT-PCR, where applicable) are in place to accurately assess these features in all new cases at presentation.

Cytogenetic features of principal prognostic significance in adult ALL are considered to be translocation t(9;22)(q34;q11), translocation t(4;11)(q21;q23), karyotype complexity and ploidy [7].

For T-lineage ALL, an abnormal karyotype is typically reported in 50-70% of cases [1]. Numerical abnormalities are less frequent than in B-cell ALL, with the exception of around 5% cases showing tetraploidy. Approximately 35% of cases show rearrangements involving the TCR loci at 7q34 (*TCRB*) or 14q11 (*TCRA/D*) [8].

The age cut-off between paediatric and adult cases can vary between clinical trials but generally ranges between 16 and 25 years. For the purposes of the testing algorithms recommended in this document, the cut-off has been set as the 25th birthday.

2.2 Diagnostic testing

2.2.1 Sample requirements

The preferred sample for cytogenetic analysis is bone marrow taken into a heparinised container and transported promptly to the laboratory. Many laboratories provide culture medium to local Haematology/Oncology units for the transport of such samples.

If bone marrow is not available, e.g. unsuccessful aspiration, then trephine biopsies or peripheral blood (if circulating blasts are present) may be cultured.

2.2.2 Cultures

It is recommended that a method of cell counting be used, so that culture densities may be optimised to approximately 1×10^6/ml.

For samples of sufficient cellularity, at least two cultures with differing durations should be initiated. Some ALL cells show an increased tendency for apoptosis, and it is recommended that one of the two cultures has an incubation time of <24hrs.

It has been reported that growth factor supplements such as SCF, interleukins, and *Flt3*-ligand may improve the quality of cytogenetic preparations and facilitate chromosome analysis in ALL [9].

For cases referred with T lineage ALL, it may be beneficial to establish additional PHA-stimulated cultures.

2.2.3   G-banded analysis

For all diagnostic cases, if no clonal abnormality is detected by G-banding or by FISH/RT-PCR, a minimum of 20 metaphases must be analysed. If fewer than 20 suitable metaphases are available, the result must be reported with a 'partial analysis' caveat. If 10 cells cannot be fully analysed in a case with a normal karyotype, then this must be considered a failed chromosome analysis.

Where an abnormal clone is detected, full analysis of 10 metaphases is recommended. Abnormal results may, if necessary, be reported on fewer metaphases, provided that the ISCN criteria for a clone are fulfilled (i.e. two or more cells with same extra chromosomes or structural rearrangements, or three or more with same chromosome loss). FISH may be useful to corroborate the presence of abnormalities tentatively identified in such restricted analyses.

Both near-haploid and low hypodiploid clones (poor prognosis) can 'double-up' to masquerade, respectively, as hyperdiploid or near-triploid metaphases [10]; the resulting karyotypes may include a number of tetrasomies representing the chromosomes which were disomic in the near-haploid stemline. Analysts should be vigilant about this possibility and, in the event of finding an atypical hyperdiploid / near-triploid karyotype with four copies of chromosomes other than 21 or the sex chromosomes, every attempt should be made to investigate an underlying near-haploid / low hypodiploid population. Investigations can include interphase FISH [11], and consideration could be given to STR analysis and zygosity testing (when derived from a doubled-up near-haploid stemline, most chromosomes will show a homozygous STR result).

2.2.4   FISH / RT-PCR analyses:

The t(9;22)(q34;q11), t(12;21)(p13;q22), iAMP(21) and *MLL* rearrangements are reported to be mutually exclusive [5]. If one of these abnormalities is detected, it is not mandatory to test for the others.

FISH and / or RT-PCR testing can be performed sequentially. The frequencies of the principal abnormalities vary with age, and the order of testing can be adjusted accordingly; *MLL* rearrangements are more common in infant cases (age <1 year), t(12;21) more common in paediatric cases (age 1-24yrs) and the t(9;22) is more common in adult cases (>25yrs).

In the absence of an 'exclusive' abnormality, FISH using an ETV6/RUNX1 probe set is mandatory in all infant and paediatric cases. In addition to detecting gene fusion resulting from the cryptic t(12;21)(p13;q22) translocation, ETV6/RUNX1 FISH will also detect
(i) amplification of *RUNX1* indicative of iAMP21, or
(ii) extra signals of *RUNX1* suggestive of a hyperdiploid karyotype.

In cases where chromosome analysis is unsuccessful, or when a normal karyotype is obtained, and interphase FISH identifies extra signals for *RUNX1*, further interphase testing for evidence for high hyperdiploidy should be performed and may include probes for chromosomes X, 4, 6, 10, 14, 17 and 18.

iAMP21 is generally defined as five or more *RUNX1* signals corresponding to three or more extra copies of the gene on a single abnormal chromosome 21 [12]. In interphase cells, the amplification will frequently be seen as a cluster of signals. However, in situations where there is uncertainty between high hyperdiploidy and iAMP21, it may be helpful to employ a chromosome 21 sub-telomeric probe: in cases of iAMP21, the number of RUNX1 signals will exceed the number of subtelomere signals.

The t(4;11)(q21;q23) is associated with a poor prognosis, and patients with this translocation may be treated on the high risk arm of MRC protocols. Currently, there are no commercial FISH probes specific for this translocation although several UK laboratories have validated in-house probes, and RT-PCR can also be used to identify the fusion transcript. If chromosome analysis is unsuccessful but FISH indicates a rearrangement of

MLL, then further attempts to identify the t(4;11) must be made, including possible onward referral to another laboratory for specialist testing.

Similarly, when TCF3 'breakapart' FISH identifies a rearrangement, it is important to distinguish between t(17;19)(q22;p13) and t(1;19)(q23;p13), the former translocation being associated with adverse prognosis. If karyotyping is unsuccessful, then further testing or onward referral is mandatory.

A possible testing strategy for ALL is given below:
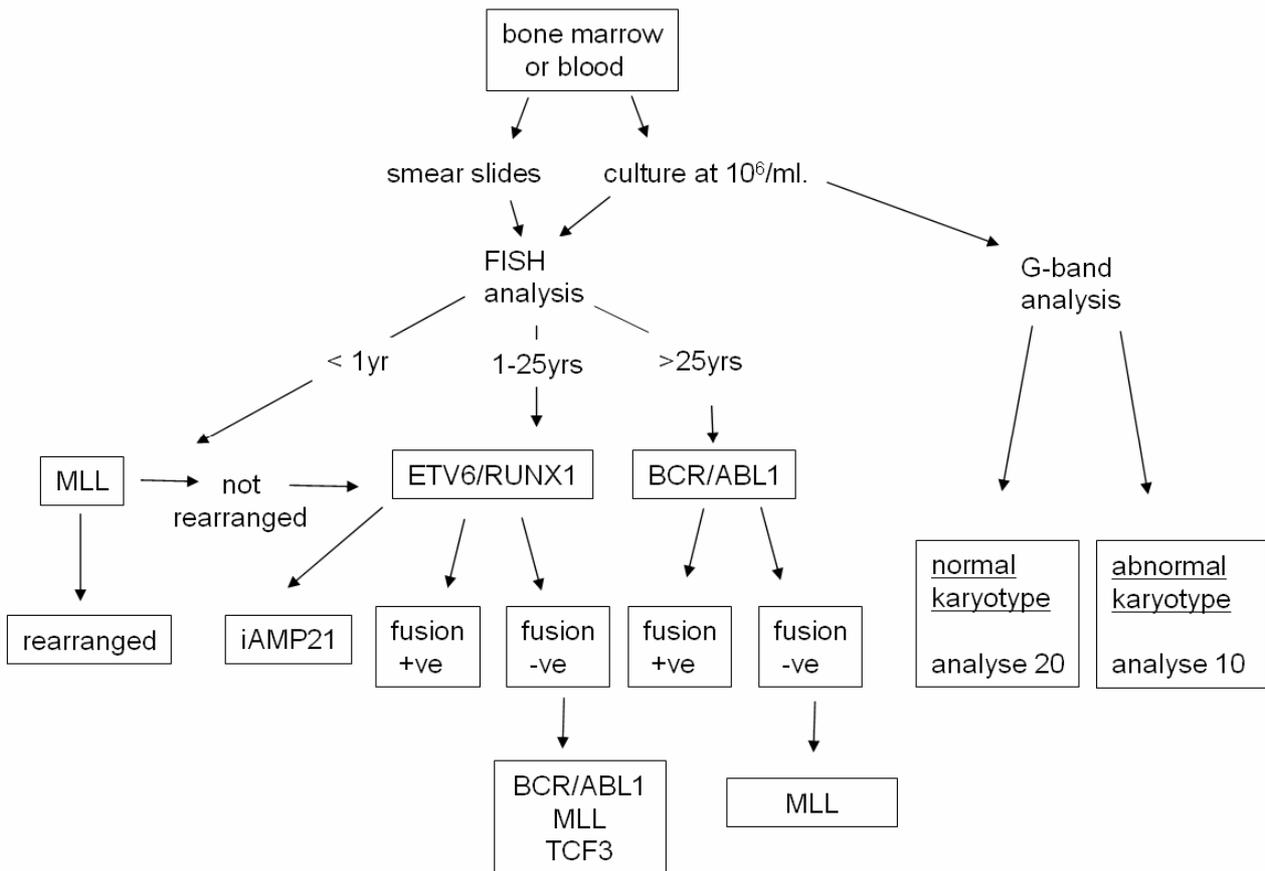
T lineage

Since no protocols currently stratify treatment on the basis of genetics, there are no FISH tests which are mandatory. Optional testing could include *TCRAD, TLX3* and *TLX1* translocations, *SIL-TAL1, LMO2* and *LMO1* rearrangements.

2.2.5   Interpretation and reporting

Abnormal results should be linked where possible to WHO classification subtypes [5].

If a patient is known to be entered onto a particular trial, e.g. UKALL 2003, then the appropriate risk stratification should be given according to the trial classification.

Information regarding prognosis may be derived from WHO [1], or from national/international studies (e.g. [13],[14]), but it should be recognised that the prognosis attached to certain abnormalities, e.g. iAMP21, is protocol-dependent.

2.3   Acute leukaemias of ambiguous lineage

By definition, this group is not classified as lymphoblastic leukaemia but is included within this set of guidelines for convenience.
Common genetic abnormalities seen in mixed phenotype acute leukaemia include t(9;22)(q34;q11.2) and *MLL* rearrangements, both of which must be investigated since either confers a poor prognosis.

2.4   Follow-up testing

Chromosome analysis in remission is not mandatory but may be undertaken subject to local agreement. Interphase FISH may have a role in monitoring specific abnormalities in individual cases.

Minimal residual disease is best monitored by molecular means, or by flow cytometry.

At relapse, chromosome analysis can be valuable to confirm the clonal provenance of the disease, identify karyotype evolution, or indicate a new secondary malignancy. Analytical requirements at relapse are the same as at diagnosis.

2.5   Reporting time guidelines

- New diagnoses must be treated as high priority, with 95% reported within 14 calendar days.
- Preliminary results conferring a poor prognosis or likely to affect treatment (e.g. Philadelphia positivity) should be communicated to the referring clinician as rapidly as possible.
- For routine follow up samples, 95% should be reported within 21 calendar days.

3   APPENDIX - Non–random abnormalities in precursor B-ALL

Abnormality	Genes	FISH	Molecular analysis	Prognosis
t(12;21)(p13;q22)	*ETV6-RUNX1*	*ETV6/RUNX1*	RT-PCR	**favourable**
t(9;22)(q34;q11)	*BCR1-ABL1*	*BCR/ABL1*	RT-PCR	**poor**
11q23 rearrangement	*MLL*	*MLL* break-apart	RT-PCR	**depends on partner gene**
t(4;11)(q21;q23)	*AFF1-MLL*		RT-PCR	**poor**
14q32 rearrangement	*IGH@*	*IGH* break-apart		**poor (preliminary data [6])**
t(X;14)(p22;q32)	*IGH@-CRLF2*	*IGH* break-apart		**poor (preliminary data [6])**
hyperdiploidy		CEP X, 4, 6, 10, 17, 18, 21	consider STR analysis	**favourable**
hypodiploidy (<44) common losses of 3 and 7		CEP 1, 3, 7, 11, 17		**poor**
t(1;19)(q23;p13)	*PBX1-TCF3*	*TCF3* break-apart, *TCF3/PBX1*	RT-PCR	**intermediate**
t(17;19)(q22;p13)	*HLF-TCF3*	*TCF3* break-apart	RT-PCR	**poor**
dic(9;20) (p11-13;q11)		CEP9 and 20		**intermediate**
dic(9;12)(p13;p13)	*PAX5-ETV6*	*PAX5* break-apart		**favourable**
iAMP21	Includes *RUNX1*	*ETV6/RUNX1*		**poor depending on protocol**
9p21 abnormalities	*P16*	*CDKN2A* deletion probe		**uncertain**
7p12 abnormalities	*IKZF1*	*IKZF1* deletion	MLPA	**poor (preliminary data)**
t(8;14)(q24.1;q32.3)	*IGH@-MYC*	*MYC* break-apart *IGH/MYC*		**poor**
complex karyotype >4 abnormalities				**poor in adults [13]**
near triploidy				**poor in adults [13]**

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Also - Clinical trials information: see http://www.ctsu.ox.ac.uk/projects/leuk/

5   Version Control

issue date	Current document	summary of changes	Version replaced
04/07/2011	**ACUTE LYMPHOBLASTIC LEUKAEMIA BEST PRACTICE GUIDELINES (2011) v1.00**	n/a	no previous version

Produced by ACC Professional Standards Committee