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**Detection of prognostically relevant genetic abnormalities in
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Detection of prognostically relevant genetic abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: recommendations from the Biology and Diagnosis Committee of iBFM-SG

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Short title: Genetic diagnosis of childhood BCP-ALL

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Key words: acute lymphoblastic leukaemia, genetics, risk stratification, RT-PCR, cytogenetics, fluorescence *in situ* hybridisation.

Summary

Treatment of childhood acute lymphoblastic leukaemia (ALL) has improved considerably in recent years. A contributing factor has been the improved stratification for treatment according to a number of factors including genetic determinants of outcome. Here we review the current diagnostic criteria of genetic abnormalities in precursor B-ALL (BCP-ALL), including the relevant technical approaches and the application of the most appropriate methods for the detection of each abnormality. The abnormalities with the most significant impact for treatment and management of BCP-ALL are $t(9;22)(q34;q11)/BCR-ABL1$, $t(4;11)(q21;q23)/MLL-AFF1$ and near-haploidy/low hypodiploidy for high risk stratification and, to a lesser extent, $t(12;21)(p13;q22)/ETV6-RUNX1$ and high hyperdiploidy for good risk management. Apart from the numerical abnormalities, these can be routinely tested for by RT-PCR, providing a basic yet informative screen. However, cytogenetics, particularly fluorescence *in situ* hybridisation (FISH) may provide reliable alternative detection methods dependent upon the preferred technical approach within each protocol.

Introduction

A European Committee for the Biology and Diagnosis of childhood acute lymphoblastic leukaemia (ALL) was initiated in 1989. The treatment protocols of the members of this committee had contributed considerably to improvements in outcome for children with ALL, which led them to publish a consensus of the minimal requirements for the diagnosis, classification and evaluation of treatment (van der Does-van den Berg, *et al* 1992). At that time, cytogenetic analysis was specified as a requirement "to support the diagnosis". The link between karyotype and prognosis had been recognised. It was known that high hyperdiploidy was associated with a favourable outcome in childhood ALL, whilst patients with hypodiploidy had a poor outcome. The translocations, $t(9;22)(q34;q11)$, giving rise to Philadelphia chromosome (Ph) positive ALL, and $t(4;11)(q21;q23)$ were associated with a high risk of treatment failure.

Since both the knowledge of genetic aberrations associated with ALL and the techniques available to detect them has expanded exponentially in recent years, the need to provide an updated description of the genetic diagnosis of BCP-ALL in children in relation to risk stratification for treatment in all protocols has emerged.

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In order to answer the questions frequently asked by haematologists: 1) which genetic aberrations are relevant to the diagnosis and risk stratification of childhood ALL; 2) which methods should be used to detect them and 3) which is the best method for the detection of each abnormality? A panel of experts was convened from within the Committee for Biology and Diagnosis and a survey was conducted among the coordinators of the leading leukaemia protocols. A list of contributing groups is provided in Supplementary Table 1. T-lineage ALL was not included as no protocol yet includes stratification of treatment based on genetics. This manuscript gives their consensus on the diagnostic criteria of genetic abnormalities in BCP-ALL, reviews the relevant technical approaches and offers guidance on the application of the most appropriate methods for the detection of each abnormality, as summarised in Table 1.

Which genetic abnormalities are relevant to diagnosis and risk stratification of BCP-ALL?

Diagnosis of ALL

Before considering the individual genetic changes in relation to prognosis, it must be highlighted that treatment is the most important prognostic factor and that specific diagnostic requirements are protocol dependent. However, there are certain essential diagnostic approaches which are consistent between treatment protocols. These include: 1) morphological and immunophenotypic classification, for which the most recent recommendations have been published by the World Health Organisation (WHO) (2008); 2) determination of minimal residual disease (MRD) status, which is a highly informative prognostic marker (Bruggemann, *et al* 2010). This is performed by clonality testing for immunoglobulin heavy chain and T cell receptor gene rearrangements using tightly standardized quantitative PCR (Q-PCR) (van der Velden, *et al* 2007) and, increasingly, flow cytometry (Basso, *et al* 2009).

Significant Genetic Abnormalities in BCP-ALL

(i) Cytogenetic structural abnormalities

In BCP-ALL, individual chromosomal abnormalities remain strong independent indicators of outcome, especially to indicate risk of relapse (Moorman, *et al* 2010). Among these abnormalities, those with the most significant impact for risk stratification for treatment are t(9;22)(q34;q11)/*BCR-ABL1* and rearrangements of the *MLL* gene. In

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3 particular this applies to t(4;11)(q21;q23)/*MLL-AFF1* (previously known as *MLL-AF4*) The
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5 prognosis of the other *MLL* partners may become significant in the future, particularly
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7 among infants (Pieters, *et al* 2007). The detection of these two abnormalities provides the
8
9 basic criteria for the classification of high risk groups which is applicable to all treatment
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11 protocols. Other significant structural abnormalities include t(12;21)(p13;q22)/*ETV6-RUNX1*
12
13 fusion, as well as t(1;19)(q23;p13.3)/*TCF3-PBX1* fusion. However, these are not used in
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15 risk stratification on all protocols. The *ETV6-RUNX1* fusion occurs in approximately 25% of
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17 younger children with BCP-ALL. These patients have an extremely good prognosis. Among
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19 patients with *TCF3* rearrangements, those with *TCF3-PBX1* were originally regarded as
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21 poor risk on some treatment protocols, but on modern therapy they are classified as
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23 standard risk (Kager, *et al* 2007, Pui, *et al* 1994). In contrast the rare variant,
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25 t(17;19)(q22;p13)/*HLF-TCF3* fusion, has a dismal outcome on all therapies (Hunger 1996).
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27 Thus its accurate identification is important.

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29 Translocations involving *IGH@* at 14q32 are emerging as a significant subgroup
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31 in childhood ALL (Akasaka, *et al* 2007, Chapiro, *et al* 2006, Russell, *et al* 2008, Russell,
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33 *et al* 2009b). It is of interest that they occur more frequently in adolescents and, although
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35 numbers are small, they appear to have an inferior outcome. Currently they are studied
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37 for research purposes only, but their strong clinical associations may lead to the need for
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39 routine screening in the future. Apart from *IGH@* translocations, these structural
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41 changes are important to a greater or lesser extent in directing the choice of treatment.

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43 Intrachromosomal amplification of chromosome 21 (iAMP21) was discovered
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45 during routine screening for the presence of the *ETV6-RUNX1* fusion by fluorescence *in*
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47 *situ* hybridization (FISH) (Harrison, *et al* 2005). Patients are negative for the *ETV6-*
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49 *RUNX1* fusion, while in addition to the two normal copies of the *ETV6* signal, show
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51 multiple *RUNX1* signals (3 or more additional signals) with this probe. In metaphase, one
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53 signal is located to the normal chromosome 21, while the others are seen in tandem
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55 duplication along an abnormal chromosome 21 (Harewood, *et al* 2003). In interphase,
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57 the signals are clustered together, except for one signal representing the normal
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59 chromosome 21 which is usually located apart. Cytogenetics, multiple colour FISH and
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array based comparative genomic hybridisation (aCGH) have shown that the
morphology of the abnormal chromosome 21 is highly variable between patients and
that the commonly amplified region always includes the *RUNX1* gene (Harewood, *et al*
2003, Robinson, *et al* 2007, Strefford, *et al* 2006). This abnormality was originally
described as poor risk (Harewood, *et al* 2003, Moorman, *et al* 2007, Robinson, *et al* 2003,

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3 Soulier, *et al* 2003), although the outcome has since been shown to be protocol dependent
4 (Attarbaschi, *et al* 2008). Thus its accurate detection is important to guide therapy, at least
5 in some protocols.
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10 (ii) *Numerical cytogenetic abnormalities*

11 Significant numerical abnormalities include: high hyperdiploidy (51-65
12 chromosomes) (Moorman, *et al* 2003), near-haploidy (24-29 chromosomes) and low
13 hypodiploidy (31-39 chromosomes) (Harrison, *et al* 2004, Nachman, *et al* 2007). High
14 hyperdiploidy accounts for approximately 30% of childhood BCP-ALL and is characterised
15 by the gain of specific chromosomes. It is associated with a good prognosis in children.
16 Near-haploidy and low hypodiploidy are rare, comprising <1% each of childhood ALL.
17 Their characteristic features are the gain of specific chromosomes onto the haploid
18 chromosome set and, in the majority of patients, the presence of a population of cells
19 with an exact doubling of this chromosome number (Harrison, *et al* 2004). Both are
20 linked to a poor outcome and are used to stratify patients as high risk.
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29 (iii) *Submicroscopic abnormalities*

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31 A significant discovery was the finding that the disruption of genes involved in B-
32 cell development played an important role in leukaemogenesis in childhood BCP-ALL
33 (Mullighan, *et al* 2007). Approximately 40% of these patients had abnormalities of genes
34 involved in the B-cell developmental pathway: *PAX5*, *TCF3*, *EBF1*, *LEF1*, *IKZF1* and
35 *IKZF3*. Other genes frequently affected were those controlling cell cycle progression:
36 *CDKN2A*, *CDKN1B* and *RB1* (Kuiper, *et al* 2007, Strefford, *et al* 2007). Whether there is
37 a link between these genes and outcome has become a critical question (Den Boer, *et al*
38 2009). In particular, the association of *IKZF1* deletions with a poor prognosis (Kuiper, *et*
39 *al* 2010, Mullighan, *et al* 2009b) requires further validation in prospective independent
40 and unselected trial based patient cohorts. Thus at present routine screening is not a
41 recommendation.
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49 Recently, a cryptic translocation, t(X;14)(p22;q32) or t(Y;14)(p11;q32), involving
50 *IGH@* and *CRLF2* in the pseudoautosomal region (PAR1) of the sex chromosomes, and
51 a deletion within PAR1, giving rise to the *P2RY8-CRLF2* fusion, have been reported
52 (Hertzberg, *et al* 2009, Mullighan, *et al* 2009a, Russell, *et al* 2009a, Yoda, *et al* 2009).
53 They lead to overexpression of *CRLF2*, which has been defined as a novel, significant
54 abnormality in BCP-ALL. *CRLF2* alterations, including activating mutations of the *CRLF2*
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3 receptor itself, are associated with activating *JAK* mutations resulting in constitutive
4 activation of the JAK-STAT signaling pathway (Chapiro, *et al* 2009, Hertzberg, *et al*
5 2009, Russell, *et al* 2009a, Yoda, *et al* 2009). Activation of this pathway has been
6 associated with a worse prognosis in adults and children (Cario, *et al* 2010, Harvey, *et al*
7 2010) and has been highlighted as an important consideration for targeted therapy.
8 Following further validation, the detection of *CRLF2* alterations may become a
9 necessary diagnostic test.
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11 The abnormalities given above are listed in Table 1 in priority order based on
12 their importance in risk stratification for treatment applicable to protocols worldwide. The
13 relationships of *IGH@* translocations, rearrangements leading to *CRLF2* deregulation,
14 deletions of the B-cell differentiation and cell cycle control genes with prognosis are
15 currently important for research only. However their true prognostic significance is soon
16 likely to emerge from further detailed studies.
17

18 ***Which techniques are recommended for the Detection of Significant Genetic*** 19 ***Abnormalities in ALL***

20 *Molecular technologies*

21 Molecular technologies make use of DNA or RNA extracted directly from bone
22 marrow. With the increasing numbers of significant chromosomal translocations
23 identified, techniques of reverse transcriptase polymerase chain reaction (RT-PCR) have
24 provided a rapid, accurate and sensitive method for the detection of their fusion
25 transcripts. One major disadvantage is that this approach is highly specific and
26 cooperating abnormalities cannot be simultaneously identified. Quantitative PCR (Q-
27 PCR) measures the relative expression levels, usually of upregulated genes.
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29 *Cytogenetics*

30 Cytogenetic analysis of bone marrow blasts has provided the gold standard
31 technique for the identification of significant numerical and structural chromosomal
32 abnormalities in leukaemia since the 1970's. Some thirty years later it remains integral to
33 the diagnosis of ALL in many laboratories. The main advantage of cytogenetics is that it
34 is well established into clinical practice, it provides a global screen of the entire genome,
35 while the disadvantages include its low resolution and restriction to the analysis of
36 abnormalities visible in metaphase.
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Flow cytometry for determination of DNA index

In a small number of institutions, determination of DNA index by flow cytometry is in routine use to indicate changes in chromosome number. Although it will not identify the specific chromosomes gained, the presence of high hyperdiploid clones can be detected (Forestier, *et al* 1998). Similarly, this approach has identified near-haploid and low-hypodiploid clones (Stark, *et al* 2001). However, there are examples where measurement of DNA index has failed to detect high hyperdiploidy, possibly due to the variable stages of the cell cycle among leukaemic blasts (Bungaro, *et al* 2009).

Fluorescence in situ hybridization

FISH was developed in the 1980's in parallel with molecular techniques. It is based on visualisation of specific DNA probes annealed to their complementary sequences in a target genome. Many probes are now commercially available, providing rapid and accurate screening for most of the significant chromosomal abnormalities in leukaemia. FISH offers a valuable substitute for cytogenetics when used to indicate the presence of specific chromosomal rearrangements, as well as deletion and gain, usually in a dual colour arrangement. One main advantage is that FISH provides information in addition to the result of the specific test for which it is applied.

High resolution array based technologies

These technologies include aCGH, single nucleotide polymorphism (SNP) arrays and gene expression profiling. Arrays for genome-wide microRNA expression, methylation status and mutation screening using deep sequencing approaches are now available. Currently these techniques provide valuable research tools in the study of the cancer genome. They are described in detail in the accompanying review article (Izraeli 2010).

Multiplex Ligation-dependent Probe Amplification (MLPA)

Multiplex Ligation-dependent Probe Amplification (MLPA) is a rapid multiplex PCR based technique that allows the comparative quantification of multiple sites in a single test (Schouten, *et al* 2002). It targets very small sequences and can distinguish those differing in a single nucleotide. Thus MLPA is able to identify those focal genetic aberrations which are too small to be detected by FISH. Although MLPA is not suitable

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3 for genome-wide research screening, it provides a low cost, simple alternative to array-
4 based techniques for many routine applications (www.mrc-holland.com). It is now used
5 routinely to identify copy number alterations in haematological malignancies for which
6 specific copy number changes are clinically significant (Coll-Mulet, *et al* 2008). MLPA
7 has been successfully applied to the simultaneous screening for deletions involving the
8 B-cell differentiation and cell cycle control genes in BCP-ALL (Schwab, *et al* 2010).
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13 14 **What is the most appropriate method to detect each abnormality?**

15 *BCR-ABL1 fusion*

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17 RT-PCR and cytogenetic analysis readily detects the *BCR-ABL1* fusion/
18 t(9;22)(q34;q11). In those cases with a failed or normal cytogenetic result, FISH provides
19 a reliable alternative method (Harrison, *et al* 2005). The use of a dual colour, dual fusion
20 probe is recommended for which the false positive rate is extremely low. At the same
21 time these probes will indicate deletions from the derivative chromosome 9 involving the
22 reciprocal *ABL1-BCR* fusion (Robinson, *et al* 2005). It could be argued that as the
23 presence of the *BCR-ABL1* fusion is the significant event, RT-PCR or FISH could
24 replace cytogenetic analysis for the detection of this abnormality.
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32 *MLL-AFF1 fusion*

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34 As for *BCR-ABL1*, the *MLL-AFF1* fusion/t(4;11)(q21;q23) is readily detectable by
35 RT-PCR and cytogenetics. A multiplex molecular approach may be applied for the
36 detection of this fusion with the advantage of detection of the other common *MLL* fusion
37 partners (Harrison, *et al* 2007). A dual colour breakapart probe provides a highly
38 effective approach for the screening of all chromosomal rearrangements involving *MLL*
39 by FISH, although the application of specific FISH probes or RT-PCR is needed for the
40 detection of partners. The *MLL* breakapart FISH probe also detects 3' deletions
41 associated with *MLL* translocations (Barber, *et al* 2004). It has been shown that not all
42 abnormalities involving 11q23 at the cytogenetic level have a rearrangement of the *MLL*
43 gene (Harrison, *et al* 2005). As such cases have an intermediate prognosis; FISH
44 provides the ideal method to verify the involvement of *MLL*.
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52 *t(17;19)(q22;p13)*

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54 Cytogenetic analysis provides the best method to identify the translocations,
55 t(1;19)(q23;p13) and t(17;19)(q22;p13), while RT-PCR will detect the fusion transcripts;
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3 *TCF3-PBX1* and *TCF3-HLF*, respectively. Alternatively both translocations can be
4 identified by FISH using a breakapart probe to *TCF3* (Barber, *et al* 2007). Generally it is
5 not necessary to screen patients for the presence of the *TCF3-PBX1* fusion. However in
6 view of the high risk associated with t(17;19)(q22;p13), it is advisable to confirm its
7 presence, by FISH or RT-PCR. This should be done in association with consideration of
8 its typical clinical presentation: disseminated intravascular coagulation (DIC) and
9 hypercalcemia, with very high levels of MRD.
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18 *ETV6-RUNX1 fusion*

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20 This fusion transcript can be readily detected by RT-PCR. As the translocation,
21 t(12;21)(p13;q22) is invisible at the cytogenetic level, an extra signal or dual colour
22 fusion probe is usually used for detection by FISH. The advantage of the FISH approach
23 is that fusions arising from rare variant breakpoints are also identified, which may not be
24 detected by RT-PCR. In *ETV6-RUNX1* positive cases, FISH provides information on the
25 status of the second *ETV6* homologue: whether it is retained or deleted, as well as
26 indicating the number of *RUNX1* and fusion signals (Harrison, *et al* 2005). Deletion of
27 the second *ETV6* allele and additional fusion signals are important secondary events in
28 this leukaemia subtype (Attarbaschi, *et al* 2004, Martineau, *et al* 2005).
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36 *High hyperdiploidy, near-haploidy and low hypodiploidy*

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38 The best method for the detection of high hyperdiploidy and near-haploidy/low
39 hypodiploidy (hypodiploid cases) is cytogenetics. Although gene expression signatures
40 have been used to predict changes in chromosome number (Hertzberg, *et al* 2007),
41 there are no truly reliable molecular methods for their detection. Among cases which are
42 *ETV6-RUNX1* negative by FISH, additional *RUNX1* signals observed in interphase often
43 correspond to the additional copies of chromosome 21 seen by cytogenetic analysis in
44 high hyperdiploidy. Thus it can be inferred that additional *RUNX1* signals seen in
45 interphase cells of patients with normal or failed cytogenetic results, when negative for
46 the three abnormalities: *ETV6-RUNX1*, *BCR-ABL1* fusions and *MLL* rearrangements,
47 represent additional copies of chromosomes 21. Hidden high hyperdiploidy has been
48 found among a high proportion of cases with failed and normal cytogenetic results
49 (Harrison, *et al* 2005). Subsequent FISH hybridisation with centromeric probes specific
50 for the trisomies of chromosomes 4, 10, 17 and 18 will identify the good risk group of
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3 high hyperdiploid patients as defined by the Children's Oncology Group (Schultz, *et al*
4 2007) and UK trials (Moorman, *et al* 2003).
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7 In near-haploidy and low hypodiploidy the abnormal clone is often accompanied
8 by a population comprising a doubling of the hypodiploid chromosome number. In such
9 cases the doubled population may represent the predominant one. In these cases the
10 gained chromosomes are usually tetrasomic in contrast to classical high hyperdiploidy in
11 which the gained chromosomes, apart from chromosome 21, are usually trisomic.
12 However the doubling from the hypodiploid clone may not always be complete and thus
13 may be misidentified as high hyperdiploidy. As these two abnormalities represent poor
14 and good risk groups, respectively, their accurate distinction is vital. The mixture of near-
15 haploid and doubled populations makes FISH detection of hidden populations difficult.
16 Probes specific for chromosomes which would be expected to be present as a single
17 copy in the hypodiploid clone need to be applied in parallel with probes specific for
18 chromosomes which would be expected to have four copies within the doubled
19 population. In these cases flow cytometry to measure the DNA index of the two
20 populations may be more appropriate.
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31 *iAMP21*

32 The finding in *iAMP21* patients that the commonly amplified region always
33 includes the *RUNX1* gene has confirmed that probes directed to *RUNX1* offers the most
34 reliable detection method for this abnormality. The *ETV6-RUNX1* probe provides a
35 suitable test. The abnormality is defined as three or more additional *RUNX1* signals. As
36 the subtelomeric region of chromosome 21 is rarely gained and often deleted
37 (Robinson, *et al* 2007, Strefford, *et al* 2006), the accurate distinction of *iAMP21* from the
38 gain of whole chromosomes 21 in interphase cells can be made by calculating the ratio
39 of *RUNX1* to subtelomeric signals. When whole chromosomes 21 are gained, the
40 number of *RUNX1* and subtelomeric signals will be equal in number. In *iAMP21* the
41 number of *RUNX1* signals will always exceed the number of signals from the
42 subtelomere. Thus application of these two probes directed to chromosome 21 provides
43 the most accurate interphase FISH diagnostic test for *iAMP21*.
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53 *IGH@ translocations*

54 FISH using a dual colour breakapart probe will reliably identify translocations
55 involving *IGH@* and in metaphase will identify their partners. Specifically the *IGH@*-
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3 *CRLF2* and the *P2RY8-CRLF2* fusions may be detected by FISH using a breakapart
4 probe flanking *CRLF2*. Alternatively the *P2RY8-CRLF2* fusion can be detected by RT-
5 PCR (Hertzberg, *et al* 2009, Mullighan, *et al* 2009a) and overexpression of *CRLF2* may
6 be demonstrated at the mRNA and protein level by Q-PCR or flow cytometry,
7 respectively (Harvey, *et al* 2010, Russell, *et al* 2009a)
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11 *Submicroscopic deletions*

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15 MLPA has been successfully applied in simultaneous screening of those genes
16 most frequently deleted in high risk BCP-ALL, including the B-cell differentiation genes:
17 *IKZF1*, *PAX5*, *EBF1* and *BTG1*; cell cycle control genes: *RB1* and *CDKN2A/B* and those
18 deleted from the PAR1 region: *CSF2RA* and *IL3RA*, as an alternative method to indicate
19 the presence of *P2RY8-CRLF2* fusion (Schwab, *et al* 2010).
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24 *Conclusions*

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26 A consensus protocol for the genetic stratification of BCP-ALL at the time of
27 diagnosis has been proposed by representatives from the Biology and Diagnosis
28 Committee of iBFM-SG. Based on their relative importance to risk stratification for
29 treatment, the abnormalities have been listed in priority order in Table 1. The algorithm
30 in Figure 2 indicates a proposed strategy for genetic testing in childhood BCP-ALL,
31 which ensures detection of the high risk abnormalities and indicates those abnormalities
32 associated with good risk if their identification is a requirement in a particular protocol.
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38 The abnormalities with the most significant impact for treatment and
39 management of BCP-ALL are *BCR-ABL1*, *MLL-AFF1* and near-haploidy/low
40 hypodiploidy for high risk stratification and to a lesser extent *ETV6-RUNX1* and high
41 hyperdiploidy for good risk management, which on some protocols are used to indicate
42 patients who are suitable for reduced therapies. Apart from the numerical abnormalities,
43 these can be routinely tested for by RT-PCR, providing a simple yet informative screen.
44 Therefore, in the routine clinical setting, where only basic risk stratification is required, it
45 is not necessary to carry out full cytogenetic analysis in addition to RT-PCR and/or FISH.
46 However, the chosen detection method depends to a certain extent upon the preferred
47 technical approach within each protocol. This may be influenced to a certain extent by
48 local expertise and cost. It is difficult to perform in depth cost analyses between
49 protocols. Generally speaking, cytogenetic preparation of samples is economical, while
50 the analysis is highly labour intensive, requiring highly skilled cytogeneticists. FISH is a
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3 reasonably inexpensive technique and, with the good quality probes now available
4 commercially, many cells can be rapidly screened to provide a timely result. RT-PCR,
5 although highly specific, also provides a rapid, relatively inexpensive test.
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8 Although we are within an era of rapidly advancing technologies, RT-PCR and
9 cytogenetics remain the gold standard methods for clinical diagnosis in BCP-ALL.
10 However, a targeted FISH approach, as illustrated in Table 2, will efficiently detect the
11 most significant chromosomal abnormalities. Table 2 also indicates whether RT-PCR
12 may provide a suitable alternative. As an illustration, if resources and time are limited,
13 the application of a single probe for *ETV6-RUNX1* will identify the presence of an
14 abnormal clone in more than 60% of childhood BCP-ALL overall, reinforcing the
15 economy of using a FISH approach. The majority of these patients will fall into three
16 distinct subgroups: *ETV6-RUNX1* positive (25%), high hyperdiploidy (30%) and iAMP21
17 (2%) (Harrison, *et al* 2005) (Figure 1). With the advent of novel technologies (Izraeli
18 2010), it is probable that we will require information on gene and miRNA expression as
19 well as methylation and mutation status in order to fully characterise the genome of
20 BCP-ALL patients for complete diagnostic and prognostic information. Ongoing research
21 into the application of these techniques will determine their future diagnostic role. Thus it
22 is imperative to store cells to enable such studies to be carried out retrospectively. As
23 further evidence is accumulating, the influence of the constitutional genotype on
24 outcome, in cooperation with the acquired genetic changes, will have to be considered in
25 relation to their relative impact on modern pharmacogenomic approaches to treatment.
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Table 1: Priority of genetic abnormalities for testing in childhood BCP-ALL

Priority	Aberration	Reason for Priority	Genetic subgroup	Molecular genetic features	Suitable methods for testing*
Highest	t(9;22)(q34;q11) and variants	High risk abnormality	<i>BCR-ABL1</i>	<i>BCR, ABL1</i>	RT-PCR, Cytogenetics, FISH
	t(4;11)(q21;q23)	High risk abnormality	<i>MLL</i> rearrangement	<i>MLL, AFF1</i>	RT-PCR, Cytogenetics, FISH
High	Near-haploidy	High risk abnormality	Numerical abnormality	Whole chromosome gains onto the haploid set, doubled population may manifest as high hyperdiploidy	Cytogenetics, FISH, DNA index
	other <i>MLL</i> partners	Potentially high risk abnormality	<i>MLL</i> rearrangement	<i>MLL</i>	FISH, Cytogenetics, RT-PCR
	t(17;19)(q22;p13)	High risk abnormality	<i>TCF3</i>	<i>TCF3, HLF</i>	Cytogenetics, FISH, RT-PCR
	t(12;21)(p13;q22)	Cryptic good risk abnormality	<i>ETV6-RUNX1</i>	<i>ETV6, RUNX1</i>	RT-PCR, FISH
	iAMP21	Potentially high risk abnormality		? <i>RUNX1</i>	FISH
	High hyperdiploidy	Good risk abnormality	Numerical abnormality	Whole chromosome gains	Cytogenetics, FISH, DNA index
	Desirable	<i>IGH@</i> translocations	Potentially high risk abnormality	<i>IGH@</i> translocation	<i>IGH@</i>
<i>IGH@-CRLF2</i>		Potentially high risk abnormality	<i>CRLF2</i> over expression	<i>CRLF2</i>	FISH, MLPA, Q-PCR, protein expression
<i>P2RY8-CRLF2</i>		Potentially high risk abnormality	<i>CRLF2</i> over expression	<i>CRLF2</i>	FISH, MLPA, Q-PCR, protein expression
<i>IKZF1</i> **		Potentially high risk abnormality	<i>IKZF1</i> deletion		MLPA

*in priority order

**may change to high priority, need to assess alongside *CRLF2* and *JAK* mutations.

Table 2: Example of a targeted FISH approach to detect the most significant chromosomal abnormalities in BCP-ALL

FISH Probe	Target Abnormality	Percentage of BCP-ALL Affected	Other Analysis	Incidentally identified changes	RT-PCR applicable
<i>ETV6-RUNX1</i>	t(12;21)(p13;q22)	25	N/A	Deletions, duplication, +21,	Yes
	High hyperdiploidy	30	Selected centromeres	Gain of chromosome 21	No
	iAMP21	3		Amplifications	No
<i>MLL 3' 5'</i>	11q23 translocations	9	Partners	Deletions	Yes
<i>BCR-ABL1</i>	t(9;22)(q34;q11)	4		Extra Ph, deletion ABL-BCR, i(9)(q10)	Yes
<i>IGH 3' 5'</i>	14q32 translocations	8	Partners	Deletions, gain of chromosome 14	Q-PCR for overexpression of partner gene
<i>TCF3 3' 5'</i> (For confirmation)	t(1;19)(q23;p13) t(17;19)(q22;p13)	6, <1	Cytogenetics	Deletions and gains	Yes

Figure 1

This figure shows the application of a probe specific for the *ETV6-RUNX1* fusion, *ETV6* is labelled in green and *RUNX1* in red. In a case with the *ETV6-RUNX1* fusion (a; interphase, b; metaphase) there is one green signal corresponding to the normal *ETV6* gene on chromosome 12, one red signal corresponding to the normal *RUNX1* gene on chromosome 21 and a diminished red signal relocated to the derived chromosome 12. A red green fusion signal is present on the derived chromosome 21. c) In a cell from a patient with high hyperdiploidy and four copies of chromosome 21, four red signals and two green signals are seen in interphase. d) In this metaphase from a different case with high hyperdiploidy, the three copies of chromosome 21 are shown each with a red signal. e) Interphase cells from a patient with intrachromosomal amplification of chromosome 21 (iAMP21). The red signal corresponding to the normal chromosome 21 is located apart, whilst the multiple signals of the abnormal chromosome 21 are clustered. f) A metaphase from a patient with iAMP21 showing the duplication of red signals along the abnormal chromosome 21 and the single signal on the normal chromosome 21.

Figure 2

An algorithm indicating the priority for genetic testing based on risk stratification. The best method for detection of hypodiploidy (near-haploidy/low hypodiploidy) is cytogenetics. For detection of *BCR-ABL1*, *MLL (MLL-AFF1)* and *ETV6-RUNX1* (green boxes) RT-PCR or FISH may be used, however alternative *MLL* partners and the detection of high hyperdiploidy and iAMP21 requires FISH. This approach will ensure the identification of the high risk abnormalities (red boxes) and indicate the good risk abnormalities if required (yellow boxes).

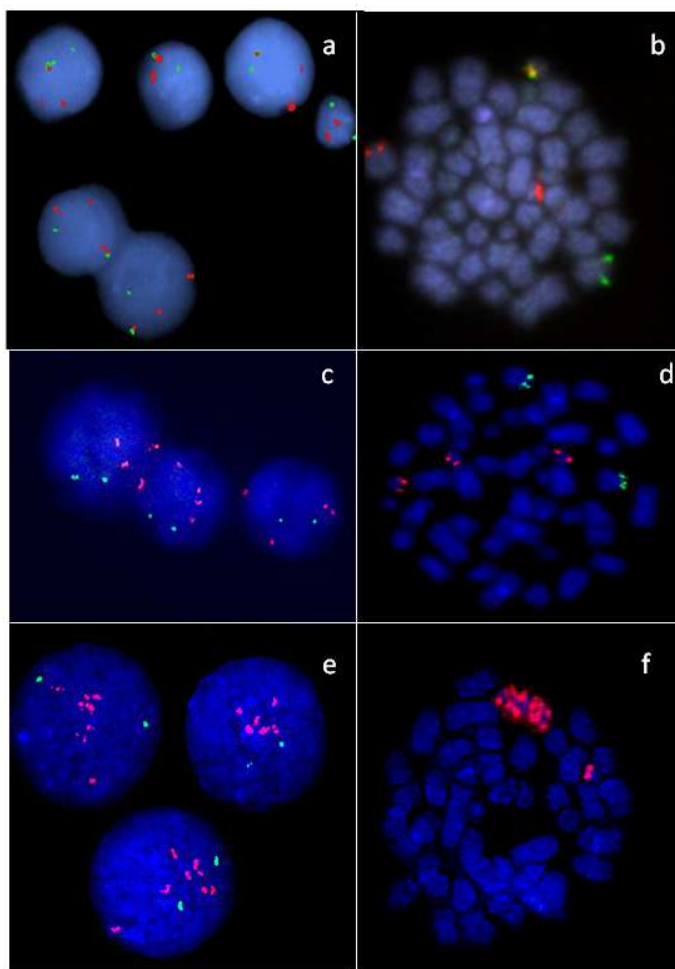


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showing the duplication of red signals along the abnormal chromosome 21 and the single signal on the normal chromosome 21.

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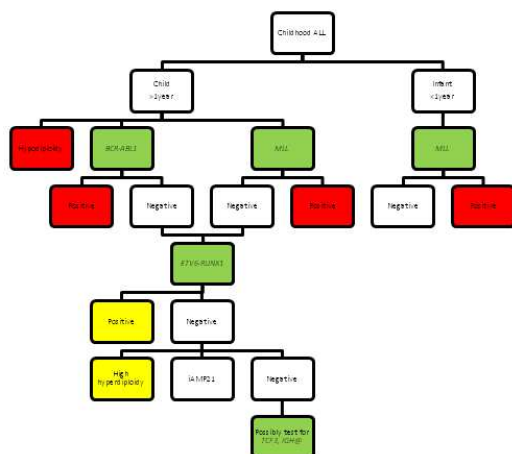


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3 The major protocols are coordinated by individual groups throughout the world:
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5 AIEOP (Associazione Italiana di Emato-Oncologia Pediatrica)
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7 BFM (Berlin-Frankfurt-Munster) Austria
8

9 BFM Germany
10

11 Croatia Haematology Oncology Study Group
12

13 CPH (Czech Pediatric Hematology)
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15 (DCOG) Dutch Childhood Oncology Group
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17 EORTC (European Organisation for Research and Treatment of Cancer) Childhood Leukaemia Group
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19 FRALLE
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21 Hungary
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23 INS (Israel National Study)
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25 JPLSG (Japanese Pediatric Leukaemia/Lymphoma Study Group)
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27 NOPHO (Nordic Society of Pediatric Hematology and Oncology)
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29 PPLG SG (Polish Leukemia/Lymphoma Study Group)
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31 TCCSG (Tokyo Children's Cancer Study Group)
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33 UK CCLG (Children's Cancer and Leukaemia Group)
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