The study of minimal residual disease in acute lymphoblastic leukaemia

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Introduction
Chemotherapy now produces haematological remission in more than 95% of children and about 70% of adults with acute lymphoblastic leukaemia (ALL). However, approximately 30% of children and 70% of adults will relapse, despite the use of post-induction chemotherapy. This is usually given in the form of blocks of consolidation followed by maintenance treatment over a period of two years. Retrospective risk factor analysis has revealed that certain groups of patients are particularly likely to relapse, but this information does not permit accurate judgement of long term prognosis for an individual patient.

Haematological remission, which is usually achieved by day 28 of therapy, pertains to that state in which no evidence of involvement of the marrow is seen using light microscopy and normal haemopoietic activity has resumed. By convention, this is defined by the blast count in marrow being less than 5% with a normal peripheral blood count. Minimal residual disease (MRD) refers to the presence of leukaemic cells in the bone marrow below the level of detection of standard morphological assessment. From animal studies, it has been estimated that in an adult human there may be 10^5 leukaemic cells present throughout the body at the time of presentation. Therefore, up to 10^6 leukaemic cells may still be present at haematological remission and it is these cells which are responsible for relapse if post-induction chemotherapy fails to eradicate them.

The study of MRD has permitted both improved understanding of the biology of ALL and a better assessment of each patient's response to treatment. The long term aim of these studies is to stratify patients into high and low risk groups and, perhaps, develop individualised treatment regimens. This could mean treatment intensification—for example, bone marrow transplantation (BMT), for some, but dose reduction for others (thereby diminishing the risk of long term sequelae of chemotherapy treatment which is of particular importance in children).

Any marker of leukaemia used to investigate MRD should fulfil the following requirements: (1) wide applicability to all subgroups of ALL; (2) stability during the course of the disease; (3) sensitive and specific detection of disease; (4) reproducibility; and (5) an estimate of quantity of disease.

The purpose of this leader is to discuss the methods available to detect these markers with particular reference to the polymerase chain reaction (PCR) and it use in the amplification of immunoglobulin and T cell receptor (TCR) gene rearrangements. This is the technique that most closely fulfils the above criteria at the present time.

Methods used for the detection of minimal residual disease in ALL
NON-MOLECULAR TECHNIQUES
The sensitivity of morphological assessment of marrow is set conventionally at 5% due to the presence of non-malignant blast cells in normal marrow. Routine marrow aspirates throughout the period of therapy are not predictive of relapse and rebound lymphocytosis, with increased numbers of blast cells at the time of marrow regeneration after chemotherapy, can be falsely interpreted as relapse.

Some leukaemic cells express combinations of immunophenotypic markers which are only rarely present in normal bone marrow or peripheral blood. Using FACS analysis these combinations of markers can be used to assess MRD with a reported sensitivity of up to 10^-5. However, overall sensitivity is usually of the order of 10^-2 to 10^-3, which is inferior when compared with PCR and this method has not been widely adopted.

Acquired, non-random chromosomal translocations occur in 30–70% of cases of ALL and can be used as markers of disease. This approach has limited sensitivity (1–5% depending on the number of metaphases analysed) and requires cell culture. Fluorescence in situ hybridisation (FISH) using chromosome specific or locus specific probes permits disclosure of abnormalities in cells at interphase and obviates the need for metaphase spreads. However, sensitivity remains at the 1% level.

MOLECULAR TECHNIQUES
Targets and methods
There are two main targets for the molecular detection of MRD: translocations and gene rearrangements. These can be detected by Southern blotting and PCR. Southern blotting relies on the ability of restriction enzymes to cut DNA consistently where they recognise specific sequences. The resultant restriction products are separated by size on an agarose gel by electrophoresis and transferred (blotted) on to a nitrocellulose or nylon membrane. Subsequent probing with a radioactively labelled probe specific to the target locus followed by autoradiography enables visualisation of the products. Translocation of chromosomal material or gene rearrangement results in deletion
or relocation of DNA segments (see later) causing a change in the distance between restriction enzyme cut sites which becomes apparent in the distance that their products travel on agarose gel. This technique has been used to recognise both tumour specific translocations and clone specific gene rearrangements at diagnosis. A major advantage of Southern blotting is that it is able to demonstrate deletion of a target allele by lack of probe hybridisation and is not influenced by PCR variables such as the requirement for specific binding sites. It therefore provides a wider picture than that provided by PCR.

Unfortunately, sensitivity of detection of a specific sequence in a polyclonal mixture of cells (as in haematological remission) is usually between 1 and 5%. Using enrichment techniques, this can be increased to 0.2% but this is still insufficient for the study of MRD. In addition, larger amounts of high quality DNA (10 μg per analysis) are needed than with PCR.

PCR is a potent technique permitting amplification of DNA defined by two known sequences at each end. Gene rearrangements generate short regions of high variability bounded by conserved sequence. As such, they are amenable to direct DNA PCR. Similarly, if both breakpoints in a particular translocation cluster within a small region then, using one primer from each of the involved genes, PCR can be performed directly on DNA and produce a translocation specific product. The situation for the majority of translocations is more complex as many translocations occur with an unclustered breakpoint within a large intronic region. The intervening segment of DNA between the primers is then too large to amplify. This problem can be overcome by designing one primer to the exon of each of the involved genes. Although these are widely separated in DNA, they become juxtaposed after splicing out of intronic segments after transcription into RNA. PCR of RNA requires an initial reverse transcriptase step yielding complementary DNA (cDNA) which is then used as template for the reaction (RT-PCR).

PCR is generally 1000 times more sensitive than Southern blotting and can be performed directly on DNA from a variety of sources including marrow slides, although DNA extracted from density gradient separated white cells yields the most reproducible results, especially from hypocalcellular samples. Meanwhile, RNA must be extracted from fresh samples because of the presence of ubiquitous RNases. However, due to the power of PCR, particular care must be taken to avoid contamination, especially where the same product is being amplified repeatedly—for example, with RT-PCR and “nested” PCR (see later).

**Tumour specific translocations**

\( t(9;22)(q34;q11) \)—Philadelphia (Ph\(^1\) chromosone positive ALL carries a poor prognosis. Molecular characterisation of this translocation (fig 1) and the development of Southern blot and PCR based systems for its detection have revealed a much higher incidence of Ph\(^1\) positive ALL than that seen by conventional karyotyping. RT-PCR yields evidence of bcr-abl transcript in 6% of children and up to 55% of adults with ALL. As yet, this

### Figure 1

The Philadelphia chromosome (Ph\(^1\)) involves the transposition of part of the abl proto-oncogene from chromosome 9 to a new position on chromosome 22. The Ph\(^1\) translocations seen in ALL are indistinguishable at the cytogenetic level; 50–80% of cases involve a breakpoint in a large intronic region \( S \) to the minor breakpoint cluster region (m-bcr) which relocates to a region \( S \) to exon 2 of the abl oncogene giving rise to the so-called ela2 transcript and a 190 kDa protein. The other 20–30% of cases have translocations involving the major breakpoint cluster region (M-bcr), with breakpoints between exons b2 and b3 and between exons b3 and b4. Both these latter translocations produce a 210 kDa protein.
marker has not been widely used for detecting MRD in ALL. From the published data so far, it appears that absence of detectable bcr-abl transcripts after chemotherapy alone is uncommon and that allogeneic BMT can lead to PCR negativity. The relative rarity of Ph+ ALL in children means that bcr-abl/PCR is not widely applicable.

**t(1;19)(q23;p13)—**Twenty five per cent of pre-B, cytogenetic μ-positive ALL (5-6% of childhood ALL overall) bear a t(1;19) translocation. This translocation results in the juxtaposition of the element of the E2A gene that codes for the transcription activating motif with the DNA binding homeodomain of the Pbx1 gene and is associated with a poor prognosis. Again, a RT-PCR approach is necessary for amplification and this correlates well with cytogenetic analysis. On occasion, it may uncover the rearrangement where cytogenetics has failed or produced a negative result. However, this approach has not yet been used for MRD analysis and, as with bcr-abl, suffers from a lack of broad applicability.

**t(17;19)(q22;p13)—**This rare translocation occurs in 1% of cases of ALL and is often associated with disseminated intravascular coagulation. The amino terminal transactivation domain of E2A is linked to the leucine zipper and basic domains of the HLF (hepatic leukemic factor) normally expressed in hepatocytes and renal cells, and not in lymphocytes. This E2A-HLF rearrangement has been monitored by three patients with this translocation. One patient had resistant disease and the other two followed a relapsing/remitting course ending in relapse after BMT. Amplifiable chimeric E2A-HLF transcript was detectable throughout the course of disease.

**t(1;14)(p33;q11) and the tal-1 rearrangement—**The t(1;14) translocation involves transposition of the Y' end of the tal-1 (also called sc1 or to1) gene onto the TCR a/b locus on chromosome 14. Although characteristic of T-ALL, this translocation is rare. However, in approximately 30% of cases of T-ALL, the tal-1 gene recombines in a site specific manner to the δ locus, on chromosome 1, resulting in a 90 kilobase deletion. In addition, random insertion and deletion of nucleotides takes place at the point of rearrangement providing a highly variable sequence that is clone specific and that can be used to track MRD. Because the rearrangement is site specific, PCR can be performed directly on DNA without a reverse transcription step.

**t(4;11)(q21;q22) and related translocations—**Translocations involving 11q23 are found in 5% of cases of childhood ALL and up to 70% of infant acute leukemia. The rearrangements most commonly involve 1p32, 4q21 and 19p13. Two groups have shown that the 11q23 breakpoints cluster in a <5 kilobase area and this area has been found to have sequence homology with the *Drosophila* trithorax gene. Although suitable for a RT-PCR approach, this rearrangement has not yet been used for the analysis of MRD.

In summary, translocation specific PCR is more sensitive than conventional karyotyping at diagnosis. While the various systems provide reproducible, specific and sensitive detection of MRD, their limited incidence, particularly in children, restricts their applicability.

**Immunoglobulin and T cell receptor gene rearrangements**

**Rationale—**Lymphoid cells make antibody or TCR proteins. These are encoded by a series of genes which, when rearranged, generate specific sequences. Very nearly all cases of ALL have undergone rearrangement of at least one of the immunoglobulin heavy chain (IgH), TCRγ or TCRδ loci. As they are the progeny of a single cell, the lymphoblasts will be characterised by at least one unique gene rearrangement which can be used to track MRD.

**Pathobiology—**It has been estimated that almost all of the genome would be used up if each possible immunoglobulin and TCR protein were individually encoded. The IgH locus illustrates how this problem is surmounted. The variable region of each antibody's heavy chain is coded for by a variable (VH), diversity (DH) and joining (JH) gene. In the germline configuration, some 200 VH, 30 DH and six JH genes (table 1) are widely separated by intronic sequences and arranged in families on chromosome 14 band q32.

The huge diversity of antibody specificity is achieved by the random recombination of one individual member of each of these families, with deletion of the intervening segments. First, a DH to JH rearrangement is formed, with the removal of bases by exonucleases and the addition of new bases by terminal deoxynucleotidyl transferase (TdT) at the DH1/JH junctions, to produce a partial -Dδ4JH rearrangement. This is followed by a similar VH to -Dδ4JH recombination resulting in a full -VH-Dδ4JH rearrangement (fig 2). The junctional sequence generated by this process encodes the antigen specific part of the antibody known as the third complementarity determining region (CDR3). The full length coding sequence for a functional IgH molecule is achieved by a last recombination with the constant region (Cμ) segment. The recombination of germline V, D and J genes alone would produce significant diversity, but the addition and/or removal of junctional ("N") nucleotides increases this considerably. These rearrangements are still subject to the antigen driven somatic mutation and secondary gene rearrangement later on in B cell maturation, which achieves improved antibody specificity.

Broadly similar events occur at the immunoglobulin light chain and TCR loci. The
latter differ in that they have fewer members of each V, D and J family and the \( \gamma \) locus does not use a D region (table 1 and fig 3). Partial rearrangements are common at the TCR\( \delta \) locus—for example, \( \psi \delta \) and \( \psi \delta \)-deficient ALL.\(^{76,77} \) In ALL, IgH and TCR rearrangements are not lineage restricted and this is referred to as lineage infidelity. Thus, clonal rearrangement of TCR\( \delta \) or \( \gamma \) loci is seen in a large proportion of B lineage ALL\(^{78,79} \) and a smaller proportion of IgH rearrangements are found in T-ALL\(^{80,81} \) (table 2). The TCR\( \alpha \) and \( \beta \) loci rearrange later in T cell ontogeny than the TCR\( \gamma \) and \( \delta \) loci and the IgH locus rearranges before the immunoglobulin light chain (IgL, and IgLa) genes in B cell ontogeny.\(^{31} \) Because of this, the IgL and TCR\( \alpha \) and \( \beta \) loci are less commonly rearranged in leukaemic lymphoid cells and will not be discussed further.

**Detection of clone specific markers in ALL**

Presentation or relapse marrow contains predominantly leukaemic cells. Therefore, PCR performed at these times, using primers designed to the conserved sequences flanking the region of rearrangement, will amplify across the rearrangement yielding a product(s) that is (are) leukaemia specific. These clonal bands can be visualised after polyacrylamide gel electrophoresis (PAGE). Because of the polyclonal nature of the content of marrows taken during

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**Table 2. Rate of success of PCR amplification using different primer combinations in B and T lineage ALL at presentation**

<table>
<thead>
<tr>
<th>PCR primer combination</th>
<th>Number positive by PCR at presentation (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>B lineage</td>
</tr>
<tr>
<td>FR3-( \psi \gamma ^{9,83} )</td>
<td>75</td>
</tr>
<tr>
<td>FR1-( \psi \alpha ^{9,85} )</td>
<td>95</td>
</tr>
<tr>
<td>V( \delta )-( \psi \alpha ^{9,87} )</td>
<td>45</td>
</tr>
<tr>
<td>D( \delta )-( \psi \gamma ^{9,89} )</td>
<td>15</td>
</tr>
<tr>
<td>V( \delta )-( \psi \delta ^{9,87} )</td>
<td>5</td>
</tr>
<tr>
<td>V( \psi \alpha ^{9,87} )</td>
<td>95</td>
</tr>
</tbody>
</table>
MRD in ALL

Figure 4 Diagram depicting the possible ways of investigating patient DNA for the presence of MRD (see text for explanation).

haematological remission, most methods require a second step to pick out the leukaemia specific PCR products from those of normal lymphocytes (fig 4).

“Gene fingerprinting” is a single step approach that relies on the resolution of radio- or fluorochrome labels of products on a sequencing gel, followed by autoradiography or gene scanning as appropriate. This provides single base separation and permits differentiation of “clonal products” from the background produced by normal rearrangements. This simple technique obtains a sensitivity of $10^{-3}$. It is not sequence specific and relies on a combination of the “fingerprint” pattern and size of rearrangement for disclosure of leukaemia.

All the other strategies make use of the leukaemia specific rearrangement to provide probes or primers for the detection of MRD in remission samples. In the methods involving probing, PCR products derived from 1 μg remission DNA, along with the products from 1 μg normal marrow DNA, are transferred to a nylon membrane before hybridisation with a radiolabelled clone specific probe (dot or electroblotting). Residual disease is considered to be present if the hybridisation of the probe is stronger with the remission DNA sample than that seen with the normal control. The probes used in this approach vary in their complexity and cost. The simplest and cheapest method involves direct use of the presentation or relapse PCR product as a probe. This can be cut out and eluted from the PAGE gel and then radiolabelled. While this is simple and cheap, its sensitivity is limited by the presence of common primer and other sequences in the leukaemic and normal products. This, in part, can be obviated by the enzymatic removal of the common primer sequence to improve specificity. The maximal sensitivity of this approach is $10^{-5}$ but this is not uniformly found. The gold standard approach using probes requires the use of 20 base junction specific oligonucleotide probes which provide maximal sensitivity and specificity.

A further method involves incorporation of the PCR products into a bacteriophage library. Leukaemic DNA is recognised by lifting off the resultant colonies on to a nitrocellulose filter and hybridising with a radiolabelled, leukaemia specific probe (phage colony assay). This method achieves a sensitivity of $10^{-4}$ to $10^{-5}$. It also has the advantage of providing quantitation of the number of leukaemic cells present as a proportion of the total number of B cells and permits confirmation of the presence of the leukaemic sequence.

Alternatively, sequence analysis of presentation specimens can be used to design clone specific primers. These can be used, later on, to amplify DNA from remission samples directly. More commonly, after a first round of PCR using the original primers, a second, “nested” or “hemi-nested” round using two or one internal, patient specific primers can be performed. These approaches have the advantage that the resultant product can be visualised on a gel without the need for blotting and avoids the use of a radioisotope. Again, a sensitivity of $10^{-3}$ is achieved routinely.

Application of gene rearrangement PCR to the detection of MRD

Which are the best loci to study?—In B-lineage ALL, the IgH locus is the most appropriate for the study of MRD. The use of up to seven family specific primers designed to the FR1 region amplifies a clonal rearrangement in 95% of cases of ALL at diagnosis. A simpler approach involves the use of a single FR3 consensus primer and produces a clonal band...
in approximately 75% of ALL cases (table 2). In T-ALL, TCRγ PCR provides the most comprehensive marker. If several loci are studied, this permits the assessment of the greatest number of patients with maximal insurance against false negativity. We now use such a multilocus IgH, TCRδ and TCRγ PCR at diagnosis and follow each unique sequence. This approach has allowed us to study 197 of 205 patients by at least one locus.

Initial data from Southern blot studies using IgH probes suggested that up to 40% of B lineage ALL showed multiple clonal rearrangements at diagnosis and that in up to 50% of patients the band pattern changed between presentation and relapse. From this information, it appeared that IgH PCR required the use of multiple probes and, in spite of this, a high rate of false negative MRD detection would occur as a consequence of changes in the marker. This would render the IgH locus, the most widely applicable gene rearrangement in ALL, a poor marker of MRD. This led several groups to use TCR PCR in preference to IgH.

However, data from more recent PCR based studies show clearly that most oligoclonality and instability at the IgH locus arises as a consequence of secondary gene rearrangement in subclones of the original leukaein. This is characterised by \( V_{\mu}D_{\mu}N_{\mu} \) disruption but preservation of the same \( D_{\lambda}N_{\lambda}H \) sequence. In our own work, of the 140 PR3 and PCR positive cases of ALL that we have now sequenced, 25% had two, and only 10% more than two rearrangements at diagnosis. In each of these patients with multiple bands nearly half of the bands shared a common \( D_{\lambda}N_{\lambda}H \) region and therefore were related. In a study of 55 patients between presentation and relapse we found that 12 of 37 (31%) of patients evaluable by IgH-PCR and six of 24 (25%) evaluable by \( V_{\delta}2-D_{\delta}3 \)-PCR relapsed with different rearrangement patterns. However, nine of 12 of the IgH-PCR group relapsed with related subclones whereas the change at the TCRδ locus was characterised either by deletion or the appearance of a completely unrelated sequence. In a similar study, Taylor et al\(^{104} \) estimated the rate of relapse with an unrelated sequence at the TCRγ locus to be 25%. Therefore, the apparent high rate of oligoclonality and instability at the IgH locus can be overcome by the design of \( D_{\lambda}N_{\lambda}H \) oligonucleotide probes. The rate of complete clonal change—that is, relapse with a completely unrelated sequence, is less at the IgH locus than at the TCR loci.

**What is the place of quantitation?**—Regardless of eventual outcome, many patients are found to have MRD when investigated during therapy. Thus, quantitation of the amount of residual disease may be desirable. Accurate quantitation is not straightforward, as PCR amplification shows sigmoid characteristics with a plateau effect at high target concentration and poor amplification at low level. It has been calculated that the linear range of amplification at the IgH locus is between 1 leukemic cell in 100 \( (10^{-2}) \) and 1 in 10 000 \( (10^{-4}) \) normal cells.\(^105\) The plateau effect can be controlled, to some degree, by reducing the cycle number and the use of nested PCR optimises amplification of the low level target. At its most simple, therefore, one can infer that the lack of detectable MRD in 1 \( \mu \)g DNA, using a fully sensitive patient specific probe, suggests that disease is not present at a level at least as low as \( 10^{-4} \). Semiquantitative assessment of MRD, and of probe sensitivity, is provided by the use of logarithmic dilutions of leukemic DNA in normal marrow DNA. This only provides quantitation accurate to within one log over the “linear” portion of amplification.

More accurate measurement may be achieved by the use of competitor assay\(^105\) or the phage colony assay.\(^106\) The only truly quantitative approach for gene rearrangements is a limiting dilution method involving multiple replicate dilutions and nested PCR.\(^107\) Such an approach is limited by the need to design clone specific primers and is time-consuming. Initial results do, however, suggest that stratification based on this type of analysis may be possible.\(^108\)

**Can MRD studies predict relapse?**—Our current understanding of the behaviour of MRD in childhood ALL comes primarily from a series of retrospective studies.\(^109 110 111 112\) Although these have been performed by a variety of techniques of differing sensitivity, and in patients treated according to varied protocols, some definite trends are becoming apparent. Initially, almost all patients remaining in remission had detectable MRD in the first six months of therapy and, although this subsequently waned, up to one third had MRD late in the second year. By contrast, all published patients with detectable MRD at or beyond the end of treatment and who have had long term follow up have relapsed.\(^107 109 111 112\) Patients with significantly rising levels of MRD at any time seemed destined to relapse. In general, these early studies were performed in patients still undergoing treatment, (and who had therefore had limited follow up) or who had already been in remission for 30 months or more.

Recently, our own results, and those of others, suggest a slightly different pattern. This may reflect improved methodology or an increase in the intensity of chemotherapy used. We have now analysed 137 serial samples retrospectively. These were taken at various times during and beyond the end of therapy from 44

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**Table 3  Number of patients positive by PCR at various stages of treatment, subdivided by outcome (137 samples were studied from 44 patients with ALL with a median follow up of 39 months from diagnosis)**

<table>
<thead>
<tr>
<th>Months from diagnosis</th>
<th>1</th>
<th>3</th>
<th>&gt;6</th>
<th>End</th>
<th>+3/12</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM relapse off treatment</td>
<td>8/10</td>
<td>7/8</td>
<td>4/6</td>
<td>3/4</td>
<td>7/15</td>
</tr>
<tr>
<td>EM relapse off treatment</td>
<td>4/4</td>
<td>2/2</td>
<td>1/2</td>
<td>1/1</td>
<td>2/8</td>
</tr>
<tr>
<td>In remission off treatment</td>
<td>9/20</td>
<td>4/14</td>
<td>1/11</td>
<td>0/0</td>
<td>0/21</td>
</tr>
</tbody>
</table>

BM = bone marrow; EM = extramedullary; End = end of therapy; +3/12 = three months after the end of therapy.
patients (table 3). In contrast to the earlier studies, we have found that a number of patients, destined to remain in remission off therapy, show relatively rapid clearance of MRD early in treatment. Those patients destined to relapse show slower clearance of disease, and this often persists into the second year. Approximately 50% of patients who relapse after completing therapy still have detectable disease at the end of treatment. It should be emphasised that this is a retrospective study performed using single round PCR and, as such, may underestimate the presence of very low level MRD. None the less, it appears that this serial semiquantitative assessment does permit a group of patients at risk of relapse, and therefore requiring further study, to be highlighted early in therapy. By contrast, the Houston group are performing a prospective study using sensitive, nested, patient specific PCR performed on replicate reactions and combined with quantitation. Again, early results have suggested that MRD is detectable in most patients during therapy regardless of outcome. However, no information is given about the level of this disease and follow up in this study is short. Fewer than 10 of the patients have reached the end of treatment.113

Single time point studies of the level of MRD at the end of induction are an attractive option as, if accurate stratification could be obtained at this time, this would provide maximal opportunity to modify therapy. Two studies have used quantitative methods to address this question.109 114 Although both could confidently predict relapse in some patients, this was only the case for a minority. Any lowering of the threshold would have led to unnecessary intensification of therapy in patients destined to remain in remission. The other obvious single time point for study is the end of treatment. Most investigators concur that PCR negativity at this time is required for continued remission.87 88 112 We have studied samples taken from 44 patients at the end of therapy. Of the 21 patients remaining in remission with a median follow up of 39 months from diagnosis, none had MRD at the end of therapy. In contrast, seven of 15 patients who relapsed had MRD at the end of treatment. In spite of this, the rate of false negative assessment at the end of therapy is unacceptably high and, although further strong prognostic information can be obtained from analysis of samples taken three months after the end of treatment, relapse often occurs soon afterwards. It can also be seen that PCR of marrow is clearly a poor predictor of extramedullary relapse (table 3).

In summary, the patterns of residual disease seen in children with ALL are dependent on the methodology and the integrity of the DNA in the samples used for its detection. In general, it appears that serial, rather than single time point, analysis will provide optimal prediction of relapse. As yet, it remains unclear whether exhaustive quantitative PCR will be clinically relevant in ALL. Any single time point study undertaken during therapy will require a quantitative approach, as will very sensitive, nested methods. It may be, however, that serial semi-

quantitative assessment with an oligoprobe based or fingerprinting method may suffice. Only large scale, prospective, blinded analyses using several standardised techniques of varied sensitivity and complexity to investigate the same samples can answer this question.

Conclusion

The study of MRD has generated exciting new insights into the behaviour of ALL. A number of reproducible and robust techniques have been developed and although these have not yet been standardised, consistent trends of clearance of MRD associated with outcome are becoming apparent. It is now time for these techniques to be used in prospective studies. These should attempt to answer three main questions. First, how sensitive and complex an approach is required to obtain acceptable predictive information for the greatest number of children? Second, can a single time point study at any time predict eventual relapse reliably and permit treatment intensification? Third, if sequential studies are required, at what interval should the samples be taken? Several such prospective analyses are now being undertaken including our own six centre study of the value of MRD detection at the end of treatment and a large European (BFM) trial. Currently, the primary goal of stratification of treatment intensity for individual patients has not been achieved, although we believe that this will become possible within the next decade.

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