Table 2. Thyroid carcinoma (TC) in the suppressed controlateral thyroid lobe in 3 patients (5%) out of the 60 patients with solitary hyperfunctioning thyroid adenoma (TA) described in Table 1

	Case 1	Case 2	Case 3
Age (years)	62	54	68
Sex	Female	Female	Female
Presence of TA (months)	8	10	12
TA size (cm)	3	2	3
TC size (cm)	0.9	0.7	0.8
TC histology	PC	PC	PC

ultrasound examination to detect carcinomatous thyroid lesions at the time of diagnosis could suggest recent tumoral growth in our 3 patients.

In conclusion, our study reports that in patients with a hot thyroid nodule, there is a 5% incidence of carcinoma in the suppressed lobe of the thyroid gland. This supports a cautious approach in the management of hot thyroid nodules. A careful, complete surgical examination of the whole thyroid gland and intraoperative biopsies of abnormal areas are advocated. Nevertheless, careful ultrasonographic examination can be helpful, as well as fine-needle aspiration cytology, when nodules outside the adenoma are identified.

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Feature Articles

The Use of the Polymerase Chain Reaction to Detect Minimal Residual Disease in Childhood Acute Lymphoblastic Leukaemia

Colin G. Steward, Nicholas J. Goulden, Michael N. Potter and Anthony Oakhill

INTRODUCTION

SEVENTY PER CENT of children with acute lymphoblastic leukaemia (ALL) can now be cured by conventional chemotherapy [1]. Successful treatment began in the 1940s when prolonged survival was first reported following the use of aminopterin [2]. The concept of a remission was thus proposed [3] and during the 1950s and 1960s the use of combination chemotherapy, for inducing remission, and maintenance therapy to consolidate that remission brought about the first true cures [4].

In adult ALL, there are an estimated 10^{12} leukaemic cells at diagnosis and a proportional number, therefore, are expected in children. Typically, induction therapy continues for 28 days, by which time the blasts have cleared from the peripheral blood and the full blood count has returned to normal. Examination of bone marrow in nearly all children at this time will reveal less than 5% blasts by light microscopy. This then is the definition of haematological remission, but the patient may still harbour up to an estimated 10^{10} malignant cells [5]. These are collectively termed minimal residual disease (MRD) and the aim of maintenance or continuing chemotherapy is the elimination of the majority of this disease.

At least 25% of patients will have a relapse in their bone

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marrow, either in isolation or in combination with extra-medullary relapse. The majority of these will occur after the cessation of treatment. Regular examination of bone marrow by light microscopy has little predictive or therapeutic value [6, 7]. Attempts at more sensitive assessment are, therefore, necessary to predict impending relapse, in the hope that a change in the treatment regime will lead to an improved chance of cure. For example, if the detection of MRD at the end of 2 years treatment were shown to herald relapse it may be appropriate to consider further therapy such as bone marrow transplantation. Before embarking on this course of action it is obviously necessary to perform prospective studies to look at the implications of detection of MRD.

A variety of cytogenic, immunological and molecular techniques for MRD detection are currently being investigated [8]. This review concentrates on the most widely researched of the molecular methods: the use of the polymerase chain reaction (PCR) to amplify immunoglobulin heavy chain (IgH) and T-cell receptor (TCR) gene rearrangements.

STRUCTURE AND FUNCTION OF IMMUNOGLOBULIN AND TCR GENE LOCI

The genes which encode antibody and TCR proteins are immensely diverse. However, if a single gene were required for each antigen-specific protein these would occupy almost the entire human genome [9]. This problem is overcome by arranging the respective genes into family regions [termed Variable (V), Diversity (D) and Joining (J)], each with many members, and recombining individual members of these in an apparently random fashion [10]. The frequency and distribution of these regions are shown in Table 1.

Figure 1 shows a schematic representation of the process of gene rearrangement at both the IgH and TCR gene loci. The more detailed mechanics of this are well illustrated by the IgH gene. During early B-cell development DNA segments within one, or both, alleles of this gene undergo recombination producing the genetic sequence(s) known as the third complementarity determining region (CDR III). One of approximately 30 D regions is firstly apposed to one of six J regions with excision of the intervening DNA segments. This D–J complex is then joined in a similar fashion to one of approximately 200 V regions forming a full V–D–J rearrangement [11]. At each stage nucleotide bases are removed from the junctional ends of these regions and new bases (termed "N"-nucleotides) are inserted by the enzyme terminal deoxynucleotidyl transferase (Tdt) [12, 13]; both involve highly variable numbers of nucleotides and are thought to be random events. The resulting V–N–D–N–J (CDR III) sequence is extremely diverse [10, 14].

Analogous events occur within the genes of the TCR subunits [15]. In general, those loci which have fewer component family

Table 1. Frequency and distribution of V, D and J segments at IgH and TCR loci. These statistics are obtained from [8, 11, 15]

	Variable (V) regions	Diversity (D) regions	Joining (J) regions
IgH	200	30	6
TCR-α	100	0	50
TCR-β	25	2	12
TCR-δ	6	3	3
TCR-γ	15	0	5

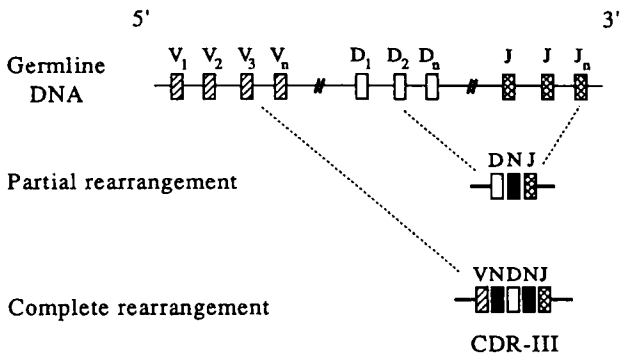

Fig. 1. A generalised scheme of gene rearrangement at IgH and TCR subunit gene loci. Individual V, D and J segments are widely separated by intronic sequences in germline DNA. Rearrangement occurs as a two step process. At the IgH gene locus D to J joining precedes complete V to DJ joining. Similar events occur at the TCR gene loci. However, in contrast to the IgH gene, partial V to D rearrangements occur at the TCRδ locus. These are exemplified by the Vδ2-Dδ3 rearrangements commonly seen in B-lineage ALL.

members (especially TCR–γ and δ) rely more on random nucleotide deletion and insertion than the recombination of different germline segments in order to generate their diversity [16].

USE OF PCR TO GENERATE MARKERS FOR M.R.D.

At diagnosis the majority of leukaemias have already undergone rearrangement of one or more of their IgH or TCR subunit genes (see Table 2). The highly variable nature of these rearrangements means that their individual DNA sequences can be effectively regarded as unique patient- or clone-specific tumour markers [10, 14, 17, 18]. In most cases the PCR can be used to amplify these rearrangements, as the DNA sequences flanking them are known and provide appropriate targets for primer design [19]. At presentation or relapse leukaemic lymphoblasts constitute the majority of bone marrow mononuclear cells. As a result the predominant products of PCR amplification of DNA extracted from these cells will be derived from the leukaemic clone [20, 21].

A wide variety of methods have been described for manipulating the PCR products in order to detect MRD in subsequent "remission" bone marrow samples. If the CDR III or its TCR analogue is sequenced, DNA probes averaging 20 bases in size (oligonucleotide probes) may be synthesised to the areas of greatest sequence diversity—at either V–N–D or D–N–J junctions [14, 22–24]. After radiolabelling these can be used to probe PCR amplification products from equivalent amounts of patient remission and control DNA. Products can be probed after either being dotted onto membranes (dot-blots) [23, 25] or incorporated into a bacteriophage library [14]. The latter, although more laborious, has the advantage of directly enabling the measurement of disease positivity via the ratio of positive to negative plaques. Sequencing may be avoided if the presentation PCR product is radiolabelled and used to probe dot-blots. Enzymatic removal of the common primer sequence is used in order to improve probe specificity [25–27]. Although these methods avoid the expense of synthesising individual oligonucleotide probes, we have found them to lack the specificity and sensitivity of such probes in some cases (unpublished observations).

Alternatively, radiolabelled products may be run on sequencing gels which very accurately distinguish products on the basis

of size ("DNA fingerprinting") [28]. If a leukaemic clone persists the amplification product will give a distinct band brighter than background on such a gel.

The sensitivity of these techniques can be assessed by log dilutions of leukaemic DNA in appropriate cellular control DNA. These suggest a typical sensitivity of residual disease detection of 1 leukaemic cell in 10^3 to 10^5 normal bone marrow mononuclear cells, i.e. on average 500 times more sensitive than light microscopy [14, 27, 29]. Unfortunately, all investigators have found blood to be a very much less sensitive tissue than bone marrow for the tracking of MRD. Other described methods rely on the use of one or two patient-specific primers for the amplification of remission specimens [22, 30–32] and could potentially achieve consistent MRD detection at the level of 1 in 10^4 to 10^6. However, such methods would be prone to false negative disease detection in the event of clonal evolution (see later). Various methods have been described for more accurate quantitation of MRD [32, 33] although these have no proven advantage at present over the simpler dilutional assays.

SPECIFIC METHODS OF PCR AMPLIFICATION

IgH rearrangements

Initial amplification may be performed using a consensus primer to the third (FR3) framework region of the V regions together with a consensus J region primer [14, 34]. This produces one or more discrete PCR products at presentation in approximately 75–80% of patients [20, 21]. The use of FR1 primers specific to each of the six V region families may increase this rate of positivity to consistently over 90% [28, 35]. Failure of amplification will occur in patients with relatively more immature leukaemias which have either not commenced gene rearrangement or have undergone only partial D–J rearrangement. It may also be caused by excessive misfit of the V region primer or varying degrees of deletion of the primer site during the rearrangement process [36].

TCR rearrangements

β *rearrangements*. The TCR β locus has a large germline repertoire which leads to difficulties in the design of consensus primers for study of this locus. PCR-based studies of the β gene have involved either anchored PCR amplification of total RNA or sub-cloning of restriction enzyme digested presentation DNA [23, 27]. Subsequent sequencing allows synthesis of a clone-specific probe [23]. Unfortunately, this approach is complex and has limited applicability considering the relatively low incidence of β rearrangement in B-lineage ALL (Table 2).

δ *rearrangements*. Southern blot analysis of leukaemic DNA has shown that the predominant pattern of TCR δ rearrangement varies with leukaemic lineage [38–41]. In B-lineage ALL 46% of a series of cases had a pattern consistent with a Vδ2–Dδ3 partial rearrangement, which is only rarely seen in T-ALL [42, 43]. In T-ALL Vδ1–Jδ1 rearrangements predominate [27, 44]. Both rearrangements are suitable for PCR amplification and this has led to the development of PCR-based studies using specific V, D and J primer sets [23, 27, 29, 44]. Full Vδ2–Dδ3–Jα rearrangements have been documented and may be studied using Vδ2 and Jα primers [42].

Table 2. Frequency of gene rearrangement as assessed by Southern blotting in ALL. Reprinted with kind permission from MN Potter [8]

	IgH	TCR β	TCR γ	TCR δ rearranged	TCR δ deleted
B-ALL	98%	29%	56%	50%	31%
T-ALL	17%	97%	96%	70%	28%

The junctional sequences of Vδ1–Jδ1 rearrangements have been shown to be extremely variable [44]. Not as much sequence data of the Vδ2–Dδ3 rearrangements is available. Although there appears to be less junctional variability at this locus this would not appear to significantly decrease the sensitivity of the relevant probes [26].

γ *rearrangements*. The Vγ genes are heterogeneous but can be subdivided into four groups. Of these the Vγl group of genes is most commonly rearranged in ALL [45]. Four methods have been described for amplification of this gene:

(a) Pre-determination by Southern blot of the V group or individual gene involved in the rearrangement prior to selection of appropriate primers [29, 44].
(b) Use of a consensus primer set to all members of the VγI subgroup and subsequent restriction digestion of the PCR amplification product to determine individual gene involvement [45].
(c) Use of primers specific to all functional members of the Vγ genes in two "cocktails" [46].
(d) Use of universal Vγ gene primers to generate an initial product from which clone-specific primers can be constructed after sequencing [31]. The same group have described an alternative PCR-mediated ribonuclease protection assay which avoids the need for sequencing and the synthesis of clone-specific primers, yet is reported to display similar sensitivity in detecting leukaemic cells [47].

The above methods have successfully achieved amplification in approximately 90% of T-ALL and 75% of B-ALL [45, 46]. Sensitivities have been assessed as 1 in 10^4 to 10^6. The analysis of MRD via PCR of this locus, therefore, has potentially widespread application.

POTENTIAL PROBLEMS

PCR methodology

The very power of amplification of the PCR technique means that "carryover" of minute amounts of previous reaction products may lead to false positive results when examining remission DNA specimens. A 0.1 μl spillage from a typical PCR reaction (such as might occur by dropping a pipette tip) has been estimated to release 10^9 PCR products into the laboratory environment [48]. Contamination problems can only be avoided by the stringent use of non-DNA-containing controls among a variety of other precautions [48].

The success of individual PCR reactions can vary depending on such factors as enzyme activity, variable DNA quality or the presence of inhibitors (e.g. heparin [49]). For this reason it is important to always perform all reactions to be analysed, including normals, at the same time. The inclusion of a set of primers for a gene present in all tissues in the same PCR tube will give a better indication of the efficiency of amplification [23, 32].

Clonal evolution

The sequential tracking of MRD through therapy is totally reliant on stability of the genetic sequences involved. This will be affected if either ongoing recombination events should occur

(clonal evolution) or if relapse develops with an entirely different clone (clonal change).

Up to 50% of patients with B-lineage ALL have been reported to show changes in their pattern of Southern blot analysis (using J region probes) between presentation and relapse [18, 50–52], casting doubt on the reliability of IgH–PCR for predicting relapse. It is now becoming clear that this probably results from the development of subclones related to the original clone and that this will not affect PCR assessment if appropriate techniques are used [53, 54].

The most common mechanism of clonal evolution at the IgH locus is termed V–V replacement [55, 56]. In this, a further recombination step(s) occurs, replacing the original V region with a new one. The effect of this is to leave the D–N–J sequence completely undisturbed but to alter the V–N sequence. This phenomenon has sometimes already occurred by the time of presentation–explaining most patients presenting with more than two bands (oligoclonality) [54].

In a study of 32 children positive by IgH–PCR at presentation we found complete clonal change (as assessed by PCR) in only 2 (6%): 1 relapsed with a completely different CDR III sequence (reported in [57]), the other had one band at presentation but was negative at relapse (unpublished observations). V–V replacement at relapse was seen in 5 (16%). Rovera *et al.* [53] by comparison reported a rate of 25% in 12 patients. Relapse could not have been predicted in such cases by any technique employing V–N–D oligonucleotides as either probes or patient-specific primers [22, 30, 32]. We also found 2 patients who relapsed with only one of two and three bands, respectively, seen at diagnosis. In order to maximise the possibility of relapse prediction by IgH–PCR and oligonucleotide probing it is, therefore, esssential to synthesise D–N–J probes to each unique D–N–J sequence seen at presentation. Related V–V replaced bands seen at presentation can be followed using a common D–N–J probe. We, therefore, estimate that an average of only 1.5 probes per patient would be required despite the high reported rate of oligoclonality. Relapse prediction would only be negated in 6% of patients as a consequence of clonal evolution events.

Southern blotting studies suggest that TCR gene rearrangements may be more stable from presentation to relapse [52]. 20 patients have so far been studied by PCR of TCR δ at both events: 2 showed changes in rearrangement at relapse which would have led to false negative results [23, 26, 29, 58]. 1 patient with T-ALL showed deletion of the Vδ1–Jδ1 rearrangement seen at presentation [29]. In a second patient with B-lineage ALL it was thought that further recombination of Vδ2–Dδ3 into Jα had occurred—this would result in deletion of the Dδ3 priming site and a negative PCR at the time of relapse [58]. We have observed a similar phenomenon in 3 out of 22 patients studied by Vδ2–Dδ3–PCR between presentation and relapse (submitted for publication). Clonal evolution has also been described at the TCR γ locus in 2 patients [29, 59]; interestingly, one of these had two new TCR γ rearrangements at relapse with complete loss of the original rearrangement, whilst the IgH CDR III sequence remained stable thoughout [59].

Clarification of the relative frequency of clonal evolution at all loci is still required from larger studies. It seems likely that the best approach will be to combine study of several different loci in the same group of patients. This will allow investigation of the vast majority of patients whilst minimising false negative relapse prediction as clonal evolution may occur at one, but not all, loci. For example, we have studied a cohort of 50 patients with B-lineage ALL. 44 (88%) were positive by either IgH–PCR or Vδ2–Dδ3–PCR at presentation: only 2 (5%) showed evolution at both loci concurrently which would have invalidated relapse prediction (submitted for publication).

Biased patterns of rearrangement

Sequence analysis of in excess of 100 leukaemic and normal IgH rearrangements have shown that V, D and J region usage are not as random as might be expected [60, 61]. This particularly affects D–N–J sequence. Of the six J segments, J4 and J6 are used in over 70% of rearrangements. Of the 30 D regions just six—D21/9, DLR2, DLR4, DN1, DXP1 and DXP'1—are used in over 60% of rearrangements. Unfortunately, these biased features of rearrangement are shared by both leukaemic and normal B-cells [61]. This is important as the tracking of MRD by IgH–PCR essentially relies on detecting the leukaemic rearrangement above a background of similar rearrangements in normal B-lymphocytes. Probe sensitivity will be reduced in rearrangements involving commonly used segments if these also happen to have small N regions.

Less sequence data is available for the various types of TCR rearrangement but similar problems may be encountered. For example, the relatively limited variability seen within Vδ2–Dδ3 rearrangements may affect probe sensitivity. It should be noted that amongst only 10 leukaemic and eight normal T-cell Vδ2–Dδ3 sequences an identical sequence has been isolated from a leukaemic rearrangement and a normal peripheral blood clone [42].

CLINICAL STUDIES

Reliable results from PCR studies are largely dependent upon high quality DNA samples obtained from bone marrow aspirates. As most research institutions are only now building up appropriate DNA banks, clinical data based on satisfactory numbers and follow-up are limited.

What follows is an attempt to collate the clinical results of small, retrospective studies using different techniques at three loci. It should be remembered that these techniques are not yet standardised and that the technology available is constantly improving.

MRD on treatment

Virtually all patients have detectable disease by PCR during the early phases of therapy. Nizet *et al.* found 15/16 patients positive during the first 3 months, Yokota *et al.* 8/8 and Yamada *et al.* 4/4 during the first 6 months of therapy [25, 26, 62]. A smaller proportion of patients remain positive as late as 19 months into therapy without subsequent relapse. Most patients who remain in remission, therefore, appear to become PCR-negative at various times between 6 and 20 months from diagnosis.

MRD in long-term remission

Yokota *et al.* [26] analysed TCR δ rearrangements in a selected group of B- and T-ALL patients in remission. 10/11 patient samples tested at intervals ranging from 6 to 41 months from the end of therapy showed no evidence of residual disease. In 1 patient, however, a positive result was obtained in isolation 3.5 years from the end of therapy. The significance of this was not clear.

Biondi *et al.* [58] recently published a study of patients in long-term clinical remission between 29 and 72 months from diagnosis. In 8 patients the last available sample was at 24 months or longer from diagnosis: none of these showed evidence

of MRD. Similarly, MRD was not detected in 4 patients in long-term remission (3.9 to 8.1 years) studied retrospectively by Neale *et al.* [23].

PREDICTION OF RELAPSE BY MRD

Relapse on treatment

One of the original aims for PCR studies of MRD was to predict the likelihood of future relapse at an early stage of treatment. This would potentially allow tailoring of treatment on an individual patient basis, e.g. selection for more intensive therapy, such as bone marrow transplantation, or reduction/curtailment of therapy for good risk patients. However, as discussed previously, there appear to be wide variations between patients in the rate of disappearance of MRD, and it seems probable that differences in the intensity of induction and consolidation therapy between protocols will act to exacerbate this variability. These factors make it unlikely that, at any given point of therapy, a threshold level of disease can be assigned above which patients are likely to relapse. For example, in morphological studies the percentage of blasts persisting early in induction therapy are thought to carry prognostic significance [63]. By extrapolation it might be expected that high levels of MRD (e.g. 1 in 10^3 or greater) at the end of induction therapy would augur relapse. However, in the largest available study, Nizet *et al.* [25] found no evidence to support this: all 6 patients positive to this degree at the end of induction remained in remission at the time of publication, although none were more than 20 months from diagnosis.

Rising levels of MRD at any stage during therapy may indicate imminent haematological relapse. IgH and TCR studies have detailed 6 patients showing such an increase: 5 of these proceeded to haematological relapse within 2–4 months [23, 25, 58, 62]. The remaining patient (C137 in Yamada *et al.* [62]) showed only a slight rise in the level of MRD which subsequently declined and that patient remained in remission at 26 months from diagnosis. This latter patient demonstrates that caution must be exercised in interpreting the results of isolated specimens unless these show a marked increase in MRD.

Relapse off treatment

The universal finding is that patients who remain in remission are PCR-negative at the end of treatment. Any patients found positive at this stage might, therefore, be expected to have a high probability of subsequent relapse. What is the evidence for this?

In a retrospective study of 4 patients relapsing off therapy we found detectable residual disease at levels of 1 in 10^3 to 10^4 in end-of-treatment bone marrow specimens in all cases. Although 2 of these children relapsed relatively soon after the end of treatment—at 6 and 8 months, respectively—the remaining 2 both relapsed very late at 8.5 and 9 years off therapy [64].

However, not all patients who subsequently relapse show disease in their end-of-treatment or off-treatment marrow samples. Tycko *et al.* describe 1 patient investigated by IgH–PCR using patient-specific primers who did not show residual disease in two samples taken during the third year of remission, yet relapse followed 5 years from diagnosis with the original clone [59]. Biondi *et al.* describe 2 patients studied by PCR of TCR δ who relapsed off treatment having been negative in marrow samples 2 and 6 months previously (patients 14 and 13, respectively [58]). Such cases may be explained by either the level of MRD dropping beneath the sensitivity of the test or the possibility that an isolated marrow sample may miss residual disease if this has a patchy distribution (as has been suggested in animal models [65]). They do, nevertheless, represent failures of relapse prediction by this technique.

In summary, a positive PCR result at the end of treatment correlates well with subsequent relapse but false negative relapse prediction does occur. We believe that larger trials are now required in order to establish the exact sensitivity and specificity of PCR performed at completion of treatment in predicting future relapse.

Extra-medullary relapse

A rise in the level of MRD has not been seen to precede extra-medullary relapse in the few patients reported. 1 patient in Yamada's series [62] had a CNS relapse off therapy without PCR evidence of disease in either the end-of-treatment or relapse marrow samples. We have studied 2 patients with CNS and 1 with testicular relapse on treatment (unpublished observations). Although all had clear evidence of bone marrow involvement by PCR at the time of relapse (in specimens judged clear by morphology) only 1 had detectable disease by PCR in the sample preceding this relapse. MRD in this patient was stable at a level of 1 in 10^3.

CHROMOSOMAL TRANSLOCATIONS IN THE STUDY OF MRD

A proportion of childhood ALL is characterised by chromosomal translocation. The breakpoints in some of these translocations occur over a large area of DNA and as a consequence of their length are not amenable to PCR amplification. However, the resulting transcript mRNA of a specific translocation is relatively constant and a PCR primer set can be designed to amplify this [8]. The translocations available to PCR-based study of MRD and their frequency in ALL are detailed in Table 3.

The Ph^1 t(9; 22) translocation has been the most intensively studied translocation in ALL. This is positive in up to 50% of adults by PCR (although only in 15% by standard cytogenetics) but in only 6% of children [66]. PCR evidence of MRD was demonstrated in 8/10 patients with Ph^1-ALL who were assessed as being in morphologic and cytogenetic remission with ALL prior to BMT [67]. 3 patients positive prior to transplant became negative following the procedure and remain in remission at 15 to 28 months. However, of the 8 patients who relapsed 6 were initially PCR-negative after transplant and MRD could be demonstrated only 3 to 9 weeks prior to eventual relapse. Parallel results were obtained in a smaller study [68].

Table 3. Type and frequency of chromosomal translocations presently amenable to investigation by PCR in childhood ALL. Reprinted with kind permission from MN Potter [8]

Chromosomal translocation	Percentage of cases amenable to PCR study
t(9;22)	6
t(8;14)	<1
t(1;19)	5
t(1;14) and *tal*-1 rearrangement	4
t(11;14)(p13;q11)	0.4
t(4;11)	0.8
Total	17.2

The t(1; 14)(p34; q11) translocation involves the first exon of the *tal* 1 gene on chromosome 1. This, together with specific deletions of this gene in the absence of cytogenetically proven translocation, can be amplified by PCR in 10 to 30% of T-ALL [44, 69]. Random deletion and insertion of nucleotides at the fusion sites result in the formation of a patient-specific sequence. PCR amplification followed by oligonucleotide probing of this site is capable of detecting MRD with a reported sensitivity of 1 in 10^5.

CONCLUSION

PCR-based assessment of MRD by amplification of either the IgH or TCR subunit loci consistently allow detection of disease with an average sensitivity of 1 in 10^4. This has improved assessment of the pattern of clearance of leukaemia and shown that a significant number of patients have clear evidence of disease well into the second year of therapy. These findings correlate well with the proven requirement for 2 full years of maintenance therapy [70]. PCR may also improve our understanding of leukaemia biology, a fact exemplified by the clear demonstration of bone marrow disease in patients thought to have isolated extra-medullary relapse.

However, PCR analyses are complex and have many potential pitfalls, relating both to the technique itself and to the inherent instability of the genes concerned. Careful control is required to eliminate false-positive results as a consequence of contamination. False-negative results may arise as a consequence of clonal evolution and further information as to the relative frequency of this event at each locus is still required.

PCR-based studies of MRD appear to be poor predictors of on-treatment relapse unless either a dramatic or sustained increase in disease is demonstrated. Early results suggest that PCR of bone marrow samples may prove to give poor indication of extra-medullary relapse. By contrast, virtually all patients who remain in remission become PCR-negative by the end of treatment and positivity at this stage shows a high correlation with relapse.

Large prospective/collaborative trials are now required using these various techniques. Ideally, these should examine the same patients by multiple loci and with all tests being performed at two different centres for purposes of quality control. All centres involved should employ the same techniques as sensitivity may vary with the methodology employed. Such trials should concentrate on two areas: firstly, an attempt to clarify the significance of high levels of MRD early in treatment as this is presently unclear. Secondly, prospective analysis assessing clinical outcome and MRD in patients at the end of treatment. The particular relevance of the latter is that patients relapsing within 12 months off treatment constitute a poor prognostic group in whom PCR could potentially allow selection for further intensive therapy.

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