Status of minimal residual disease testing in childhood haematological malignancies

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Summary

In children with acute leukaemia, measurements of minimal residual disease (MRD) provide unique information on treatment response and have become a crucial component of contemporary treatment protocols. In acute lymphoblastic leukaemia (ALL), the most useful MRD assays are based on polymerase chain reaction (PCR) amplification of antigen-receptor genes, and on flow cytometric detection of abnormal immunophenotypes. The latter is the only MRD assay available for most patients with acute myeloid leukaemia (AML). PCR amplification of chromosomal breakpoints and fusion transcripts can also be used to track MRD in a minority of patients with ALL or AML. Because of the strong correlation between MRD levels and risk of relapse, several ongoing regimens include treatment intensification for children with higher MRD. Treatment de-intensification for patients with early MRD clearance is also being tested. In addition to their direct clinical application, MRD measurements can be used to better understand the molecular and cellular mechanisms of drug resistance in vivo. The identification of new markers of leukaemia and the use of increasingly sophisticated technologies for detection of rare cells should further facilitate routine monitoring of MRD and elucidate the features of drug-resistant leukaemic cells.

Keywords: acute lymphoblastic leukaemia, acute myeloid leukaemia, flow cytometry, polymerase chain reaction, treatment response.

Rationale for minimal residual disease testing

Monitoring response to treatment by periodic examination of bone marrow aspirates is an integral part of the clinical management of patients with acute leukaemia. The presence of residual leukaemia and the overall status on normal haematopoiesis, as determined by the cellular appearance of bone marrow smears, provide an indication of the sensitivity of leukaemic cells to chemotherapy and of the degree of haematopoietic regeneration occurring during treatment intervals. Because the morphology of leukaemic cells generally resembles that of normal lympho-haematopoietic progenitors, it is difficult to identify leukaemic cells with confidence. In fact, identification of individual leukaemic cells scattered among normal bone marrow cells might not be possible, even for an experienced haemopathologist, unless leukaemic cells have striking morphological traits, such as Auer rods. Therefore, a patient can have nearly 5% leukaemic cells that morphologically appear as leukaemic blasts and yet be considered in remission. Only a minority of patients have leukaemic cells above this threshold at the end of remission induction therapy but those with a lower proportion of blasts may still harbour a considerable leukaemic burden (Campana & Pui, 1995). Hence, a significant proportion of patients are likely to receive postremission therapy that is less intensive than required. The morphological similarities between leukaemic cells and normal haematopoietic cells may also lead to the opposite error, i.e. an overestimation of the leukaemia burden due to the mistaken identification of normal cells as leukaemic cells. This error could trigger unnecessary treatment intensification and toxicities.

Over the last 2–3 decades there has been an intense effort to develop methods that could determine the degree of residual leukaemic cells present in patients considered to be in morphological remission, that is to measure minimal residual disease (MRD). These efforts have resulted in assays whose sensitivity is much higher (100 times or more) than that of morphology (Szczepanski et al, 2001; Campana, 2003). MRD assays are also more objective because they rely on specific leukaemia markers rather than on the subjective recognition of morphological patterns. Studies reported during the last decade, discussed below, have unequivocally demonstrated the prognostic importance of MRD in childhood leukaemia. Therefore, the more stringent definition of remission provided by MRD assays is now preferentially used at many cancer centres.
Methodologies to detect MRD

Targets and methods

The common principle underlying all MRD assays is that the leukaemogenic process has resulted in molecular and cellular changes that distinguish leukaemic cells from their normal counterparts (Szczepanski et al, 2001; Campana, 2003). These leukaemia-associated features are identified at diagnosis (or at relapse) and then used to monitor MRD. Table I summarizes the applicability and sensitivity of the most widely used assays.

One of the distinguishing features of leukaemic cells is the expression of cell markers in abnormal patterns. These abnormal cell profiles are best detected with multiparameter flow cytometry (Campana, 2003). The sensitivity of this approach depends on two main factors: the degree of dissimilarity between the immunophenotypes of leukaemic cells and those of normal cells, and the number of cells available for study. In nearly all patients with acute lymphoblastic leukaemia (ALL), leukaemic lymphoblasts express immunophenotypes that are sufficiently distinct to allow the detection of one leukaemic cell among 10 000 normal cells (Campana & Coustan-Smith, 1999). Distinctive markers can also be identified in most patients with acute myeloid leukaemia (AML), although in approximately 40% of patients the routine sensitivity that can be achieved is not higher than one in 1000, owing to a partial overlap between the phenotype of leukaemic cells and those of normal haematopoietic cells (Coustan-Smith et al, 2003).

Flow cytometry-based assays are rapid and provide an accurate quantitation of MRD while gaining information on the status of normal haematopoietic cells at the same time. The number of antibody combinations used to identify leukaemic cells and the stability of the markers targeted are important factors for the reliability of this approach. In general, it is advisable to use multiple sets of antibodies to compensate for immunophenotypic switches (Van Wering et al, 1995; Coustan-Smith et al, 1998; Baer et al, 2001; Gaipa et al, 2005). In addition to the skills necessary for reliable leukaemia immunophenotyping, productive MRD studies by flow cytometry require great care to avoid sample contamination at all stages of processing as well as a solid knowledge of the immunophenotypic patterns found in normal and regenerating bone marrow cells, particularly of immature myeloid and lymphoid cells (Campana & Coustan-Smith, 1999).

A second distinguishing feature of leukaemic cells is the clonal rearrangement of the genes encoding immunoglobulin (Ig) and T-cell receptor (TCR) proteins. This leukaemia-specific molecular signature can be found in the majority of cases of ALL (Pongers-Willemse et al, 1999), but in less than 10% of AML cases (Boeckx et al, 2002). ‘Real-time’ polymerase chain reaction (PCR) is the preferred method for the detection of cells with such rearrangements because it allows a precise quantitation of the PCR product (van der Velden et al, 2003), hence of MRD (each cell has one copy of the rearranged gene and the PCR product is directly proportional to the leukaemic cell number). PCR analysis of the genes encoding Ig and TCR proteins allows the routine detection of one leukaemic cell in 10 000–100 000 normal cells.

Monitoring the persistence of clonal antigen-receptor genes during treatment provides a sensitive and objective assessment of MRD. The reliability of the method can be affected by the presence of multiple rearrangements in the same leukaemic cell population. Thus, a minor clone at diagnosis may become predominant during the course of the disease and remain undetected because only a major clone present at diagnosis is being monitored (Szczepanski et al, 2002; van der Velden et al, 2004). To prevent this potential problem, it is advisable to use sets of probes matching two or more different rearrangements (Pongers-Willemse et al, 1999; Flohr et al, 2008). Flohr et al (2008) reported that among 3341 patients studied, two or more targets that allowed PCR analysis with a one in 10 000 sensitivity or better were found in 2365 (71%); 671 (20%) additional patients had only one such target. Alternatively, the use of two independent MRD methods (e.g., PCR and flow cytometry) should greatly reduce the risk of false-negative results. Extensive standardization of the methods for PCR amplification of antigen-receptor genes has been performed by the BIOMED collaborative group, which has published specific guidelines for optimal performance in a clinical setting (van der Velden et al, 2007).

A third leukaemia-associated feature that can be used to distinguish leukaemic from normal cells is represented by chromosomal abnormalities and resulting gene fusions (van Dongen et al, 1999; Gabert et al, 2003). Fusion transcripts, such as BCR-ABL1, MLL-AFF1, TCF3-PBX1, and

<table>
<thead>
<tr>
<th>Method</th>
<th>ALL % of cases with marker</th>
<th>Sensitivity</th>
<th>AML % of cases with marker</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow cytometric detection of abnormal phenotypes</td>
<td>98%</td>
<td>$10^{-4}$</td>
<td>93%</td>
<td>$10^{-3}$–$10^{-4}$</td>
</tr>
<tr>
<td>PCR amplification of genes encoding Ig and TCR proteins</td>
<td>90%</td>
<td>$10^{-4}$–$10^{-5}$</td>
<td>&lt;10%</td>
<td></td>
</tr>
<tr>
<td>RT-PCR amplification fusion transcripts</td>
<td>&lt;50%</td>
<td>$10^{-3}$–$10^{-5}$</td>
<td>&lt;20%</td>
<td>$10^{-3}$–$10^{-5}$</td>
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Table I. Methods for monitoring MRD in childhood leukaemia.
ETV6-RUNX1 in ALL, and RUNXI-RUNXIIT1, CBFB-MYH11 and PML-RARA in AML can be used as target for amplification; real-time PCR provides the most accurate way to measure their level (van Dongen et al., 1999; Gabert et al., 2003). Overall, less than one-third of patients with ALL or AML have leukaemic cells with genetic abnormalities that can be studied with the typical assays performed in molecular pathology laboratories, allowing the detection of one leukaemic cell in 1000 to 100 000 normal bone marrow cells.

An advantage of monitoring MRD by targeting fusion transcripts is the strong association between the molecular abnormality and the leukaemic clone, irrespective of the presence of intrachromosomal differentiation and cellular changes caused by therapy. Although earlier studies had reported the detection of leukaemia gene fusions in apparently healthy individuals (Bose et al., 1998), this does not seem to be a major problem, particularly at the detection levels used to monitor MRD clinically. The main disadvantage of targeting fusion transcripts is that the number of transcripts per leukaemic cell may vary from patient to patient with the same genetic leukaemia subtype and among different cells within the leukaemic clone, and might be affected by therapy (Gabert et al., 2003). Therefore, precise quantitation of MRD with this technique can be difficult.

It has been suggested that overexpression of WT1 could be used as a leukaemia marker for MRD studies (Ogawa et al., 1998). Because WT1 is also expressed in normal CD34+ bone marrow cells (Maurer et al., 1997), the use of this approach in a clinical setting may not be straightforward. The ever-increasing understanding of the molecular lesions that participate to leukaemogenesis might reveal new genomic alterations that can be used as targets for MRD assays (Mullighan et al., 2007).

**Consideration for clinical use of MRD assays**

If the signal detected by an MRD assay corresponds closely to the number of leukaemic cells present in the sample tested, it is expected that different MRD assays would yield concordant MRD estimates. Indeed, we and others found that flow cytometry and PCR amplification of the genes encoding Ig and TCR proteins estimated similar levels of MRD in most remission samples obtained from children with ALL (Neale et al., 2004; Kerst et al., 2005). Given that the two techniques yield similar results when MRD is present at levels of 0.01% or above, which is the best method for routine monitoring of MRD? Flow cytometry is more likely to be readily available (flow cytometers and methods for leukaemia immunophenotyping are used at virtually every cancer centre) but MRD monitoring requires expertise beyond that needed for leukaemia immunophenotyping. Without such specific expertise, the likelihood of errors in MRD estimates is very high; laboratories that are unprepared to perform the assay correctly should resist the pressure to deliver MRD results until the methodology has been validated. Flow cytometry is generally quicker than PCR but both methods can produce MRD estimates within 24 h of sample collection. For studies at early time points during therapy, e.g. day 15, flow cytometry has an advantage, as the development of a patient-tailored PCR assay currently requires more than 2 weeks. Conversely, PCR may be preferable for studies at the end of therapy, when the higher sensitivity of PCR might reveal MRD undetectable by flow cytometry. Although flow cytometry is often regarded to be less expensive, many variables must be factored in, such as the type of antibody panels used, the number of molecular targets studied, and the cost of sequencing and probe preparation for PCR. Based on our experience, we estimate the cost of the two methods to be similar overall. In sum, it is difficult to pick a clear winner on the basis of speed, accuracy, complexity and cost for MRD studies in ALL. Because either assay must be performed in specialized laboratories with proven expertise, the type of expert laboratory available to a cancer centre or a cooperative group may ultimately be the decisive factor in selecting the method to be used. The strategy currently used at our institution, where both methods are available, is outlined in Fig 1. In the case of AML there is no contest because flow cytometry is the only method that can study MRD in most patients.

While flow cytometry and PCR amplification of antigen-receptor genes typically yield similar MRD estimates in patients with ALL, the relation between these estimates and those obtained by PCR amplification of fusion transcripts is not entirely clear because systematic comparisons including the latter technique have not yet been performed. Such comparisons might provide unique insights in leukaemia biology. For example, clinically silent preleukaemic clones in patients with ETV6-RUNX1 ALL (Hong et al., 2008) (and possibly other leukaemia subtypes) might be detectable by PCR targeting of the fusion transcript but may lack the abnormal phenotypes and clonal antigen-receptor gene rearrangements observed in the leukaemic cells at diagnosis. Persistent stem cell populations bearing BCR-ABL1 might become undetectable during treatment with methods that do not directly target the gene fusion.

![Flowchart](image-url) **Fig 1.** MRD monitoring strategy used in the current Total XVI study at St. Jude Children’s Research Hospital.
Minimal residual disease assays are complex, expensive and time-consuming in relation to other routine diagnostic assays for leukaemia, which might preclude the wider application of MRD-directed therapy. The observation that normal lymphoid progenitors in the bone marrow, identified by the expression of CD19, CD10 and/or CD34, are exquisitely sensitive to corticosteroids and other antileukaemic drugs (Coustan-Smith et al, 2006), suggested to us that this immunophenotype could be useful to monitor early response to therapy in patients with B-lineage ALL. Given that lymphoblasts in most cases of this leukaemia subtype express CD19, CD10 and/or CD34, the detection of such cells during remission induction therapy should reflect persistent disease. We therefore developed a three-antibody assay and studied MRD in bone marrow samples collected on day 19 of remission induction therapy from 380 children with B-lineage ALL (Coustan-Smith et al, 2006). The results of the simplified assay correlated well with those of the standard flow cytometric assay and those of PCR amplification of antigen-receptor genes. We expect that this assay will facilitate the implementation of MRD measurements in centres that have limited resources to invest in MRD testing.

Must MRD studies be performed in bone marrow or can peripheral blood be used instead? In patients with B-lineage ALL (Briscoto et al, 1997; Coustan-Smith et al, 2002b; van der Velden et al, 2002), and in those with AML (E. Coustan-Smith, D. Campana, et al, unpublished observations) MRD is usually detected at higher levels in bone marrow. Therefore, studies of blood might be less informative about the patient remission status than those in marrow. It is possible, however, that detection of MRD in peripheral blood may indicate a higher risk of relapse, as suggested by preliminary observations in patients with B-lineage ALL (Coustan-Smith et al, 2002b). The pattern of MRD distribution is different in patients with T-lineage ALL, where MRD levels in peripheral blood are similar to those in bone marrow (Coustan-Smith et al, 2002b; van der Velden et al, 2002). In these patients, sequential MRD testing can be performed in blood, a practice that we currently follow in the current St Jude Total XVI study (Fig 1).

Prognostic significance of MRD in ALL

Many studies have demonstrated the prognostic importance of MRD as detected by flow cytometry in children with ALL (Coustan-Smith et al, 1998, 2000, 2002a; Dworzak et al, 2002; Borowitz et al, 2008). We found that patients who had MRD of 0.01% or higher in bone marrow at any of the time points during treatment had a significantly higher risk of relapse (Coustan-Smith et al, 1998, 2000, 2002a). Patients with MRD 1% or higher at the end of remission induction therapy and those with MRD 0.1% or higher during continuation therapy had an extremely high relapse hazard. MRD testing also identified a group of patients with a particularly favourable prognosis. Thus, among 112 patients studied on day 19 of remission induction therapy, the 53 who were MRD negative had a 3-year cumulative incidence of relapse of less than 5% (Coustan-Smith et al, 2002a). Investigators of the Children’s Oncology Group (COG) monitored MRD in peripheral blood specimens collected on day 8 and in bone marrow specimens collected on day 29 (end of remission induction therapy) in over 2000 children with B-lineage ALL (Borowitz et al, 2008). The presence of MRD (0.01% or higher) at either interval predicted a poorer outcome. The MRD results obtained in the day 29 bone marrow were the strongest prognostic indicator, superior to other commonly used prognostic parameters in childhood ALL. Of note, MRD predicted both early and late relapses.

Studies of MRD by PCR also showed clearly its prognostic importance (Brisco et al, 1994; Cave et al, 1998; van Dongen et al, 1998; Zhou et al, 2007; Flohr et al, 2008). Investigators of the International Berlin-Frankfurt-Munster (I-BFM) Study Group found that by combining the MRD information from day 33 and day 78 they could identify three groups of patients with a significantly different outcome: 43% of patients had MRD negative results at both time points and a 3-year relapse rate of only 2%; 15% of patients had MRD levels of 0.1% or higher at both time points and a relapse rate of 75%; the remaining patients (43%) had a relapse rate of 23%. These data were recently updated by Flohr et al (2008) who reported 10-year event-free survivals of 93% for the low MRD risk group, 16% for the high MRD risk group and 74% for the intermediate risk group. Investigators of the Dana-Farber Cancer Institute ALL Consortium studied MRD in 284 children with B-lineage ALL. The 5-year risk of relapse was 5% in 176 children with no detectable MRD at end of remission induction and 44% in the 108 children with detectable MRD (P < 0.001) (Zhou et al, 2007). An MRD cut-off level of 0.1% was found to be the one that best predicted 5-year relapse hazard: 72% for patients with higher levels of MRD and 12% for those with lower levels.

Minimal residual disease was also an independent predictor of second relapse in patients with ALL who had a relapse and then achieved a second remission, irrespective of whether MRD was measured by PCR amplification of antigen-receptor genes (Eckert et al, 2001), or by flow cytometry (Coustan-Smith et al, 2004). We studied 35 patients with first relapsed ALL in second remission and detected MRD 0.01% or higher in 19 (54%). The 2-year cumulative incidence of second leukaemia relapse was 70% for the MRD-positive patients and 28% for MRD-negative patients (P < 0.01). Among patients with a first relapse off therapy, 2-year second relapse rates were 49% in the 12 MRD-positive and 0% in the 11 MRD-negative patients (P = 0.014); among those who received only chemotherapy after first relapse, the 2-year second relapse rates were 82% (n = 12) and 25% (n = 13), respectively (P < 0.01). Time of first relapse and MRD were the only two significant predictors of outcome in a multivariate analysis.

Minimal residual disease monitoring using PCR amplification of antigen-receptor gene rearrangements predicts outcome in patients undergoing haematopoietic stem cell transplantation (HSCT) (Knechtli et al, 1998; Uzunel et al,
2001; van der Velden et al, 2001; Bader et al, 2002; Goulden et al, 2003; Krejci et al, 2003). In patients receiving T-cell-depleted grafts, high levels of MRD-PCR positivity (0-1-1%) before HSCT were consistently associated with relapse post-transplant, and patients with lower levels of MRD had a 35-50% 2-year event-free survival as compared to 70% for MRD-negative patients (Knechtli et al, 1998; Uzunel et al, 2001; Bader et al, 2002; Krejci et al, 2003).

Prognostic significance of MRD in AML

Initial MRD studies in AML were performed in adult patients by using either reverse transcription (RT)-PCR amplification of fusion transcripts (Tobal et al, 2000; Marcuccio et al, 2001; LoCoco & Ammatuna, 2007), or flow cytometry (Campana et al, 1990; San Miguel et al, 2001; Venditti et al, 2003; Buccisano et al, 2006). These studies demonstrated the potential clinical usefulness of monitoring MRD in AML.

Children’s Oncology Group investigators detected MRD in the bone marrow of 41 of 252 children with AML, all of whom had achieved remission (Sievers et al, 2003). These patients had a 48% higher relapse hazard in a multivariate model, with MRD being the strongest prognostic factor. We studied MRD by flow cytometry in 46 children with de novo AML enrolled in the St. Jude Children’s Research Hospital AML97 study and observed that the mean 2-year survival estimates for patients with MRD positivity (0.1% or higher) after induction therapy was 33% compared to 72% for those with lower levels or no detectable MRD. MRD was also the strongest predictor of outcome in this cohort. Among patients tested after the first cycle of remission induction therapy, those in morphological remission but with detectable MRD were 3-8 times more likely to die than those who were MRD negative. Similar observations were made for tests postinduction two: MRD-positive patients were 6-2 times more likely to die than those with undetectable disease. Langebrake et al (2006) studied residual disease by flow cytometry in 150 children enrolled in the AML-BFM 98 study. Detection of residual disease was significantly associated with a lower event-free survival, with positive patients at the earlier time points having a more than two-fold risk of relapse. When considered in combination with other prognostic factors, however, residual disease findings lost statistical significance in this series.

Clinical applications of MRD assays in childhood leukaemia

In childhood ALL, slow clearance of leukaemic cells during remission induction therapy as assessed by morphological examination of peripheral blood or bone marrow predicts an inferior treatment outcome (Gajjar et al, 1995; Steinherz et al, 1996; Gaynon et al, 2000; Schrappe et al, 2000; Sandlund et al, 2002). The application of MRD assays to measure early response to therapy is considerably more powerful because a substantial proportion of poor responders by MRD criteria would not have been identified by morphological analysis. In the recently completed Total XV study for newly diagnosed children with ALL at our institution, remission induction therapy was intensified for patients who had MRD 1% or higher on day 19 of remission induction therapy; postremission therapy was intensified for standard risk patients who had MRD 0.01% or higher on day 46 (Pui et al, 2001). Moreover, any patient with MRD 1% or higher on day 46, or 0.1% or higher during continuation therapy was considered as candidate for allogeneic HSCT. The BFM group uses MRD levels on days 33 and 78 as a guide for treatment intensification (Flohr et al, 2008), and other groups worldwide are planning to introduce MRD in their risk-assignment schema.

Early clearance of MRD indicates a high chemosensitivity of the leukaemic clone, and was associated with an excellent overall outcome in correlative studies (Panzer-Grumayer et al, 2000; Coustan-Smith et al, 2002a). Following this observation and considering that in past trials nearly half of children with ALL could be cured with therapy less intensive than that of today (Rivera et al, 1993), we hypothesized that patients who achieve MRD negativity after 2-3 weeks of remission induction chemotherapy can be cured with less intensive therapy. The need for treatment deintensification is particularly pressing in developing countries, where contemporary therapies for childhood ALL may have unacceptably high toxicities (Eden, 2002; Howard et al, 2004; Ribeiro & Pui, 2005). To this end, a protocol that incorporates reduction in treatment intensity for patients with negative MRD in bone marrow on day 19 as determined by a simplified flow cytometric assay (Coustan-Smith et al, 2006) has been implemented in Recife (Brazil). These studies are ongoing and the validity of this approach awaits evaluation.

In addition to measuring early response to chemotherapy, MRD assays have several other applications in the clinical management of children with ALL. For example, they can uncover impending relapse, thus giving a head start in the planning of salvage therapy and/or HSCT. Since the risk of relapse after HSCT is strongly related to levels of MRD before transplant (Goulden et al, 2003; Krejci et al, 2003), MRD measurements can also be used to determine the timing of HSCT. MRD measurements postHSCT can be used to guide the administration of donor lymphocyte infusions or other agents. Finally, in children who relapse and achieve a second remission, MRD assays can be used to help select the optimal postremission treatment, i.e. chemotherapy versus HSCT.

In patients with AML, MRD assays can also be applied to guide treatment decisions (Goulden et al, 2006). In our recently completed AML02 study, patients with MRD 1% or higher at the end of remission induction therapy were classified as high-risk and offered HSCT. In addition, patients with MRD 0.1% or higher received gentuzumab ozogamicin; if MRD persisted, they became candidates for HSCT. In addition, as described for childhood ALL, MRD can be used to optimized timing of HSCT and selection of post HSCT therapy. It should be clear, however, that the overall clinical benefits of

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changes in therapy based on MRD findings remain to be proven.

**Use of MRD for correlative studies with cellular and molecular features of leukaemic cells**

Minimal residual disease measurements provide an indication of the drug sensitivity of leukaemic cells. Therefore, they can be used to identify genes that are associated with multiagent chemoresistance in vivo.

Cario *et al* (2005) compared gene expression profiles of lymphoblasts in 21 B-lineage ALL patients with high MRD and 30 with low MRD enrolled in the BFM ALL-2000 protocol; leukaemic cells in all patients lacked known genetic abnormalities predictive of outcome. Several genes whose expression was strongly associated with MRD were found; those with low expression in high-MRD cases were predominantly associated with cell-cycle progression and apoptosis.

We analyzed gene expression of diagnostic lymphoblasts from 189 children with ALL and compared the findings with MRD on days 46 of remission induction treatment (Flotho *et al*, 2006). The gene encoding caspase 8 associated protein 2 (*CASP8AP2*) was of particular interest because of its strong association with MRD (patients with lower *CASP8AP2* had higher MRD) and its reported role in apoptosis and glucocorticoid signaling. Low levels of *CASP8AP2* expression were associated with a lower propensity of leukaemic lymphoblasts to undergo apoptosis and predicted a lower event-free survival and a higher rate of leukaemia relapse. We also compared gene expression in the same cohort with MRD results obtained on day 19 of remission induction treatment (Flotho *et al*, 2007). We identified 674 probe sets that were associated with MRD on day 19; 40 of the identified genes predicted relapse in the independent cohort of 99 patients. Among these, 14 showed independent prognostic significance. More than half of the 40 genes and nearly all of the 14 genes were functionally related, as indicated by their roles in the regulation of cell proliferation. Underexpression of genes promoting cell proliferation was associated with resistance to chemotherapy.

**Conclusions**

Currently available MRD assays ensure the objective and sensitive assessment of treatment response in most patients with acute leukaemia. For example, in our recently closed Total XV study, 481 of 482 (99.8%) children with ALL could be monitored by flow cytometry and/or PCR amplification of the genes encoding Ig and TCR proteins; the only patient whose cells lacked suitable markers for these techniques had *MLL-AFF1* and response to therapy could be monitored by PCR amplification of the corresponding fusion transcript. It is now important to further simplify the assays so they can be implemented widely. To this end, new markers emerging from genome-wide expression studies might help reducing the number of antibodies required for flow cytometric analysis of MRD (Chen *et al*, 2001).

Minimal residual disease studies can be used to quickly assess the effectiveness of novel antileukaemic agents, and support innovative designs for Phase II studies. In this regard, an exciting opportunity is the possibility of determining the status of cell signalling pathways in the leukaemic cell population (Irish *et al*, 2006). Taking advantage of the new flow cytometers, which are capable of detecting nine or more parameters, it should be possible to assess whether the signalling pathways targeted by tyrosine kinase inhibitors are affected in the MRD cell population.

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