Acute lymphoblastic leukemia (precursor lymphoblastic leukemia/lymphoma, WHO classification) is the most common malignancy of childhood. Over the past few decades, incremental improvements in therapy derived from clinical trials have resulted in dramatic improvements in the clinical outcome for these patients. Nevertheless, subsets of patients remain with poor outcome. In the last decade, the detection of minimal residual disease (MRD) by flow cytometry or molecular techniques has come to be recognized as one of the most important measures of clinical response to therapy and is now routinely evaluated in experimental clinical treatment protocols. In comparison with molecular techniques, the flow cytometric detection of MRD has the advantage of general applicability, speed and lower cost, and hence has been the preferred method used for MRD detection by the Children’s Oncology Group (COG).

Standard good risk factors identified in acute lymphoblastic leukemia include age between 1 and 9 years, white blood cell count of less than 50,000, less than 5% blasts at days 8, 15 or day 29 by morphology after therapy, absence of testicular or central nervous system involvement, and cytogenetics showing trisomy 4 or 10 or t(12;21). Bad prognostic factors include age less than one year, T cell immunophenotype, hypodiploid DNA content (<44 chromosomes), and cytogenetics showing MLL rearrangement or t(9;22). Use of these prognostic factors in combination in prior COG trials has allowed the identification of low, standard, high, and very-high risk groups. At the time of planning for the last generation of COG trials, evidence from a number of groups suggested that MRD detection by flow cytometry might add additional information for prognostic stratification.

The detection of MRD by flow cytometry presupposes an understanding of the patterns of antigen expression seen at each stage of normal B and T cell maturation. Consequently, the identification of a discrete population of cells whose immunophenotype is different from normal progenitors after therapy can be taken as evidence of residual disease. Knowledge of the immunophenotype of the leukemic population at diagnosis can be helpful in directing the search for residual disease; however, immunophenotypic change after therapy is now well documented and slavish adherence to detection of the diagnostic immunophenotype is not appropriate. In particular, the use of steroids during induction chemotherapy has been shown to result in the apparent maturation of the leukemic population as indicated by increased expression of CD20 and CD45 and reduced expression of CD10 and CD34 in B lineage ALL. Similar loss of immature T cell antigens have been demonstrated in T lineage ALL. Changes in immunophenotype between diagnosis and relapse have also been described in many patients. Despite these challenges, relatively simple antibody combinations have been shown to be suitable for use in minimal residual disease detection in the vast majority of cases.

The sensitive flow cytometric detection of MRD requires evaluation of a suitably large number of cells, roughly 1,000,000 cells in order to achieve a sensitivity of 0.01% of white cells. Once this criterion has been satisfied, the sensitivity of the assay largely depends on the interplay between the degree of immunophenotypic deviation of the leukemic cells from normal progenitors and the number of normal cells of similar immunophenotype in the specimen. The consequence is that the
sensitivity of the assay varies depending on the time point from therapy that is evaluated, being greater earlier in therapy such as at the end of induction and is reduced further from therapy when the number of normal B cell progenitors is increased such as after the end of consolidation. As a general rule, the sensitivity for the detection of B lineage ALL at the end of induction is better than 0.01% while after the end of consolidation may be no better than 0.1%.

The most recent generation of COG clinical trials has firmly established the prognostic importance of MRD detection. Increasing levels of MRD from 0.01% to 0.1% to 1.0% at end of induction therapy are associated with increasingly poor clinical outcome. The presence of MRD at end of induction is also associated with both early (< 3 years) and late (> 3 years) relapse. MRD is not simply a surrogate for other good prognostic risk factors such as cytogenetics, as the presence of MRD in patients with good risk cytogenetics, i.e. t(12;21) and trisomy 4 and 10, also identifies patients with poor outcome. The kinetics of blast eradication adds additional prognostic information, as patients who achieve an MRD negative status at end of induction and were also negative for MRD at day 8 in peripheral blood have an excellent outcome and likely require no additional therapy. Further from the therapy, the identification of MRD positivity at the end of consolidation is a particularly poor prognostic factor and additional therapy may be appropriate. Speaking to the importance of MRD determination, in a multivariate analysis MRD detection at end of induction was the single most important prognostic factor identified, trumping other clinical parameters and cytogenetics.

Although MRD detection is clearly of prognostic importance in ALL, no studies to date have been conducted to demonstrate that modification of therapy based on MRD detection is capable of improving outcome for these patients. The current generation of COG trials attempts to answer this question by incorporating MRD detection into risk stratification and giving additional therapy to those that have levels of MRD above 0.1% at the end of induction. If a high level of MRD persists after additional therapy, the patients are taken off study and more aggressive therapies are administered. Consequently, MRD detection has now reached the point where it is being incorporated in frontline clinical trials and is likely to become part of the standard of care for the treatment of pediatric patients with acute lymphoblastic leukemia.

References