

# ADVANCES IN PEDIATRIC HEM/ONC

Current Developments in the Management of Childhood Malignancies

Section Editor: Mitchell S. Cairo, MD

---

## Monitoring Minimal Residual Disease in Pediatric Hematologic Malignancies

Dario Campana, MD, PhD  
Vice Chair for Laboratory Research, Oncology  
Member, Oncology and Pathology  
St. Jude Children's Research Hospital  
Professor of Pediatrics  
University of Tennessee  
Memphis, Tenn.

### **H&O** Why is the measurement of minimal residual disease important during the treatment of children with hematologic malignancies?

**DC** The clinical significance of minimal residual disease (MRD) in children with acute lymphoblastic leukemia (ALL), which is the most common form of cancer in children, was conclusively demonstrated in the late 1990s. In studies at that time, measurements of MRD emerged as the most important prognostic factors, more than any other presenting features such as age, cytogenetics, or leukocyte count. This finding has been confirmed and reproduced with various protocols using different methodologies that assess MRD at different timepoints. As a consequence, most childhood leukemia protocols today use MRD to guide the intensity of therapy. MRD can be of particular significance when measured early during therapy, for example after 2 or 4 weeks of remission-induction chemotherapy. These are important timepoints in children with ALL, wherein the degree of early leukemic cyto-reduction is a very important prognostic indicator. Studies done in children with acute myeloid leukemia (AML) indicate that MRD is the most important prognostic indicator in this setting as well. Some protocols are now using assessment of MRD to guide the intensity of therapy in children with AML.

### **H&O** Is measurement of MRD useful in less common pediatric hematologic malignancies?

**DC** Chronic leukemias, such as chronic myelogenous leukemia, are very rare in children. There is not enough experience to say whether measurement of MRD is useful in this setting, but based on the experience with adult patients, one would conclude that measuring MRD in children with these diseases could also be clinically useful. Lymphomas are also relatively rare in children. Studies are ongoing to determine the prognostic significance of MRD in children with lymphoblastic lymphoma. At the moment, there is not enough data to suggest that this is going to be an important prognostic factor. Further research is needed.

### **H&O** What assays are used to measure MRD in children?

**DC** There are three main assays that can be used to monitor MRD in acute leukemia in both children and adults. One is flow cytometric detection of aberrant immunophenotypes, which are found in 90% or more of patients with ALL or AML. The sensitivity of this method is approximately 1 leukemic cell in 10,000 normal bone marrow cells. The second method is polymerase chain reaction (PCR) amplification of immunoglobulin or T-cell receptor genes. This method can be applied to 90% of children with ALL but almost no children with AML because antigen receptor genes are typically not rearranged in AML. The sensitivity of this method is

approximately 1 leukemic cell in 100,000 normal cells, 1 log higher than flow cytometry. The third method is PCR amplification of fusion transcripts resulting from chromosomal abnormalities. These are currently found in approximately one third of patients with ALL and AML. The sensitivity of this method ranges from 1 in 1,000 to 1 in 100,000 depending on the molecular abnormalities studied. By using these methods together, MRD can be monitored in every patient, but the level of positivity required to define a patient as MRD-positive varies according to disease. In a child with ALL, St. Jude Children's Research Hospital uses the cutoff point of 1 leukemic cell in 10,000 normal cells because this is the level of sensitivity we can achieve with flow cytometry and PCR in every patient. In the case of AML, we are not confident that flow cytometry can detect 1 in 10,000 cells in all patients, so we use a higher cutoff point of 1 in 1,000 cells to define MRD positivity. Improvements in technology will hopefully allow use of a lower cutoff point in the future. The sensitivity of the method dictates the cut-off point, but 1 leukemic cell in 10,000 normal cells seems to be a clinically informative cutoff point. There is no evidence that detectable disease lower than 1 in 10,000 cells has clinical significance in the early phases of therapy.

### **H&O** Could you discuss the research into genes linked to MRD?

**DC** In addition to its use as a clinical prognostic factor, MRD can be used as an endpoint of sorts in the research setting to assess genes that may be associated with drug sensitivity or resistance in vivo. Some of the work done has looked at gene-expression arrays at diagnosis and sought genes that would be associated with the presence of MRD during early remission-induction therapy. By doing so, a number of genes strongly associated with MRD were found. The prognostic validity of these genes was then tested in an independent cohort of patients not included in the initial research. It was possible to identify a handful of genes that are very strong prognostic indicators in ALL. These genes include some that support the apoptotic process and others that are involved in cell proliferation. Patients who had measurable MRD during remission-induction therapy had a lower expression of genes associated with cell proliferation, suggesting a higher proportion of quiescent cells.

### **H&O** What other research into MRD is ongoing?

**DC** There are ongoing studies looking at the association between MRD and the presence of polymorphisms of genes associated with drug metabolism. Another appli-

cation of MRD could be to alert us about overlooked drug-resistant subtypes of leukemia. For example, it may be observed that certain subtypes of leukemia are more commonly associated with MRD than others, which may prompt more in-depth studies of those specific subtypes. Finally, in the clinical setting, MRD can be used not only to intensify therapy as is common practice now; it can also be used as a way to identify candidates for reduced therapy early on. We implemented a research protocol based on the use of MRD to identify children with ALL who are candidates for treatment reduction in Recife, Brazil. In that setting, as in many centers located in areas with limited resources, the toxicity of chemotherapy can be unacceptably high. If the protocols used at St. Jude Children's Research Hospital were used in these centers, approximately 15% of children with ALL would be likely to have fatal infections as a result of the immunosuppressive chemotherapy received. Therefore, it is urgent in this patient population to reduce therapy as early as possible. The hypothesis underlying the Recife protocol is that patients who have a very good early response to therapy (ie, MRD-negative within 2 weeks of therapy) can be cured with less intensive therapy, similar to the treatment regimens used in the 1970s, which could cure 30–40% of patients. This protocol uses a simplified measurement of MRD based on flow cytometry, which requires a low number of antibodies and is much more affordable than the typical tests. Over 70 patients have been accrued to this study, and though results are not yet available, preliminary observations have been encouraging.

### **H&O** What is the future outlook of research in this setting?

**DC** The currently available methodology for MRD detection is good, but it needs further improvement. With respect to flow cytometry, improvement could take two forms. One improvement would be to discover new markers of leukemia. It would be helpful to develop antibody panels that are more simple, easy, and reliable than previous incarnations. Comparisons of gene-expression profiles of leukemic cells and their normal counterparts (lymphoid or myeloid cells) will enable researchers to find new markers of leukemia that should improve the currently available panels. The second improvement relates to new instruments that allow the simultaneous study of 10 or 15 markers on a cell, rather than only four, as previous technology has allowed. With such technology, not only can MRD be identified, but the biologic features of the MRD cell population could be identified, such as expression of multidrug resistance genes or the quiescent proliferation status mentioned earlier. I believe much more about the biology of MRD will be learned

*(Continued on page 915)*

with new instruments and new antibodies. Another area of research that requires attention is the discovery of why there is a subset of cells resistant to chemotherapy. Are these resistant cells leukemic stem cells or are they cells that interact preferentially with the microenvironment and become protected from chemotherapy by virtue of this interaction? There is a good deal of room for learning in this regard, and once more is known, mechanisms can hopefully be developed to eradicate MRD.

### Suggested Readings

Cavé H, van der Werff ten Bosch J, Suciú S, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer—Childhood Leukemia Cooperative Group. *N Engl J Med*. 1998;339(9):591-598.

Coustan-Smith E, Behm FG, Sanchez J, et al. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. *Lancet*. 1998;351:550-554.

Coustan-Smith E, Ribeiro RC, Stow R, et al. A simplified flow cytometric assay identifies children with acute lymphoblastic leukemia who have a superior clinical outcome. *Blood*. 2006;108:97-102.

Floho C, Coustan-Smith E, Pei D, et al. Genes contributing to minimal residual disease in childhood acute lymphoblastic leukemia: prognostic significance of CASP8AP2. *Blood*. 2006;108:1050-1057.

Floho C, Coustan-Smith E, Pei D, et al. A set of genes that regulate cell proliferation predicts treatment outcome in childhood acute lymphoblastic leukemia. *Blood*. 2007;110:1271-1277.

van Dongen JJ, Seriu T, Panzer-Grumayer ER, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. *Lancet*. 1998;352:1731-1738.