The patient received two cycles as consolidation therapy (methotrexate, idarubicin) but we observed hematologic relapse after three months of CR; FISH analysis showed the same chromosomal abnormality. Reinduction therapy was started with the FLANG regimen. The girl died of resistant disease in December 1999.

Several groups have reported a higher incidence of abnormal/complex karyotypes in AML-M0, particularly deletion -7/7q and/or -5/5q and trisomy 8, 4 and 13, which are all frequently associated with poor prognosis.[1,6] To our knowledge, this t(8;13;14) is the first described in the literature.[7] In this three-way translocation between chromosomes 8, 13 and 14 we also analyzed whether the regions of chromosomes 8 and 14 involved were the same as those found in the t(8;14) reciprocal translocation commonly associated with ALL L3.[8,9] Our data showed that C-MYC was not involved in the rearrangement (data not shown). Larger studies are needed to clarify which chromosome abnormalities contribute to the poor prognosis of this disease.

Tiziana Clelia Storlazzi, Vincenzo Liso,° Francesco Albano,° Gianluigi Castoldi,# Mariano Rocchi,* Giorgina Specchia°*

**Genetic Institute and °Department of Hematology, University of Bari; #Institute of Hematology, University of Ferrara, Italy*

Key words

Acute myeloid leukemia, minimal myeloid differentiation, FISH, t(8;13;14).

Funding

The financial support of AIL "Trenta Ore per la Vita" and AIRC is gratefully acknowledged.

Correspondence

Giorgina Specchia, M.D., Department of Hematology, University of Bari, Policlinico, piazza G. Cesare 11, 70124 Bari, Italy. Phone: internaional +39-080-5478711 – Fax: international+39-080-5428978 – E-mail: emadhba@cimedoc.uniba.it

References

1. Cuneo A, Ferrant A, Michaux JL, et al. Cytogenetic profile of minimally differentiated (FAB M0) acute myeloid leukemia: correlation with clinicobiologic findings. Blood 1995; 85:3688-94.
2. Lee EJ, Pollak A, Leavitt RD, Testa JA, Schiffer CA. Minimally differentiated acute nonlymphocytic leukemia: a distinct entity. Blood 1987; 70:1400-6.
3. Villamor N, Zarco MA, Rozman M, Ribera JM, Feliu E, Montserrat E. Acute myeloblastic leukemia with minimal myeloid differentiation: phenotypical and ultrastructural characteristics. Leukemia 1998; 12:1071-5.
4. Bennett JM, Catovsky D, Daniel MT, et al. Proposal for the recognition of minimally differentiated acute myeloid leukemia (AML-MO). Br J Haematol 1991; 78:325-9.
5. Antonacci R, Marzella R, Finelli P, et al. A panel of subchromosomal painting libraries representing over 300 regions of the human genome. Cytogenet Cell Genet 1995; 68:25-32.
6. Venditti A, Del Poeta G, Stasi R, et al. Minimally differentiated acute myeloid leukaemia (AML-M0): cytochemical, immunophenotypic and cytogenetic analysis of 19 cases. Br J Haematol 1994; 88:784-93.
7. Mitelman F. Catalog of chromosome aberrations in cancer. Sixth edition. (CD-Rom) New York: Wyley-Liss; 1998.
8. Navid F, Mosijczuk AD, Head DR, et al. Acute lymphoblastic leukemia with the (8;14) (q24;q32) translocation and FAB L3 morphology associated with a B-precursor immunophenotype: the Pediatric Oncology Group experience. Leukemia 1999; 13:135-41.
9. Dalla Favera R. The causes and consequences of chromosomal aberrations. Kirsch IR ed. Boca Raton: CRC Press FL; 1993: p. 312-32.

Monitoring of minimal residual disease and mixed chimerism in a case of high-risk TEL/AML1-positive acute lymphocytic leukemia

We report the case of a child with acute lymphocytic leukemia with a combination of positive (TEL/AML1 positivity, age, low level minimal residual disease before bone marrow transplantation) and negative (poor prednisone response, high leukocytosis) prognostic features. We used molecular-genetic techniques and flow cytometry for the follow-up of minimal residual disease.

Sir,

Children with TEL/AML1-positive acute lymphocytic leukemia (ALL) generally have an excellent prognosis. This fusion gene, resulting from t(12;21), is usually found in children aged 2-5 years, with non-hyperdiploid DNA content and low leukocyte count at presentation[1] and very low expression of CD66c.[2] Nevertheless, rare cases have been described in the literature of TEL/AML1 together with a WBC higher than $50x10^9$/L or age over 10 years at diagnosis.[3] It has been documented that relapses do occur in children with TEL/AML1-positive ALL, although their frequency is still discussed.[4,5] Minimal residual disease (MRD) monitoring is a valuable predictor of prognosis throughout conventional chemotherapy as well as before and after bone marrow transplantation (BMT).[6] We combined molecular-genetic techniques and flow cytometry for MRD monitoring in a 4-year old child with TEL/AML1-positive ALL with initial hyperleukocytosis and involvement of the central nervous system. Leukemic blasts were classified as $CD10^+$ with aberrant expression of CD33 on 15% of blasts. Other myeloid markers were negative.[2]

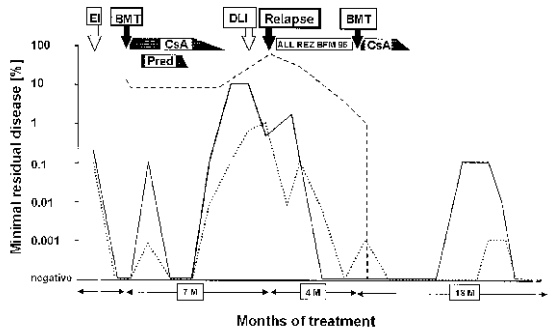

Figure 1

..... MRD levels estimated using IgH DIG-labeled clonospecific probe and/or IgH specific primer (maximum sensitivity is 0.1%)

..... MRD levels estimated using RT-PCR for TEL/AML1 (maximum sensitivity of the approach is 0.001%);

——MRD levels estimated by flow-cytometry (combinations: CD10+CD19+; CD10+CD33+CD19+; CD10++CD20-CD19+; maximum sensitivity is 0.01%);

--------chimerism studies: percentage of autologous hematopoiesis evaluated by PCR (DNA polymorphism of ApoBII, Col2Aa1, TPO: maximum sensitivity of the method is approximately 5%). Unfortunately, separate lineage-specific chimerism was not evaluated during the pre-relapse period. Both molecular-genetic methods for MRD detection are limited by the sensitivity threshold, therefore, "negativity" using the IgH approach means MRD levels < 0.1% and using RT-PCR, levels < 0.001%. Note that RT-PCR demonstrates the expression of TEL/AML1 fusion mRNA, not the actual number of leukemic cells. EI, end of induction therapy.

Cytogenetic studies and cytometric DNA analysis showed a normal karyotype and euploidy. Reverse-transcriptase polymerase chain reaction (RT-PCR) analysis showed TEL/AML1 positivity and FISH analysis confirmed the presence of t(12;21). The patient was treated according to the ALL-BFM 95 protocol – he had only a partial response to prednisone, but hematologic remission was achieved by the end of induction therapy. However, molecular studies using both RT-PCR for TEL/AML1 gene and PCR for IgH rearrangement followed by hybridization with clonospecific probes detected MRD levels higher than 0.1% (Figure 1); cytometric analysis confirmed a high proportion of $CD10^+CD19^+$ cells. The poor prednisone response indicated the patient for BMT from an identical sibling in first complete remission after completing four cycles of chemotherapy for high-risk disease.[7] Immediately before BMT MRD was not detectable. Chimerism studies done weekly post BMT showed weak but continuous mixed chimerism (<10% of autologous hematopoiesis). A bone marrow sample taken on day +28 showed threshold 2nd round RT-PCR positivity ~0.001%) for the TEL/AML1 fusion, IgH PCR/hybridization demonstrated levels lower than 0.1%. Both approaches revealed completely negative results on day +64. Six months after transplantation molecular-genetic relapse of leukemia was diagnosed and despite donor lymphocyte infusion (DLI) with 1×10^8/kg $CD3^+$ cells, hematologic relapse developed. Chemotherapy according to protocol ALL-REZ BFM 96 (group S4) was given and after two cycles the patient achieved a second remission. After a third cycle, eleven months after the first transplantation, in remission without detectable levels of MRD, the patient underwent a second transplantation from the same donor. The patient now remains in remission 1.5 years after the second transplantation. The MRD levels fluctuate below and slightly above the threshold of detection of molecular-genetic methods. In this case negative prognostic features apparently outweighed several favorable factors including TEL/AML1 positivity and molecular remission before BMT.[8] We conclude that evaluation of a single prognostic parameter may be misleading in terms of overall prognosis. Moreover, rapid development of relapse documented by independent methods at frequent time-points over 50 days raises questions about limited time-frames for possible effects of adoptive immunotherapy. The effect of immunotherapy in such settings remains unclear despite being indicated early on the basis of MRD before the onset of hematologic relapse.

Petr Sedlácek, Jan Trka,* Jan Zuna,* Ondrej Hrusák,° Lenka Honzátková-Krsková,# Jan Stary* for the Czech Pediatric Haematology Working Group*

**2nd Dept. of Pediatrics and °Institute of Immunology, 2nd Medical School, Charles University Prague; #Institute of Hematology and Blood Transfusion, Prague, Czech Republic*

Funding

Supported by grant # 3425-3 from the Czech Ministry of Health and by the Czech Ministry of Education Programs #111300001 and #111300003.

Acknowledgments

We would like to thank Dr K. Michalova and Dr Z. Zemanova for the FISH analyses and Dr R. Formankova and J. Madzo for their help with chimerism and MRD analyses.

Correspondence

Petr Sedlácek, MD, 2nd Department of Paediatrics, BM Transplant Unit, 2nd Medical School, Charles University Prague, V úvalu 84, CZ-150 06, Prague 5, Czech Republic. Phone: international +420-2-24432201 – Fax: international +420-2-24432220 – E-mail: petr.sedlacek@lfmotol.cuni.cz

References

1. Trka J, Zuna J, Hrusák O, et al. Impact of TEL/AML1-positive patients on age distribution of childhood acute lymphoblastic leukemia in Czech Republic. Pediatric Haematology Working Group in the Czech Republic. Leukemia 1998; 12:996-7.
2. Hrusák O, Trka J, Zuna J, Housková J, Bartùnková J, Stary J. Aberrant expression of KOR-SA3544 antigen in childhood acute lymphoblastic leukemia predicts

TEL-AML1 negativity. The Pediatric Hematology Working Group in the Czech Republic. Leukemia 1998; 12:1064-70.
3. Rubnitz JE, Shuster JJ, Land VJ, et al. Case-control study suggests a favorable impact of TEL rearrangement in patients with B-lineage acute lymphoblastic leukemia treated with antimetabolite-based therapy: a Pediatric Oncology Group study. Blood 1997; 89: 1143-6.
4. Seeger K, Adams HP, Buchwald D, et al. TEL/AML1 fusion transcript in relapsed childhood acute lymphoblastic leukemia. The Berlin-Frankfurt-Münster Study Group. Blood 1998; 9:1716-22.
5. Zuna J, Hrusák O, Kalinová M, Muzíková K, Stary J, Trka J. TEL/AML1 positivity in childhood ALL: average or better prognosis? Czech Paediatric Haematology Working Group. Leukemia 1999; 13:22-4.
6. Knechtli CJ, Goulden NJ, Hancock JP, et al. Minimal residual disease status before allogeneic bone marrow transplantation is an important determinant of successful outcome for children and adolescents with acute lymphoblastic leukemia. Blood 1998; 92:4072-9.
7. Ladenstein R, Peters C, Gadner H. The present role of bone marrow and stem cell transplantation in the therapy of children with acute leukemia. Ann NY Acad Sci 1997; 824:38-64.
8. Biondi A, Masera G. Molecular pathogenesis of childhood acute lymphoblastic leukemia. Haematologica 1998; 83:651-9.

Use of molecular techniques to confirm true re-emergence of an original clone and to track minimal residual disease in acute lymphoblastic leukemia

We report the case of a young girl whose acute lymphoblastic leukemia (ALL) recurred as lymphoblastic lymphoma 6.6 years after diagnosis. Analysis of minimal residual disease (MRD) allowed tracking of the leukemic clone during the phases of therapy, confirming that relapse was a true re-emergence of the original clone.

Sir,

Relapse of childhood lymphoblastic leukemia 5 or more years after diagnosis is a very rare event. When it happens, a true relapse of the original clone should be confirmed to exclude a secondary malignancy. Confirmation of the relapse can be obtained by assessing the presence of the same features in the clone at diagnosis and at recurrence.[1-5] The patient, a 7-year old girl, was admitted to our hospital for the first time in November 1992, at the age of 7 years. Main symptoms included fever and intercostal pain; the total leukocyte count was 3.3×10^9/L with 13% abnormal cells, Hb was 4.2 g/dL and the platelet count was 82×10^9/L. Bone marrow aspiration showed a subtotal infiltration by leukemic lymphoblasts (L1, PAS+, $CD10^+$, $CD19^+$, $HLA\text{-}DR^+$, TdT 95%). Karyotype was normal. The patient was treated according to the AIEOP 9101 protocol for low risk acute lymphoblastic leukemia (ALL).[7] Cytological remission was achieved on day +42, at the end of the induction phase. After two years of maintenance treatment she discontinued all chemotherapy and remained in continuous remission until June 1999 (6 years and 6 months after the original diagnosis), when she presented with massive lateral cervical lymphadenopathy and a retroperitoneal abdominal mass. Biopsy of a lateral cervical lymph node was consistent with high grade lymphoblastic lymphoma. The DNA index of lymphoblasts was 1.20 and cytogenetic analysis revealed a hyperdiploid pattern in the majority of metaphases. Surface markers were consistent with the original leukemia. Bone marrow aspiration from four different sites and the spinal fluid did not show any leukemic infiltration. The patient was treated according to the AIEOP NHL non-B protocol. After the induction phase she achieved a complete clinical remission confirmed by a CT scan. She subsequently received consolidation treatment and is now in reinduction phase.

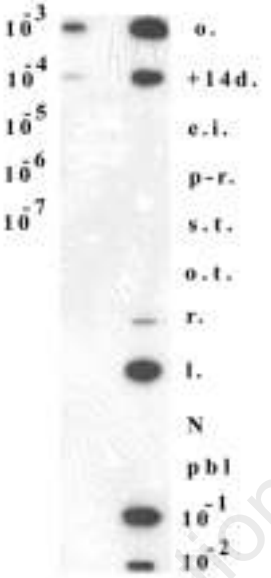

Figure 1. Dot blot of DNA of the patient, hybridized with the patient specific probe, at different time points. O, onset; + 14 d., +14 day; e.i., end of induction; p-r., pre-reinduction; s.t., stop therapy; o.f. off therapy; r., BM at time of relapse; l., lymph node at relapse; N, negative control; pbl, normal peripheral blood: $10^{-1,-2,-3,-4,-5,-6,-7}$ indicate serial dilutions of the onset DNA to test the sensitivity of the probe. The signal is detected until a dilution of 10^{-4}.

DNA at diagnosis was investigated by standard techniques for T-cell receptor gene rearrangements.[6] The Vδ2Dδ3 rearrangement detected was sequenced and a 20mer primer spanning the junctional region was designed to monitor minimal residual disease (MRD) at day +14, end of induction, pre-reinduction, stop therapy, off therapy (+38 months), and relapse. DNA was probed with the patient specific primer. Serial dilutions of the onset DNA were analyzed to test the sensitivity of the probe, which was about 10^{-4}. The patient was positive at day +14 of therapy, but