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 this three-way translocation between chromosomes 8, 13 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.

References

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

We report the case of a child with acute lymphoblastic 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 lymphoblastic 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 presentation1 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 50x10⁹/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
nosed and despite donor lymphocyte infusion molecular-genetic relapse of leukemia was diag-
on day +64. Six months after transplantation approaches revealed completely negative results
demonstrated levels lower than 0.1%. Both for the TEL/AML1 fusion, IgH PCR/hybridization
threshold 2nd round RT-PCR positivity ~0.001% (Figure 1); cytometric analysis con-
firmation of a high proportion of CD10+CD19+ cells. More than 0.1% (Figure 1); cytometric analysis con-
proved the presence of CD10+CD33+CD19+; CD10++CD20-CD19+; maximum sensitivity is 0.01%);———
MRD levels estimated using IgH DIG-labeled clonospecific probe
......
MRD levels estimated using RT-PCR for TEL/AML1 (maximum sensi-
tivity of the approach is 0.1%);———
MRD levels estimated by flow-cytometry (combinations: CD10+CD19+;
CD10+CD3+CD19+; CD10++CD20-CD19+; maximum sensitivity is 0.01%);———
chimerism studies: percentage of autologous hematopoiesis evaluated
by PCR (DNA polymorphism of ApoBII, COL2A1, 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 expres-
sion of TEL/AML1 fusion mRNA, not the actual number of leukemic cells. El.
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 remis-
sion 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 con-
firmed a high proportion of CD10+CD19+ cells. The poor prednisone response indicated the patient for BMT from an identical sibling in first
remission. 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 under-
went 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 factors
precluded the MRD approaches indicating early on the basis of M RD before the onset of hematologic relapse.

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. 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-DR+, TdT 95%). Karyotype was normal. The patient was treated according to the AIEOP 9101 protocol for low risk acute lymphoblastic leukemia (ALL). 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.

DNA at diagnosis was investigated by standard techniques for T-cell receptor gene rearrangements. The V62D83 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. The signal is detected until a dilution of 10^-4.