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Molecules and Cells

Molecular Detection of TEL-AML1 Transcripts as a Diagnostic Tool and for Monitoring of Minimal Residual Disease in B-lineage Childhood Acute Lymphoblastic Leukemia

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The chromosomal translocation t(12;21) (p12;q22) which results in the TEL-AML1 fusion gene is the most frequent genetic rearrangement in childhood B-lineage acute lymphoblastic leukemia (ALL). The rearrangement in this locus, however, is only rarely observed by routine karyotypic analysis. We established a nested-reverse transcriptase-polymerase chain reaction (nested-RT-PCR) technique for the detection of the TEL-AML1 transcript, and used this to investigate the incidence of the rearrangement, and to characterize the disease present in TEL-AML1-positive B-lineage ALL patients. The TEL-AML1 fusion transcript was detected in nine of fourteen patients. These patients were relatively homogeneous in that they were young and had low presenting leukocyte counts, both features of which are associated with a favorable prognosis. Furthermore, we could detect the TEL-AML1 transcript in the peripheral blood of t(12;21)-positive patients and we used this to assess minimal residual disease (MRD) in patients during chemotherapy. The data demonstrate that nested-RT-PCR is a suitable tool for diagnosing t(12;21)-positive ALL, that these patients constitute a clinically distinct subgroup of ALL patients, and that the method could also be used to monitor MRD in these patients.

Keywords: Acute Lymphoblastic Leukemia; CBFA; PEBP2; TEL-AML1.

Introduction

Numerous cytogenetic studies have demonstrated that most human neoplasms carry chromosomal aberrations. Recent developments in molecular biological techniques have permitted an understanding of the pathological consequences of such genetic events, including the chromosomal translocations which are a common characteristic of leukemias (Nucifora and Rowley, 1995). One such translocation, the TEL-AML1 fusion associated with t(12;21) (p13;q22), occurs in approximately 25% of pediatric acute lymphoblastic leukemia (ALL), making it the most common gene rearrangement in childhood leukemia. The *AML1* gene was originally identified at the breakpoint of t(8;21), the chromosomal translocation associated with acute myeloid leukemia (AML, subtype M2). *AML1* was found to encode the DNA-binding subunit of the protein PEBP2 (also called CBF) (Bae *et al*., 1993; Miyoshi *et al*., 1993). The non-DNA binding component of this protein, PEBP2β/CBFβ (Ogawa *et al*., 1993), has also been found to be rearranged in acute myeloid leukemia with inv(16) (Liu *et al*., 1993). Subsequently, *AML1* was found to be involved in several other chromosomal translocations, such as t(3;21) in therapy-related AML of myelodysplastic syndrome (MDS) and chronic myeloid leukemia at the blastic phase. The translocations involving *AML1* produce chimeric proteins: AML1-Evi1, AML1-MDS1 or AML1-EAP (out of frame fusion) in t(3;21) (Mitani *et al*., 1994; Nucifora *et al*., 1994), and AML1-

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Abbreviations: ALL, acute lymphoblastic leukemia; BM, bone marrow; MRD, minimal residual disease; PB, peripheral blood; RT-PCR, reverse transcriptase-polymerase chain reaction.

ETO in t(8;21) (Erickson *et al*., 1992; Miyoshi *et al*., 1993). An additional AML1 chimera is made with the TEL protein. The TEL gene is a member of ETS gene family, and it was initially identified at the breakpoint of t(5;12) (q34;p13) which generated TEL-PDGFRβ (Golub *et al*., 1994). The amino terminal part of the TEL protein, which contains a putative protein-protein dimerization domain, was found to be fused to *AML1* by t(12;21) and the fusion transcript is expressed from the TEL promoter (Golub *et al*., 1995; Romana *et al*., 1995a; 1995b).

The molecular structure of these fusion proteins and their contributions to leukemogenesis have been reviewed previously (Bae and Ito, 1999; Ito and Bae, 1997). In addition, transgenic mice heterozygously knocked-in with chimeric genes have been shown to be defective in definitive hematopoiesis (Castilla *et al*., 1996; Yergeau *et al*., 1997). These observations have led to the general notion that the formation of chimeric genes is primarily responsible for leukemogenesis. Accurate detection of the chimeric genes could thus provide a highly useful diagnostic tool for leukemia.

Currently, treatment of children with acute lymphoblastic leukemia (ALL) is dependent on the presence of distinct prognostic factors. Thus, the disease is treated with a therapy either with reduced toxicity in low risk patients, or with an intensive approach in high risk patients. In newly diagnosed ALL, the patient's age and white blood cell (WBC) counts are the most important criteria for risk assessment (Smith *et al*., 1996), but the patient's response to therapy subsequently also plays a role in determining the treatment required (Steinherz *et al*., 1996). Until 1995, genetically defined risk factors could account for only 35% of childhood ALL patients. These included hyperdiploidy, which is identified as a subset of ALL with a favorable prognosis, and the t(9;22), t(1;19), t(4;11) translocations associated with a poor prognosis. The recent identification of TEL-AML1 from the breakpoint of t(12;21) (p13;q22) of childhood B-lineage ALL has expanded this genetically characterizable group to 60%. It appears that t(12;21) is associated with specific presenting features, namely young age and low presenting WBC count, and that patients with this translocation appear to have a favorable prognosis (Rubnitz *et al*., 1997).

Although the TEL-AML1 fusion associated with t(12;21) is the most common gene rearrangement in childhood leukemia (occurring in approximately 25% of pediatric B-lineage ALL), t(12;21) is rarely detected (0.05% of patients) by routine cytogenetic methods (Romana *et al*., 1995b). Since the fusion transcript is only present in leukemic cells, reverse transcriptase-polymerase chain reaction (RT-PCR) might be a better method for the molecular characterization of leukemia and for monitoring minimal residual disease (MRD) during treatment (Satake *et al*., 1997). In this study, we analyzed newly diagnosed B-lineage childhood ALL cases by a modified method of RT-PCR known as nested-RT-PCR, and used this method to investigate the frequency and clinical impact of t(12;21). We also applied this technique to detect MRD in children with t(12;21)-positive ALL during chemotherapy so as to evaluate the clinical value of this diagnostic tool.

Materials and Methods

Sample preparation Bone marrow (BM) and/or peripheral blood (PB) samples were obtained from fourteen B-lineage ALL patients diagnosed at Chungbuk National University Hospital and Seoul National University Children's Hospital from September 1996 to September 1998. Three of the patients were relapse cases. Diagnosis of ALL was made by standard procedures. Immunophenotyping was performed by flow cytometry (Chen *et al*., 1986). Complete remission was defined by clinical and morphological criteria. All children were treated according to the treatment protocol of the Children's Cancer Group. BM and/or PB were collected before and during chemotherapy. Mononuclear cells (MNC) obtained from BM and/or PB samples were isolated by RNeasy blood kit (QIAGEN) and frozen at −70°C until use. PB mononuclear cells (PBMC) from a healthy child and BM mononuclear cells (BMMC) from a patient of idiopathic thrombocytopenic purpura (ITP) were used as negative controls, while Reh cells bearing t(12;21) served as a positive control.

Cytogenetic studies Cytogenetic studies were systematically performed on BM cells at initial presentation or relapse. Metaphases were obtained from short-term unstimulated cultures (24 and 48 h). Chromosomes were identified using RHG and/or trypsin-Gimesa banding techniques and classified according to the International System for Cytogenetic Human Nomenclature (Mitelman, 1991).

Nested RT-PCR Total RNA was extracted from cryo-preserved cells with the RNeasy blood kit (QIAGEN) according to the manufacturer's instructions, and the quality of RNA was checked by agarose gel electrophoresis. cDNA was synthesized from 1 μg of total RNA using oligo-dT primers (Gibco-BRL, Superscript kit). The first PCR amplification was performed with primers p4 (5′-CCCTGGACAACATGATCC-3′) and m0 (5′-GCTGGCATCGTGGACGTC-3′) and 0.5 units of *Taq* polymerase (Perkin-Elmer) in the buffer recommended by the supplier (Kim *et al*., 1999). The reaction mixtures were placed in a thermal cycler (Perkin-Elmer Cetus model-9600). An initial 2 min denaturation at 95°C was followed by 30 cycles of 15 s at 95°C, 40 s at 55°C, and 1 min at 72°C. One hundredth of the reaction product from this PCR was subjected to a second PCR amplification performed as before except that the p5 (5′- CAGGAGAGCACACGCGTG-3′) and m0 primers were used. The PCR products were analyzed on 2% agarose gel. A schematic diagram of the TEL-AML1 protein and the relative positions of the oligonucleotide primers used are shown in Fig. 1.

Southern blotting analysis PCR products were analysed by Southern blot hybridization as described by Sambrook *et al*. (1989). Briefly, PCR products were electrophoresed on 2% agarose gel and transferred to Hybond-N^+ membrane (Amersham) by the alkaline blotting method recommended by the

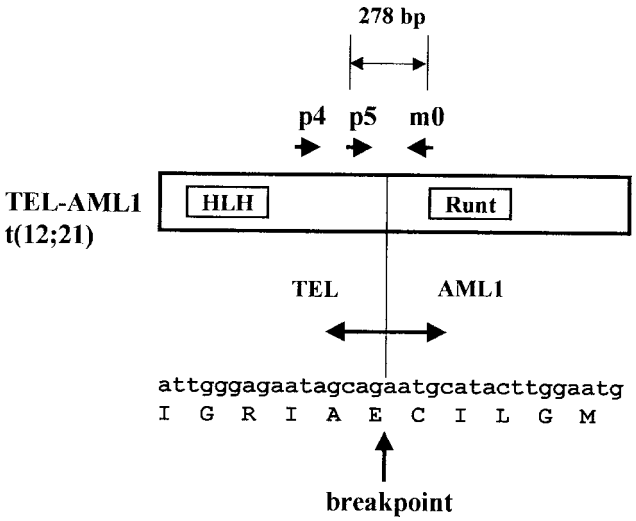

Fig. 1. The structure of the TEL-AML1 fusion protein resulting from t(12;21). Nucleotide and amino acid sequences around the junction region are indicated. Relative positions of the p4, p5 and m0 oligonucleotide primers used for PCR are shown. The runt domain of AML1 and the HLH domain of TEL protein are marked by boxes.

manufacturer. Probe DNA was prepared from TEL-AML1 cDNA and radioactively labeled with [^{32}P]dCTP (3,000Ci/mmole) by the random primer labeling kit (Amersham). Unincorporated nucleotides were removed by Sephadex G-50 spun columns (Sambrook *et al*., 1985). Southern hybridization was performed for 16 h at 65°C. The membrane was washed three times in 0.1× SSC, 0.1% SDS at 65°C for 15 min and exposed to Kodak XAR-5 film for 2 h.

Results

Detection of TEL-AML1 transcripts by nested-RT-PCR TEL-AML1 transcripts were detected in nine of fourteen B-lineage ALL patients, namely in the bone marrow aspirate before induction of chemotherapy in three cases, in the peripheral blood before induction of chemotherapy in two cases, in the bone marrow aspirate after induction of chemotherapy in two cases, and in the bone marrow aspirate in two relapse cases. An RNA extract from Reh cells was used as a positive control for all amplifications, while PBMC from a healthy child and BMMC from an ITP patient were used as negative controls. The nested-RT-PCR products were checked by Southern blot hybridization, and all positive bands were found to bind to the TEL-AML1 cDNA probe (Fig. 2). That RT-PCR amplified the correct sequence was further confirmed by direct sequencing of the PCR products (data not shown).

Cytogenetic analysis of TEL-AML1 positive patients The cytogenetic data of the nine patients who were positive for TEL-AML1 are shown in Table 1. Karyotype analysis was not available for patient No. 9. The t(12;21) could not be detected by conventional banding techniques in any of our patients. The Philadelphia chromosome and its resulting BCR-ABL fusion transcript was detected in two out of 14 patients, both of whom were negative for TEL-AML1.

Clinical Data of TEL-AML1-positive patients All TEL-AML1-positive cases (four boys and five girls) were aged between 3.2 and 15 years (median, 5 years) old at presentation (Table 2). Eight cases were aged between 2 and 10 years old. The presenting leukocyte counts were 1.29-109.8 $\times$ 10^9/L (median 5.44 $\times$ 10^9/L). In eight of the nine cases, the presenting leukocyte counts were below 10 $\times$ 10^9/L. B cells of all patients had a precursor phenotype (HLA-DR+CD19+CD10+). The myeloid marker CD13 was expressed in one case. The TEL-AML1 transcript was not found in the one surface membrane Ig+ B-ALL patient. All patients achieved complete remission after induction of chemotherapy, including the two relapsed patients. Since then, six patients remain in continuous complete remission for a median of 22 months. Two patients, one in CNS and one in BM, relapsed, and two patients died from sepsis due to intensive multiagent chemotherapy.

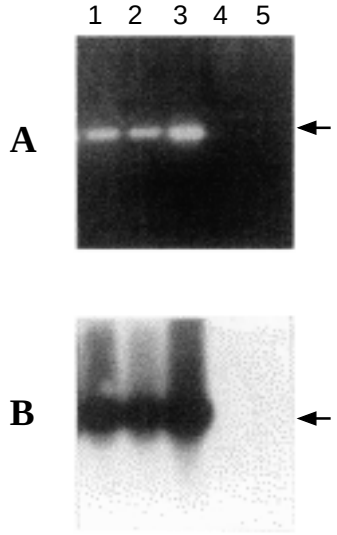

Fig. 2. Detection of the TEL-AML1 transcript in BM or PB samples from leukemic patient 2 by nested-RT-PCR. Testing was initiated after induction of chemotherapy. 1, BM sample immediately after chemotherapy began; 2, PB sample 5 months after diagnosis; 3, PB sample 6 months after diagnosis; 4, PB sample 15 months after diagnosis; 5, PB sample 18 months after diagnosis. The TEL-AML1 transcripts could be detected in PB samples up until 5 months after obtaining complete remission.

Table 1. Cytogenetic Data of TEL-AML1 positive patients

Patient No.	Karyotype
1	27-44, XX, −4,−5,−10,−15,−16,−21,−22[cp8]/46, XX[2]
2	46,XY[2]/44,XY[1]/43,XY[1]/40,XY[1]
3	43,XY[1]/42,XY[2]/46,XY[2]
4	46,XX
5	46,XY,add(8),del(9),t(9;17),del(22)[2]/46, der(9)t(9;17),del(22)[9]/ 45,der(9)t(9;17),−17,del(22)[7]
6	46,XY
7	44-45,XX,−X4],add(10)[5],222[4],2m2-3[1]/ 46,XX[5]
8	46,XX
9	NA

NA, not available.

Detection of MRD in patients with TEL-AML1 using nested RT-PCR MRD was evaluated in BM or PB samples collected from four TEL-AML1-positive patients during chemotherapy. Results of the nested-RT-PCR study and the clinical course of patients are summarized in Table 2 and Fig. 3. Patient 1 became negative for the TEL-AML1 transcript immediately after obtaining complete remission. In chemotherapy. In patients 2 and 3, TEL-AML1 transcripts were still detected in BM samples after achieving complete remission. These patients remained in complete remission which were maintained until the completion of this study, for 2 and 15 months, respectively. After this, the TEL-AML1 transcripts could no longer be detected. In patient 5, an isolated CNS relapse occurred and remission of CNS disease was achieved by craniospinal irradiation. Remission of the BM in this patient was confirmed by light microscopy of the BM three months after the CNS relapse. At this time, the TEL-AML1 transcript became negative. This patient is in continuous complete remission for 10 months at present.

Discussion

Identification of fusion genes has enabled us not only to study the molecular mechanisms of leukemogenesis but also to develop molecular tools for the diagnosis of leukemia. The TEL-AML1 fusion associated with the t(12;21) (p13;q22) translocation is specific for B lineage ALL. It is the most common gene rearrangement in

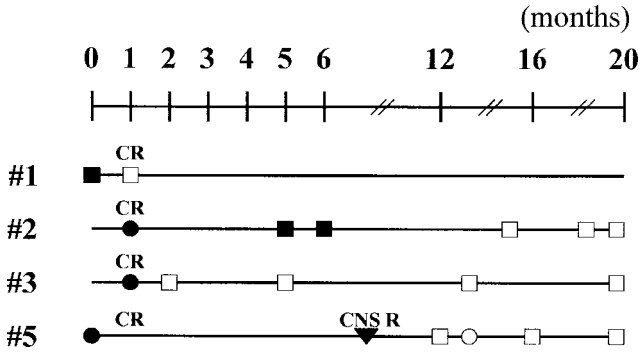

Fig. 3. Testing for the presence of the TEL-AML1 transcript during the long term follow-up of ALL patients. BM or PB samples were obtained from patients No. 1, 2, 3 and 5 and the presence of TEL-AML1 transcripts were analysed by nested-RT-PCR. Closed circle, TEL-AML1-positive BM; open circle, TEL-AML1 negative-BM; closed rectangle, TEL-AML1-positive PB; open rectangle, TEL-AML1-negative PB; closed triangle, CNS R (isolated craniospinal relapse); CR, complete remission.

Table 2. Clinical data of TEL-AML1 positive patients.

No.	Age(yr)/Sex	WBC (10^9/L)	Immunophenotype	Disease status	Clinical course*	Survival (months)$^+$
1	4.3/F	5.44	DR, CD19, CD10	D	CCR	20
2	3.8/M	5.6	DR, CD19, CD10, CD13	D	CCR	23
3	3.2/M	6.5	DR, CD19, CD10	D	CCR	23
4	7.6/F	109.8	DR, CD19, CD10	D	CCR	18
5	5/M	3.2	DR, CD19, CD10	D	CR→CNS R→CR	22(10)
6	5.7/M	1.29	CD10	R	CCR	23
7	15/F	7.7	DR, CD19, CD10	R	CR→Death	3
8	9.4/F	4.15	DR, CD19, CD10	D	CR→BM R→CR→Death	12(10)
9	3.5/F	1.65	DR, CD19, CD10	D	CCR	17

D, diagnosis; R, replapse; CCR, continuous complete remission; CR, complete remission; CNS R, isolated craniospinal relapse; BM R, bone marrow relapse; $^+$Numbers in parentheses indicates months from diagnosi to the first relapse.

childhood ALL and is predictive of a favorable treatment outcome (Borkhardt *et al*., 1997; Liang *et al*., 1996). In this study, we have established a nested-RT-PCR technique which allows detection of the TEL-AML1 fusion in pediatric B-lineage ALL patients. The modification of the standard RT-PCR method (see Materials and Methods) significantly improved the results in that nonspecific bands were removed and the authentic band was intensified. With this method, we were able to calculate that 64% of our pediatric ALL patients (9 of 14) were TEL-AML1-positive. This incidence is quite high compared to previous reports (16–36%), but it should be noted that our sample size is quite small and this could have contributed to a misleading high incidence. It is unlikely that some patients were false positives since the authenticity of the PCR products was verified by both Southern blot hybridization and DNA sequence analysis. Cross contamination was also strictly monitored by including positive and negative controls in all PCR reactions. The positive control consisted of Reh cells which contain t(12;21), while PBMC from a healthy child and BMMC of an ITP patient served as negative controls. In all experiments, the TEL-AML1 transcript was detected in the Reh cells but not in the negative controls (data not shown).

Our data suggest that patients who are TEL-AML1-positive are a homogeneous subgroup of ALL patients characterized by a young age (between 2 and 10 years) and a lack of hyper-leukocytosis in nearly all cases. Both of these features are associated with a good prognosis. Our data are thus in line with previous observations regarding t(12;21)-positive patients (Rubnitz *et al*., 1997). Immunophenotypic analysis did not reveal any remarkable features other than the expression of myeloid-associated antigen (CD13) in one of the nine cases.

We could detect TEL-AML1 transcripts in PBMC before and during chemotherapy, highlighting an advantage of the nested-RT-PCR method. That this method is sensitive enough to detect MRD from PBMC is important because it is difficult to obtain multiple BM samples during chemotherapy. It has previously been shown that RT-PCR detection of MRD by amplification of chimeric mRNAs produced by chromosomal translocations provides clinically useful information which may have therapeutic implications (Campana and Pui, 1995; Lion, 1996). Regarding t(12;21)-positive patients with MRD, the efficacy of and indications for subsequent treatment are presently unknown and carefully implemented long-term studies (Cayuela *et al*., 1996) are required.

The t(12;21) is particularly difficult to detect by classic cytogenetics. Although screening assays using fluorescence *in situ* hybridization (FISH) or PCR reveal that 25% of pediatric B-lineage ALL patients are positive for this fusion, only 0.05% of patients are shown to be positive by routine cytogenetic methods. We also could not detect the t(12;21) by conventional banding techniques in any of our cases, confirming previous reports of the inadequacy of this method for this purpose. While detection of antigen-receptor gene rearrangements is also a specific and sensitive tool for the diagnosis of B-lineage ALLs, this method is quite tedious and cumbersome. Given that we can detect the TEL-AML1 fusion reliably and easily with the nested RT-PCR method, we believe this could be a powerful diagnostic tool which could simplify diagnosis and evaluation of MRD in childhood B-lineage ALLs, thus leading to more effective therapeutic strategies.

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References

Bae, S.-C. and Ito, Y. (1999) Regulation mechanisms for the heterodimeric transcription factor, PEBP2/CBF. *Histol. Histopathol.* **14**, 1213–1221.

Bae, S.-C., Yamaguchi-Iwai, Y., Ogawa, E., Maruyama, M., Inuzuka, M., Kagoshima, H., Satake, M., and Ito, Y. (1993) Isolation of PEBP2αB cDNA representing the mouse homolog of human acute myeloid leukemia gene *AML1*. *Oncogene* **8**, 809–814.

Borkhardt, A., Cazzaniga, G., Viehmann, S., Valsecchi, M. G., Ludwig, W. D., Burci, L., Mangioni, S., Schrappe, M., Riehm, H., Lampert, F., Basso, G., Giuseppe, M., Harbott, J., Biondi, A. (1997) Incidence and clinical relevance of TEL/AML1 fusion genes in children with acute lymphoblastic leukemia enrolled in the German and Italian multicenter therapy trials. *Blood* **90**, 571–577.

Campana, D. and Pui, C. H. (1995) Detection of minimal residual disease in acute leukemia: methodologic advances and clinical significance. *Blood* **85**, 1416–1434.

Castilla, L. H., Wijmenga, C., Wang, Q., Stacy, T., Speck, N. A., Eckhaus, M., Marin-Padilla, M., Collins, F. S., Wynshaw-Boris, A., and Liu, P. P. (1996) Failure of embryonic hematopoiesis and lethal hemorrhages in mouse embryos heterozygous for a knocked-in leukemia gene CBFB-MYH11. *Cell* **87**, 687–696.

Cayuela, J. M., Baruchel, A., Orange, C., Madani, A., Auclerc, M. F., Daniel, M. T., Schaison, G., and Sigaux, F. (1996) TEL-AML1 fusion RNA as a new target to detect minimal residual disease in pediatric B-cell precursor acute lymphoblastic leukemia. *Blood* **88**, 302–308.

Chen, Z., Sigaux, F., Miglierina, R., Valensi, F., Daniel, M. T., Ochoa-Noguera, M. H., and Flandrin, G. (1986) Immunological typing of acute lymphoblastic leukemia: oncurrent analysis of flow cytofluorometry and immunocytology. *Leuk. Res.* **10**, 1411.

Erickson, P., Gao, J., Chang, K. S., Look, T., Whisenant, E., Raimondi, S., Lasher, R., Trujillo, J., Rowely, I., and Drabkin, H. (1992) Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, AML1/ETO, with similarity to *Drosophila* segmentation gene, runt. *Blood* **80**, 825–1831.

Golub, T. R., Barker, G. F., Lovett, M., and Gilliland, D. G. (1994) Fusion of PDGF receptor beta to a novel ets-like gene,

tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell* **77**, 307–316.

Golub, T. R., Barker, G. F., Bohlander, S. K., Ward, D. C., Bary-Ward, P., Morgan, E., Raimondi, S. C., Rowely, J. D., and Gilliland, D. G. (1995) Fusion of the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. *Proc. Natl. Acad. Sci. USA* **92**, 4917–4921.

Ito, Y. and Bae, S.-C. (1997) The Runt domain transcription factor, PEBP2/CBF, and its involvement in human leukemia; in *Oncogenes as Transcription Regulators*, Yaniv, M. and Ghysdael, J. (eds.), Vol. 2, pp. 107–132, Birkhauser Basel, Switzerland.

Kim, S. J., Park, J. G., and Lee, C. C. (1999) Transcript titers of ecdysteroid receptor components vary between tissues and stages during *Drosophila* development. *Mol. Cells* **9**, 61–66.

Liang, D. C., Chou, T. B., Chen, J. S., Shurtleff, S. A., Rubnitz, J. E., Downing, J. R., Pui, C. H., and Shih, L. Y. (1996) High incidence of TEL/AML1 fusion resulting from a cryptic t(12;21) in childhood B-lineage acute lymphoblastic leukemia in Taiwan. *Leukemia* **10**, 991–993.

Lion, T. (1996) Monitoring of residual disease in chronic myelogenous leukemia: methodological approaches and clinical aspects. *Leukemia* **10**, 896–906

Liu, P. P., Tarle, S. A., Hajra, A., Claxton, D. F., Marlton, P., Freedman, M., Siciliano M. J., and Collins, F. S. (1993) Fusion between transcription factor CBFβ/PEBP2β and a myosin heavy chain in acute myeloid leukemia. *Science* **261**, 1041–1044.

Mitani, K., Ogawa, S., Tanaka, T., Miyoshi, H., Kurokawa, M., Mano, H., Yazaki, Y., Ohki, M., and Hirai, H. (1994) Generation of the AML1-EVI1 fusion gene in the t(3;21) causes blastic crisis in chronic myelocytic leukemia. *EMBO J.* **13**, 504–510.

Mitelman, F. (1991) *ISCN*: Guidelines for Cancer Cytogenetics, Supplement to an International System for Human Genetic Nomenclature. Basel, Switzerland, Karger.

Miyoshi, H., Ohira, M., Shimizu, K., Enomoto, K., Maseki, N., Kaneko, Y., Kamada, N., and Ohki, M. (1993) The t(8;21) translocation acute myeloid leukemia results in production of an AML1-MTG8 fusion transcript. *EMBO J.* **12**, 2715–2721.

Nucifora, G. and Rowley, J. D. (1995) AML1 and the 8;21 and 3;21 translocation in acute and cronic myeloid leukemia. *Blood* **86**, 1–14.

Nucifora, G., Begy, C. R., Kobayashi, H., Roulston, D., Claxton, D., Pedersen-Bjergaard, J., Parganas, E., Ihle, J. N., and Rowley, J. D. (1994) Consistant intergenic splicing and production of multiple transcripts between AML1 at 21q22 and unrelated genes at 3q26 in (3;21) (q26;q22) translocations. *Proc. Natl. Acad. Sci. USA* **91**, 4004–4008.

Ogawa, E., Inuzuka, M., Maruyama, M., Satake, M., Naito-fujimoto, M., Ito, Y., and Shigesada, K. (1993) Molecular cloning and characterization of PEBP2β, the heterodimeric partner of a novel *Drosophila* runt-related DNA binding protein PEBP2a. *Virology* **194**, 314–331.

Romana, S. P, Mauchauffe, M., Chumakov, I., Le Paslier, D., Berger, R., and Bernard, O. A. (1995a) The t(12;21) of acute lymphoblastic leukemia results in a TEL-AMLI gene fusion. *Blood* **85**, 3662–3670.

Romana, S. P., Poirel, H., Leconiat, M., Flexor, M. A., Mauchauffe, M., Jonveaux, P., Macintyre, E. A., Berger, R., and Bernard, O. A. (1995b) High frequency of t(12;21) in childhood B-lineage acute lymphoblastic leukemia. *Blood* **86**, 4263–4269.

Rubnitz, J. E., Downing, J. R., Pui, C. H., Shurtleff, S. A., Raimondi, S. C., Evans, W. E., Head, D. R., Crist, W. M., Rivera, G. K., Hancock, M. L., Boyett, J. M., Buijs, A., Grosveld, G., and Behm, F. G. (1997) TEL gene rearrangement in acute lymphoblastic leukemia: a new genetic marker with prognostic significance. *J. Clin. Oncol.* **15**, 1150–1157.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Satake, N., Kobayashi, H., Tsunematsu, Y., Kawasaki, H., Horikoshi, Y., Koizumi, S., and Kaneko, Y. (1997) Minimal residual disease with TEL-AML1 fusion transcript in childhood acute lymphoblastic leukaemia with t(12;21). *Br. J. Hematol.* **97**, 607–611.

Smith, M., Arthur, D., Camitta, B., Carroll, A. J., Crist, W., Gaynon, P., Gelber, R., Heerema, N., Korn, E. L., Link, M., Murphy, S., Pui, C. H., Pullen, J., Reamon, G., Sallan, S. E., Sather, H., Shuster, J., Simon, R., Trigg, M., Tubergen, D., Uckun, F., and Ungerleider, R. (1996) Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. *J. Clin. Oncol.* **14**, 18–24.

Steinherz, P. G., Gaynon, P. S., Breneman, J. C., Cherlow, J. M., Grossman, N. J., Kersey, J. H., Johnstone, H. S., Sather, H. N., Trogg, M. E., Chappell, R., Hammond, D., and Bleyer, W. A. (1996) Cytoreduction and prognosis in acute lymphoblastic leukemia — The importance of early marrow response: report from the Childrens Cancer Group. *J. Clin. Oncol.* **14**, 389–399.

Yergeau, D. A., Hetherington, C. J., Wang, Q., Zhang, P., Sharpe, A. H., Binder, M., Marin-Padilla, M., Tenen, D. G., Speck, N. A., and Zhang, D. E. (1997) Embryonic lethality and impairment of haematopoiesis in mice heterozygous for an AML1-ETO fusion gene. *Nat. Genet.* **16**, 307–310.