# The TEL-AML1 translocation in two paediatric cases of acute lymphoblastic leukemia

**Gill HK[1], Ten SK[2], Dhaliwal JS[1], Tan SK[3], Roshida H[3], Yasmin A[4], Zubaidah Z[2], Nasuruddin BA[1], Yong ASM[5], Chang KM[5], Hishamshah I[6], Eni J[6], Purushotaman V[5], and Ho CML[6]** *[1]Immunology Division, Institute for Medical Research; [2]Haematology Division, Institute for Medical Research; [3]Department of Pathology, Kuala Lumpur Hospital; [4]Blood Bank, Kuala Lumpur Hospital; [5]Clinical Haematology Unit, Kuala Lumpur Hospital; [6]Paediatric Institute, Kuala Lumpur Hospital. (Correspondence: Dr Harvindar Kaur Gill; e-mail: gillhk@imr.gov.my)*

**Abstract**

The TEL-AML1 translocation was detected in two paediatric cases of acute lymphoblastic leukemia. Case 14 was a three year old boy with a WBC of 5.0 x $10^9$/l who was diagnosed with ALL-L1, while case 28 was a five year old girl with a WBC of 22.1 x $10^9$/l who was also diagnosed with ALL-L1. The karyotype of case 14 was 46XY[3] and that of case 28 was 46XX[4]/46XX, -5,add (9q),+mar[2]. Immunophenotyping of case 14 classified the case as CALLA-ALL with co-expression of the CD13/CD33 myeloid antigens. RT-PCR on RNA from both cases generated a 464 hp fusion product which sequencing confirmed as the TEL gene joined to exon 2 of the AML1 gene. Case 28 also displayed a minor band of the 425 bp shorter fusion product in which the TEL gene is joined to exon 3 of the AML1 gene. A less intense band of the reciprocal AML1-TEL translocation was also detected in both cases. The presence of the fusion transcript was also confirmed by Fluorescence *In Situ* Hybridization (FISH). In case 14, FISH further revealed that a proportion of the cells with a TEL-AML1 fusion signal showed an associated loss of the non-translocated TEL gene.

Key Words: Translocation; TEL-AML1; ALL; RT-PCR; FISH

## Introduction

The fusion of the TEL, or ETV6, gene on chromosome 12 to most of the AML1, or CBFα, gene on chromosome 21 results in the TEL-AML1 t (12; 21)(p13; q22) translocation (Golub *et al.*, 1995; Romana *et al.*, 1995). This fusion transcript is found in about 20% of all childhood leukemias (Borkhardt *et al.*, 1999). It is exclusively associated with B-lineage acute lymphoblastic leukemia (ALL) and in this particular group, its occurrence maybe as high as 35% (Garcia-Sanz *et al.*, 1999). Thus t (12; 21) is widely accepted as the most frequent genetic rearrangement in childhood leukemias. Its frequency in Malaysian childhood ALL is unknown. But two studies, one in Spain (Garcia-Sanz *et al.*, 1999) and another in India (Inamdar *et al.*, 1998) revealed that TEL-AML1 incidence in these populations is much lower, 2 % and 9 % respectively, than hitherto found in most studies.

The TEL-AML1 translocation segregates with B-lineage ALL, either C-ALL or B precursor ALL, and is associated, to a significant extent, with the co-expression of myeloid markers CD13/CD33 (Baruchel *et al.*, 1997; Borkhardt *et al.*, 1997). It also segregates with non-hyperdiploidy that is to say leukemia cells which have less than 50 chromosomes per cell (Borkhardt *et al.*, 1999). Hyperdiploidy is associated with a good prognosis in childhood leukemias (Chessels *et al.*, 1997). The t (12; 21) is generally, but not always, found in patients classified as standard risk (Kersey, 1997) that is to say, those 1) between 1 and 10 years of age and 2) with a total white blood cell count < 50 x $10^9$/l. Whilst generally favourable, the above features convey a mixed picture insofar as prognosis is concerned. Given that genetic translocations per se are important prognostic indicators (Ma *et al.*, 1999), the crucial issue is the prognosis conferred by the TEL-AML1 translocation independently of the features outlined above. TEL-AML1 has been reported to confer a good prognosis independent of other risk factors (Rubnitz *et al.*, 1997). Furthermore it has been reported that the relapse rate in TEL-AML1 positive patients compared to TEL-AML1 negative patients, is absent *i.e.* 0% versus 29.6% (McLean *et al.*, 1996) to low ie. 3.0% versus 11.1% (Borkhardt *et al.*, 1997). However, this has been challenged by two studies (Harbott *et al.*, 1997; Seeger *et al*, 1998) which showed that t (12; 21) occurs at the same

high frequency in relapse cases *i.e.* about 20 %, that it does in new cases.

The TEL-AML1 translocation is a cryptic translocation. The similar banding patterns of the chromosomal segments involved make it difficult to detect by conventional cytogenetic techniques. Consequently, the first reports of t (12; 21), in the middle of the last decade (Romana *et al.*, 1994, Golub *et al.* 1995, Romana *et al.*, 1995), employed FISH and/or RT-PCR in the detection of this translocation. This is part of a study to determine the frequency of this translocation in Malaysian ALL patients and in this paper we describe the detection of the TEL-AML1 translocation, by RT-PCR and FISH, in two Malaysian paediatric ALL patients.

## Materials and Methods

### *RT-PCR Assay*

Total RNA was extracted from fresh bone marrow or blood samples with the Rneasy Mini Blood Kit (Qiagen, Germany) as recommended by the manufacturer. First strand cDNA was synthesised with 1µg of total RNA using Superscript™ II Rnase H- Reverse Transcriptase (Gibco BRL, USA) and random hexamers (Promega, USA) incubated at 42°C for 60 min.

PCR amplification to detect the TEL-AML1 fusion transcript was carried out as described by Nakao *et al.* (1996) using the TL667 and 3HO combination of primers. Essentially, cDNA corresponding to 40ng of RNA was amplified in a 50µl reaction containing 200µM of each dNTP, 1 x PCR buffer, 2 U of AmpliTaq DNA Polymerase (Perkin Elmer, USA), 4% DMSO and 40pmol of each primer. The cycling conditions were as follows: 94°C for 5 min, followed by 40 cycles of 20s at 61°C, 60s at 72°C, 30s at 94°C, with a 10 minute final extension at 72°C. Detection of the reciprocal AML1-TEL fusion transcript, using the 1H and ETS primers (Nakao *et al.*, 1996), was carried out under the same conditions as that for the TEL-AML1 transcript, except that DMSO was omitted from the reaction mix.

RNA integrity was ascertained by amplification of an exon 5-6 fragment of the AML1 gene, under identical conditions using primers 5H and 6H (Nakao *et al.*, 1996). Both negative and positive controls were used in all assays. S. Moore (Institute of Medical & Veterinary Sciences, Adelaide) kindly provided the RNA from the PER-145 cell line, raised from an 11 year old boy with ALL (Kees, 1987) which carries the t (12; 21) and was used as a positive control. Rigorous precautions were taken to prevent cross contamination.

PCR products (7.2µl) were resolved on a gel of 2.5 % Nusieve Agarose (FMC, USA), which was stained with ethidium bromide and then photographed under ultraviolet light.

### *Sequencing of the PCR product*

The TEL-AML1 PCR product obtained using TL667 and 3HO primers was diluted 1:1,500 and cycle sequenced in both the forward and reverse directions with Cy5 labelled nested primers, TL841 and 3HI. Cycling was carried out using the Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech). The cycling conditions were as follows: 94°C for 120s, followed by 25 cycles of 95°C at 30s, 60°C at 30s, 72°C at 30s. The resulting products were processed according to the manufacturer's instructions and sequenced on Long Ranger gels (FMC, USA) in a Pharmacia Alf Express sequencer.

### *Cytogenetic study*

Chromosome analysis was performed on trypsin-Giemsa banded bone marrow spreads harvested after 24 hours of culture. The analysis of the metaphases was done in accordance with the International System for Human Cytogenetic Nomenclature (ISCN 1995).

### *FISH study*

FISH analysis was carried out on stored cell suspensions (-20°C) using the LSI TEL-AML1 extra signal (ES) dual colour probe (Vysis Inc., Downers Grove, IL, U.S.A.). The rapid method of the Birmingham's Womens Hospital, with minor modifications, was used in the hybridization and detection of the TEL & AML1 genes. Slides were counterstained with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) and visualised on an Olympus fluorescence microscope. The FISH images were acquired, digitised and analysed using the Cytovision Ultra (Applied Imaging).

## Results

### *Patients*

#### *Case 14*

Case 14 is a 3 year old Malay boy who presented with pallor (1 month) and fever (2 weeks). He had a hepatomegaly of 7 cm and a splenomegaly of 4 cm. The Hb was 5.2 gm %, WBC 5.0 x $10^9$/l, platelets 30 x $10^9$/l and 55% blasts in peripheral blood. The bone marrow aspirate showed a hypercellularity with 98%

blasts. The leukemia was classified as acute lymphoblastic leukemia with L1 morphology. The immunophenotye was CD10+, CD19+, CD22+, HLA-DR+, CD13+, CD33+ and CD 34+. The karyotype was that of a normal male *i.e.* 46XY[3]. The patient was placed on chemotherapy for standard risk ALL and is presently in the maintenance phase after receiving induction, two blocks of intensification and high dose methotrexate for central nervous system prophylaxis. Remission was achieved after the induction phase, and the patient will undergo a two-year treatment regimen.

*Case 28*

Case 28 was a 5 year old Malay girl, who presented with fever, pallor and easy bruising, which she had suffered for a week. She had a hepatomegaly of 4 cm and a splenomegaly of 2 cm. Haematological data was as follows: Hb 3.9 gm%, WBC 22.1 x $10^9$/l, platelets 3 x $10^9$/l and 88% blasts in peripheral blood. The case was classified as acute lymphoblastic leukemia of the L1 type. Immunophenotyping was not possible as the sample was inadequate. The karyotype analysis revealed two clones, an apparently normal 46XX[2], and abnormal clone showing 46XX, -5,add (9q), +mar [2]. The child was placed on induction chemotherapy comprising prednisolone, vincristine, L-asparaginase and intrathecal methotrexate. Unfortunately, the patient acquired a *Klebsiella* septicaemia in the second week of therapy, which progressed, despite appropriate antibiotics, and died three weeks after diagnosis.

***RT-PCR Detection of the TEL-AML1 translocation***

The TL667 and 3HO primers generate a long 464-bp TEL-AML1 fusion product in which the TEL gene is fused to exon 2 of the AML1 gene. However, a shorter fusion product of 425 bp is found in a minor subset of cases, in which the TEL gene is fused to exon 3 of the AML1 gene (Nakao *et al.*, 1996, Aguiar *et al.*, 1996) . Fig. 1 shows that PCR generated the same product in the two cases, 14 & 28, and the PER-145 cell line. This was shown to be the long product by sequencing (see below). Both cases were positive for the reciprocal AML1-TEL, although both bands were less intense then those for the TEL-AML1 product. The reciprocal translocation was absent in the PER-145 cell line. Fig 2 which gives a more detailed picture of case 28, shows an interesting phenomenon in that while the major product for the TEL-AML1 transcript is of the 464 bp type, there is also a minor band of the shorter 425 bp product. Both cases also display the same phenomenon for the reciprocal translocation *ie.* a major band for the shorter product and a minor band for the longer product (Fig. 1). cDNA from case 14 was sent to S. Moore, at the IMVS in Adelaide, to confirm the presence of the fusion transcript. S Moore confirmed that case 14 was positive for t(12;21) using the TELa and AML1a primers of Aguiar *et al.* (1996) which generated a long 269 bp product.

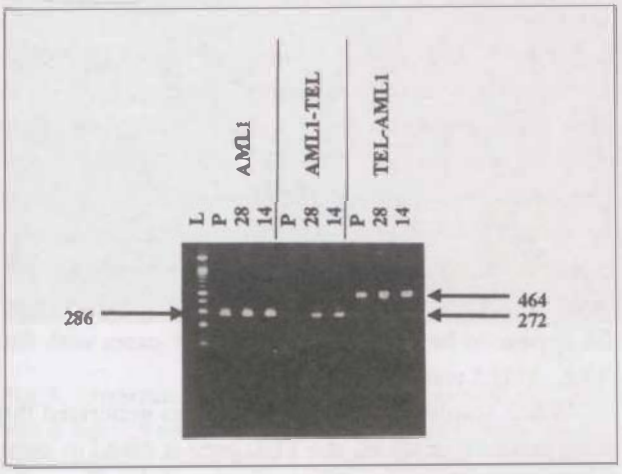


Fig. 1. Detection of TEL-AML1, AML1-TEL & AML1 transcripts by RT-PCR. In both the cases, 14 & 28, & the PER-145 cell line the TEL-AML1 product detected is the long 464 bp product. The AML1-TEL product detected in cases 14 & 28 is the short 272 bp product. Both cases also display a faint band for the long 311 bp AML1-TEL product. An exon 5-6 fragment (286 bp) of AML1 was amplified to check the integrity of the RNA.

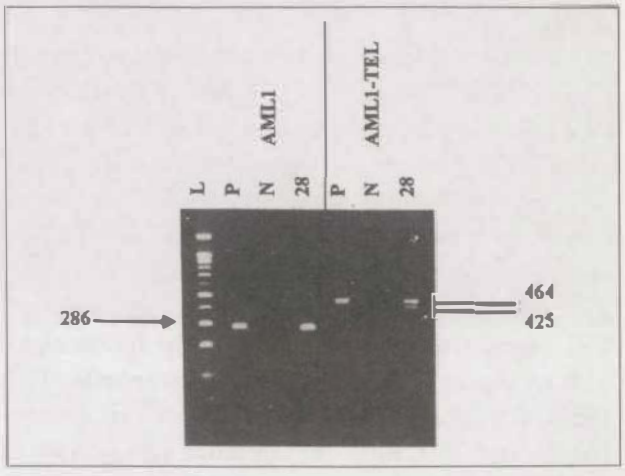


Fig. 2. Detection of TEL-AML1 & AML1 transcripts by RT-PCR. Case 28 shows a major band for the long 464 bp TEL-AML1 product & a minor band for the TEL-AML1 short 425 hp product (P = PER-145 cell line, N = Negative Control). An exon 5-6 fragment (286 hp) of AML1 was amplified to check the integrity of the RNA.

### *Sequencing of the product*

Sequence analysis of the TEL-AML1 chimeric products revealed, Fig. 3, that in both cases 14 and 28, the TEL nucleotide 1033 (Accession no. NM001987) was fused to the nucleotide 503 on exon 2 of the AML1C (Accession no. D43969) sequence. The motif at the breakpoint therefore was GGGAGAATACAG/AATGCATACTT with nucleotides to the left of the breakpoint being part of the TEL gene and nucleotides to the right being part of the AML1 gene (Nakao *et al.*, 1996; Aguiar *et al.*, 1996).

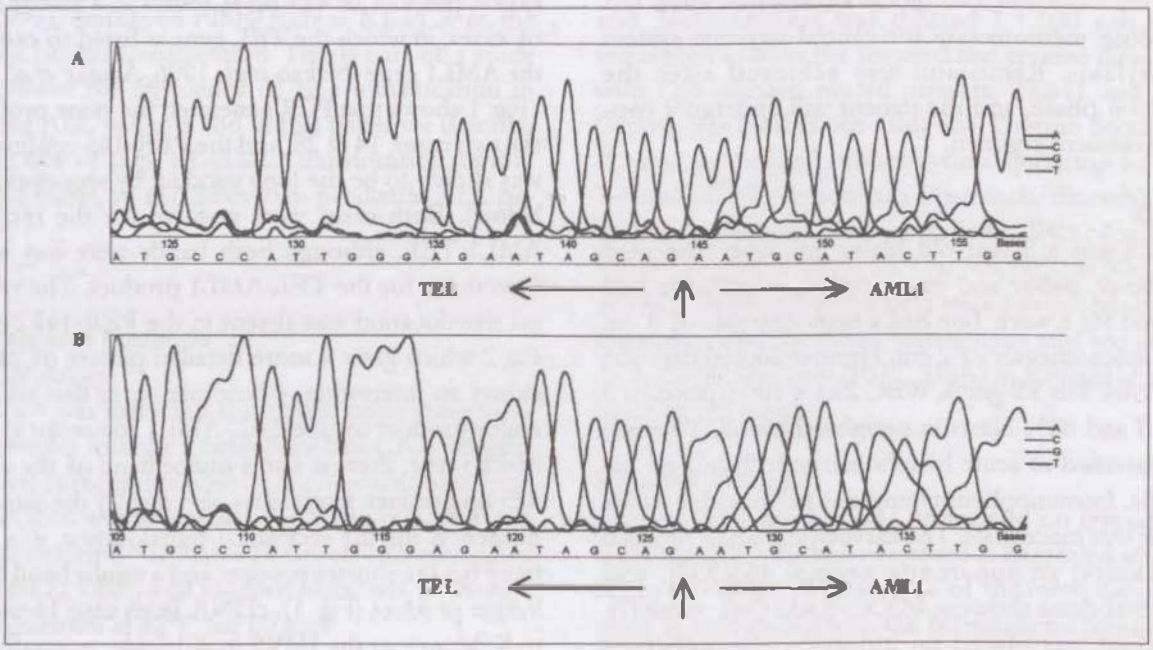


Fig. 3. Sequence analysis of the TEL-AML1 transcripts of case 14 (A) and case 28 (B). Both sequences show that the TEL gene (nt 1033) joins exon 2 of the AML1 gene.

### *FISH*

In case 14, FISH analysis of 3 metaphase spreads showed fusion of the LSI TEL-AML1 translocation probe on one chromosome 21q, with absence of the other TEL signal on the normal chromosome 12 (Fig. 4a). Ten metaphases displayed separate TEL and AML1 signals indicating the absence of the TEL-AML1 rearrangement. Fifty-four percent, of 300 nuclei screened, displayed the TEL-AML1 fusion signal and a normal TEL signal (Fig. 4b), 25% showed the fusion signal with an absent TEL, and 21% showed separate TEL and AML1 signals. These results indicate that the non-translocated TEL allele was deleted in a proportion of the TEL-AML1 fusion positive cells. In case 28, FISH analysis on 10 metaphases showed a TEL signal on the normal 12, a native AML1 signal on the normal 21, a smaller AML1 translocated signal on the der (12), and a yellow TEL-AML1 fusion signal on the der (21) (Fig. 5a). Eighty-eight per cent, of the 200 nuclei screened, displayed the TEL-AML1 fusion signal (Fig. 5b).

## Discussion

In terms of age and cell count, both cases 14 and 28 would be classified as standard risk patients. Both cases also have leukemia cells with non-hyperdiploid chromosomal content. Case 14 has a CALLA-positive immunophenotype with co-expression of the myeloid antigens CD13/CD33, which is not uncommon in patients with the TEL-AML1 translocation (Baruchel *et al.*, 1997; Borkhardt *et al.*, 1997). Thus, case 14 and 28 appear to be quite characteristic of cases with the TEL-AML1 translocation.

When amplified by PCR, both cases generated the long product, in which the TEL gene is fused to exon 2 of the AML1 gene. Interestingly, whilst the major band in case 28 was the long 464 bp product, a minor band of the shorter 425 bp product was clearly visible. This phenomenon was also observed by Borkhardt *et al.* (1997) and Seeger *et al.* (1998) who explained it as alternate splicing of the AML1 gene. Most studies report that majority of TEL-AML1 positive patients

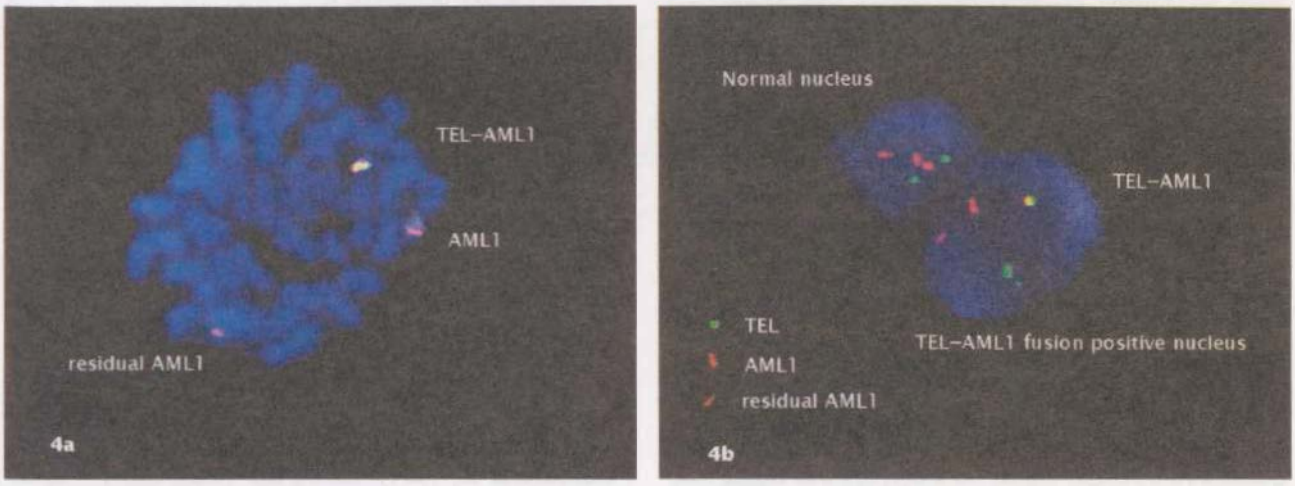


Fig. 4. Detection of the TEL-AML1 fusion transcript in Case 14 by FISH. 4a depicts a metaphase spread with a yellow TEL-AML1 fusion signal, a native AML1 signal and a residual AML1 signal. 4b depicts a normal interphase nucleus with two TEL (green) signals and two AML1 (red) signals. The adjacent fusion-positive nucleus shows one TEL-AML1 (yellow) fusion signal, one TEL signal, one large native AML1 signal and one smaller residual AML1 signal.

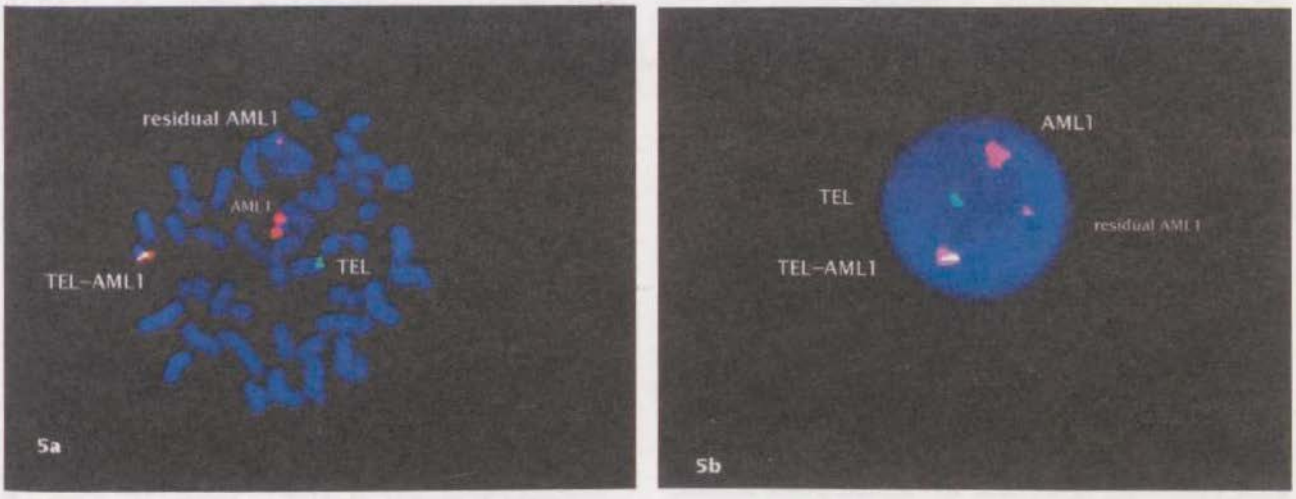


Fig. 5. Detection of the TEL-AML1 fusion transcript in Case 28 by FISH. 5a depicts a metaphase spread with a TEL-AML1 fusion signal, a native AML1 signal, a residual AML1 signal and a native TEL signal. 5b depicts a nucleus with a TEL-AML1 fusion signal, a native AML1 signal, a residual AML1 signal and a native TEL signal.

carry the long amplified product where the TEL gene is fused to exon 2 of the AML1 gene (Nakao *at al.*, 1996; Seeger *et al.*, 1998). The significance of this remains unclear. While both the cases in this study generated the reciprocal AML1-TEL product, most authors reported that this product was not always found in TEL-AML1 positive cases (Nakao *et a.*, 1996; McLean *et al.*, 1996). The general consensus regarding the reciprocal product is that it is not biologically relevant and that it is the TEL-AML1 fusion protein that is responsible for leukemogenesis.

A very striking feature of t (12;21) is its very strong association with the loss of heterozygosity in the TEL gene. Whilst both cases display an AML1-TEL reciprocal translocation band, it is very much less intense than the TEL-AML1 band. Whilst, a smaller translocated AML1 signal on a der(12) was observed, the reciprocal fusion *per se* was not observed by FISH. However FISH identified a loss of TEL in 25 % of the nuclei in case 14. This loss of heterozygosity of the TEL gene was observed in the first four cases in which the TEL-AML1 translocation was detected (Golub *et al.*, 1995; Romana *et al.*, 1995). This was studied further by Raynaud *et al.* (1996) who showed that 15 of 16 t (12:21) positive cases had an associated allelic loss of TEL. They further postulated that this allelic loss of TEL is secondary to the t (12; 21) translocation as it is only seen in a minor subset of leukemic cells.

The value of a genetic translocation lies not only in the prognosis it confers but also in the clues it provides on leukemogenesis. Both TEL (Wang *et al.*, 1998) and AML1 (Okuda *et al.*, 1996) code for transcription genes which are important in hematopoiesis. They feature recurrently in an increasing number of translocations with a variety of partners e.g. t (3;12), t (7;12), t (9;12) (Wlodarska *et al.*, 1998), t (12;22) (Buijs *et al.*, 1995), t (3;21) and t (5:21) (Golub *et al.*, 1996), which are associated with a cross section of leukemias. Given that they both code for transcription factors, leukemogenesis could conceivably be explained by altered transcription in the chimeric TEL-AML1 gene (Rabbitts, 1994). However, it is also important to consider the role that the allelic loss of TEL may play in leukemogenesis. Whilst leukemogenesis following a loss of a gene implies a suppressor function, the TEL gene does not function as a suppressor gene in the classical sense. A study by McLean *et al.* (1996) showed that the fusion gene TEL-AML1 and the normal TEL gene form heterodimers via the HLH domain on 5' end of the TEL gene. They postulate that this heterodimerization suppresses the transcription of the fusion gene. The loss of the TEL gene is therefore seen as a permissive event in leukemogenesis.

We agree with Borkhardt *et al.* (1997) that t (12;21) testing should be integrated into routine patient care. The case for this rests on the following arguments. Firstly, TEL-AML1 is the most common genetic rearrangement found in childhood leukemias. Whilst the frequency of this translocation in ALL cases in Malaysia is unknown, the work by Thong *et al.* (manuscript in prep.) at the University Hospital, Kuala Lumpur and our work at the Kuala Lumpur Hospital indicate that it is found in paediatric cases here. Secondly, t(12;21) defines a group of patients whose prognosis, while leaning towards favourable, needs to be ascertained. This is particularly important for treatment (Rubnitz *et al.*, 1997). Lastly, the TEL-AML1 translocation is a cryptic translocation that is, nevertheless, easily detected by RT-PCR.

**Acknowledgements**

The authors thank Dr. Kok TW (IMVS, Adelaide), Ms. S. Moore (IMVS, Adelaide), Dr. P. Kearney (Garvan Inst., Sydney), Dr. Thong MK (UH, KL) and Dr. U. Kees (ICHR, Perth) for helpful discussions & advice. The technical assistance of Thilagavathi S, Maimonah R (Haemotology Div, IMR), Wan Nor Azma WS, Nadarajah P (Blood Bank, KLH) and Saraswathi R, Fazli MY, Maimunah K (Path. Dept., KLH) is gratefully acknowledged. The authors thank the Director, Institute for Medical Research for permission to publish. This project was funded by IRPA Grant 06-05-01-0128. This study was initiated by Prof. John Bosco, UM, who passed away in April 1999; this paper is dedicated to his memory.

**References**

Aguiar RC, Sohal J, Van Rhee F, Carapeti M, Franklin IM, Goldstone AH, Goldman JM & Cross NCP (1996). TEL-AML1 fusion in acute lymphoblastic leukemia of adults. *British Journal of Haematology* **95**, 673-677.

Baruchel A, Cayuela JM, Ballerini P, Landman-Parker J, Cezard V, Firat H, Haddad E, Auclerc MF, Valensi F, Cayre YE, Macintyre EA & Sigaux F (1997). The majority of myeloid-antigen-positive ($My^+$) childhood B-cell precursor acute lymphoblastic leukemias express TEL-AML1 fusion transcripts. *British Journal of Haematology* **99**, 101-106.

Borkhardt A, Cazzaniga G, Viehmann S, Valsecchi MG, Ludwig WD, Burci L, Mangioni S, Schrappe M, Riehm H, Lampert F, Basso G, Masera G, 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.

Borkhardt A, Harbott J & Lampert F (1999). Biology and clinical significance of the TEL-AML1 rearrangement. *Current Opinion in Pediatrics* **11**, 33-38.

Buijs A, Sherr S, van Baal S, van Bezouw S, van der Plas D, van Kessel, AG, Rieginan P, Deprez R, Zwarhoff E, Hagemeijer A & Grosfeld G (1995). Translocation t(12;22) (p13;q11) in myeloproliferative disorders results in fusion of the ets-like TEL gene on 12p13 to the MN1 gene on 22q11. *Oncogene* **10**, 1511-1519.

Chessells JM, Swansbury GJ, Reeves B, Bailey CC & Richards SM (1997). Cytogenetics and prognosis in childhood lymphoblastic leukemia: results of MRC UKALL X. *British Journal of Haematology* 99, 93-100.

Garcia-Sanz R, Alaejos I, Orfao A, Rasillo A, Cbillon MC, Tabernero MD, Mateos MV, Lopez-Perez R, Gonzales D, Balanzategui A, Gonzalez M & San Miguel JF (1999). Low frequency of the TEL/AML1 fusion gene in acute lymphoblastic leukemia in Spain. *British Journal of Haematology* **107**, 667-669

Golub TR, Barker GF, Stegmaier K & Gilliland DG (1996). The TEL gene contributes to the pathogenesis of myeloid and lymphoid leukemias by diverse molecular genetic mechanisms. *Current Topics in Microbiology & Immunology* **211**, 67- 79.

Golub TR, Barker GF, Bohlander SK, Hiebert SW, Ward DC, Bray-Ward P, Morgan E, Raimondi SC, Rowley JD & Gilliland GD (1995). Fusion of TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. *Proceedings of the National Academy of Sciences, USA* 92, 4917-4921.

Harbott J, Viebmann S, Borkhardt A, Henze G & Lampert F (1997). Incidence of TEL/AML1 fusion gene analysed consecutively in children with acute lymphoblastic leukemia in relapse. *Blood* 90, 4933-4937.

Inamdar N, Kumar SA, Banavali SD, Advani S, Magrath I & Bhatia K (1998). Comparative incidence of the rearrangements of TEL/AML1 and ALL1 genes in pediatric precursor B acute lymphoblastic leukemias in India. *International Journal of Oncology* **13**, 1319-1322.

ISCN (1995) *An International System for Human Cytogenetic Nomenclature*. Mitelman F (Ed). S. Karger, Basel

Kees UR (1987). Resistance to 1-β-D-arabinofuranosyl-cytosine after high dose treatment of childhood lymphoblastic leukemia: Isolation of a drug resistant and a sensitive cell line. *Cancer Research* **47**, 3088-3091.

Kersey JH (1997). Fifty years of studies of the biology and therapy of childhood leukemia. *Blood* 90, 4243-4251.

Ma SK, Wan TSK & Chan LC (1999). Cytogenetics and molecular genetics of childhood leukemia. *Hematological Oncology* 17, 91-105.

McLean TW, Ringold S, Neuberg D, Stegmaier K, Tantravahi R, Ritz J, Koeffler HP, Takeuchi S, Janssen JWG, Seriu T, Bartram CR, Sallan SE, Gilliland DG & Golub TR (1996). TEL/AML-1 dimerizes and is associated with a favourable outcome in childhood acute lymphoblastic leukemia. *Blood* **88**, 4252-4258.

Nakao M, Yokota S, Horiike S, Taniwaki M, Kashima K, Sonoda Y, Koizumi S, Takaue Y., Matsushita T, Fujimoto T & Misawa S (1996). Detection and quantification of TEL/AML1 fusion transcripts by polymerase chain reaction in childhood acute lymphoblastic leukemia. *Leukemia* **10**, 1463-1470.

Okuda T, van Deursen J, Hiebert SW, Grosveld G & Downing JR (1996). AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal hematopoiesis. *Cell* **84**, 321-330.

Rabbitts TH (1994). Chromosomal translocations in human cancer. *Nature* 372, 143-149.

Raynaud S, Cave H, Baens M, Bastard C, Cacheux V, Grosgeorge J, Guidal-Giroux C, Guo C, Vilmer E, Marynen P & Grandchamp B (1996). The 12:21 translocation involving TEL and deletion of the other TEL allele: Two frequently associated alterations found in childhood acute lymphoblastic leukemia. *Blood* 87, 2891-2899.

Romana SP, Le Coniat M & Berger R (1994). t(12;21): a new recurrent translocation in acute lymphoblastic leukemia. *Genes, Chromosomes & Cancer* 9, 186-191.

Romana SP, Mauchauffe M, Le Coniat M, Chumakov I, Lepaslier D, Berger R & Bernard OA (1995). The t(12;21) of acute lymphoblastic leukemia results in TEL-AML1 gene fusion. *Blood* 85, 3662-3670.

Rubnitz JE, Downing JR, Pui CH, Shurtleff SA, Raimondi SC, Evans W, Head DR, Crist WM, Rivera GK, Hancock ML, Boyett JM, Buijs A, Grosveld G and Behm FG (1997). TEL gene rearrangement in acute lymphoblastic leukemia: a new genetic marker with prognostic significance *Journal of Clinical Oncology* 3, 1150-1157.

Rubnitz JE, Shuster JJ, Land VJ, Link MP, Pullen J, Camitta BM, Pui C-H, Downing JR & Behm FG (1997). Case-control study suggests a favourable impact of TEL rearrangement in patients with B-lineage acute lymphoblastic leukemia treated with antimetabolite-based therapy: A pediatric oncology group study. *Blood* 89, 1143-1146.

Seeger K, Adams H-P, Buchwald D, Beyermann B, Kremens B, Niemeyer C, Ritter J, Schwabe D, Harms D, Schrappe M & Henze G (1998). TEL-AML1 fusion transcript in relapsed childhood acute lymphoblastic leukemia. *Blood* 91, 1716-1722.

Wang LC, Swat W, Fujiwara Y, Davidson L, Visvader J, Kuo F, Alt FW, Gilliland DG, Golub TR & Orkin SH (1998). The TEL/ETV6 gene is required specifically for hematopoiesis in the bone marrow. *Genes & Development* 12, 2392-2402.

Wlodarska I, La Starza R, Baens M, Dierlamm J, Uyttebroek A, Selleslag D, Francine A, Mecucci C, Hagemeijer A, Van den Berghe H & Marynen P (1998). Fluorescence in situ Hybridization characterization of new translocations involving TEL (ETV6) in a wide spectrum of hematologic malignancies. *Blood* 91, 1399-1406.