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**Short communication**

# Molecular follow up of donor lymphocyte infusion in CML children after allogeneic bone marrow transplantation

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**Abstract.** In this study we present the applicability of fluorescence in situ hybridisation (FISH) and RT-PCR to detect the minimal residual disease (MRD) in relapsed Ph+ children after donor lymphocyte infusion (DLI) post bone marrow transplantation (BMT). In both patients *BCR/ABL* fusion was detected and its transcript at the moment of relapse. After the DLI treatment in short time intervals a decreasing number of cells with *BCR/ABL* fusion were noticed and the expression of the hybrid gene disappeared. These results demonstrate that all the methods presented in this study provide a feasible, rapid and accurate way for the detecting of the minimal residual disease after DLI in Ph positive CML patients.

**Key words:** bone marrow transplantation, donor lymphocyte infusion, fluorescence in situ hybridisation, minimal residual disease , RT-PCR.

Chronic myeloid leukaemia (CML) is a clonal disease of hematopoietic stem cells (HSC) with incidence in children of 1 per 100 000 per year. The chromosomal abnormality occurring in HSC in 90% of CML regardless of age is a reciprocal translocation between chromosomes 9 and 22 t(9; 22)(q34; q11) (NOWELL, HUNGERFORD 1960, RASKIND et al. 1998). This chromosomal abnormality results from the fusion of a part of the Abelson oncogene (*ABL*) of 9q34 with the breakpoint cluster region (*BCR*) at 22q11.2 (WESTBROOK et al. 1995). The fu-

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sion creates a tumour-specific marker and functionally results in an increased tyrosine kinase activity (RABBITS 1994).

For the purpose of monitoring the therapeutic response in Ph-positive CML patients, a standard cytogenetic analysis can be used. After the allogeneic bone marrow transplantation (BMT) a majority of patients achieve a sustained cytogenetic remission, but approximately 30-40% of them will relapse (GOLDMANN 1988). Response to the therapy in these patients may be evaluated at the cytogenetic and/or molecular level by methods for the detection of leukaemic cells, such as fluorescence in situ hybridization (FISH) (ARNOLDUS et al. 1990, ŁADOŃ et al. 2000) or reverse transcription-polymerase chain reaction (RT-PCR) (CROSS et al. 1993, LIN et al. 1995).

Several authors have shown that the analysis of *BCR-ABL* mRNA alone is applicable in the individual management of CML patients after allogeneic BMT (MIYAMURA et al. 1994). CROSS et al. (1993) described that increasing levels of *BCR-ABL* mRNA could be observed several months prior to the detection of Ph-positive metaphases in the marrow of patients who relapse after BMT. Several authors described the presence of *BCR-ABL* transcripts in CML children after BMT (VETTENRANTA et. al. 1998, BAURMANN et al. 1998, LEDA et al. 2001).

The main aim of this study was to compare the usefulness of fluorescent in situ hybridisation (FISH) and RT-PCR for the estimation of the level of the minimal residual disease (MRD) in children with CML Ph+ after donor lymphocyte infusion (DLI) administrated as an adoptive immunotherapy of post-BMT leukaemia relapse.

Samples of 5-10 ml of peripheral blood from CML patients were collected at one-month intervals after DLI until both cytogenetic and molecular remission occurred, and then every 3 months.

Fluorescent in situ hybridisation experiments were performed on metaphase and/or interphase nuclei obtained from standard short culturing of fresh whole blood samples, with probes specific for *ABL*-oncogene (on chromosome 9) and *BCR*-breakpoint cluster region (on chromosome 22) (Cytocell Ltd.). The probe detects the fusion gene *BCR/ABL*, whether the chromosome 22 breakpoint occurs in either the minor or major breakpoint cluster region (m-*BCR/ABL* or M-*BCR/ABL*). FISH procedure according to Cytocell Ltd. was used.

Total RNA extraction according to CHOMCZYŃSKI with some modification was applied in the study (CHOMCZYŃSKI et al. 1987). Peripheral blood cells were fractionated on Ficoll gradients and a total RNA was isolated by guanidinum isothiocyanate solubilization/LiCl precipitation procedure. cDNA synthesis was performed according to the procedure described in the Promega Inc. commercial kit.

Multiplex PCR for the detection of m-*BCR/ABL* (e1a2), M-*BCR/ABL* (b3a2 or b2a2) and *BCR* mRNA (internal control) were performed with the use of oligonucleotide primers described by CROSS et. al. (1994), 31 cycles of 95°C 30 s, 60°C 45 s, 72°C 50 s, followed by 10 min extension at 72°C; samples were analysed on 1.8% agarose gel.

As a positive control of *BCR/ABL* fusion gene, K562 cell line was used carrying b3a2 junction like in Ph+ CML (p210) patients.

Two patients M.L.: (UPN 18) and E.M.: (UPN 45), 11- and 15-year old females, suffering from CML were transplanted with bone marrow from HLA-identical brothers. At 23 months and 76 months after BMT, respectively, haematological relapse was diagnosed and *BCR/ABL* fusion was detected by FISH and by RT-PCR. To reduce the mass of leukaemic cells, the treatment of relapse started with hydroxyurea, and two weeks from the diagnosis of relapse the patients received the first infusion of bone marrow donor $CD3^+$ lymphocytes (DLI).

In the patient UPN 18: 23% of *BCR/ABL* positive cells by FISH and the presence of b2a2 transcript by RT-PCR were detected at the time of haematological posttransplant relapse (23 months after BMT). At day +120 and +180 post DLI *BCR/ABL* positive cells detected by FISH on interphase nuclei were reduced to 20.5% and 6%, respectively. *BCR/ABL* transcripts at the same time post DLI were not detected. Laboratory findings showed that this patient achieved haematological remission 119 days post DLI, molecular remission 180 days, while cytogenetic remission as late as 593 days post DLI (Figures 1A, B).

In the patient UPN 45: 23.1% of *BCR/ABL* positive cells by FISH and the presence of b3a2 transcript were detected at the time of posttransplant relapse (76 months after BMT). On day +30 and +45 post DLI, 37.3% and 51.3% *BCR/ABL* positive cells, respectively, were detected by FISH; at the same time (+30, +45 days post DLI) the presence of b3a2 transcript was found. On day +90 post DLI the number of *BCR/ABL* positive cells detected by FISH was reduced to 2.2% and the presence of b3a2 transcript was not detected (molecular remission). *BCR/ABL* positive cells detected by FISH were not found (cytogenetic remission) on day +115 post DLI. Peripheral blood parameters showed that in this patient the haematological remission was achieved 115 days post DLI.

The presence or absence of residual disease is an important prognostic indicator of response to DLI given for the treatment of post BMT relapse. Several authors reported that in adult patients after BMT, the presence of residual disease can precede clinical relapse even by a few months (LIN et al. 1995, ROTH et al. 1992). FISH on interphase nuclei with the probe for *BCR/ABL* provides information on the detection of this rearrangement in dividing and nondividing cells. Qualitative multiplex RT-PCR for *BCR/ABL* on RNA extracted from total peripheral blood leukocytes is more sensitive than FISH analysis, but in this study FISH gives quantitative information on the number of cells carrying this translocation.

VETTENRANTA et al. (1998) reported that children at posttransplant relapse demonstrating positivity for FISH and RT-PCR may be considered candidates for DLI. In our study at the time of relapse in both patients *BCR/ABL* fusion gene was detected by FISH and RT-PCR, during therapy a decreasing number of cells carrying *BCR/ABL* fusion and a decreasing level of *BCR/ABL* transcripts were ob-

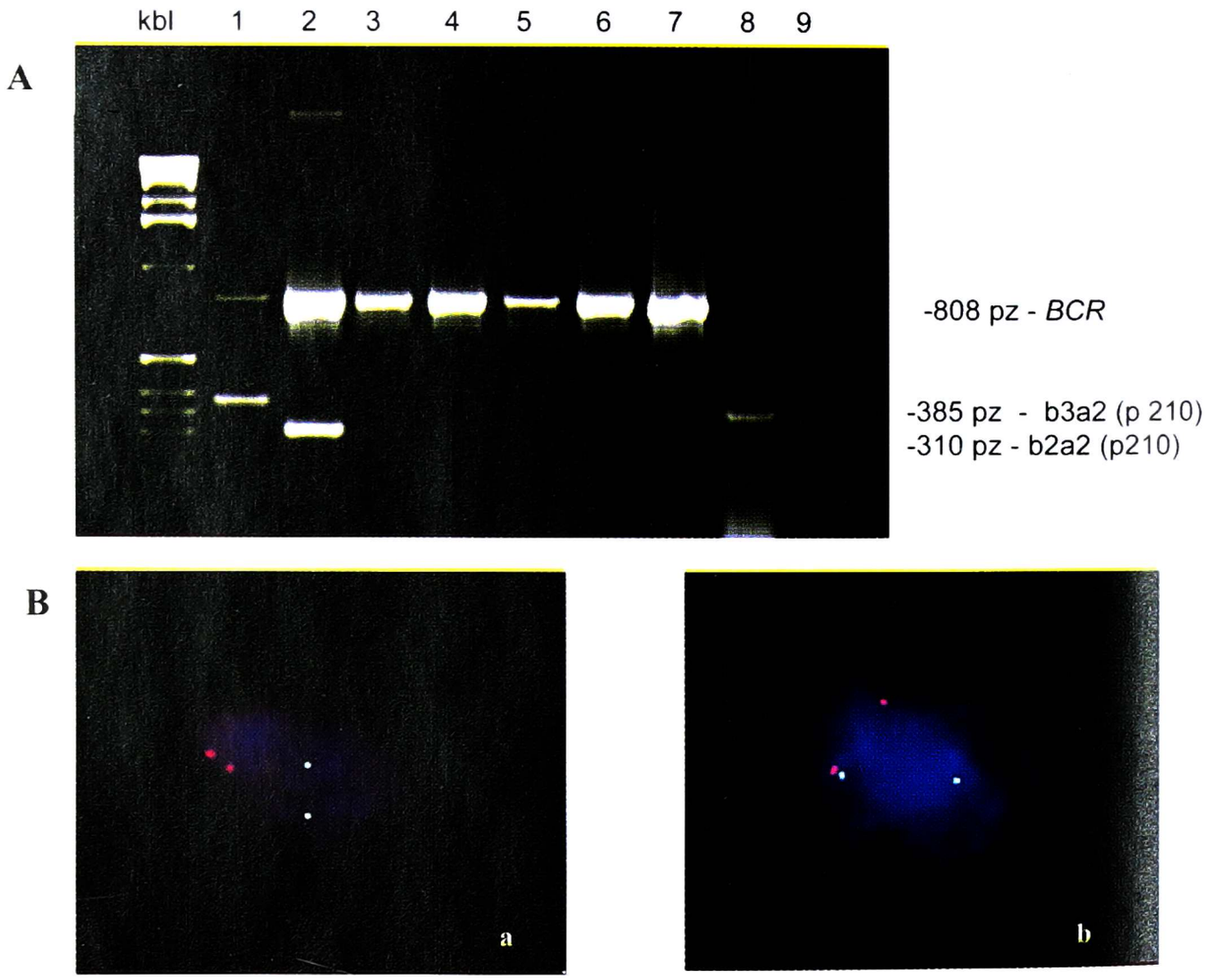


Figure 1. A) Detection of *BCR/ABL* transcripts by RT-PCR method in patient UPN 18 at various intervals post BMT; 1 = K 562 (control), 2 = + 60 post BMT, 3 = + 120 post BMT, 4 = + 160 post BMT, 5 = + 180 post BMT, 6 = + 270 post BMT, 7 = healthy (negative control), 8 = CML b3a2 (p 210) (control), B) Detection of *BCR/ABL* fusion by FISH method: a = interphase nuclei of a healthy individual (negative control), b = interphase nuclei of patient UPN 18.

served. In both patients (UPN 18 and UPN 45) the level of *BCR/ABL* transcript decreased earlier than the number of cells with *BCR/ABL* fusion.

Other authors described a similar molecular scenario where results of RT-PCR, the most sensitive method for the detection of *BCR/ABL* mRNA, were found to be negative, but interphase FISH was clearly positive in several patients after BMT or IFNα treatment (ZHANG et al. 1996, BRIZARD et al. 1998). Attempts to explain the reason for the persistence of quiescent malignant cells after IFNα treatment were made by several authors (FURUKAWA et al. 1997, LENGYel et al. 1993). These investigations revealed that INFα is able to down-modulate the activity of E2F transcription factor acting on genes involved in the progression of the cell cycle. Decreases in the expression of E2F might maintain malignant cells in the quiescent state.

Donor leukocyte infusions (DLI) can provide a direct graft-versus-leukaemia effect, which is sustained in the majority of patients who reach a complete remis-

sion (ROMAN et al. 2000). Monitoring of MRD in these patients has relevant therapeutic implications in this context. Our experience suggests that a combined evaluation of the minimal residual disease by FISH and RT-PCR methods gives more informative details concerning therapy response in patients with *BCR/ABL* fusion gene after DLI.

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