Post-transplantation lymphoproliferative disorder of recipient origin in a boy with acute T-cell leukemia with detection of B-cell clonality 3 months before stem cell transplantation

Post-transplantation lymphoproliferative disorder is an infrequent complication after hematopoietic stem cell transplantation. It is hypothesized that lack of T-cell surveillance following transplantation permits reactivation of latent EBV leading to polyclonal B-cell expansion and finally outgrowth of a predominant clone. Most cases are of donor origin. Here, we describe an 8-year-old boy with early onset post-transplantation lymphoproliferative disorder following matched-unrelated stem cell transplantation for high-risk T-cell leukemia whose disease was unusual for two reasons. First, his B-cell clone was of host origin and, in contrast to the few PTLD of host origin described so far, not associated with autologous reconstitution. Secondly, using clonal analysis, we could retrospectively show that the B-cell clone emerged during consolidation chemotherapy for T-cell leukemia, 3 months before stem cell transplantation.

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Post-transplant lymphoproliferative disorder (PTLD) is a rare complication following organ and stem cell transplantation (SCT).[1] Most cases are associated with EBV-infection and are of B-cell origin.[2] Whereas PTLD following solid organ transplantation is predominantly of host origin, B-cells in PTLD after SCT almost exclusively arise from the donor[1;3] It is hypothesized that the development of lymphoid transformation is a multistep process.[4] Immunosuppressive therapy such as T-cell depletion leads to a lack of T-cell control and permits the polyclonal expansion of EBV-infected and immortalized B-cell clones. Certain clones will acquire genetic changes and outgrow the other clones leading to oligoclonality and eventually monoclonality. The clonal development of PTLD is associated with distinct morphological features ranging from[1] plasmacytic hyperplasia which is usually polyclonal to[2] polymorphic B-cell hyperplasia which is nearly always monoclonal and to[3] immunoblastic lymphoma or multiple myeloma which is monoclonal.[4;5] We present an 8-year-old boy with early-onset PTLD after allogeneic stem cell transplantation, whose disease was unusual because of host origin of the PTLD and detection of the B-cell clone giving rise to PTLD already before SCT.

Material and Methods

DNA extraction. Genomic DNA was extracted from frozen peripheral blood specimen and bone marrow using the GFX genomic blood purification kit (Amersham Pharmacia Biotech GmbH, Freiburg, Germany) according to the manufacturer's instructions. Informed consent of the parents was obtained.

PCR. In order to look for rearranged immunoglobuline genes in the patient a PCR was performed with a set of family specific primers that amplify the heavy and light chain variable regions together with a set of JH-primers (Table 1). 250 µg of DNA was used per PCR reaction. For heavy chain amplification 0,35 pmol of each heavy chain variable primer was combined with 0,3 pmol of joining 1-5 primers and 0,6 pmol joining 6 primer. For kappa light chain amplification 1 µmol of each kappa variable primer was combined with 0,6 µmol of joining 1,2,4 primers and 0,2 µmol joining 3 and 5 primers.

Table 1. Primers used for detection of rearrangement in immunoglobuline genes.

a) Family specific primers for the heavy chain variable region.

Gene	*Sequence*
VH1	5'-CCTCAGTGAAGT<CT>TCCTGCAAGGC-3'
VH2	5'-GTCCTGCGCTGGTGAAA<GC>CCACACA-3'
VH3	5'-GGGGTCCCTGAGACTCTCCTGTGCAG-3'
VH4	5'-GACCCTGTCCCTCACCTGC>AG<CTGTC-3'
VH-5	5'-AAAAAGCCCGGGGAGTCTCTGA<AG>GA-3'
VH6	5'-ACCTGTCCATCTCCGGGGACAGTG-3'
JH1-5	5'-GGTGACCAGGGT<TGC>CC<TC<TGGCCCCAG-3'
JH6	5'-GGTGACCGTGGTCCCTTGCCCCCAG-3'

b) Family specific primers for the kappa light chain variable region.

Gene	*Sequence*
VK1	5'-ACATCC<AG>G<TA>TGACCCAGTCTCC<AT>TC -3'
VK2	5'-CAG<TA>CTCCACTCTCCCTG<CT>CCGTCA -3'
VK3	5'-TCTCCATG<GC>CACCCTGTCT<TG>TGTCTC -3'
VK4	5'-AGACTCCCTGGCTGTGTGTCTCTGGGC -3'
VK5	5'-CAGTCTCCAGCATTCATGTCAGCGA-3'
VK6	5'-TTTCT<GC>TCTGTGACTCCA<GA><GA>GGAGAA -3'
JK1,2,4	5'-ACTCACGTTTGAT<TC>TCCA<GC>CTTGGTCC -3'
JK3	5'-GTACTTACGTTTGATATCCACTTTGGTCC-3'
JK5	5'-GCTTACGTTTAATCTCCAGTCGTGTCC-3'

c) Family specific primers for the lambda light chain variable region.

Gene	*Sequence*
Vï1	5'-GGTCCTGGGCCCAGTCTGTG-3'
Vλ2	5'-CAGTCTGCCCTGACTCAGCCT-3'
Vλ3	5'-CTCAGCCACCCTCAGTGTCCGT-3'
Vλ4	5'-TTTCTTCTGAGCTGACTCAGGAC-3'
Vλ7	5'-GTGGTGACTCAGGAGCCCTCAC-3'
Vλ8	5'-ACTGTGGTGACCCAGGAGCCA-3'
Vλ9	5'-GCTGACTCAGCCACCTTCTGCA-3'
Jλ1	5'-GCCACTTACCTAGGACGGTGAC-3'
Jλ2,3	5'-GAAGAGACTCACCTAGGACGGTC-3'
Jλ6,7	5'-GGAGACTCACCGAGGACGGTC-3'

For lambda light chain amplification 1 µmol of each lambda variable primer was combined with 0,5 µmol of joining 1 primer, 1 µM of joining 2,3 primer and 0,5 µmol of joining 6,7 primer. The PCR reaction consisted of a first activation step for ampli Taq gold at 95°C for 7 min. followed by 95°C for 90 s, 61°C for 30 s, 72°C for 80 s for a total of 40-45 cycles and a final extension step for 5 min. at 72°C. PCR products were loaded in an ethidium bromide stained 2% agarose gel.

Cloning and sequencing of PCR products. Bands obtained at the estimated bp level were cut and isolated using a QIAquick gel extraction kit according to the manufacturer's instruction (QIAGEN GmbH, Hilden, Germany). PCR products were cloned into the linearized pCR2.1-vector using the TA-cloning system (Invitrogen GmbH, Karlsruhe, Germany) following the manufacturer's instructions. The DNA sequencing was performed directly from a small scale plasmid preparation after determining the presence of an insert using the ABI-310 sequencer (Perkin-Elmer, Weiterstadt, Germany).

Southern blot hybridization. The PCR product was run on a 1,4% agarose gel, denatured with alkali, neutralized, and transferred to nitrocellulose filters according to the method of Southern.[6] The filters were hybridized with a radiolabeled 17-mer oligonucleotide designed from the immunoglobuline variable region of the patient. The

images were visualized on a phosphoimager (FIJIX BAS 1000).

Mutational Analysis for ras and p53. 50 ng of DNA were used as template for PCRs specific for KRAS 2 exon 1 (*GTAAAACGACGGCCAGT*TTATAAGGCCTGCTGAA, *CAGGAAACAGCTATGAC*GTC CTGCACCAGTAAT), *KRAS2* exon 2 (*GTAAAACGACGGCCAGT*GACTGT-GTTTCTCCCTTCTCA GGAT, *CAGGAAACAGCTATGA-C*ACTCCTTAATGTCAGCTTATTATATTC), *NRAS* exon 1 (*GTAAAA CGACGGCCAGT*TGTGGCTCGCCAATTA, *CAGGAAACAGCTATGACAAAGA*TGATCCGACAAG), *NRAS* exon 2 (*GTAAAACGACGGCCAGT*CCCTTACC-CTCCACA, *CAGGAAACAGCTATGAC*CCTCA TTTCCC-CATAA), *p53* exon 5 (TGTTCACTTGTGCCCTGACT, CAGCCCTGTCGTCTCTCCAG), p53 exon 6 (GTCC-CCAGGCCTCTGATTC, TCAAATAAGCA GCAGGA-GAAAGC), p53 exon 7 (*GTAAAACGACGGCCAGT*GCG-CACTGGCCTCATCT, *CAGGAAACAGCTATGAC C*ACAGCAGGCCA GTGTG), and *p53* exon 8 (CTGATTTCCTTACTGCCTCTTGC, CTTGGTCTC-CTCCACCGCT). The primers specific for *KRAS2, NRAS* and *p53* exon 7 were tailed (tail is given in italics). Subsequently, 2 μL of crude PCR products were used as template for sequencing reactions employing Big-Dye Terminator sequencing kits (Applied Biosystems, Darmstadt, Germany) according to the manufacturer's instructions and 18 pmol of the primers specific for the tails (*GTAAAACGACGGCCAG, CAGGAAACAGCTATGAC*) or one of the PCR primers, respectively. Sequencing reaction products were purified using DyEx v. 2.0 kits (QIAGEN) according to the manufacturer's instructions. 2 μL of the eluate were then mixed with 18 μL of highly deionized formamide (HiDi, Applied Biosystems) heated for 2 minutes at 90°C, snap frozen on ice and then analysed employing the ABI310 Genetic Analyzer (Applied Biosystems).

Chimerism Analysis. Post-transplant chimerism was analyzed by a semi-quantitative PCR approach for the amplification of STR markers as described in detail elsewhere.[7] After identifying an informative locus, sequential patient samples of the peripheral blood and bone marrow were investigated. The analysis was performed in the peripheral blood weekly. Fluorescent-based PCR analysis after capillary gel electrophoresis allowed the semi-quantitative characterization of post-transplant chimerism samples. Analysis of different cell subpopulations was performed using the *magnet-activated cell sorting technique (MACS)* as described previously.[8]

Case report

An 8-year-old boy was diagnosed with acute lymphoblastic leukemia (ALL) of T-cell origin. Treatment was started according to the ALL-BFM 2000 protocol using a 4-drug induction with vincristine, daunorubicin and L-asparaginase and randomising prednisone against dexamethasone.[9] Because of prednisone-poor response on day 8, allogeneic SCT was planned as per protocol. On day 33 of induction chemotherapy, morphological remission was achieved. After 3 blocks of consolidation chemotherapy, SCT from a 10/10 matched-unrelated donor was performed. Donor as well as host pre-transplant serological studies were consistent with past EBV-infection. The conditioning regimen according to the protocol consisted of TBI (12 Gy), Etoposide (40 mg/kg of body weight) and anti-thymocyte globulin (Merieux) (30 mg/kg of body weight). A total of 20×10^6 $CD34^+$ cells/kg of body weight were infused. Cyclosporine and methotrexate (day 1, 3, 6) were given for GvHD-prophylaxis. On day 15 after SCT, neutrophil engraftment occured. On day 21, acute GvHD Grade II affecting the gastrointestinal tract was diagnosed. Treatment was started with prednisolone (2 mg/kg of body weight), leading to a prompt response. On day 36, after having suffered from bloody vomiting and diarrhea, CMV-esophagitis was diagnosed by biopsy. Treatment was started with ganciclovir and CMV-hyperimmunoglobuline. On day 43, the patient developed fever. Over the following days, liver and spleen size enlarged. On day 45, after suffering from a reversible episode of intestinal invagination, EBV-LPD was diagnosed by flowcytometry demonstrating increasing numbers of plasma cells in the peripheral blood. The cells expressed CD19, CD20, the plasma-cell antigens CD38 and CD138 and were restricted for kappa-light-chains. Hematopoietic chimerism analysis by PCR after separation of peripheral blood subpopulations by immunobeads showed that the B-lymphocytes were of host origin. Cyclosporine was discontinued. On day 48, with number of plasma cells continuing to rise, anti-CD20 monoclonal antibody was administered. On day 50 the boy was transferred to the intensive care unit and intubated for respiratory failure. Extracorporal dialysis was started because of renal failure secondary to Bence-Johnes proteinuria of kappa-type. With plasma cells in the peripheral blood rising to 6765/μL, chemotherapy containing cyclophosphamide, Ara-C, etoposide and dexamethasone was administered over a period of 5 days. The plasma cell count fell and the EBV copy number declined from 600.000 on day 50 to 35.000 copies/μg DNA on day 53. On day 55, anti-CD20 antibody was readministered followed by the infusion of donor lymphocytes (1×10^5 CD3 cells/kg of body weight) the next day. On day 64, plasma cells reappeared in the peripheral blood while the patient was neutropenic and no signs of graft recovery were seen in the bone marrow. In addition, the EBV-copy number increased to 600.000 copies/μg. Conditioning for a second SCT from the haploidentical father was started on day 67. Three days later, the EBV-copy number increased to 12 Mio copies/μg. On day 72, the patient died of cardiac failure. Autopsy showed infiltration of multiple organs by plasma cells expressing kappa-light chains and EBV-LMP1, but with only 10-20% of cells bearing CD20.

Mutational analysis of RAS genes and p53

Since the B-cell lymphoma was of host origin, we wanted to investigate whether there were common abnormalities between the initial T-cell leukemic clone and the B-cell lymphoma. Sequence analysis of PCR-reactions using primers specific for *K-RAS* exon 1 and 2, and *N-RAS* exon 1 and 2 revealed a mutation in *N-RAS* exon 1 in peripheral blood cells at diagnosis of the T-cell leukemia. The mutation could also be detected in a bone marrow specimen obtained on day 14 after start of the inital chemotherapy when there were still 70% blast cells (Table 2). The mutation consisted of a substitution of GGT to GTT at codon 13. No mutation was found in specimen obtained at a later time point when there was also no evidence of an increased blast count as well as in the cells of the B-cell lymphoma. Sequence analysis of PCR-reactions using primers for *p53* exon 5,6,7, and 8 did not demonstrate any mutation in the initial T-cell leukemia clone as well as in the B-cell-lymphoma.

Rearrangement of immunoglobulin chains

PCR using heavy and light chain family specific primers demonstrated clonality of the B-cell lymphoma belonging to the VH4-family. This immunoglobulin rearrangement

could not be detected in the initial T-cell leukemia clone. However, when samples of peripheral blood and bone marrow obtained during initial chemotherapy were examined, the VH-4 clonal rearrangement could be detected in bone marrow cells obtained after two cycles of consolidation chemotherapy, 100 days before SCT (Table 2).

Table 2. Time course of RAS-mutation and IGH-gene rearrangement.

RAS-mutation:	+	+	-	-	-	ND	-
IGH-gene rearrangement:	ND	-	-	+	ND	+	+
Blast:	18%	70%	< 5%	< 5%	< 5%	18%	76%
Lineage:	T-LB	T-LB				PC	PC
Origin:	PB	BM	BM	BM	BM	PB	PB
Days after dx of ALL:	3	14	33	52	182	204	220
Days before SCT:		-158	-129	-100	+30	+52	+68

Abbreviations: T-LB, T-lymphoblasts; PC, plasma cells; PB, peripheral blood; BM, bone marrow.

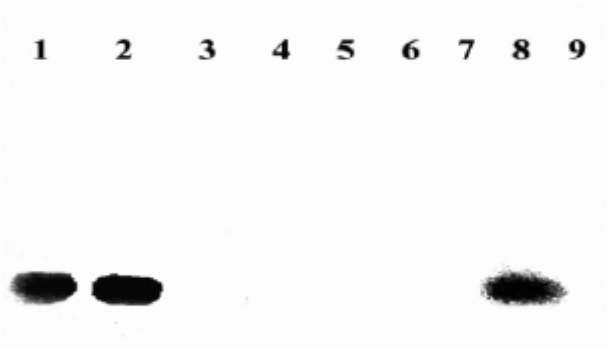

Figure 1. Southern blot with 17-mer radiolabelled oligo on PCR products with primers designed from the heavy chain variable region of the patient's B-cell lymphoma demonstrating the presence of the same clonal rearrangement already 100 days before SCT. Lane 1: Plasmid containing case study patient's B-cell lymphoma Ig variable sequence. Lane 2: Patient's B –cell lymphoma DNA day 52 after SCT. Lane 3-5: B-cell lymphoma DNA of three other patients. Lane 6-8: DNA from case study patient's peripheral blood 158 days (lane 6), 129 days (lane 7) and 100 days before SCT (lane 8). Lane 9: water used as control.

The specificity of the PCR was confirmed by Southern blot using a 17-mer radioactive-labeled oligonucleotide which was derived from the interior part of the cloned PCR-product (Figure 1). This clonal rearrangement could not be detected in samples obtained prior to the second cycle of consolidation therapy or in samples from other patients with B-cell lymphoma. Whereas clonal rearrangement of B-cells could already be detected by PCR 100 days before SCT, EBV-latent membrane protein 1 (LMP-1), which is central for the induction of B-cell proliferation, was only detectable by specific immunostaining when overt EBV-PTLD was diagnosed.

Discussion

Most cases of PTLD after SCT occur within the first year after transplantation with a cumulative incidence of 1% at 10 years, with up to 24% in patients at high-risk.[10;11] In a large retrospective analysis the strongest risk factor for the development of PTLD after SCT was T-cell depletion *ex vivo* or *in vivo* using antithymocyte globulin or anti-CD3-monoclonal.[10] Also, an association has been established between the development of PTLD and the use of stem cells from an HLA-unrelated or an HLA-mismatched related donor, the occurence of acute GvHD grades II to IV, and with conditioning regimens including TBI. For our patient, most of the described risk factors were present, such as the use of antithymocyte globulin and TBI, the use of an HLA-unrelated donor, and the occurence of acute GvHD grade II.

Treatment of early PTLD after SCT is unsatisfactory with a mortality rate of up to 90%.[1] Therapy consists of reduction of immunosuppression, immunotherapy with monoclonal antibodies such as anti-CD20, transfer of cytotoxic T-cells or lymphoma-like-chemotherapy.[12] In our patient, only chemotherapy led to a response which, however, lasted only for several days. Treatment with anti-CD20 was unsuccessful, since it selected for tumor cells not expressing CD20. Generation of EBV-specific donor CTLs which are more potent than donor lymphocytes was started when the diagnosis of PTLD was made. Because of the patient's rapid disease progression, sufficient number of EBV-specific T-cells could not be generated. Nowadays, partly HLA-matched allogeneic EBV-specific T-cells from a frozen bank may be of benefit by allowing the early application of specific T-cell therapy.[12]

In contrast to solid organ transplantation, cases of PTLD after allogeneic SCT are almost exclusively of donor origin, since recipient lymphopoiesis is thought to be greatly diminished if not ablated by the conditioning regimen.[13] In our patient, the PTLD was of recipient origin which implies that the conditioning regimen of TBI and etoposide had not been able to eradicate all of the host's B-cells in our patient. Evidence that B-cells of host origin may survive intensive conditioning in allogeneic SCT, derives also from cases of pure red cell anemia following SCT when host B-cells produce antibodies against donor red cell antigens.[14] So far, only five cases of PTLD of host origin following allogeneic SCT have been described.[11;15;16] In contrast to our patient, PTLD in these 5 patients was preceded by autologous bone marrow reconstitution or mixed chimerism and developed late after transplantation (5-49 months).

The fact that the B-cell clone in our patient survived intensive conditioning and was not preceded by mixed chimerism prompted us to examine whether this clone had features of the initial leukemic clone which might have confered resistance to the conditioning regimen. Using mutational analysis we were able to detect a *K-RAS* mutation in the initial T-cell leukemia. *RAS*-mutations have been described in lymphoblasts of patients with ALL at a frequency of 6-20 % and also in patients with multiple myeloma (17-19). Though two earlier studies demonstrated that mutations in *RAS* in ALL were associated with an inferior outcome, this could not be confirmed in two larger, recently published studies (17;18;20;21). The *RAS*-mutation could still be detected on day 14 of treatment, when there were 70% blast in the bone marrow, but no longer on day 33, when the patient's bone marrow was in remission. No RAS-mutations was noted in the B-cell lymphoma cells.

A second clonal marker was obtained after identifying the Ig-chain rearrangement of the B-cell lymphoma cells. While we could not detect common rearrangements of antigen-receptors in both T-cell blasts and B-cell lymphoma cells, we were able to demonstrate that the clone giving rise to the PTLD had already emerged during con-

solidation chemotherapy. Clonal expansion of B-cells in the peripheral blood can be readily observed in patients with autoimmune disorders such as rheumatoid arthritis or in patients with HIV and hypergammaglobulinemia but not in healthy individuals.[22;23] Data on patients undergoing chemotherapy are not available. However, there have been few cases of EBV-PTLD arising in children with acute lymphoblastic leukemia reported.[24;25] In these cases EBV-PTLD arises during maintenance therapy and it is speculated that methotrexate induced T-cell dysfunction might play a central role in the development of PTLD.

We hypothesize that dysregulation of T-cell function by the T-cell leukemia and chemotherapy led to the emergence of B-cell clonality already during consolidation chemotherapy. Whereas B-cells usually get destroyed through conditioning chemotherapy, cells of this clone survived, and got expanded through the lack of T-cell function after transplantation. It remains speculative, whether the B-cells giving rise to the PTLD had an intrinsic abnormality allowing them to have a growth advantage already during consolidation chemotherapy and protecting them against the cytotoxic effect of conditioning therapy. The fact that the T-cell leukemia cells poorly responded to induction therapy and that PTLD of host origin following SCT is exclusively rare may point to such an intrinsic defect which even might have affected both, B-cells and T-cells.

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References

1. Loren AW, Porter DL, Stadtmauer EA, Tsai DE. Post-transplant lymphoproliferative disorder: a review. Bone Marrow Transplant 2003; 31:145-55.
2. Deeg HJ, Socie G. Malignancies after hematopoietic stem cell transplantation: many questions, some answers. Blood 1998; 91:1833-44.
3. Ng IO, Shek TW, Thung SN, Ye MM, Lo CM, Fan ST et al. Microsatellite analysis in post-transplantation lymphoproliferative disorder to determine donor/recipient origin. Mod Pathol 2000; 13:1180-5.
4. Knowles DM, Cesarman E, Chadburn A, Frizzera G, Chen J, Rose EA et al. Correlative morphologic and molecular genetic analysis demonstrates three distinct categories of posttransplantation lymphoproliferative disorders. Blood 1995; 85:552-65.
5. Nalesnik MA. The diverse pathology of post-transplant lymphoproliferative disorders: the importance of a standardized approach. Transpl Infect Dis 2001; 3:88-96.
6. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975; 98:503-517.
7. Kreyenberg H, Holle W, Mohrle S, Niethammer D, Bader P. Quantitative analysis of chimerism after allogeneic stem cell transplantation by PCR amplification of microsatellite markers and capillary electrophoresis with fluorescence detection: the Tuebingen experience. Leukemia 2003; 17:237-40.
8. Bader P, Stoll K, Huber S, Geiselhart A, Handgretinger R, Niemeyer C et al. Characterization of lineage-specific chimaerism in patients with acute leukaemia and myelodysplastic syndrome after allogeneic stem cell transplantation before and after relapse. Br J Haematol 2000; 108:761-8.
9. Attarbaschi A, Mann G, Kronberger M, Witt V, Gadner H, Dworzak M. Effects of dose-reduced Medac L-asparaginase on coagulation in trial ALL-BFM 2000. Klin Padiatr 2003; 215:321-6.
10. Gross TG, Steinbuch M, DeFor T, Shapiro RS, McGlave P, Ramsay NK et al. B cell lymphoproliferative disorders following hematopoietic stem cell transplantation: risk factors, treatment and outcome. Bone Marrow Transplant 1999; 23:251-8.
11. Shapiro RS, McClain K, Frizzera G, Gajl-Peczalska KJ, Kersey JH, Blazar BR et al. Epstein-Barr virus associated B cell lymphoproliferative disorders following bone marrow transplantation. Blood 1988; 71:1234-43.
12. Haque T, Wilkie GM, Taylor C, Amlot PL, Murad P, Iley A et al. Treatment of Epstein-Barr-virus-positive post-transplantation lymphoproliferative disease with partly HLA-matched allogeneic cytotoxic T cells. Lancet 2002; 360(9331):436-442.
13. Chadburn A, Suciu-Foca N, Cesarman E, Reed E, Michler RE, Knowles DM. Post-transplantation lymphoproliferative disorders arising in solid organ transplant recipients are usually of recipient origin. Am J Pathol 1995; 147:1862-70.
14. Klumpp TR, Block CC, Caligiuri MA, Rabinowe SN, Soiffer RJ, Ritz J. Immune-mediated cytopenia following bone marrow transplantation. Case reports and review of the literature. Medicine (Baltimore) 1992; 71:73-83.
15. Zutter MM, Martin PJ, Sale GE, Shulman HM, Fisher L, Thomas ED et al. Epstein-Barr virus lymphoproliferation after bone marrow transplantation. Blood 1988; 72:520-9.
16. Au WY, Lie AK, Lee CK, Ma SK, Wan TS, Shek TW et al. Late onset post-transplantation lymphoproliferative disease of recipient origin following cytogenetic relapse and occult autologous haematopoietic regeneration after allogeneic bone marrow transplantation for acute myeloid leukaemia. Bone Marrow Transplant 2001; 28:417-9.
17. Yokota S, Nakao M, Horiike S, Seriu T, Iwai T, Kaneko H et al. Mutational analysis of the N-ras gene in acute lymphoblastic leukemia: a study of 125 Japanese pediatric cases. Int J Hematol 1998; 67:379-87.
18. Perentesis JP, Bhatia S, Boyle E, Shao Y, Shu XO, Steinbuch M et al. RAS oncogene mutations and outcome of therapy for childhood acute lymphoblastic leukemia. Leukemia 2004; 18:685-92.
19. Hallek M, Bergsagel PL, Anderson KC. Multiple myeloma: increasing evidence for a multistep transformation process. Blood 1998; 91:3-21.
20. Lubbert M, Mirro J, Jr., Miller CW, Kahan J, Isaac G, Kitchingman G et al. N-ras gene point mutations in childhood acute lymphocytic leukemia correlate with a poor prognosis. Blood 1990; 75:1163-9.
21. Rodenhuis S, Bos JL, Slater RM, Behrendt H, van ', V, Smets LA. Absence of oncogene amplifications and occasional activation of N-ras in lymphoblastic leukemia of childhood. Blood 1986; 67:1698-704.
22. Itoh K, Patki V, V, Furie RA, Chartash EK, Jain RI, Lane L et al. Clonal expansion is a characteristic feature of the B-cell repertoire of patients with rheumatoid arthritis. Arthritis Res 1999; 2:50-8.
23. Chong Y, Ikematsu H, Ariyama I, Chijiwa K, Li W, Yamaji K et al. Evidence of B cell clonal expansion in HIV type 1-infected patients. AIDS Res Hum Retroviruses 2001; 17(16):1507-1515.
24. Lehrnbecher T, Trusen A, Deinlein F, Hocht B, Marx A, Kuhl J. B-Cell lymphoproliferative disorder not associated with Epstein-Barr Virus in a child with relapsed acute lymphoblastic leukemia. Med Pediatr Oncol 2003; 40:13-7.
25. Mustafa MM, Winick NJ, Margraf LR. Epstein-Barr virus lymphoproliferative disorder in children with leukemia: case report and review of the literature. J Pediatr Hematol Oncol 1997; 19:77-81.