thropoiesis observed in one of our patients is only speculative, since we have no formal evidence that this phenomenon was really due to administration of CTM.

Our conclusion is that the use of CTM in MM patients with progressive disease cannot be recommended.

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Key words: myeloma, clarithromycin.

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Manuscript processing
This manuscript was peer-reviewed by two external referees and by Professor Mario Boccadoro, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Professor Boccadoro and the Editors. Manuscript received January 18, 2002; accepted April 11, 2002.

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Relapse of chronic myeloid leukemia in lymphoid crisis after allogeneic bone marrow transplantation in chronic phase with a busulfan plus cyclophosphamide regimen

We report 3 cases of atypical relapse of chronic myeloid leukemia (CML) that presented with a sudden increase of lymphoid blasts, without preceding signs of chronic phase relapse, and viral infections after allogeneic bone marrow transplantation following conditioning with a busulfan plus cyclophosphamide regimen for treatment of CML in chronic phase.

haematologica 2002; 87:659-661
(*http://www.haematologica.ws/2002_06/659.htm*)

The leukemic cells of the 3 patients at relapse expressed CD10, CD19, CD34 and HLA-DR, and were negative for peroxidase.

The first case was a 39-year old male. Monthly follow-up of the peripheral blood count and a bone marrow aspirate every 6 months were normal for 18 months after a first bone marrow transplant (Table 1).One month later (19 months post-BMT), the white blood cell count (WBC) had risen to 23.3 × 10^9/L with 77% atypical cells. Chemotherapy and subsequent donor lymphocyte infusion from the bone marrow donor produced no effect. He received an allogeneic peripheral blood stem cell transplant conditioning with total bosy irradiation (TBI) (12 Gy) and etoposide 60 mg/kg, but he died of veno-occlusive disease 83 days after the second transplant.

The second case, a 31-year old woman, showed normal blood counts for 38 months after BMT. When she came to our clinic after a 2-month interval, her WBC was 34.5 × 10^9/L with 55% atypical cells. She achieved complete remission after an allogeneic transplant with CA/CY/TBI. However, she relapsed with CML in lymphoid crisis 6 months after the second transplantation. Although chemotherapy and donor lymphocyte infusion induced temporary remission of leukemia as well as chronic graft-versus-host disease (GVHD) in the skin and liver, she died of acute respiratory distress syndrome 14 months after the second transplant.

In the third case, a 19-year old man, the fluorescence *in situ* hybridization (FISH) analysis of BM cells obtained every 6 months post-BMT showed essentially all cells to be of donor origin. Relapse of CML was not detected for 39 months after BMT. One month later, the patient suddenly developed severe bone pain in the right tibia. Bone marrow examination revealed hypercellularity with atypical cells occupying 96% of nucleated cells. The patient underwent allogeneic PBSCT conditioned with CA/CY/TBI. He developed grade II acute GVHD a month after transplantation, and was in molecular remission until 11 months after the second transplantation. The patient relapsed again 12 months after transplantation, and died of refractory disease within a month.

Although a recent report showed that approximately one-third of CML patients relapsing in advanced phase were transplanted while in chronic phase, this paper did not distinguish relapse into myeloid or lymphoid blast crisis.[2] Relapse with lymphoid crisis does not seem so uncommon after BMT, but reports in the literature are scarce. To our knowledge, only 3 cases have been described.[1,3,4]

This atypical form of relapse may be ascribed to the conditioning regimen we used for allografting. Between April 1986 and May 2000, 30 consecutive patients with chronic phase CML were treated with allogeneic transplantation at our facility (Table 2). We have used the Bu/CY regimen for patients with CML in chronic phase since 1989 based on accumulated evidence that the regimen is as potent as CY/TBI in eliminating CML cells.[5,6] Among 16 patients transplanted during the last 11 years, three patients (19%) relapsed with lymphoid crisis of CML. In contrast, there was no advanced phase relapse among 14 CML patients who were transplanted during the same period after condition-

Table 1. Characteristics of the three patients.

	Case 1	*Case 2*	*Case 3*
Age at transplant	39	31	19
Sex (patient/donor)	M/M (identical sibling)	F/F (identical sibling)	M/F (identical sibling)
Treatment before BMT	IFNα, HU	IFNα	IFNα, HU
Months from diagnosis to BMT	8	24	5
Conditioning regimen	Bu/CY	Bu/CY	Bu/CY
Marrow cells infused ($\times 10^8$/kg)	1.8	3.8	4.0
Acute GVHD	no	no	no
Chronic GVHD (onset)	extensive (at 12 months)	no	no
Months from BMT to relapse	19	40	40
Karyotype at transplant/relapse	Ph^+/Ph^+	Ph^+/Ph^+	Ph^+/Ph^+, +4, +8
Survival (months)	24	55	58

F, female; M, male; IFN, interferon; HU, hydroxyurea.

Table 2. Patient characteristics.

	No.
Patients	30
BMT	26
PBSCT	4
Donor	
HLA-identical sibling	21
HLA one-locus mismatched relative	2
HLA-identical unrelated donor	7
Conditioning	
CA/CY/TBI	11
CY/TBI	2
CA/TBI	1
Bu/CY	16
GVHD prophylaxis	
Short-term methotrexate and cyclosporine	30

BMT, represents bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation; CA 2 g/m² of cytarabine every 12 hours over 2 days, CY 60 mg/kg of cyclophosphamide on each of 2 consecutive days, TBI 12 Gy total body irradiation in 4 fractions over 4 days, and Bu 4 mg/kg of busulfan daily in 4 doses on each of 4 successive days. Unmanipulated BM or PBSC was infused.

ing with TBI-based regimens. This trend was seen in the incidence of chronic phase relapse after transplantation; chronic phase relapse developed in 3 of the 16 CML patients with Bu/CY regimen and in 1 of the 14 CML patients with TBI-based regimens. The high probability of acute phase relapse in our patients may have been due to insufficient blood levels of Bu because low Bu plasma levels are associated with an increased risk of relapse in patients with CML.[7] However, this hypothesis does not account for the fact that all of the acute phase relapses were due to lymphoid crisis.

One plausible explanation is that the Bu/CY regimen may have induced additional damage to the DNA of CML stem cells that favor proliferation of lymphoid crisis clone.[8,9] Testoni *et al.*[8] reported the new and additional clonal cytogenetic abnormalities as seen in the third case were found in 8 (27%) out of 30 patients with AML who underwent autologous BMT after conditioning with the Bu/CY regimen. When damaged CML stem cells are not eliminated by the graft-versus-leukemia (GVL) effect, they may potentially expand to develop blast crisis. The other factor that may have influenced the lymphoid crisis relapse of CML is the absence of acute GVHD. The probability of relapse with lymphoid crisis in 14 Bu/CY-treated patients who did not develop acute GVHD reached 21% while 2 patients developing acute GVHD remain in remission. The incidence of acute GVHD ≥ grade II in our series of Japanese patients was only 13%. This is in sharp contrast to the high incidence of acute GVHD (29-37%) in previous studies that documented an efficacy of the Bu/CY regimen comparable to that of the TBI-based regimen.[5,6]

The present study indicates a possibility that non-TBI regimens containing alkylating agents such as Bu may allow stem cells to persist and also to evolve into lymphoid crisis in the absence of acute GVHD after allogeneic transplantation. These findings suggest caution against the application of non-myeloablative stem cell transplantation (NST) with Bu to CML patients eligible for conventional myeloablative transplantation because CML stem cells are more likely to persist after NST and the incidence of acute GVHD is similar or not so high in NST as in myeloablative transplantation.

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Key words: BMT; CML; lymphoid crisis; busulfan plus cyclophosphamide regimen

Manuscript processing
This manuscript was peer-reviewed by two external referees and by Franco Dazzi, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Dazzi and the Editors. Manuscript received December 4, 2001; accepted April 11, 2002.

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Absence of structural mutations of the BAK gene in B-cell lymphomas

Bak is a Bcl-2 family member with pro-apoptotic activity. Mutations in the coding region of Bak have been described in human gastrointestinal cancers. We examined the status of the Bak gene in ninety-two B cell lymphomas. We found that structural mutations in the BAK gene are not involved in B cell lymphomagenesis.

haematologica 2002; 87:661-662
(*http://www.haematologica.ws/2002_06/661.htm*)

Evading apoptosis is a key feature in the malignant transformation of normal B-cells to lymphoma cells. BAK, a Bcl-2 family member, is the principal antagonist of the anti-apoptotic protein BCL-XL and interacts with BAX to form membrane pores leading to mitochondrial dysfunction and release of cytochrome c.[1-3] Mutations in the coding region of Bak have recently been described in human gastric and colorectal cancers.[4] Therefore, we examined the status of the Bak gene in 92 paraffin-embedded B-cell lymphomas, peripheral blood samples from 20 healthy donors and 10 paraffin-embedded samples of non-neoplastic oral tonsils for mutations in the five coding exons (exon 2–6). Genomic DNA extraction, SSCP-PCR analysis and direct sequencing of samples that showed mobility-shifted bands on the gel were performed as previously described.[5] Primers located within the intron sequences were used to amplify the five coding exons of BAK.[4] Bak protein expression was evaluated using immunohistochemical analysis with polyclonal anti-human Bak antibody (DAKO, Copenhagen, Denmark) in all samples, as previously described.[5] SSCP analysis of exons 3, 5 and 6 did not show any abnormal mobility indicative of sequence alteration in normal peripheral blood, non-neoplastic tonsils or in B-cell lymphomas. An abnormal mobility was, however, frequently observed in exon 2 of samples from peripheral blood, non-neoplastic tonsils, and lymphomas of germinal and post-germinal center origin (i.e. follicular lymphomas, diffuse large B-cell lymphomas and extra-nodal marginal zone lymphomas, MALT-type), but not in lymphomas of pre-germinal center origin (mantle cell and small lymphocytic lymphomas) (Figure 1A and Table 1). This alteration, monoallelic or biallelic, consists of a C to T transition in codon 14 (TGC to TGT), without translation into an amino acid substitution. In 6 patients demonstrating the exon 2 sequence alteration, epithelial tissue was available as a non-lymphoid control. In contrast to the lymphoma samples, epithelial tissues did not show exon 2 mutations (Figure 1B). In the SSCP analysis of exon 4 we found a different migration pattern of the PCR fragments in one case with follicular lymphoma, consisting in a biallelic T to C transition in the non-coding region of the intron sequence flanking exon 4 (data not shown). Immunohistochemical analysis did not show any different expression of Bak protein between exon 2 mutated and exon 2 non-mutated samples. Our study shows that structural mutations in the BAK gene are not involved in B-lymphomagenesis. The distribution of BAK exon 2 mutation in different lymphoma types suggests a segregation of this mutation with a germinal/post-germinal center origin. We observed this mutation with comparable frequency in normal peripheral blood and non-neoplastic tonsils (Table 1), which contain predominantly B-cells of post-germinal center origin. In contrast we found no mutation in normal epithelial tissues of patients with B-cell lymphoma containing the exon 2 mutation. Taken together these data indicate the germinal center origin of the exon 2 mutation. The germinal center is the site of somatic hypermutation, contributing to the diversity of the variable region of the immunoglobulin (IgV) genes.[6] Recently, other genetic regions have been identified as targets for the somatic hypermutation machinery.[7,8] These include the 5' region of the Bcl-6 gene. The Bak mutation, as Bcl-6 and IgV mutations, is located within 2 Kb from the transcriptional initiation site. The distribution and frequency of Bak and Bcl-6 mutations appear to be similar. However, the Bak mutations occur at one hotspot in the coding region and can be found in a heterozygous or homozygous state, whereas Bcl-6 mutations are distributed within a 740 bp region of the 5' non-coding sequence and were all found in heterozygosity.[7] The Bcl-6 mutations show a preferential targeting for the characteristic hotspot of the IgV hypermutation, the RGYW motif (R= purine, Y= pyrimidine and W= A or T).[7,8] This motif is not present in the mutation site of Bak. It is, therefore, unlikely that Bak and Bcl-6 mutations in the germinal center are caused by the same mechanism. As the Bak mutation is a silent base change in the coding region there is no evident functional significance, but in combination with other gene mutations it may serve as an indicator for the germinal center transition of both normal and malignant B-cells. In conclusion, the absence of mutations in BAX and BAK and their ubiquitous expression in B-cell lymphomas suggest that cell survival in this type of neoplasia does not depend on the alteration of apoptotic agonists, but is most probably due to alterations of the apoptotic antagonists. However, further studies are needed

Table 1. Exon 2 Bak mutational analysis in B-cell non-Hodgkin's lymphomas and normal lymphoid tissues.

	Number	*Normal of cases*	*Monoallelic*	*Biallelic mutation*	*Mutated (%) mutation*
Mantle cell lymphoma	7	7	–	–	0
Small lymphocytic lymphoma	7	7	–	–	0
Follicular lymphoma	32	20	10	2	37.5
Diffuse large B-cell lymphoma	27	20	7	–	26
Marginal zone lymphoma	17	9	7	1	47
Lymphoplasmocytoid lymphoma	2	1	1	–	50
Peripheral blood	20	11	8	1	45
Non-neoplastic tonsil	10	6	4	-–	40