Donor cell leukemia after umbilical cord blood transplantation—recurrent? or *de novo*? The importance of diagnosis for therapeutic decision-making

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Relapse of the underlying host leukemia is the most frequent cause of treatment failure after allogeneic stem cell transplantation (SCT). However, secondary neoplastic complications, including posttransplant lymphoproliferative disorders, therapy-related *de novo* malignancies and, less commonly, donor cell leukemia (DCL) [1], can also occur in SCT patients. Cord blood (CB) is now recognized as a feasible alternative source for SCT. More than 10,000 CB transplants (CBT) have been performed worldwide, and only ten cases of DCL following CBT have been reported. We report a new case of DCL in an adult patient with a history of refractory acute lymphoblastic leukemia (ALL) that manifested as recurrent ALL seven months after CBT.

A 31-year-old woman was hospitalized with a complaint of severe back pain, and results of blood tests revealed a markedly reduced platelet count and elevated lactate dehydrogenase. She had a long history of her disease prior to this event as shown in Table 1. She was initially diagnosed as having ALL (L2, pro-B cell), and she underwent allogeneic bone marrow transplantation (BMT) and achieved complete remission (CR). Three years later, she underwent non-myelo-ablative CBT to treat a recurrence of the disease, and she again achieved CR and was followed as an outpatient for seven months. At the time of her admission, numerous blasts were seen in a bone marrow biopsy and accompanying aspirate smear. Blast cells accounted for 84.4% of cells in bone marrow, and flow cytometric analysis revealed that these leukemic cells were positive for CD19 (97.1%), CD34 (96.6%), and CD38 (99.5%), consistent with the results of analysis performed in the recurrent state prior to CBT, with the exception of HLA-DR, which had become negative. Immunophenotypic change can occur in the context of recurrent leukemia, and such change is not a reliable indicator of a *de novo* process [2]. Cytogenetic analysis showed a normal karyotype. Wilms tumor 1 (WT1) mRNA in bone marrow was markedly elevated. Taken together, the findings suggested recurrent ALL. We further performed molecular analysis by short tandem repeat

(STR) analysis that we previously reported for evaluation of donor-type chimerism [3]. Purified $CD19^+$ leukemic cells from bone marrow revealed complete donor-type chimerism. We also performed HLA-typing of DNA from purified bone marrow $CD19^+$ cells using a reverse sequence-specific oligonucleotide (PCR-rSSO; SRL, Tokyo, Japan), and the genotypes were determined to be HLA-A2402/1101 and HLA-B0702/6701, which were identical to those of the CB cells. This lead to our reinterpretation of the patient's disease as donor-derived leukemia. The patient underwent re-induction therapy and achieved partial remission. She is in generally good condition and will undergo further induction and consolidation treatments.

The development of DCL after SCT is a very rare event. The estimated incidence of DCL is approximately 0.1% [4]. According to a report by Tokyo Cord Blood Bank, the estimated incidence of DCL after CBT is about 1% [5]. To date, approximately 10 cases of DCL following CBT have been reported, as clearly summarized by Crow et al [6]. Judging from these reports, there does not appear to be a relationship between the lineage of the original disease and that of DCL, and to our knowledge, this is the first report of donor-derived ALL from an ALL patient after CBT. The incidence of DCL after SCT may be underestimated, as chimerism analysis can be difficult and the results of cytogenetic studies alone are sometimes misleading [7, 8]. Numerous mechanisms for the genesis of DCL, including (1) occult leukemia or a pre-leukemic state in the donor, (2) a defect in immune surveillance, (3) transfer of oncogenic material from the host to donor cells, (4) therapy-related stromal abnormalities, and (5) excess cytokine stimulation and DNA replication and/or repair errors associated with post-transplant expansion of stem/progenitor cells, have been reported [9–12]. However, because of the rarity and heterogeneity of cases of DCL, the causal mechanisms may prove elusive. Moreover, the prognosis of DCL has not yet been established.

The percentage of leukemic cells in bone marrow cells is an important factors for distinguishing leukemia from hematogones [13]. In our case, the bone marrow was occupied by nearby 85% blasts, indicating a possible malignant state. Although we could not perform additional analysis for B-cell receptor rearrangement nor prove aberrant surface marker expression of $CD19^{+}$ cells, such data should provide further confirmation of donor cell-derived lymphoblastic leukemia.

Our case demonstrates that DCL can be easily diagnosed as a mere recurrence, which can affect the therapeutic decision-making. Given that the patient's disease was refractory to the series of BMT and CBT, it appeared that additional chemotherapy was unlikely to be effective, and the choice of the best supportive care rather than chemotherapy was justified. However, we were ultimately able to correctly diagnose the patient as having DCL through careful analysis of chimerism and HLA-typing of leukemic cells. These two methods provided sufficient and compelling proof of DCL, and we decided to begin induction chemotherapy for the patient. This case highlights the importance of close coordination of all aspects of clinical testing for transplant patients, including molecular studies, for distinguishing the very common complication of recurrent disease from the very rare complication of DCL. The detection and reporting of additional cases will provide more information on prognosis and possible treatment outcomes and may provide further clues into leukemogenesis.

Table 1. Treatment history

Table 1. Treatment history

Transplantation	1st: BMT	2nd: CBT	At Relapse (Blasts from patient)
Conditioning regimen	VP+CY+TBI 12Gy	FLU+LPAM+TBI 2Gy	
Cell number	NCC $3.7x10^8$/kg	NCC $4.9x10^7$/kg $CD34^+$ $2.5x10^5$/kg	
GVHD prophylaxis	FK+sMTX	FK+sMTX	
HLA mismatch	None	HLA-A and B	
Blast phenotyoe	CD19+, CD34+, HLA-DR+	CD19+, CD34+, CD38+, HLA-DR+	CD19+, CD34+, CD38+, HLA-DR-
Cytogenetics	46,XX,t(5;13)(q13;q14) [3/20]	46,XX	46,XX
Donor HLA	A2402/2603 B0702/1501 DR0101/0901	A2402/1101 B0702/6701 DR0101/0901	A2402/1101* B0702/6701* DR0101/0901*

VP, VP-16; CY, cyclophosphamide; TBI, total body irradiation; FLU, fludarabine; LPAM, melphalan; GVHD, graft-versus-host disease; FK, FK506; sMTX, short term methotrexate; HLA, human leukocyte antigen

*Analyzed from DNA samples from blasts.

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