(Table 1). The immunosuppressive therapy to prevent graft rejection consisted of tacrolimus, prednisolone, and azathioprine. Signs of acute rejection appeared after LDLLT, but rejection was successfully avoided by the intravenous administration of methylprednisolone and mycophenolate mofetil. The hypercapnia rapidly improved, and the vital capacity increased immediately after LDLLT. This progress allowed the patient to be weaned from mechanical ventilation on day 15, and she was discharged from the hospital on day 64 after LDLLT. BBO has not recurred in the transplanted lungs, and tests of pulmonary function have shown improvements in both the FVC and FEV1.0 for more than 30 months following LDLLT, despite the persistence of dry eyes and impaired lacrimal secretion. A pathologic examination of the explanted lungs revealed focal desquamation of the broncho-bronchiolar epithelium with an aggregate of foamy macrophages and lymphocytes and occlusions of the broncho-bronchiolar lumen, observations compatible with a diagnosis of either BBO or BO [16].

## 3. Discussion

BO is one of the LONIPCs and occurs 3 to 15 months following allogeneic HSCT [4,5,7,8]. The clinical symptoms of BO include a nonproductive cough, rapidly progressive dyspnea, and wheezing. The incidence of BO following allogeneic HSCT varies from 2.7% to 7.6% according to the published studies [4,7,8]. BO is one of the most devastating complications of allogeneic HSCT. Once it occurs in a patient, the prognosis tends to be extremely poor. The mortality rate reportedly varies from 14% to 100% [7,17,18]. The response to BO treatment has a significant effect on survival: 79% of responders survived more than 5 years from the diagnosis of BO, but only 13% of nonresponders survived [8]. Patients with LONIPCs have been treated with immunosuppressive agents such as antithymocyte globulin, methylprednisolone, prednisolone, CsA, tacrolimus, and azathioprine. Most patients with LIP and BOOP responded well to such treatments, whereas only 16% to 49% of BO patients improved owing to these treatments [4,7,8]. Consequently, no standard therapy for BO has yet been established.

Because BO often develops in association with chronic GVHD, this complication is thought to be one of the pulmonary manifestations of chronic GVHD [7,8,19]. However, the development of BO following autologous bone marrow transplantation in 2 patients [20] and a lower incidence of BO in recipients of allogeneic HSCT following reduced-intensity conditioning than in HSCT recipients with myeloablative conditioning [21] suggest that tissue damage due to high-dose radiochemotherapy plays a role in the development of BO. Our patient had extensive chronic GVHD, as manifested by sicca symptoms, at the time of LDLLT, and these symptoms persisted for more than 30 months, even after LDLLT. Because her lungs were suspected to be a target of the chronic GVHD, an LT from the donor of the allogeneic HSCT was thought to be ideal; however, because the donor and patient's family did not consent, she received an LT from 2 family members who were mismatched at 3 HLA loci. Despite the presence of HLA mismatches between the lung donors and the patient and the persistence of chronic GVHD, there has been no recurrence of BO in the transplanted lungs.

Fifteen cases of LT for post-SCT pulmonary complications following allogeneic HSCT, including 10 BO patients, have been reported (Table 2) [22-29]. The indications for LT in patients demonstrating BO following allogeneic HSCT are limited by many factors, such as the criteria of the donor and recipient, the existence of a suitable donor, the status of hematologic disease, and the timing of LT, especially for LDLLT [30]. Four of the 10 BO patients died after LT [24,27,29]. The patient in case 7 died of chronic rejection 6 years after undergoing LT, patient 11 died of pulmonary hemorrhaging 3 weeks after receiving LT, and patient 3, who received a single LT from a cadaver, died of BO on day 271, following recurrent episodes of both perivascular and bronchial rejection. Pechet et al [29] described 2 patients who had complications of BO long after LT; however, the authors did not report the details of these patients. The development of post-LT BO was thought to be due in part to a manifestation of chronic rejection [31,32]; however, preexisting chronic GVHD may have played some role in the development of BO in the transplanted lung early after transplantation. The other 5 BO patients (except for those described by Pechet et al [29]) and our patient have not experienced BO in the transplanted lungs from more than 15 months to 6 years after LT, despite the existence of chronic GVHD. These findings indicate that factors other than chronic GVHD, such as high-dose chemoradiotherapy and viral infections, may contribute to the development of BO following allogeneic HSCT. Another possibility is that the development of BO may require the presence of specific minor histocompatibility antigen (mHa) mismatches between the donor of the LT and the patient's immune system. The transplanted lungs may have evaded the immune attack responsible for BO because of a lack of mHas mismatches. LT is therefore considered to be a promising therapy for BO, even for patients associated with active chronic GVHD.

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CASE REPORT

# Graft rejection and hyperacute graft-versus-host disease in stem cell transplantation from non-inherited maternal antigen complementary HLA-mismatched siblings

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**Abstract**

Human leukocyte antigen (HLA)-mismatched stem cell transplantation from non-inherited maternal antigen (NIMA)-complementary donors is known to produce stable engraftment without inducing severe graft-versus-host disease (GVHD). We treated two patients with acute myeloid leukemia (AML) and one patient with severe aplastic anemia (SAA) with HLA-mismatched stem cell transplantation (SCT) from NIMA-complementary donors (NIMA-mismatched SCT). The presence of donor and recipient-derived blood cells in the peripheral blood of recipient (donor microchimerism) and donor was documented respectively by amplifying NIMA-derived DNA in two of the three patients. Graft rejection occurred in the SAA patient who was conditioned with a fludarabine-based regimen. Grade III and grade IV acute GVHD developed in patients with AML on day 8 and day 11 respectively, and became a direct cause of death in one patient. The findings suggest that intensive conditioning and immunosuppression after stem cell transplantation are needed in NIMA-mismatched SCT even if donor and recipient microchimerisms is detectable in the donor and recipient before SCT.

**Key words** graft-versus-host disease; rejection; graft failure; non-inherited maternal antigen; fetomaternal microchimerism

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In allogeneic stem cell transplantation (SCT) from human leukocyte antigen (HLA)-mismatched donors, severe acute graft-versus-host disease (GVHD) and graft rejections occur at a higher rate than SCT from HLA-matched donors (1, 2). Recently, allogeneic SCT from non-inherited maternal antigen (NIMA)-complementary donors has received attentions as one of the methods that potentially overcome the barrier of HLA incompatibility. It is well known that a small number of maternal blood cells exist in the newborn's blood, and in turn, blood cells derived from children can be detected in the mother's blood long after labor. This phenomenon is referred to as fetomaternal microchimerism. The existence of fetomaternal microchimerism suggests that immunological tolerance of hematopoietic cells takes place both in mother and child. Van Rood *et al.* (3) reported that the incidence of chronic GVHD was significantly lower in mother-to-child SCTs than in father-to-child SCTs. Their findings also suggest that HLA-haploidentical NIMA complementary siblings can be alternate donor candidates when there is no HLA-matched donor. Shimazaki *et al.* (4) reported five patients treated with allogeneic SCT from two or three loci-mismatched familiy donors who had a small number of recipient-derived cells in their blood before SCT. Engraftment occurred in all patients, and although acute GVHD developed in all five, their

severity was grade I or II except for one patient who developed grade III acute GVHD. Ichinohe *et al.* (5) reported that in HLA-haploidentical SCTs, NIMA mismatches in the graft-versus-host (GVH) direction, was associated with a lower risk of severe acute GVHD compared with IPA mismatches.

Based on these backgrounds, we treated two acute myeloid leukemia (AML) patients and one severe aplastic anemia (SAA) patient with SCT from NIMA-mismatched sibling donors. Graft rejection and severe acute GVHD occurred despite the fact that donor and recipient-derived microchimerisms were shown in donor and recipient.

## Case report

### Case 1

A 27-yr-old man was diagnosed as having chronic myloid leukemia (CML) in myeloid crisis. He underwent an HLA-matched unrelated bone marrow transplantation in September 1999. However, he relapsed with CML in blastic crisis in October 2000. He received allogeneic peripheral blood hematopoietic stem cell transplantation from a NIMA-complementary dizygotic sibling in November 2002. A fever of 38°C occurred on day 3 after transplantation and erythema developed in upper and lower extremities on day 8. A diagnosis of acute GVHD, which met the criteria of hyperacute GVHD (6, 7) was made through skin biopsy findings. The patient's GVHD responded to the treatment and both erythema and icterus disappeared on day 26. The complete donor chimerism was confirmed on day 17 by microsatellite marker analysis. Imatinib mesylate was administered on day 21 and he was in molecular remission on day 58. However, CML recurred as subcutaneous nodules on day 153 and the patient died of CML on day 203.

### Case 2

A 15-yr-old woman was diagnosed as having SAA in 2000. She did not respond to all kinds of therapy including ATG and anabolic steroids, and required frequent transfusions. An HLA-matched donor was absent either in relatives or in the bone marrow banks. Allogeneic bone marrow transplantation from the NIMA-complementary sister was performed in September 2003. Microchimerism was revealed in both the patient and donor (8). Her neutrophil count rose to 750/μL on day 25, but it became 0/μL following high fever associated with hyperferritinemia (24 490 ng/dL). Virus-associated hemophagocytic syndrome was suspected and foscarnet was administered without any effect. A chimerism analysis performed on day 34 revealed the absence of donor-derived cells in both the peripheral blood and bone marrow, thus leading to the diagnosis of secondary graft failure. She received an infusion of $1.65 \times 10^6$/kg of peripheral blood $CD34^+$ cells collected from the marrow donor without conditioning due to the deteriorating clinical condition, but no hematological recovery occurred. She underwent a cord blood cell transplantation (CBT) following conditioning with fludarabine 125 mg/m$^2$; melphalan, 160 mg/m$^2$ and total body irradiation at 4 Gy on day 89 after the first transplantation. She achieved a complete reconstitution of hematopoiesis after CBT and remains well 33 months after CBT.

### Case 3

In January 2002, a 32-yr-old man was diagnosed to have AML with a normal karyotype. He achieved a complete remission following standard chemotherapy. A year later, he relapsed with acute lymphocytic leukemia with the Philadelphia chromosome ($Ph^+$ALL). He was treated with chemotherapy consisting of daunorubicin, vincristine, L-asparaginase, and prednisolone, followed by the administration of imatinib mesylate, but did not achieve a complete remission. There was no HLA-matched family member. The microchimerism by NIMAs possessed by the patient was documented in the blood of one brother. He received allogeneic SCT from this NIMA-complementary brother. He became febrile from day 2 and erythema appeared diffusely on the generalized skin. He was diagnosed to have hyperacute GVHD. His skin GVHD deteriorated thus leading to a diagnosis of grade III acute GVHD. Bohrus methylprednisolone therapy could not improve the symptoms of acute GVHD. As a result, the patient died of thrombotic microangiopathy associated with acute GVHD on day 47.

## Results and discussion

This study is observational. The incidence of grade II to IV acute GVHD and graft failure in patients who were transplanted from HLA-haploidentical NIMA-complementary siblings has been reported to be 40–50% and 0–18% respectively (3, 5). Based on the results of these studies, HLA-haploidentical siblings whose NIMA is complementary to that of a patient are thought to be a possible donor candidate when HLA-matched donors are unavailable. However, our experience of hyperacute GVHD and graft rejection in the present report raises a concern about the efficacy of HLA-mismatched SCT from NIMA-complementary siblings.

Tables 1 and 2 summarize the patient characteristics and outcome of SCT for the three patients. Although the HLA disparity was one locus in the GVH direction in case 1, acute GVHD appeared on day 8 before

**Table 1** Patient Characteristics

| Case | Age | Sex | Diagnosis | Status at SCT | Preconditioning regimen | GVHD prophylaxis | CD34+ cells ($\times 10^8$/kg) | HLA (A, B, DR) | | Microchimerism | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Recipient | Donor | patient | Donor |
| 1 | 27 | M | CML | BC, relapse after UR-BMT | Flu 150 mg/m$^2$ + BU 8 mg/kg + ATG 40 mg/kg | CSA | 12.5 | 2/-, 51/38, 4/8 | 2/33, 51/44, 4/8 | ND | ND |
| 2 | 15 | F | AA | Refractory to immunosuppressive therapy | Flu 150 mg/m$^2$ + CY 120 mg/kg + ATG 25 mg/kg + TBI 2Gy | CSA + sMTX | 1.4 | 11/-, 55/67, 4/- | 11/24, 52/67, 4/15 | + | + |
| 3 | 32 | M | Ph + ALL | Resistant | TBI 12 Gy + CY 120 mg/kg | FK506 + sMTX | 3.1 | 2/-, 52/51, 9/8 | 2/11, 51/60, 8/14 | - | + |

CML = chronic myeloid leukemia; AA = aplastic anemia; Ph + ALL = Philadelphia chromosome positive acute lymphoblastic leukemia; BC = blastic crisis; UR-BMT = unrelated bone marrow transplantation; Flu = fludarabine; BU = busulfan; ATG = antithymocyte globulin; TBI = total body irradiation; CY = cyclophosphamide; CSA = cyclosporine; FK506 = tacrolimus; sMTX = short term methotrexate; NIMA = non-inherited maternal antigens; ND = not done.

**Table 2** Clinical outcome

| Case | Engraftment | | aGVHD | | GVHD stage | Treatment of GVHD | Complications | Outcome | Survival after SCT (d) |
|---|---|---|---|---|---|---|---|---|---|
| | Neu (d) | Plt (d) | Onset (d) | Grade | | | | | |
| 1 | 9 | 9 | 8 | 3 | Skin 2, Liver 2 | 2 mg/kg of mPSL started on day 9, 15 mg/m$^2$ of MTX on day 11 and 1000 mg/d of MMF started on day15 | - | Relapse on day 131 | 203 |
| 2 | 22 | NR | - | - | - | - | HPS on day 23, graft rejection on day 27 | CBT on day 89 | 993+ |
| 3 | 13 | 15 | 11 | 4 | Skin 4, Liver 4, Gut 3 | 1 g/d of mPSL for 3 d | TMA, convulsion | Death by GVHD | 47 |

Neu = neutrophil; Plt = platelet; G = granulocyte; T = T lymphocyte; aGVHD = acute graft-versus-host disease; HPS = homophagocytic syndrome; SCT = stem cell transplantation; mPSL = methylprednisolone; MTX = methotrexate; MMF = mycophenolate mofetil; TMA = thrombotic microangiopathy; CMV = cytomegalovirus.

neutrophil engraftment and rapidly progressed to grade III. Case 3 also developed hyperacute GVHD despite the fact that microchimerism was documented in the donor's blood. Acute GVHD is known to occur frequently before engraftment of neutrophil in recipient of HLA-mismatched SCT (6, 7). In the analysis of SCTs between NIMA-complementary family members described by Ichinohe *et al.* (5), the presence of acute GVHD was observed from day 10. The presence of the recipient-specific microchimerism did not necessary predict low incidence of acute GVHD in this study, in line with our experience. Our findings suggest the necessity of intensive immunosuppressive therapy to prevent acute GVHD such as ATG (9, 10) or alemtuzumab (11) even when a donor shows recipient-specific microchimerism. As case 1 had received HLA-matched unrelated bone marrow transplantation before undergoing the second SCT from a NIMA complementary sibling, recipient dendritic cells were probably replaced by the cells of the unrelated donor. The dendritic cells of a recipient play an important role in the development of acute GVHD (12, 13). The absence of the patient-derived dendritic cells, which were educated to tolerate donor T cells, may be responsible for hyperacute GVHD of the patients.

Case 2 was conditioned with fludarabine-based regimen which was known to ensure engraftment of bone marrow from HLA-matched unrelated donors in AA patients (14). Microchimerism by donor cells was documented in the patient. Nevertheless, SCT from the donor ended up with secondary graft rejection. Although the number of of $CD34^+$ cells infused ($1.4 \times 10^6$/kg) was relatively low, the minimal number of $CD34^+$ cells in the successful SCT was $1.26 \times 10^6$/kg in the report by Shimazaki *et al.* (4) and $1.3 \times 10^6$/kg in the report by Ichinohe *et al.* (5). It is therefore necessary to intensify the conditioning regimen to prevent graft rejection when HLA-mismatched SCTs from NIMA-complementary siblings are administered to patients with AA even if microchimerism by donor cells is documented in the recipient.

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ELSEVIER

# Safety and Efficacy of Foscarnet for Preemptive Therapy Against Cytomegalovirus Reactivation After Unrelated Cord Blood Transplantation

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ABSTRACT

In association with the increased use of unrelated cord blood transplantation (UCBT) in adults, numerous patients have developed cytomegalovirus (CMV) reactivation concomitant with cytopenia. Although foscarnet appears to offer similar efficacy and higher safety as a preemptive therapy against CMV infection than ganciclovir, little is known about the usefulness of foscarnet in such patients. Foscarnet was administered as preemptive therapy against CMV antigenemia in 10 UCBT recipients who were unable to receive ganciclovir due to cytopenia or poor response to ganciclovir. Fatal CMV disease developed in one patient, whereas CMV antigenemia resolved without progression to CMV disease in the remaining nine patients. Foscarnet was well tolerated without serious hematotoxicity and was not discontinued due to adverse events in any patient. Foscarnet represents a safe and effective agent for preemptive therapy against CMV infection and may offer a feasible alternative to ganciclovir in UCBT recipients.

COMPARED TO ALLOGENEIC bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT), the advantages of unrelated cord blood transplantation (UCBT) include ease and safety of cell collection, low risk of transmitting viral infections, prompt availability of stem cells, and reduced incidence and severity of graft-versus-host disease (GVHD). Conversely, UCBT is disadvantageous in that slow marrow recovery and immunological immaturity after transplantation lead to an increased risk of infectious complications, and these account for most transplant-related deaths in adult patients receiving UCBT. Patients undergoing UCBT not only develop cytomegalovirus (CMV) reactivation more frequently but also earlier posttransplant compared to patients undergoing BMT or PBSCT.[1,2] For patients who develop CMV reactivation in the early post-UCBT period, preemptive treatment with ganciclovir might promote neutropenia that could place the patient at risk of fatal infectious complications. In contrast to ganciclovir, the antiviral drug foscarnet (trisodium phosphonoformate) appears to lack significant hematotoxicity in allograft recipients.[3-5] Dose-limiting toxicities of foscarnet are nephrotoxicity and neurotoxicity. Foscarnet might thus represent a feasible alternative for preemptive therapy against CMV reactivation in patients after UCBT. To evaluate the safety and efficacy of foscarnet for preemptive therapy against CMV infection after UCBT, the present study used this drug as an alternative to ganciclovir for patients who were unable to receive ganciclovir due to cytopenia or poor response to ganciclovir.

## MATERIALS AND METHODS

### Patients

Patients with CMV-positive antigenemia detected at least once were considered eligible for the study if absolute neutrophil count (ANC) was $<1.0 \times 10^9$/L or platelet count was $<20 \times 10^9$/L or response of ganciclovir against CMV antigenemia was tardy as assessed by a physician. Exclusion criteria were serum creatinine clearance <40 mL/min or therapy with foscarnet before study inclusion. Among 28 consecutive adult patients receiving UCBT at Kanazawa University Hospital between 2001 and 2005, 10 patients entered this study. Written informed consent was obtained from all patients. Foscarnet was administered as initial preemptive therapy in 5 of the 10 patients due to cytopenia. Pharmacotherapy was switched from ganciclovir to foscarnet due to ganciclovir-induced

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cytopenia in three patients and tardy response to ganciclovir in two patients.

### CMV Antigenemia Monitoring

Monitoring CMV-pp65 antigen levels assessed CMV antigenemia. CMV antigenemia assays were performed as previously described.[6] Degree of CMV antigenemia was expressed as the number of CMV antigen-positive cells per $5 \times 10^4$ leukocytes, and CMV antigenemia was defined as ≥1 antigen-positive cell.

### Preemptive Therapy With Foscarnet for Prevention of CMV Disease

Foscarnet was intravenously administered over 2 hours at 60 mg/kg every 12 hours for 14 days as induction treatment. Treatment was stopped if two consecutive CMV antigenemia assays yielded negative results. When CMV antigenemia remained detectable in peripheral blood assay after induction treatment, patients then received intravenous foscarnet at a maintenance dosage of 90 mg/kg/d over 3 hours while antigenemia persisted. Dosage of foscarnet was adjusted according to decreased renal function based on predetermined guidelines. Renal impairment was considered present for an increase in serum creatinine ≥100% or a decrease in creatinine clearance ≥50% from baseline values.

### Statistical Analysis

Parameters in the two groups were compared by Fisher exact test.

## RESULTS

### Efficacy of Foscarnet

The 10 patients had a total of 28 episodes of CMV antigenemia one to five episodes a patient). Median duration from transplantation to initiation of foscarnet treatment was 43 days (range, 14 to 52 days; Table 1). Median duration of exposure to foscarnet in one episode was 10 days (range, 7 to 20 days). In 9 of the 10 patients CMV antigenemia ended, which was defined as attainment of no CMV antigenemia for ≥3 months without antiviral treatment, at a median of 60 days (range, 7 to 104 days) after starting foscarnet treatment. The remaining one patient received foscarnet starting on day 14 due to ganciclovir-associated neutropenia, but it resulted in no response and the patient died of interstitial pneumonia (IP) on day 83.

### Safety of Foscarnet

Although one patient developed impaired renal function during treatment with foscarnet, only dose adjustment was required. Although hypocalcemia, hypomagnesemia, hypokalemia, and hypophosphatemia occurred in seven, five, seven, and one patients, respectively, electrolyte disturbances were improved by supplementation through intravenous infusions, and no clinical symptoms or signs attributable to changes in serum electrolyte levels were identified. No seizures or parasthesias occurred. Nausea and vomiting occurred in two patients and were resolved using antiemetic treatments. Median ANC on onset of foscarnet was $1.3 \times 10^9$ (range, $0.4 \times 10^9$/L to $4.3 \times 10^9$/L), and median maximal drop in ANC was $0.2 \times 10^9$/L (range, $-1.6 \times 10^9$/L to $12.9 \times 10^9$/L). No patient developed foscarnet-induced severe neutropenia ($<0.5 \times 10^9$/L), although one patient whose ANC was $0.4 \times 10^9$/L at the start of foscarnet therapy received transient treatment with granulocyte colony-stimulating factor in association with foscarnet. No adverse events required discontinuation of foscarnet treatment.

### Comparison Between UCBT Patients and Unrelated BMT Patients

When the UCBT patients were compared to seven unrelated BMT patients who received foscarnet as preemptive therapy against CMV infection, there was no statistical difference between the two groups in parameters on the efficacy and toxicity of foscarnet (Table 1).

## DISCUSSION

CMV infection is still a major concern following allogeneic hematopoietic transplantation because CMV pneumonia is fatal in 70% of patients, even when treated with a combination of antiviral therapies and CMV hyperimmune immunoglobulin.[7] The determinants for development of CMV infection and disease are seropositivity for CMV prior to transplant, GVHD, HLA-mismatched donor, unrelated bone marrow or cord blood donor, treatment with steroids, and modifications of the graft such as in vitro and in vivo T-cell depletion. In contrast to patients treated with high-

**Table 1. Efficacy and Toxicity of Foscarnet**

| | UCBT ($n$ = 10) | Unrelated BMT ($n$ = 7) |
|---|---|---|
| Median time from SCT to treatment start with foscarnet, d (range) | 43 (14–52) | 34 (25–70) |
| Median time of foscarnet treatment, d (range) | 10 (7–20) | 8 (6–60) |
| Patients with clearance of CMV antigenemia (%) | 90 | 100 |
| Patients with CMV disease (type) | 1 (IP) | 1 (enteritis, before the initiation of foscarnet) |
| Transplant-related deaths | | |
| Infections | 2 | 1 |
| CMV-induced IP | 1 | 0 |
| Median ANC on onset of foscarnet (range), $\times 10^9$/L | 3 (0.4–15.4) | 3.1 (1.7–10.5) |
| Median platelet counts on onset of foscarnet (range), $\times 10^9$/L | 26 (13–113) | 75 (40–223) |
| Median maximal drop in ANC from the start of foscarnet during foscarnet treatment (range), $\times 10^9$/L | 0.2 (−1.6–12.9) | 0 (−2.4–6.9) |
| Patients with discontinuation of foscarnet | 0 | 0 |

dose chemotherapy and autologous stem cell transplantation, patients after allogeneic stem cell transplantation are at a much higher risk of development of CMV infection because of the delayed recovery of T- and B-cell functions. Thus the rate by which immune function recovers after hematopoietic reconstitution significantly influences the incidence of CMV infection and disease after stem cell transplantation.

The current study has shown that foscarnet can be effective as a preemptive therapy against CMV reactivation after UCBT. Only 1 of 10 patients developed CMV disease and CMV antigenemia resolved in the remaining nine patients during the study period, appearing comparable to treatment results with ganciclovir and foscarnet for recipients of allogeneic BMT and PBSCT.[3,5,8–10]

Despite effectiveness in preventing CMV disease after BMT and PBSCT, use of ganciclovir is associated with marked toxicity to the bone marrow, and severe neutropenia is reported in up to 35% of patients who receive ganciclovir treatment after allogeneic BMT or PBSCT.[11–14] This long duration of neutropenia places patients receiving ganciclovir at significant risk of bacterial and fungal infection,[14] and 8% also develop CMV disease.[13] Furthermore, neutropenia in ganciclovir recipients has been shown to represent an independent risk factor for mortality.[11]

Foscarnet was well tolerated and was not associated with any serious hematotoxicity, even in patients with cytopenia at the start of treatment. In addition, foscarnet did not interfere with hematopoietic engraftment despite being administered in the early posttransplant period after UCBT. These results suggest that foscarnet not only can be used from the very early phases of engraftment but also might be started before transplantation for prophylaxis against CMV reactivation. Of note is the fact that foscarnet was not discontinued due to adverse events in any patient. All adverse events, including nephrotoxicity, were resolved with supportive medication or a reduction in foscarnet dose.

In conclusion, the present results may suggest that foscarnet offers an effective and safe alternative to ganciclovir for CMV prophylaxis in recipients of allogeneic UCBT who are unable to receive treatment with ganciclovir due to cytopenia or poor response to ganciclovir. Randomized, comparative studies between ganciclovir and foscarnet are warranted for better evaluation of the preemptive and treatment roles of these agents in patients after UCBT.

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# Individual Dose Adjustment of Oral Busulfan Using a Test Dose in Hematopoietic Stem Cell Transplantation

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**Abstract**

Maintaining the appropriate average steady-state plasma concentrations (Css) of busulfan (BU) is critical for both successful engraftment and minimizing toxicity in hematopoietic stem cell transplantation (HST). We therefore performed a prospective trial with 50 adult Japanese patients that involved adjusting the BU dose in accordance with individual BU pharmacokinetics (PK). After administering a 0.5-mg/kg test dose of oral BU, we analyzed individual BU PK parameters and calculated an adjusted BU dose that would achieve a target BU Css of 850 ng/mL. Thirty-nine patients (78%) required a BU dose decrease, and the median adjusted BU dose was 0.81 mg/kg (range, 0.51-1.29 mg/kg). All patients who underwent allogeneic HST received the adjusted BU dose. After administering the sixth BU dose, we measured the plasma BU concentration. The actual BU concentration was significantly correlated with the expected BU concentration, and the predictability of the BU Css was 103% ± 19%. The incidence of toxicity excluding oral mucositis was low, and there was no regimen-related toxicity–associated mortality. Engraftment was achieved in 98% of the patients. This study showed that our method for adjusting the BU dose facilitated reliable prediction of the actual BU Css and that individualized BU dose adjustment was able to improve clinical outcomes in HST recipients treated with a BU-containing conditioning regimen.
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*Key words:* Busulfan; Conditioning; Hematopoietic stem cell transplantation; Pharmacokinetics; Dose adjustment

## 1. Introduction

Oral busulfan (BU) in combination with cyclophosphamide (CY) is generally used as a myeloablative conditioning regimen for allogeneic hematopoietic stem cell transplantation (HST). The standard BU dose is 1 mg/kg body weight administered orally every 6 hours. The therapeutic effects of BU are related to the area under the plasma concentration–time curve (AUC) or the average steady-state plasma concentration (Css) [1]. Excessively high BU concentrations are associated with an increase in hepatic veno-occlusive disease (VOD) [2], whereas low levels are associated with a high relapse rate in chronic myeloid leukemia (CML) patients [3] and with graft rejection in children [4,5]. Adjustment of the BU dose according to individual BU pharmacokinetics (PK) has been shown to be critical for achieving successful HST results for myelodysplastic syndrome (MDS) [6] and CML [7].

BU PK and BU dose adjustment have been extensively investigated in Caucasians, but few studies have focused on Asian people. We previously showed that the BU Css varied widely (745-2422 ng/mL) after 1 mg/kg of oral BU was administered to adult Japanese patients and that excessively high BU Css were associated with the development of hepatic VOD [8], indicating that BU dose adjustment is needed in Japanese patients to minimize toxicities and improve clinical outcomes. We therefore performed a prospective clinical trial in

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Japanese patients that used a test dose to adjust the BU dose in accordance with individual BU PK, and we used our method to evaluate the predictive performance of the BU Css.

## 2. Materials and Methods

### 2.1. Patients

All consecutive patients who underwent allogeneic HST with BU-based conditioning regimens at 9 institutions in Kyushu were enrolled in this prospective study. Informed and written consent was obtained from all patients who participated in this study. This study was approved by each hospital's institutional review board.

### 2.2. Individual Adjustment of BU Dose with a Test Dose

A 0.5-mg/kg test dose of BU was administered orally approximately 1 week prior to the HST preparative regimen. Blood samples were collected in heparinized tubes for PK analysis at 30, 60, 120, 240, and 360 minutes after oral BU administration, and BU concentrations were measured by a high-performance liquid chromatographic method, as previously described [9]. The population parameter and variance values published by Sandstrom et al were used [10]. We calculated individual BU PK parameter values with a 1-compartment model using the Bayesian modeling program PEDA [11]. BU Css levels were determined by the ratio of the BU AUC over the dosing interval to the time between doses. A targeted dose was calculated to achieve a BU Css of 850 ng/mL.

### 2.3. Hematopoietic Stem Cell Transplantation

The HST conditioning regimen consisted of either a conventional conditioning regimen of dose-adjusted BU administered every 6 hours for 16 doses over 4 days followed by 120 mg/kg CY or a reduced-intensity preparative regimen of dose-adjusted BU administered every 6 hours for 8 doses over 2 days plus 180 mg/kg fludarabine (FLU) with or without 2 Gy total body irradiation (TBI). The graft used for HST was bone marrow (BM) cells or peripheral blood stem cells (PBSC). During BU administration, all patients received either sodium valproate or phenytoin as prophylaxis against BU-related seizures. Either fluconazole or itraconazole, either ciprofloxacin or levofloxacin, and acyclovir were administered as prophylaxis against fungal, bacterial, and herpesvirus infection. Prophylaxis against graft-versus-host disease (GVHD) consisted of a combination of cyclosporine and methotrexate (MTX) in patients who underwent transplantation from HLA-matched siblings, and tacrolimus and MTX were given to those who underwent HST from unrelated donors or HLA-mismatched siblings.

### 2.4. Comparison of Expected and Observed BU Concentrations

The primary end point of this study was to investigate whether target BU concentrations can be achieved by administering adjusted BU doses calculated with our method. The predictive performance of our method was evaluated in 2 ways. First, we investigated whether the BU concentration expected after the test dose (expected BU concentration) could be used to predict the actual BU concentration measured after dose-adjusted BU administration (actual BU concentration) by analyzing the mean prediction error. Blood samples were collected at 30, 60, 120, 240, and 360 minutes after the sixth adjusted BU dose was administered, and actual BU concentrations were measured. We calculated expected BU concentrations at each time point, on the basis of the individual BU PK data obtained with the test dose. We analyzed 5 blood samples collected from each of the 50 patients and analyzed the correspondence between actual and expected BU concentrations in the 250 samples. Actual BU concentrations were plotted against expected BU concentrations, and the mean prediction error was calculated as follows:

Mean Prediction Error = Σ[(Actual BU Concentration − Expected BU Concentration)/Actual BU Concentration]/(Sample Number).

Second, we investigated whether the actual BU Css were able to reach the target level of 850 ng/mL. Actual BU Css were calculated from the BU PK data obtained after administrating adjusted BU doses. The predictability of Css was calculated as follows:

Css Predictability = (Actual BU Css)/850 ng/mL.

### 2.5. Clinical Evaluation

Because an insufficient BU Css increases the incidence of graft rejection and the intensity of regimen-related toxicities (RRT) and because these events influence early mortality after HST, we evaluated engraftment, RRT, and overall survival at day 100 as a secondary end point of this study. Although low BU Css were related to the high incidence of relapse in CML patients, we did not analyze either the recurrence rate or disease-free survival in this study because the enrollment of patients with various diseases and with varying disease status made it difficult to analyze the relationship between BU Css and disease recurrence. Engraftment was defined as a neutrophil count $>0.5 \times 10^9$/L for 3 consecutive days with evidence of donor cells either by conventional restriction fragment length polymorphism analysis or by in situ hybridization analysis for X and Y chromosomes in cases of sex-mismatched HST. RRT was scored by means of the criteria described by Bearman et al [12]. The overall toxicity grade was given by the maximum toxicity grade for the bladder, renal, pulmonary, hepatic, central nervous, mucosal, and gastrointestinal organ systems experienced by the patient during the first 28 days after transplantation. A diagnosis of VOD was made clinically on the basis of the standard criteria of a bilirubin concentration >2 mg/dL and at least 2 of the following signs or symptoms: hepatomegaly with right upper-quadrant pain, ascites, or weight gain of greater than 5% from the baseline [13].

## 3. Results

### 3.1. Patient Characteristics

Fifty patients 16 to 64 years old (median, 45 years) underwent allogeneic HST between May 2004 and

**Table 1.**
Patient Characteristics*

| UPN | Age, y/Sex | Height, cm | Weight, kg | Disease | Cell Source | HLA Compatibility | Conditioning Regimen |
|---|---|---|---|---|---|---|---|
| 1 | 20/M | 165 | 54 | AML/1CR | Sibling BM | Matched | BU + CY |
| 2 | 39/F | 156 | 44 | AML/1CR | Sibling BM | Matched | BU + CY |
| 3 | 64/M | 180 | 64 | MDS | Sibling BM | Matched | BU + CY |
| 4 | 31/M | 176 | 72 | AML/Ref | Unrelated BM | Matched | BU + CY |
| 5 | 35/M | 169 | 53 | ML/Ref | Sibling PB | Matched | BU + CY |
| 6 | 46/M | 172 | 77 | MF | Sibling PB | Matched | BU + CY |
| 7 | 32/M | 163 | 65 | AML/1CR | Sibling BM | Matched | BU + CY |
| 8 | 57/M | 163 | 44 | ATL/1CR | Unrelated BM | Matched | BU + CY |
| 9 | 43/F | 164 | 50 | AML/Ref | Sibling PB | Matched | BU + CY |
| 10 | 51/M | 176 | 75 | MDS | Sibling PB | Matched | BU + CY |
| 11 | 47/M | 162 | 60 | CML/CP | Sibling BM | Matched | BU + CY |
| 12 | 50/F | 150 | 43 | AML/1CR | Sibling BM | Matched | BU + CY |
| 13 | 45/F | 157 | 46 | ML/Ref | Sibling PB | Matched | BU + CY |
| 14 | 53/M | 170 | 57 | AML/1CR | Sibling PB | Matched | BU + CY |
| 15 | 30/M | 171 | 75 | ML/Ref | Sibling PB | Matched | BU + CY |
| 16 | 38/M | 180 | 74 | AML/2CR | Unrelated BM | 5/6 | BU + CY |
| 17 | 56/M | 169 | 77 | AML/4CR | Unrelated BM | Matched | BU + CY |
| 18 | 45/M | 175 | 67 | AML/2CR | Sibling PB | Matched | BU + CY |
| 19 | 48/M | 178 | 72 | MF | Sibling PB | Matched | BU + CY |
| 20 | 41/M | 174 | 55 | AML/Ref | Sibling BM | Matched | BU + CY |
| 21 | 57/M | 163 | 49 | ATL/1CR | Unrelated BM | Matched | BU + CY |
| 22 | 30/F | 168 | 53 | AML/1CR | Sibling PB | Matched | BU + CY |
| 23 | 19/F | 166 | 65 | AML/2CR | Sibling PB | Matched | BU + CY |
| 24 | 32/M | 168 | 55 | AML/1CR | Unrelated BM | Matched | BU + CY |
| 25 | 51/F | 158 | 51 | MDS | Sibling BM | Matched | BU + CY |
| 26 | 57/M | 167 | 57 | AML/1CR | Sibling PB | 5/6 | BU + CY |
| 27 | 55/M | 168 | 57 | AML/2CR | Unrelated BM | 5/6 | BU + CY |
| 28 | 27/M | 172 | 65 | MDS | Sibling PB | 5/6 | BU + CY |
| 29 | 27/F | 149 | 49 | CML/AP | Sibling PB | Matched | BU + CY |
| 30 | 45/M | 171 | 71 | AML/2CR | Unrelated BM | Matched | BU + CY |
| 31 | 54/M | 163 | 51 | MDS | Sibling BM | Matched | BU + CY |
| 32 | 57/M | 172 | 71 | AML/Ref | Unrelated BM | Matched | BU + CY |
| 33 | 61/M | 162 | 57 | AML/Ref | Unrelated BM | Matched | BU + CY |
| 34 | 42/F | 151 | 45 | ALL/2CR | Unrelated BM | 5/6 | BU + CY |
| 35 | 20/M | 171 | 74 | MDS | Sibling BM | Matched | BU + CY |
| 36 | 41/M | 172 | 66 | ATL/PR | Unrelated BM | 5/6 | BU + CY |
| 37 | 54/M | 159 | 52 | MM/Ref | Unrelated BM | 5/6 | BU + FLU + TBI |
| 38 | 63/M | 160 | 60 | AML/Ref | Unrelated BM | Matched | BU + FLU + TBI |
| 39 | 37/F | 149 | 45 | ALL/Ref | Unrelated BM | Matched | BU + FLU + TBI |
| 40 | 52/M | 171 | 55 | AML/Ref | Unrelated BM | Matched | BU + FLU + TBI |
| 41 | 58/F | 149 | 55 | AML/Ref | Unrelated BM | Matched | BU + FLU + TBI |
| 42 | 38/M | 165 | 73 | MDS | Sibling PB | Matched | BU + FLU + TBI |
| 43 | 28/F | 157 | 56 | AML/Ref | Unrelated BM | 5/6 | BU + FLU + TBI |
| 44 | 16/M | 167 | 40 | AML/2CR | Unrelated BM | Matched | BU + FLU + TBI |
| 45 | 51/F | 154 | 47 | MM/CR | Sibling BM | Matched | BU + FLU |
| 46 | 32/M | 175 | 93 | AML/2CR | Sibling PB | Matched | BU + FLU |
| 47 | 30/F | 165 | 52 | Ewing sarcoma/Ref | Sibling PB | Matched | BU + FLU |
| 48 | 34/M | 181 | 81 | MDS | Sibling BM | Matched | BU + FLU |
| 49 | 53/M | 164 | 47 | PLL/Ref | Unrelated BM | Matched | BU + FLU |
| 50 | 60/F | 156 | 57 | ML/2CR | Unrelated BM | Matched | BU + FLU |

*UPN indicates unique patient number; AML, acute myeloid leukemia; 1CR, first complete remission; BM, bone marrow; BU, busulfan; CY, cyclophosphamide; MDS, myelodysplastic syndrome; Ref, refractory; ML, malignant lymphoma; MF, myelofibrosis; PB, peripheral blood; ATL, adult T-cell leukemia; CML, chronic myeloid leukemia; CP, chronic phase; AP, accelerated phase; ALL, acute lymphocytic leukemia; PR, partial remission; MM, multiple myeloma; FLU, fludarabine; TBI, total body irradiation; PLL, prolymphocytic leukemia.

**Table 2.**
Busulfan Pharmacokinetic Parameters and Clinical Outcomes*

| UPN | $k_a$, $h^{-1}$ | Vd/F, L/kg | CL/F, L/h per kg | $t_{1/2}$, h | Adjusted BU Dose, mg/kg | Actual BU Css, ng/mL | Day of Early Death | Cause of Death |
|---|---|---|---|---|---|---|---|---|
| 1 | 3.13 | 0.89 | 0.18 | 3.45 | 0.89 | 825 | — | |
| 2 | 1.46 | 0.85 | 0.20 | 3.02 | 1.00 | 795 | — | |
| 3 | 1.05 | 0.64 | 0.16 | 2.81 | 0.76 | 802 | — | |
| 4 | 1.92 | 0.76 | 0.15 | 3.58 | 0.79 | 858 | — | |
| 5 | 3.40 | 0.75 | 0.16 | 3.29 | 0.79 | 715 | Day 82 | Progressive disease |
| 6 | 1.65 | 0.63 | 0.09 | 4.91 | 0.51 | 801 | — | |
| 7 | 5.07 | 0.82 | 0.16 | 3.55 | 0.93 | 745 | — | |
| 8 | 2.07 | 0.65 | 0.20 | 2.24 | 0.95 | 767 | — | |
| 9 | 4.61 | 0.60 | 0.14 | 2.91 | 0.74 | 807 | — | |
| 10 | 0.77 | 0.74 | 0.16 | 3.27 | 0.88 | 964 | — | |
| 11 | 1.68 | 0.63 | 0.11 | 2.95 | 0.63 | 835 | — | |
| 12 | 2.93 | 0.47 | 0.12 | 2.72 | 0.60 | 650 | — | |
| 13 | 0.83 | 0.57 | 0.17 | 2.27 | 0.87 | 833 | — | |
| 14 | 0.53 | 0.67 | 0.21 | 2.17 | 1.09 | 1010 | — | |
| 15 | 2.58 | 0.71 | 0.18 | 2.68 | 1.09 | 1140 | — | |
| 16 | 2.35 | 0.64 | 0.15 | 3.32 | 0.57 | 630 | Day 64 | Engraftment failure, sepsis |
| 17 | 4.85 | 0.60 | 0.15 | 2.82 | 0.94 | 960 | — | |
| 18 | 3.90 | 0.70 | 0.16 | 2.96 | 0.82 | 740 | — | |
| 19 | 2.59 | 0.71 | 0.24 | 2.05 | 1.23 | 920 | — | |
| 20 | 4.69 | 0.67 | 0.18 | 2.60 | 0.91 | 850 | Day 100 | Progressive disease |
| 21 | 0.97 | 0.82 | 0.16 | 3.65 | 0.80 | 890 | Day 15 | Sepsis |
| 22 | 4.34 | 0.80 | 0.22 | 2.54 | 1.11 | 1240 | — | |
| 23 | 1.89 | 0.62 | 0.23 | 1.89 | 1.29 | 900 | — | |
| 24 | 1.88 | 0.70 | 0.14 | 3.42 | 0.71 | 910 | — | |
| 25 | 6.03 | 0.97 | 0.34 | 1.98 | 0.88 | 700 | — | |
| 26 | 1.06 | 0.86 | 0.14 | 4.14 | 0.74 | 690 | — | |
| 27 | 5.34 | 0.47 | 0.10 | 3.14 | 0.54 | 720 | Day 21 | Sepsis |
| 28 | 0.95 | 0.62 | 0.15 | 2.95 | 0.74 | 780 | Day 48 | GVHD |
| 29 | 2.06 | 0.41 | 0.12 | 2.30 | 0.70 | 750 | — | |
| 30 | 2.71 | 0.69 | 0.16 | 2.94 | 0.91 | 910 | — | |
| 31 | 5.11 | 0.64 | 0.15 | 3.00 | 0.76 | 930 | — | |
| 32 | 2.74 | 0.74 | 0.13 | 4.03 | 0.71 | 1067 | Day 45 | Sepsis |
| 33 | 2.58 | 0.71 | 0.19 | 2.55 | 1.00 | 895 | — | |
| 34 | 1.60 | 0.73 | 0.15 | 3.31 | 0.80 | 848 | — | |
| 35 | 4.21 | 0.63 | 0.12 | 3.60 | 0.70 | 920 | — | |
| 36 | 1.18 | 0.71 | 0.14 | 3.47 | 0.72 | 830 | Day 16 | Sepsis |
| 37 | 1.11 | 0.69 | 0.22 | 2.23 | 1.10 | 914 | — | |
| 38 | 0.76 | 0.66 | 0.17 | 2.62 | 0.98 | 1100 | — | |
| 39 | 1.48 | 0.73 | 0.22 | 2.28 | 1.14 | 1600 | Day 43 | Progressive disease |
| 40 | 4.92 | 0.64 | 0.16 | 2.87 | 0.82 | 830 | — | |
| 41 | 5.11 | 0.58 | 0.16 | 2.47 | 1.02 | 913 | — | |
| 42 | 5.20 | 0.64 | 0.12 | 3.70 | 0.76 | 840 | — | |
| 43 | 4.06 | 0.57 | 0.18 | 2.20 | 0.98 | 665 | — | |
| 44 | 3.62 | 0.46 | 0.10 | 3.34 | 0.55 | 1070 | — | |
| 45 | 1.89 | 0.81 | 0.22 | 2.54 | 1.11 | 1040 | — | |
| 46 | 4.49 | 0.66 | 0.14 | 3.27 | 0.97 | 960 | — | |
| 47 | 3.11 | 0.52 | 0.12 | 2.98 | 0.63 | 880 | Day 53 | Progressive disease |
| 48 | 1.97 | 0.91 | 0.09 | 6.96 | 0.51 | 720 | Day 24 | Sepsis |
| 49 | 0.92 | 0.64 | 0.14 | 3.10 | 0.72 | 995 | Day 17 | Sepsis |
| 50 | 1.03 | 0.57 | 0.12 | 3.38 | 0.68 | 730 | — | |

*UPN indicates unique patient number; $k_a$, mean absorption rate constant; Vd/F, volume of distribution; CL/F, clearance; $t_{1/2}$, elimination half-life; BU, busulfan; Css, steady-state plasma concentration; GVHD, graft-versus-host disease.

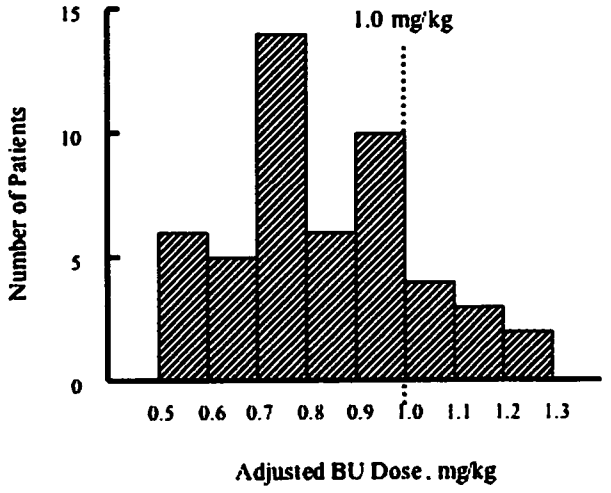


**Figure 1.** Distribution of the adjusted busulfan (BU) dose to achieve a target steady-state plasma concentration of 850 ng/mL based on individual pharmacokinetic analysis with a test dose.

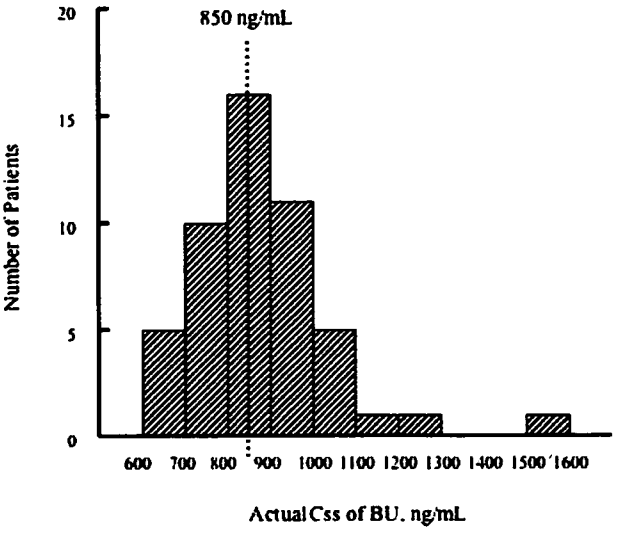


**Figure 3.** Distribution of actual steady-state plasma concentrations (Css) of busulfan (BU) after administering dose-adjusted BU as a conditioning regimen for hematopoietic stem cell transplantation.

April 2005. Indications for HST were acute myeloid leukemia (n = 25), acute lymphocytic leukemia (n = 2), CML (n = 2), prolymphocytic leukemia (n = 1), MDS (n = 8), myelofibrosis (n = 2), malignant lymphoma (n = 4), adult T-cell leukemia (n = 3), multiple myeloma (n = 2), and Ewing sarcoma (n = 1). Twenty-nine patients underwent transplantation of either BM or PBSC from sibling donors, and 21 patients received BM from unrelated donors. HLA compatibility was 6 of 6 antigens in 42 patients, and 5 of 6 antigens in 8 patients. Thirty-six patients received dose-adjusted BU and CY as a conventional conditioning regimen for HST, and 14 patients received dose-adjusted BU plus FLU with or without TBI as a reduced-intensity preparative regimen. Patient characteristics are summarized in Table 1.

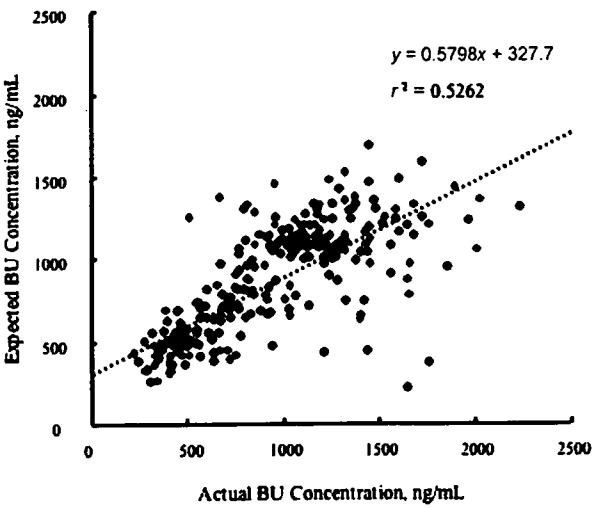


**Figure 2.** Linear regression of the actual versus expected busulfan (BU) concentration following dose-adjusted BU administration.

### *3.2. Individual PK Analysis and BU Dose Adjustment*

The PK data obtained after administering 0.5 mg/kg of oral BU as a testing dose are shown in Table 2. The mean (±SD) absorption rate constant ($k_a$) was 2.73 ± 1.57/hour, the mean volume of distribution (Vd/F) was 0.68 ± 0.12 L/kg, the mean clearance (CL/F) was 0.16 ± 0.05 L/h per kg, and the mean elimination half-life was 3.05 ± 0.83 hours. BU Css ranged widely (246-1263 ng/mL).

Individual BU doses were calculated to achieve a target BU Css of 850 ng/mL. Compared with the conventional dose of 1 mg/kg per administration, the BU dose was decreased in 39 (78%) of the 50 patients, increased in 9 patients (18%), and unchanged in 2 patients (4%) (Figure 1). The median adjusted dose was 0.81 mg/kg per administration (range, 0.51-1.29 mg/kg per administration).

### *3.3. Actual BU Concentrations after Administration of the Adjusted BU Dose*

All patients were administered the adjusted oral BU dose as conditioning for HST. After the sixth BU dose was administered, plasma samples were collected from each patient at 5 time points, and the BU concentrations were measured. A linear regression plot of the expected BU concentration versus the actual BU concentration is shown in Figure 2. A significant

**Table 3.**
Relationship between the Steady-State Plasma Concentration (Css) of Busulfan (BU) and Regimen-Related Toxicity (RRT)*

| | | Patients Developing Grade 2/3 RRT, n | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BU Css, ng/mL | Total No. | Heart | Bladder | Kidney | Lung | Liver | CNS | Oral Mucosa | GI Tract |
| Patients treated with conventional conditioning regimen: BU + CY (UPN 1-36) | | | | | | | | | |
| 601-700 | 4 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 3/0 | 0/0 |
| 701-800 | 8 | 1/1 | 0/0 | 1/0 | 0/1 | 0/0 | 0/0 | 3/0 | 0/0 |
| 801-900 | 13 | 0/0 | 0/0 | 1/0 | 0/2 | 0/0 | 0/0 | 5/0 | 0/0 |
| 901-1000 | 7 | 0/0 | 0/0 | 1/0 | 0/0 | 0/0 | 0/0 | 2/0 | 0/0 |
| 1001-1100 | 2 | 0/0 | 0/0 | 0/1 | 0/0 | 0/0 | 0/0 | 1/0 | 0/0 |
| >1100 | 2 | 0/0 | 0/0 | 1/0 | 0/0 | 0/0 | 0/0 | 2/0 | 0/0 |
| Patients treated with reduced-intensity conditioning regimen: BU + FLU ± TBI (UPN 37-50) | | | | | | | | | |
| 601-700 | 1 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| 701-800 | 2 | 0/0 | 0/0 | 0/0 | 0/1 | 0/0 | 0/0 | 1/0 | 0/0 |
| 801-900 | 3 | 0/0 | 0/0 | 1/0 | 0/0 | 0/0 | 0/0 | 1/0 | 0/0 |
| 901-1000 | 4 | 0/0 | 0/0 | 0/1 | 0/0 | 0/1 | 0/0 | 1/0 | 0/0 |
| 1001-1100 | 3 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 3/0 | 0/0 |
| >1100 | 1 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 1/0 | 0/0 |

*CNS indicates central nervous system; GI, gastrointestinal; CY, cyclophosphamide; UPN, unique patient number; FLU, fludarabine; TBI, total body irradiation.

relationship between the 2 parameters was found; the mean prediction error ranged from −10.8% to +8.3%.

Actual BU Css are shown in Table 2 and Figure 3. Forty (80%) of the 50 patients achieved target levels of 850 ng/mL ± 20% (680-1020 ng/mL). When the data are expressed as the mean ± SD, the mean BU Css was 878 ± 167 ng/mL, and the predictability of Css was 103.2% ± 19.7%.

### *3.4. Clinical Outcomes after HST*

Thirty-six patients were treated with 16 BU doses in combination with 120 mg/kg CY as a conventional conditioning regimen (BU + CY group), and 14 patients received a reduced-intensity conditioning regimen of 8 BU doses plus 180 mg/kg FLU with or without 2 Gy TBI (BU + FLU ± TBI group). Because BU toxicity was dependent on not only the BU Css but also the total BU dose administered, RRT was analyzed separately in the BU + CY and the BU + FLU ± TBI groups (Table 3). In the BU + CY group, RRT of grades 2 to 3 was observed in 17 (47%) of 36 patients, and grade 3 RRT was observed in 5 patients (14%). In the BU + FLU ± TBI group, RRT of grades 2 to 3 was observed in 7 (50%) of 14 patients, and grade 3 RRT was observed in 2 patients (14%). No grade 4 RRT developed in either the BU + CY group or the BU + FLU ± TBI group. The incidence of stomatitis was high: grade 2 oral mucositis occurred in 44% (16/36) and 50% (7/14) of the patients in the BU + CY and the BU + FLU ± TBI groups, respectively; however, there was no clear relationship between the actual BU Css and the development of mucosal toxicity. RRT of grades 2 to 3 excluding stomatitis developed in 19% (7/36) and 21% (3/14) of patients, respectively. The incidences of RRT of grades 2 to 3 were 6%, 14%, 8%, and 0% in the heart, kidney, lung, and liver, respectively, in the BU + CY group and were 0%, 14%, 7%, and 7%, respectively, in the BU + FLU ± TBI group. No RRT of grades 2 to 3 was observed in the bladder, central nervous system, or gastrointestinal tract. There was no significant trend toward a higher BU Css with increasing RRT incidence. None of the patients developed hepatic VOD.

Five patients (unique patient numbers [UPN] 21, 27, 36, 48, and 49) died of sepsis on days 13, 15, 17, 21, and 24, respectively, before hematologic recovery was attained. Engraftment was observed in 44 (98%) of the remaining 45 patients. The one patient who developed graft failure was a 38-year-old male patient with acute myeloid leukemia (UPN 16). He underwent allogeneic HST in a second remission from an unrelated donor mismatched at 1 HLA locus. He received 16 doses of targeted-dose BU and 120 mg/kg CY. The actual BU Css was 630 ng/mL. He received $0.6 \times 10^8$/kg of BM mononuclear cells. The patient's blood cell counts did not recover until day 67, however, and he died of sepsis.

Twelve patients died within 100 days after HST. The causes of death were sepsis in 7 patients (UPN 16, 21, 27, 32, 36, 48, and 49), progressive disease in 4 patients (UPN 5, 20, 39, and 47), and acute GVHD in 1 patient (UPN 28). No treatment-related death was observed within 100 days after HST.

## 4. Discussion

BU Css have been shown to be critical for successful clinical outcomes in patients who receive oral BU as a conditioning regimen for HST. A BU Css of <600 ng/mL is significantly correlated with autologous recovery or mixed chimerism in pediatric patients [4,5]. Severe RRT is observed when BU Css are higher than 900 ng/mL [14]. Dose adjustment to maintain the BU Css in the range of 800 to 900 ng/mL reduces nonrelapse mortality and improves relapse-free survival in allogeneic HST for MDS [6]. We thus decided in this study to adjust the BU dose to achieve a target BU Css of 850 ng/mL.

The primary goal of this study was to evaluate the predictive performance of the BU test dose. The mean prediction error of the BU concentration was between −10.8%

and +8.3%, and the mean predictability of Css was 103.2% ± 19.7%. This latter value, which is similar to the value of 101.9% ± 17.9% previously reported by Bleyzac et al [15], indicates that our BU dose–adjusting method facilitated a reliable prediction of the actual BU Css.

Although the test dose reliably predicted an actual BU Css in the majority of patients, some patients showed different PK behavior at the time of the pretransplantation conditioning regimen. BU is mainly metabolized in the liver, and the BU PK are influenced by other drugs that use a common metabolic pathway, such as phenytoin, itraconazole, and metronidazole [16-18]. When BU was administered as a conditioning regimen for HST in this study, all patients simultaneously received either sodium valproate or phenytoin as a prophylaxis to prevent BU-related seizures, and fluconazole or itraconazole, ciprofloxacin or levofloxacin, and acyclovir were used as prophylaxis against fungal, bacterial, and herpesvirus infection, respectively. None of these drugs were given when BU was administered as a test dose. It is conceivable that interaction of the anticonvulsants and antimicrobials with BU used as a conditioning regimen for HST might have altered the hepatic clearance of BU and led to the intraindividual variability observed in this study between the BU PK in the test and preparative conditioning for HST.

In the Fred Hutchinson Cancer Research Center, individual BU PK analysis and dose adjustment were performed with blood samples collected after the first BU dose was administered in the HST preparative regimen. BU PK analysis was repeated after each morning dose on days 2 and 3, and the BU dose was subsequently adjusted if the BU Css was <800 ng/mL or >1000 ng/mL. This strategy achieved the expected target BU range of 800 to 1000 ng/mL in 35 of 40 patients [19]. This method is very reliable but was not suitable for our study because BU PK analysis can take place only in the laboratory in the Faculty of Pharmacology, Fukuoka University; however, the 9 transplantation centers that participated in this study are scattered throughout Kyushu island and on Okinawa, which is located more than 300 km south of Fukuoka. It is too difficult to send blood samples from the transplantation center to the laboratory by express transportation, start BU PK analysis immediately after sample arrival (which may be at night), report the recommended BU dose to each transplantation center by fax, and administer the adjusted BU dose to each patient within a day or two. All procedures should be done as scheduled; otherwise, BU dose adjustment will be delayed in the pretransplantation conditioning regimen for HST. To avoid such an impossible schedule, we performed BU PK analysis with a test dose approximately a week prior to the HST preparative regimen. As the test dose, 0.5 mg/kg of BU was given in addition to the HST preparative regimen. This dose constituted only 3% of the 16-mg/kg standard BU dose given in the conventional conditioning regimen. The test dose was well tolerated by all patients, and no adverse event was observed.

A high incidence of oral mucosal toxicity was observed in both the BU + CY and BU + FLU ± TBI groups; however, no clear correlation was found between the actual BU Css and the occurrence of toxicity. Stomatitis was thought to be caused by other agents, such as the CY administered as part of one of the conditioning regimens or the MTX given for the prevention of GVHD. The incidence of RRT excluding oral mucositis was low, and none of the patients developed hepatic VOD. Engraftment was achieved in 98% of the patients, and there was no RRT-associated mortality. Bleyzac et al retrospectively carried out a matched-group analysis to compare the clinical outcomes of HST recipients who received dose-adjusted BU and recipients who received the standard BU dose [15]. These investigators showed that the incidence of hepatic VOD was lower in the dose-adjusted group (3.4%) than in the standard-dose group (24.1%); however, the incidences of stomatitis in the 2 groups were not significantly different. All patients who received dose-adjusted BU achieved engraftment, whereas graft failure was observed in 12% of the patients treated with the standard BU dose. Our clinical findings are quite similar to these investigators' data for the patients who received dose-adjusted BU, indicating that the incidence of RRT observed in this study is reasonable. This result suggests that the clinical outcomes in HST recipients who receive BU-containing conditioning regimens could be improved by adjusting the BU dose.

Engraftment was not obtained in 1 patient. The actual BU Css for this patient (630 ng/mL) was less than the 850-ng/mL target level but greater than 600 ng/mL. A BU Css of less than 600 ng/mL was previously defined as the concentration associated with an increased risk of graft rejection [4,5]. On the other hand, this patient received only $0.6 \times 10^8$/kg of BM mononuclear cells from an unrelated donor mismatched at 1 HLA locus. The number of cells transplanted was far smaller than the optimal recommended BM cell dose for successful engraftment from an unrelated donor, which is greater than $3.6 \times 10^8$/kg [20]. It is unlikely that the engraftment failure in this case was caused primarily by a low BU Css.

Twelve patients died within 100 days after transplantation. The most frequent cause of death was sepsis, which was observed in 7 (58%) of the 12 patients. Although individually adjusting the BU dose was expected to improve early mortality related to RRT, infection-related mortality still remained.

This report is the first of a study to apply BU dose adjustment in a Japanese adult population. Oral BU is generally administered at a dose of 1 mg/kg. We found, however, that 1 mg/kg constituted an overdose in 78% of the Japanese patients, and the median recommended BU dose was 0.81 mg/kg per administration. Bolinger et al showed in the United States that BU dose reduction was required in only 29% of pediatric patients; the dose was increased in 13% of patients and was unchanged in 55% [21]. Deeg et al reported that 78% of 785 adult MDS patients in the United States required dose reduction but that the mean adjusted BU dose was 0.9 mg/kg per administration [6]. BU is metabolized mainly in the liver through conjugation with glutathione by glutathione-*S*-transferase (GST). Hepatic GST activity has been shown to be correlated with BU clearance [22], and GST gene polymorphisms have been associated with the development of hepatic VOD [23]. Ethnic variation in GST polymorphism has also been demonstrated [24], suggesting that BU metabolism in the Japanese population might be different from that in Caucasians. Intravenous administration of BU

has recently been approved in Japan [25]. For a conventional conditioning regimen, the original intravenous BU regimen was administration in a 0.8-mg/kg dose in a 2-hour infusion every 6 hours for 4 consecutive days followed by 120 mg/kg CY. Recently, a modified protocol, such as using intravenous BU in a twice-daily or once-daily schedule or using FLU instead of CY, has been evaluated in the United States and Europe. The present study suggests that race as well as age should be considered in deciding the dose and administration schedule of intravenous BU.

In conclusion, we performed a prospective clinical study that involved adjusting the BU dose to achieve a target BU Css of 850 ng/mL on the basis of individual PK parameter values. This study showed that our method for calculating individual BU doses by evaluating the results of a test dose allowed reliable prediction of actual BU Css and successful clinical outcomes by reducing early adverse events in HST recipients treated with BU-containing conditioning regimens. It is apparent from this study that oral BU at a dose of 1 mg/kg constitutes an overdose for Japanese patients and that the BU dose should be reduced to approximately 0.8 mg/kg if dose adjustment based on individual PK analysis is not possible.

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# Perforin gene mutations in adult-onset hemophagocytic lymphohistiocytosis

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## ABSTRACT

Perforin gene (PRF1) mutations cause the primary form of hemophagocytic lymphohistiocytosis (HLH). We report a genetic defect of PRF1 in a 62-year-old Japanese man with recurrent episodes of HLH. Sequencing of PRF1 from both peripheral blood mononuclear cells and nail clippings showed compound heterozygous mutation, including deletion of two base pairs at codons 1090 and 1091 (1090–1091delCT) and guanine-to-adenine conversion at nucleotide position 916 (916G→A). Although primary HLH has been detected in infants and children, genetic mutation of PRF1 or other genes should be considered a differential diagnosis of HLH even in the elderly.

Key words: perforein mutations, hemophagocytic lymphohistiocytosis

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The primary form of hemophagocytic lymphohistiocytosis (HLH),[1-3] also known as familial hemophagocytic lymphohistiocytosis (FHL), shows autosomal recessive inheritance which is fatal unless aggressively treated. Mutations in the perforin gene (PRF1) were first documented in patients with FHL by Stepp *et al.*, and have been classified as FHL type2.[4] The onset of FHL typically occurs within the first year of life in 70-80% of cases, and the number of patients under 6 months of age is especially high.[5-7] We report a case of HLH at 62 years of age, referable to compound heterozygous PRF1 mutation.

A 62-year-old man was referred to us on August 18, 2004 because of persistent fever. There was no family history of unexplained fever, pancytopenia, or lymphoid malignancies. He had had tuberculosis-related pleurisy at the age of 23. He had no history of any cancer. Late in July 2004, the patient had been admitted to a regional hospital after onset of fatigue and high fever. Physical examination demonstrated marked hepatosplenomegaly. Laboratory results included hemoglobin, 10.6 g/dL; white blood cell (WBC) count, $2.9\times10^9$/L; platelet count $50\times10^9$/L; lactate dehydrogenase (LDH) 1,054 U/L (normal 220 to 430); ferritin 13,078 ng/mL (normal 5 to 120); and soluble interleukin-2 receptor 9,044 U/mL (normal 190 to 650). A bone marrow aspirate was reported to show normocellular marrow without hemophagocytosis. The patient was treated with broad-spectrum antibiotics without improvement. When his condition deteriorated and fever persisted, he was referred to us. Re-evaluation of the previously obtained bone marrow aspirate detected hemophagocytosis. On admission, his temperature was 37.1°C, with a gradual improvement in his general condition. The spleen was palpable 7 cm below the left costal margin. Laboratory results showed moderate pancytopenia (WBC $2.27\times10^9$/L; hemoglobin, 9.9 g/dL; platelets $6.8\times10^9$ /L); LDH 313 U/L (normal 119 to 229); and elevated ferritin 6,306 ng/mL. Serologic tests for herpes simplex virus (HSV), Epstein Barr virus (EBV), human herpes virus-6 (HHV-6), cytomegalovirus (CMV), and parvovirus B19 showed past infection, and PCR blood examinations were negative for HSV, EBV,

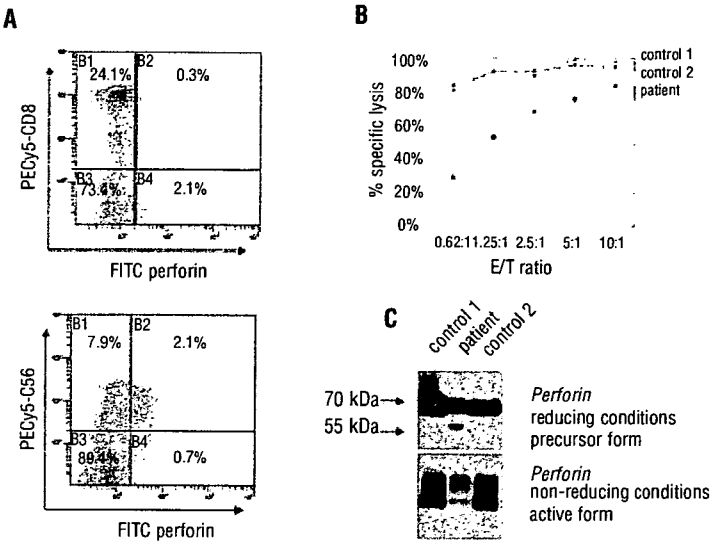


**Figure 1. Perforin expression and function.** A. Flow cytometric analysis of intracellular perforin protein expression. Peripheral blood mononuclear cells were stained with R-phycoerythrin Cy-Chrome (PECy5)-conjugated anti-CD8 or anti-CD56 monoclonal antibody (mAb) followed by intracellular staining with fluorescein isothiocyanate (FITC)-conjugated anti-perforin mAb after lysing and permeabilizing procedures. B. Cytotoxic activity of alloantigen-specific CD8⁺ T-cell lines generated from peripheral blood mononuclear cells of the patient and healthy controls by stimulation with allogeneic B-LCL (KIN-LCL). Cytotoxicity against allogeneic KIN-LCLs was compared between the patient and controls. C. Perforin expression by Western analysis under reducing and non-reducing conditions. Alloantigen-specific CD8⁺ T-cell lines from the patient and healthy controls were cultured. Extracts of these cells (20 mg each) were analyzed by Western blot with anti-perforin antibody.

HHV-6, CMV and parvovirus B19. Eleven days after admission his general condition recovered and palpable splenomegaly had resolved. He was discharged with a diagnosis of HLH of undetermined etiology.

Late in September 2005, fever developed following symptoms of an upper respiratory infection and the patient consulted our hospital on October 18. On physical examination, body temperature was 38.5°C and the spleen was palpable 6 cm below the left costal margin. On readmission, laboratory data were WBC $3.34\times10^9$/L; hemoglobin 11.4 g/dL; platelets $8.6\times10^9$/L and LDH 510 U/L. A bone marrow examination again showed hemophagocytosis. Symptoms resolved in a week and he was discharged on November 11. However, he soon developed another upper respiratory infection and again developed HLH in mid-November. When the patient was readmitted on November 20, his body temperature was 39.5°C and the spleen was palpable at 15 cm. Hydrocortisone 100 mg was administered for 2 days, and the symptoms resolved completely. He was discharged on December 1. At the time of this report, in December 2006, he remains asymptomatic. Laboratory values are within normal limits.

## Design and Methods

### Flow cytometric analysis

We obtained peripheral blood mononuclear cells (PBMNC) and performed flow cytometric analysis as previously described.[8]

### Assay for NK cell activity

NK cell activity among PBMNC was measured by incubating the cells with K562 targets for 4 hours at an effector-target (E/T) cell ratio of 20:1 (normal >18%).[9]

### Generation of alloantigen-specific cytotoxic T cell (CTL) lines and analysis of CTL cytotoxicity

Alloantigen-specific CD8⁺ CTL lines were generated and analyzed as previously described.[10]

### Sequencing of PRF1

This analysis was approved by the Institutional Review Board, and written informed consent was obtained from the patient. Genomic DNA was extracted from the patient's PBMNC and nail clippings. The coding region of PRF1 in exons 2 and 3 was sequenced as previously described.[4,9]

### Western analysis

CTL lines were subjected to Western analysis as previously described.[11]

## Results and Discussion

Flow cytometry showed markedly decreased intracellular perforin expression in both CD8⁺ and CD56⁺ cells. CD8⁺/perforin⁺ cells were 0.3% (normal 16.8%±6.0%) and CD56⁺/perforin⁺ cells 2.1% (normal 20.0%±7.2%) (Figure 1A).

NK cell activity of the patient had moderately decreased to 12.7%. Cytotoxic activities of CD8⁺ alloantigen-specific bulk T-cell lines generated from the patient and two healthy controls are shown in Figure 1B. Tested at an E/T ratio of 1.25:1, cytotoxicity of CTL generated from the patient was clearly low (53%) compared with CTL from two healthy controls (92% and 93%). Sequencing of *PRF1* detected compound heterozygous mutation in both PBMNC and somatic nail cells. As shown in Figure 2, one mutation was a deletion of two base pairs at codons 1090 and 1091

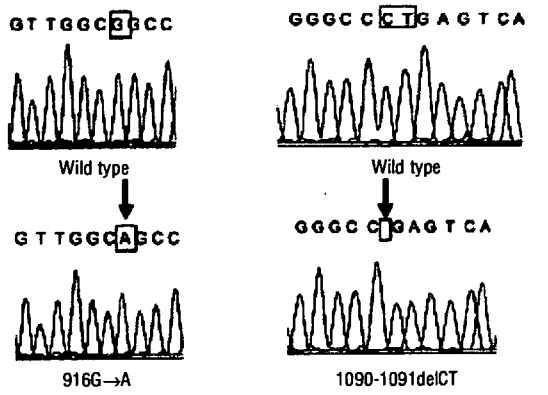


**Figure 2. Sequence analysis of the perforin gene. Sequencing demonstrated compound heterozygous mutation in both the patient's peripheral blood mononuclear cells and nail clippings. One mutation was deletion of two base pairs at codon 1090 to 1091 (1090–1091delCT), while the other was a guanine-to-adenine conversion at nucleotide position 916 (916G→A).**

(1090–1091delCT), while the other was a guanine-to-adenine conversion at nucleotide position 916 (916G→A). In studies on the PRF1 abnormality in Japan, the incidence of 1090-1091delCT observed in our patient was high and characteristic in Japanese people.[12]

Perforin in T-cells from healthy controls migrated to show a molecular mass of approximately 65 to 70 kDa under reducing conditions. The gene product associated with the PRF1 nonsense mutation 1090–1091delCT present in this patient has been reported to produce a band of 55 kDa under reducing conditions and no band under non-reducing condition.[8,9] Under reducing conditions, the patient's CTL showed a slightly smaller amount of normal-size perforin than controls, plus a weak band at 55 kDa. Under non-reducing conditions to detect an active form of perforin, this active mature form was markedly reduced in the patient (Figure 1C). Thus, the PRF1 missense mutation 916G→A in this patient causes the amino acid substitution V306I, inhibiting proteolytic cleavage of perforin. The marked decrease in perforin in $CD8^+$ cells and $CD56^+$ cells and their reduced cytotoxic activity, together with the result of Western analysis, exclude the possibility that V306I may be influential as a simple polymorphism.

The primary form of HLH is genetically determined while the secondary form is triggered by infection, malignancies, and autoimmune diseases. However, the cause of secondary HLH cannot always be determined. In fact, the causes of primary and secondary HLH are sometimes difficult to define. There are only a few reports of adult HLH patients with genetic mutation of PRF1, but most were in their twenties.[13-15] Recently, Mancebo *et al.* reported a 49-year-old male who developed HLH with a tuberculosis infection.[16] The PRF1 mutation has never been detected in a patient over 49 years old. Our 62-year-old patient had compound heterozygous mutation of PRF1 (1090-1091delCT and 916G→A) with decreased but residual perforin activity.[17]

Our patient raises important issues. First, even in the elderly, the differential diagnosis of HLH causation should include PRF1 mutations. HLH with genetic mutation was thought to be a disease experienced in infancy. But the introduction of molecular diagnosis has led to a recent increase in the number of confirmed cases among older children and young adults.[13-16] Thus, in the future, screening for PRF1 mutations is expected to yield more cases involving older patients among individuals presenting HLH.[18] The second issue raised is that PRF1 mutation alone might not be sufficient to cause HLH. In fact, the perforin defect in our patient is supposed to be present since birth, yet he had been asymptomatic for more than six decades. It is well known that viral infections may elicit a FHL episode in genetically predisposed individuals.[19] Although we could not detect the association of viral infection in this patient, it is still possible that a late viral infection also induces symptoms of HLH in the elderly. Alternatively, additional genetic or environmental factors may contribute importantly to the pathogenesis of HLH. A homozygous mutation of PRF1 is considered a reason for stem cell transplantation (SCT).[20] Our patient's decreased but residual perforin activity by compound heterozygous mutation of PRF1 may contribute to his relatively mild presentation of HLH. A very short course of corticosteroids treatment has maintained remission for more than 1 year. Some primary HLH with residual perforin activity may be treated without SCT.

In conclusion, since adult-onset of HLH in our patient was associated with compound heterozygous mutation of PRF1, PFR1-mutated primary HLH may occur beyond infancy and childhood.

### Authors' Contributions

*KN wrote the paper; KN, AN and KT were responsible for patient care; TK, YK, GY, and KS carried out perforin gene analysis; SO analyzed cytotoxic T-cell positivity; HH carried out Western analysis of perforin expression; EI and MH prepared this report.*

### Conflict of Interest

*The author reported no potential conflicts of interest.*