Ⅲ. 研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版
Kobayashi S, Ito M, Sano H, Mochizuki K, Akaihata M, Waragai T, Ohara Y, Hosoya M, Kikuta A.	Idiopathic Hyperammonemia That Developed During Initial Treatment With Steroid in a Patient With Newly Diagnosed Leukemia.	J Pediatr Hematol Oncol	Epub ahead of print		2014
Kobayashi S, Ito M, Sano H, Mochizuki K, Akaihata M, Waragai T, Ohara Y, Hosoya M, Ohto H, Kikuta A.	T-cell-replete haploidentical stem cell transplantation is highly efficacious for relapsed and refractory childhood acute leukaemia.	Transfusion Med	24 (5)	305-310	2014
Kobayashi S, Waragai T, Sano H, Mochizuki K, Akaihata M, Ohara Y, Hosoya M, Kikuta A.	Malignant peritoneal mesothelioma in a child: chemotherapy with gemcitabine and platinum was effective for the disease unresponsive to other treatments.	Anticancer Drugs	25 (9)	1102-1105	2014
Kobayashi S, Kikuta A, Ito M, Sano H, Mochizuki K, Akaihata M, Waragai T, Ohara Y, Ogawa C, Ono S, Ohto H, Hosoya M.	Loss of mismatched HLA in myeloid/NK cell precursor acute leukemia relapse after T cell-replete haploidentical hematopoietic stem cell transplantation.	Pediatr Blood Cancer	61 (10)	1880-1882	2014

Koh K, Tomizawa D, Moriya Saito A, Watanabe T, Miyamura T, Hirayama M, Takahashi Y, Ogawa A, Kato K, Sugita K, Sato T, Deguchi T, Hayashi Y, Takita J, Takeshita Y, Tsurusawa M, Horibe K, Mizutani S, Ishii E.	Early use of allogeneic hematopoietic stem cell transplantation for infants with MLL gene-rearrangement-positive acute lymphoblastic leukemia.	Leukemia	29 (2)	290-296	2015
Hayakawa F, Sakura T, Yujiri T, Kondo E, Fujimaki K, Sasaki O, Miyatake J, Handa H, Ueda Y, Aoyama Y, Takada S, Tanaka Y, Usui N, Miyawaki S, Suenobu S, Horibe K, Kiyoi H, Ohnishi K, Miyazaki Y, Ohtake S, Kobayashi Y, Matsuo K, Naoe T; Japan Adult Leukemia Study Group (JALSG).	Markedly improved outcomes and acceptable toxicity in adolescents and young adults with acute lymphoblastic leukemia following treatment with a pediatric protocol: a phase II study by the Japan Adult Leukemia Study Group.	Blood Cancer J	4	e252	2014
Kaida K, Ikegame K, Ikemoto J, Murata R, Irie R, Yoshihara S, Ishii S, Okada M, Inoue T, Tamaki H, Soma T, Fujimori Y, Kai S, Ogawa H.	Soluble interleukin-2 receptor level on day 7 as a predictor of graft-versus-host disease after HLA-haploidentical stem cell transplantation using reduced intensity conditioning.	Int J Hematol	99 (4)	463-470	2014

Konuma T, Ooi J, Uchida N, Ogawa H, Ohashi K, Kanamori H, Aotsuka N, Onishi Y, Yamaguchi H, Kozai Y, Nagamura-Inoue T, Kato K, Suzuki R, Atsuta Y, Kato S, Asano S, Takahashi S.	Granulocyte colony-stimulating factor combined regimen in cord blood transplantation for acute myeloid leukemia: a nationwide retrospective analysis in Japan.	Haematologica	99 (12)	e264-e268	2014
Matsuoka Y, Nakatsuka R, Sumide K, Kawamura H, Takahashi M, Fujioka T, Uemura Y, Asano H, Sasaki Y, Inoue M, Ogawa H, Takahashi T, Hino M, Sonoda Y.	Prospectively isolated human bone marrow cell-derived MSCs support primitive human CD34-negative hematopoietic stem cells.	Stem Cells	In press		2014
Fukano R, Mori T, Kobayashi R, Mitsui T, Fujita N, Iwasaki F, Suzumiya J, Chin M, Goto H, Takahashi Y, Hara J, Park YD, Inoue M, Koga Y, Inagaki J, Sakamaki H, Adachi S, Kawa K, Kato K, Suzuki R.	Haematopoietic stem cell transplantation for relapsed or refractory anaplastic large cell lymphoma: a study of children and adolescents in Japan.	Br J Haematol	168(4)	557-563	2015
Kawashima N, Ito Y, Sekiya Y, Narita A, Okuno Y, Muramatsu H, Irie M, Hama A, Takahashi Y, Kojima S.	Choreito formula for BK virus-associated hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation.	Biol Blood Marrow Transplant	21(2)	319-325	2015

Kobayashi R, Yabe H, Kikuchi A, Kudo K, Yoshida N, Watanabe K, Muramatsu H, Takahashi Y, Inoue M, Koh K, Inagaki J, Okamoto Y, Sakamaki H, Kawa K, Kato K, Suzuki R, Kojima S.	Bloodstream infection after stem cell transplantation in children with idiopathic aplastic anemia.	Biol Blood Marrow Transplant	20(8)	1145-1149	2014
Umeda K, Adachi S, Tanaka S, Ogawa A, Hatakeyama N, Kudo K, Sakata N, Igarashi S, Ohshima K, Hyakuna N, Chin M, Goto H, Takahashi Y, Azuma E, Koh K, Sawada A, Kato K, Inoue M, Atsuta Y, Takami A, Murata M.	Comparison of continuous and twice-daily infusions of cyclosporine A for graft-versus-host-disease prophylaxis in pediatric hematopoietic stem cell transplantation.	Pediatr Blood Cancer	62	291-298	2015
Yoshida N, Kobayashi R, Yabe H, Kosaka Y, Yagasaki H, Watanabe K, Kudo K, Morimoto A, Ohga S, Muramatsu H, Takahashi Y, Kato K, Suzuki R, Ohara A, Kojima S.	First-line treatment for severe aplastic anemia in children: bone marrow transplantation from a matched family donor versus immunosuppressive therapy.	Haematologica	99 (12)	1784 -1791	2014
Goto H, Kaneko T, Shioda Y, Kajiwara M, Sakashita K, Kitoh T, Hayakawa A, Miki M, Kato K, Ogawa A, Hashii Y, Inukai T, Kato C, Sakamaki H, Yabe H, Suzuki R, Kato K.	Hematopoietic stem cell transplantation for patients with acute lymphoblastic leukemia and Down syndrome.	Pediatr Blood Cancer	62 (1)	148-152	2015

Ⅳ. 研究成果の刊行物・別刷

Idiopathic Hyperammonemia That Developed During Initial Treatment With Steroid in a Patient With Newly Diagnosed Leukemia

Shogo Kobayashi, MD, Masaki Ito, MD,* Hideki Sano, MD,† Kazuhiro Mochizuki, MD,† Mitsuko Akaihata, MD,* Tomoko Waragai, MD,* Yoshihiro Ohara, MD,* Mitsuaki Hosoya, MD,* and Atsushi Kikuta, MD†*

Summary: Idiopathic hyperammonemia (IHA) has been described as a complication of intensive chemotherapy for the treatment of hematologic malignancy but has subsequently been found in patients undergoing bone marrow transplantation and in those with solid tumors treated with 5-fluorouracil. Although IHA is a rare complication, it is sometimes associated with high mortality in hematologic malignancies. Here we report the case of a 15-year-old boy in whom hyperammonemia developed during the initial treatment with prednisolone for newly diagnosed acute lymphoblastic leukemia and who survived after early detection and oral lactulose therapy. To the best of our knowledge, this is the first report of IHA that was not induced by intensive chemotherapy, stem cell transplantation, or asparaginase therapy in a patient with newly diagnosed leukemia, but developed during an initial treatment with a steroid. Early detection of IHA by measuring the plasma ammonia level in patients with neurological symptoms may improve the outcome.

Key Words: idiopathic hyperammonemia, encephalopathy, acute lymphoblastic leukemia, children

(*J Pediatr Hematol Oncol* 2014;00:000–000)

Hyperammonemia occurs as a complication in a diverse range of disorders such as liver failure, Reye syndrome, and inborn errors of urea synthesis. It may also occur as a complication of urinary tract infection, asparaginase therapy, valproic acid therapy, and systemic carnitine deficiency.[1] Regarding cases of hyperammonemia that developed during the treatment of acute leukemia, most of such cases were reported as a side effect of asparaginase therapy.[2–4] However, hyperammonemia may also be idiopathic (here after, IHA). IHA is a diagnosis of exclusion. It has been defined as a plasma ammonia level greater than 2-fold the upper normal limit, with a relatively normal liver function and without inborn errors of metabolism or other identifiable causes.[5,6] IHA was first reported in the 1980s as a complication of intensive chemotherapy in leukemia patients,[6–8] but it can also occur after stem cell transplantation for hematologic malignancies or solid organ malignancies treated with 5-fluorouracil.[9] The main manifestations are confusion, lethargy, hallucinations, and seizures, and the outcomes are frequently intractable coma and death.[5,6] The prognosis is generally poor in hematologic malignancies; about 80% of patients in a previous series died.[10] We report the case of a 15-year-old boy who developed IHA while receiving an initial treatment with a steroid for newly diagnosed acute lymphoblastic leukemia (ALL). He survived the episode of IHA and made a complete neurological recovery without obvious immediate adverse effects, although a previous series reported a poor prognosis of IHA in hematologic malignancies.

CASE REPORT

A 15-year-old boy presented with malaise and palpitation. The patient had no preexisting illnesses, personal or family history of liver disease, or known exposure to occupational or environmental toxins. Physical and ultrasound examinations revealed no evidence of lymphadenopathy or hepatosplenomegaly. Hepatic and renal functions were normal. An initial complete blood test showed a hemoglobin level of 4.6 g/dL, a leukocyte count of 11.9×10^9/L, and a platelet count of 373×10^9/L. A bone marrow aspirate was analyzed, revealing a hyperplastic bone marrow with malignant cells. A diagnostic workup revealed precursor B-lymphoblastic leukemia. Cerebrospinal fluid at diagnosis was negative for blasts.

After obtaining informed consent, the patient was enrolled in induction chemotherapy under the Children's Cancer and Leukemia Study Group high-risk ALL 2004 protocol. On hospital day 9, he initially received prednisolone (80 mg/d) and triple intrathecal therapy (methotrexate, hydrocortisone, and cytarabine). Although his liver function was normal on admission, aspartate aminotransferase and alanine aminotransferase rose to 219 IU/L (normal, 13 to 33 IU/L) and 249 IU/L (normal, 8 to 42 IU/L) by day 13, respectively. The patient did not have fever or show any clinical or laboratory signs of infection. On hospital day 15 (day 7 after the start of prednisolone), he became disoriented, lethargic, irritable, and delirious. No meningeal signs or focal neurological signs were found after repeated examinations. The total aspartate aminotransferase and alanine aminotransferase levels decreased to 69 IU/L and 159 IU/L, respectively. Blood gas analysis revealed a pH of 7.334, a CO_2 partial pressure of 26.5 mm Hg, and a bicarbonate level of 13.7 mmol/L. The initial levels of serum urea nitrogen, creatinine, and electrolytes, coagulation parameters, and blood glucose level were within normal limits. Computed tomography showed no intracranial hemorrhage or cerebral ischemia. Electroencephalogram was diffusely slow, but no epileptiform discharges or electrographic seizures were observed. Screening for possible metabolic causes of encephalopathy was unrewarding except that the serum ammonia level was markedly elevated at 235 μmol/L (normal, 12 to 66 μmol/L) and over the next 12 hours reached a maximum of 311 μmol/L. Plasma amino acid levels were within normal limits except for high levels of glutamine and phenylalanine. Other tests required for the determination of plasma

Received for publication June 22, 2014; accepted August 8, 2014.
From the Departments of *Pediatrics; and †Pediatric Oncology, Fukushima Medical University School of Medicine, Fukushima, Japan.
The authors declare no conflict of interest.
Reprints: Shogo Kobayashi, MD, PhD, Department of Pediatrics, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima City, Fukushima 960-1295, Japan (e-mail: shogo@fmu.ac.jp).

acylcarnitine, organic acid, urine organic acid, and orotic acid levels revealed them to be within normal limits. He was treated conservatively with oral lactulose and adequate hydration with close monitoring of his mental status. His ammonium level decreased spontaneously to within normal limits over the next 86 hours and his mental status concurrently returned to normal. He made a complete neurological recovery with no obvious acute confusional state as an immediate adverse effect, and he tolerated subsequent chemotherapy without further episodes of hyperammonemia. He remains on maintenance treatment for ALL and free of any other disease, and he showed a performance status of 0 at his last follow-up visit, 30 months after induction chemotherapy.

DISCUSSION

In the case under discussion, IHA developed in a previously healthy 15-year-old boy while receiving an initial treatment with a steroid for newly diagnosed ALL. He survived the episode of IHA after treatment with lactulose alone.

It was reported that hyperammonemia in multiple myeloma may develop in the presence of factors that induce hyperammonemia in the patient's plasma or ammonia production by myeloma cells[11]; however, the etiology of IHA in leukemia patients remains uncertain. There has been no report of hyperammonemia developing as a symptom of tumor lysis syndrome, although our patient developed it during the early treatment of acute leukemia. Hyperammonemia has infrequently been reported after bone marrow transplantation and high-dose chemotherapy, but our patient did not previously receive intensive chemotherapy. Several drugs such as valproate and asparaginase have been associated with hyperammonemia in the absence of fulminant hepatic failure,[1] but our patient received none of them. Furthermore, there was no recurrence with subsequent treatment with asparaginase. Although he also received triple intrathecal therapy, there was no report of IHA caused by intrathecal injection of anticancer drugs.

Inborn errors of metabolism involving enzymes of the urea cycle also lead to hyperammonemia, but these errors usually cause severe symptoms during infancy and childhood. Certain urea cycle disorders (UCDs) and other defects of amino acid metabolism or fatty acid oxidation had been suggested as a possible mechanism.[12] In our patient, the normal levels of glycine and citrulline, the absence of argininosuccinic acid, and the normal orotic acid excretion exclude the presence of a UCD including ornithine transcarbamylase deficiency (OTCD), which is the most common UCD that develops in adulthood.[13]

It has been suggested that there might be temporary impairment of urea synthesis in a highly catabolic setting.[7] Other factors such as infections, mucositis, gastrointestinal bleeding, dehydration, tissue breakdown, changes in the intestinal flora, treatment with corticosteroids and total parenteral nutrition might contribute to an increased ammonia load.[5,9] There are several reports of OTCD in adults who developed acute hyperammonemic coma after steroid administration.[14] High circulating concentrations of glucocorticoids are known to have a general catabolic effect by primarily enhancing protein turnover.[15] Although OTCD was excluded and there was no recurrence of hyperammonemia with subsequent treatment with steroids for ALL, we hypothesize that the administration of prednisolone may have induced protein catabolism, resulting in endogenous nitrogen release and worsening hyperammonemia. Our patient also presented mild elevation of liver enzymes before hyperammonemia developed. However, no obvious signs of infection were observed in our patient, although he received steroid treatment. Therefore, a combination of factors such as steroid administration and some degree of damage to the liver might have contributed to the development of hyperammonia by enhanced tissue catabolism, which induces prerenal azotemia leading to increased nitrogen load and ammonia production.

Because of the high fatality rate associated with this syndrome, early recognition and rapid reduction in ammonia level are the most important steps in managing patients with IHA. A review of previous cases suggests that early treatment before the ammonia level reaches 350 μmol/L may improve the prognosis.[8] Although there is no consensus regarding the therapeutic approach to IHA, therapies aimed at temporarily decreasing the ammonia level using agents such as lactulose and neomycin should be considered first.[16] When these agents fail to produce an appreciable decrease in the serum ammonia level within a few hours, dialysis should be considered.[17] Ammonia-trapping agents such as sodium phenylacetate and sodium benzoate have also been used successfully to reduce the serum ammonia level in patients with IHA as well as in patients with hereditary defects in urea synthesis.[8] In our patient, there was a substantial initial decrease in the ammonia level with lactulose therapy alone and dialysis was not necessary, although we would have performed dialysis and therapy with ammonia-trapping agents if his ammonia level had increased further. It is not clear why our patient survived the episode of IHA, despite the poor prognosis of hematologic malignancies; about 80% of the patients in a previous series died.[5,6] It was reported that patients with 5-fluorouracil-related or asparaginase-related IHA recover within a few days of a specific therapy with a favorable prognosis.[3,4,18,19] It is speculated that the pathologic mechanism underlying IHA development induced by intensive chemotherapy or bone marrow transplantation might differ from that induced by other factors.

To the best of our knowledge, this is the first case of IHA in a patient with newly diagnosed leukemia that was not induced by intensive chemotherapy, stem cell transplantation, or asparaginase therapy but developed during an initial treatment with a steroid. In patients with acute leukemia and an altered mental status without a clear cause, IHA should be considered in the differential diagnosis. The pathologic mechanism underlying the development of IHA in acute leukemia patients after intensive chemotherapy might differ from that in patients not receiving intensive chemotherapy. Further studies are required to elucidate the pathology, and prognostic factors, and to determine whether certain subsets of patients are at an increased risk for IHA.

REFERENCES

1. Clay AS, Hainline BE. Hyperammonemia in the ICU. *Chest*. 2007;132:1368–1378.
2. Leonard JV, Kay JD. Acute encephalopathy and hyperammonaemia complicating treatment of acute lymphoblastic leukaemia with asparaginase. *Lancet*. 1986;1:162–163.
3. Jörck C, Kiess W, Weigel JF, et al. Transient hyperammonemia due to l-asparaginase therapy in children with acute lymphoblastic leukemia or non-Hodgkin lymphoma. *Pediatr Hematol Oncol*. 2011;28:3–9.

4. Heitink-Pollé KM, Prinsen BH, de Koning TJ, et al. High incidence of symptomatic hyperammonemia in children with acute lymphoblastic leukemia receiving pegylated asparaginase. *JIMD Rep*. 2013;7:103–108.
5. Davies SM, Szabo E, Wagner JE, et al. Idiopathic hyperammonemia: a frequently lethal complication of bone marrow transplantation. *Bone Marrow Transplant*. 1996;17:1119–1125.
6. Mitchell RB, Wagner JE, Karp JE, et al. Syndrome of idiopathic hyperammonemia after high-dose chemotherapy: review of nine cases. *Am J Med*. 1988;85:662–667.
7. Watson AJ, Chambers T, Karp JE, et al. Transient idiopathic hyperammonaemia in adults. *Lancet*. 1985;2:1271–1274.
8. del Rosario M, Werlin SL, Lauer SJ. Hyperammonemic encephalopathy after chemotherapy. Survival after treatment with sodium benzoate and sodium phenylacetate. *J Clin Gastroenterol*. 1997;25:682–684.
9. Nott L, Price TJ, Pittman K, et al. Hyperammonemia encephalopathy: an important cause of neurological deterioration following chemotherapy. *Leuk Lymphoma*. 2007;48:1702–1711.
10. Metzeler KH, Boeck S, Christ B, et al. Idiopathic hyperammonemia (IHA) after dose-dense induction chemotherapy for acute myeloid leukemia: case report and review of the literature. *Leuk Res*. 2009;33:69–72.
11. Matsuzaki H, Uchiba M, Yoshimura K, et al. Hyperammonemia in multiple myeloma. *Acta Haematol*. 1990;84:130–134.
12. Cohn RM, Roth KS. Hyperammonemia, bane of the brain. *Clin Pediatr (Phila)*. 2004;43:683–689.
13. Felig DM, Brusilow SW, Boyer JL. Hyperammonemic coma due to parenteral nutrition in a woman with heterozygous ornithine transcarbamylase deficiency. *Gastroenterology*. 1995;109:282–284.
14. Cavicchi C, Donati M, Parini R, et al. Sudden unexpected fatal encephalopathy in adults with OTC gene mutations-Clues for early diagnosis and timely treatment. *Orphanet J Rare Dis*. 2014. [Epub ahead of print].
15. Löfberg E, Gutierrez A, Wernerman J, et al. Effects of high doses of glucocorticoids on free amino acids, ribosomes and protein turnover in human muscle. *Eur J Clin Invest*. 2002;32:345–353.
16. Flannery DB, Hsia YE, Wolf B. Current status of hyperammonemic syndromes. *Hepatology*. 1982;2:495–506.
17. Liaw CC, Liaw SJ, Wang CH, et al. Transient hyperammonemia related to chemotherapy with continuous infusion of high-dose 5-fluorouracil. *Anticancer Drugs*. 1993;4:311–315.
18. Liaw CC, Wang HM, Wang CH, et al. Risk of transient hyperammonemic encephalopathy in cancer patients who received continuous infusion of 5-fluorouracil with the complication of dehydration and infection. *Anticancer Drugs*. 1999;10:275–281.
19. Yeh KH, Cheng AL. High-dose 5-fluorouracil infusional therapy is associated with hyperammonaemia, lactic acidosis and encephalopathy. *Br J Cancer*. 1997;75:464–465.

TRANSFUSION MEDICINE Official Journal of the British Blood Transfusion Society
Transfusion Medicine | SHORT COMMUNICATION

T-cell-replete haploidentical stem cell transplantation is highly efficacious for relapsed and refractory childhood acute leukaemia

S. Kobayashi,[1] M. Ito,[1] H. Sano,[1] K. Mochizuki,[1] M. Akaihata,[1] T. Waragai,[1] Y. Ohara,[1] M. Hosoya,[2] H. Ohto[3] & A. Kikuta[1]

[1]*Department of Pediatric Oncology,* [2]*Department of Pediatrics, and* [3]*Division of Blood Transfusion and Transplantation Immunology, Fukushima Medical University School of Medicine, Fukushima, Japan*

Received 1 April 2014; accepted for publication 11 August 2014

SUMMARY

Background: Despite improvements in first-line therapies, the outcomes of relapsed or refractory childhood acute leukaemia that has not achieved complete remission after relapse, has relapsed after stem cell transplantation (SCT), has primary induction failure and has relapsed with a very unfavourable cytogenetic risk profile, are dismal.

Objectives and Methods: We evaluated the feasibility and efficacy of T-cell-replete haploidentical peripheral blood stem cell transplantation (haplo-SCT) with low-dose anti-human thymocyte immunoglobulin (ATG), tacrolimus, methotrexate and prednisolone (PSL) in 14 paediatric patients with high-risk childhood acute leukaemia.

Results: All patients achieved complete engraftment. The median time to reaching an absolute neutrophil count of more than 0.5×10^9 L^{-1} was 14 days. Acute graft-vs-host disease (aGVHD) of grades II–IV and III–IV developed in 10 (71%) and 2 (14%) patients, respectively. Treatment-related mortality and relapse occurred in one (7%) patient and six (43%) patients, respectively. Eleven patients were alive and seven of them were disease-free with a median follow-up of 36 months (range: 30–159 months). The probability of event-free survival after 2 years was 50%.

Conclusion: These findings indicate that T-cell-replete haplo-SCT, with low-dose ATG and PSL, provides sustained remission with an acceptable risk of GVHD in paediatric patients with advanced haematologic malignancies.

Key words: children, graft-vs-leukaemia effect, HLA-haploidentical stem cell transplantation, refractory leukaemia, T-cell-replete haploidentical stem cell transplantation.

Correspondence: Atsushi Kikuta, MD, PhD, Department of Pediatric Oncology, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima City, Fukushima 960-1295, Japan.
Tel: +81 24 547 1295; fax: +81 24 548 6578; e-mail: akikuta@fmu.ac.jp

INTRODUCTION

The most common type of cancer in patients under the age of 18 is acute leukaemia (McNeil *et al.*, 2002). Although recent years have seen advances and improvements in effective therapies and outcomes, some cases remain persistently difficult to treat. Overall, the 5-year survival rates for patients with acute lymphoblastic leukaemia (ALL) and patients with acute myelogenous leukaemia (AML) are nearly 85% and 50–60%, respectively (Jemal *et al.*, 2008). Among children with relapsed acute leukaemia, 30–50% can be treated successfully with a combination of chemotherapy and allogeneic stem cell transplantation (SCT) (Hijiya *et al.*, 2004; Raetz *et al.*, 2008; Parker *et al.*, 2010; Tallen *et al.*, 2010). Despite these successes, children who fail to attain complete remission after relapse, relapse after an SCT, experience a primary induction failure, or relapse with a very unfavourable cytogenetic risk profile have very poor prognoses (Gaynon, 2005). With this in mind, it is crucial that new therapies and regimens be developed to meet the needs of this vulnerable population.

It is most challenging when a patient experiences a significant number of reinduction failures, particularly with an early recurrence of the disease. Furthermore, even when remission is achieved in these instances, it is less likely to persist. Gaynon *et al.* (2006) reported subsequent relapses in up to one-third of these patients within the median time to allogeneic SCT (allo-SCT). Longer-term event-free survival (EFS) rates for this population of high-risk acute leukaemia patients do not exceed 20% (Einsiedel *et al.*, 2005; Gaynon *et al.*, 2006), even with the use of intensive salvage strategies, including allo-SCT. Furthermore, patients with fms-like tyrosine kinase 3/internal tandem duplication (FLT3/ITD) positive or other NK-lineage leukaemia also have a very poor prognosis by conventional allo-SCT even if remission is achieved at transplantation (Suzuki *et al.*, 2006; Sengsayadeth *et al.*, 2012).

One form of immunotherapy that has proven to be highly efficacious for haematological malignancies that are primarily attributed to T-cell-mediated responses to human leukocyte

doi: 10.1111/tme.12150

antigen (HLA) disparities between donors and leukaemic cells is T-cell-replete haploidentical SCT (haplo-SCT) (Kolb, 2008).

Severe graft-vs-host disease (GVHD), rejection and a high risk of early death are related to major complications associated with haplo-SCT (Henslee-Downey *et al.*, 1997; Mehta *et al.*, 2004). The high risks of both graft failure and acute GVHD (aGVHD) associated with haplo-SCT have been mitigated in the past by infusing megadoses of purified *ex vivo* T-cell-depleted $CD34^+$ peripheral blood stem cells (PBSCs) (Aversa *et al.*, 1998, 2005). However, because the patients lost almost all of their T-cells, their immune reconstitution was slow. This led to high incidences of mainly viral and fungal infectious complications and high relapse rates. In recent studies, enabling haplo-SCT with a T-cell-replete graft using a vigorous pre- and post-transplantation pharmacologic GVHD prophylactic regimen has proven to be an encouraging alternative treatment for the patients (Huang, 2008; Chang & Huang, 2012). However, particularly among high-risk groups, the threat of relapse remains, and the treatment can fail.

Previously, we have reported on the safety profile of a GVHD prophylactic regimen consisting of anti-human thymocyte immunoglobulin (ATG), tacrolimus, methotrexate (MTX) and prednisolone (PSL) in T-cell-replete haplo-SCT and demonstrated that haplo-SCT with our GVHD prophylaxis regimen is as feasible as HLA matched unrelated SCT in terms of GVHD and treatment-related mortality (TRM) (Mochizuki *et al.*, 2011). Using a retrospective approach, we assessed this protocol and found that we were able to achieve an optimal graft-vs-leukaemia (GVL) effect on T-cells by performing T-cell-replete haplo-SCT using a myeloablative preconditioning regimen, accompanied by intensive GVHD prophylaxis, including low-dose ATG and steroids. Patients, even those at an advanced stage of the disease, responded to the treatment and relapse rates decreased.

PATIENTS AND METHODS

Between August 2000 and April 2011, 14 consecutive patients with high-risk acute leukaemia underwent peripheral blood SCT from an HLA-haploidentical related donor at Fukushima Medical University Hospital. All patients were with a Karnofsky or Lansky performance score greater than or equal to 50 at transplantation. High-risk acute leukaemia was defined as primary refractory acute leukaemia not in remission or relapse. Those leukaemia are with poor prognostic features such as positivity for the Philadelphia chromosome, mixed lineage leukaemia gene rearrangements, FLT3/ITD and with early relapse ALL (<36 months from initial diagnosis). NK-lineage leukaemia is included because it has a very poor prognosis even after conventional SCT. The institutional review board approved the protocol, and written informed consent was obtained from the patients or their guardians and family donors. Data were analysed on 1 November 2013. The patient characteristics are shown in Table 1. Donors and patients shared one HLA haplotype, but differed in the other haplotype. For donor selection, there should only be at least three or four incompatible HLAs, and we also preferred donors with the best possible health, who have the same blood type, and are not suffering from anaemia. Donors included fathers (nine), mothers (four) and siblings (one). HLA-A, HLA-B, HLA-C and HLA-DRB1 typing was performed by intermediate-resolution DNA typing (Genosearch HLA; Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). HLA disparities in the graft-vs-host directions included four loci mismatches in 10 patients and three loci mismatches in four patients. The median patient age was 7.3 years (range: 0.8–17.9 years). Myeloablative conditioning was administered to 13 patients (total-body-irradiation-based, nine patients; Busulfan-based, four patients), and one patient with organ dysfunction received reduced intensity conditioning. The conditioning regimen of each patient is described in Table 2. Rabbit ATG (Genzyme Japan K. K., Tokyo, Japan) was used to treat 12 patients at a total dose of 2.5 mg kg^{-1} body weight. GVHD prophylaxis was conducted using tacrolimus, MTX administered in a short term, and PSL. Tacrolimus was started on day −1, and was continuously administered intravenously. The concentration of tacrolimus in peripheral blood was adjusted to be between 7 and 15 ng mL^{-1}. Three or four weeks after transplantation, the route of tacrolimus administration was changed to oral, with the target trough level within the range of 5–10 ng mL^{-1}. MTX (10 mg m^{-2}) was administered intravenously on day +1 and was reduced to 7 mg m^{-2} on days +3 and +6 after transplantation. PSL was begun on day +0 at an initial dose of 1 mg kg^{-1} body weight day^{-1}. When there was no sign of aGVHD, from day +29, the PSL dose was tapered every week and was discontinued within 2 or 3 months after transplantation. All patients received granulocyte-colony-stimulating factor (G-CSF) intravenously on day +1 until sustained granulocyte recovery was achieved. Posttransplantation G-CSF was administered to all 14 patients.

The presence of minimal residual disease (MRD) was analysed in bone marrow samples at diagnosis of ALL in patients by using sensitive quantitative real-time reverse transcriptase polymerase chain reaction (RT-qPCR) methods. A tumour specific primer could be obtained in three patients (Patients 5, 8, 10), and MRD was detected in all three patients using a cut-off point of 0.001% at haplo-SCT.

Each patient was isolated in a laminar air-flow room, and standard decontamination procedures were followed. Intravenous immunoglobulin was administered at a minimum dose of 100 mg kg^{-1} body weight every week until day +100. TMP/SMX was administered for at least 1 year for prophylaxis against *Pneumocystis* infections. Acyclovir was administered at 10 mg kg^{-1} body weight for 35 days after transplantation to prevent herpes simplex infections. CMV-pp65 antigen testing of peripheral blood was performed once weekly. After grafting, ganciclovir or foscarnet administration was initiated in patients with CMV antigenemia.

RESULTS

All the patients received G-CSF-mobilised PBSCs. The patients received PBSCs containing a median of 9.5×10^6 (range:

Table 1. Patient, donor and graft characteristics

Patient	Age (year/sex)	Diagnosis	Cytogenetics	Status at SCT (time point of relapse)	Donor	HLA disparity in GVH	Stem cell source	$CD34^+$ cells ($\times 10^6\ kg^{-1}$)	$CD3^+$ cells ($\times 10^8\ kg^{-1}$)
1	0.8/M	ALL	t(4;11)	CR2 (VER)	Mother	4/8	PB	11	NT
2	1.8/F	AML		Refractory relapse after CBT	Mother	4/8	PB	10.49	5.36
3	7.7/M	AML		Refractory relapse	Father	3/8	PB	8.43	3.79
4	12.0/M	ALL		Refractory relapse after BMT	Father	3/8	PB	13.2	4.36
5	11.9/F	ALL		Refractory relapse, MRD+ (ER)	Father	4/8	PB	11.5	5.25
6	6.8/M	ALL		CR2 (ER)	Father	4/8	PB	8.32	1.18
7	6.0/M	M/NK-AL		Primary refractory	Father	4/8	PB	13	3.86
8	5.0/M	ALL	t(9;22)	Relapse after BMT, MRD+	Mother	3/8	PB	10	5.51
9	13.9/M	ALL		Refractory relapse after BMT	Father	4/8	PB	7.14	2.74
10	9.8/M	ALL	t(9;22)	Relapse after BMT, MRD+	Father	4/8	PB	7.8	5.22
11	2.9/M	ALL		Refractory relapse (VER)	Mother	4/8	PB	5.65	5.01
12	13.8/M	AMoL	FLT3/ITD	Primary refractory	Father	4/8	PB	8.9	5.84
13	17.9/M	M/NK-AL	t(4;11)	Primary refractory	Sibling	4/8	PB	12.7	3.71
14	6.1/F	ALL	t(4;11)	CR2, relapse after BMT	Father	3/8	PB	6.3	1.83

AMoL, acute monocytic leukaemia; BMT, bone marrow transplantation; CR2, second complete remission; CBT, cord blood transplantation; ER, early relapse at least 18 months after diagnosis but less than 6 months after cessation of chemotherapy; F, female; M, male; M/NK-AL, myeloid NK precursor acute leukaemia; NT, not tested; PB, peripheral blood stem cells; VER, very early relapse within 18 months after diagnosis.
Patients are sorted by the day of transplantation.

$6.3-13.2\times10^6$) $CD34^+$ cells kg^{-1} body weight without T cell depletion. Consequently, a median of 4.36×10^8 (range: $1.18-5.84\times10^8$) $CD3^+$ cells kg^{-1} body weight were transfused. All the patients were engrafted with a median time of 14 days (range: 11–15 days) for neutrophil recovery (absolute neutrophil count $>0.5\times10^9\ L^{-1}$). The platelet recovery to more than $20\times10^9\ L^{-1}$ was achieved in 13 patients in a median time of 28 days (range: 18–93 days). All the patients who began in non-remission achieved complete remission on day +30 and showed complete donor chimerism by day +30. Acute GVHD occurred in 12 of the 14 patients. Acute GVHD was grade I in two patients, grade II in eight patients and grade III in two patients. Of the two patients with grade III GVHD, one did not receive ATG. The other patient, who had received ATG, improved symptoms of gut GVHD by temporary augmentation with PSL and oral administration of beclomethasone dipropionate (BDP). Chronic GVHD (cGVHD) developed in 11 of 12 evaluable patients. Relapse occurred in six patients (43%) on days +45, +117, +159, +405, +600 and +670. Among the six patients who relapsed, one (Patient 6) received additional chemotherapy, one (Patient 11) received a second haplo-SCT from another haploidentical donor and one (Patient 13) received donor lymphocyte infusion for his bone marrow (BM) relapse. Another one (Patient 10) received radiation therapy for his local bone relapse. All four of these patients achieved complete remission and survived. TRM occurred in one patient (7%) as a result of Epstein–Barr virus (EBV)-associated lymphoproliferative disorder (Patient 4). Seven patients survived and were free of disease between 30 and 159 months after transplantation (Table 2). With a median follow up of 36 months, the probability of 2-year EFS and the overall survival (OS) rate were 50% and 79%, respectively (Fig. 1).

DISCUSSION

Seeking to achieve an optimal GVL effect on T-cells, in this study we investigated the technique of T-cell-replete haplo-SCT using a myeloablative preconditioning regimen accompanied by intensive GVHD prophylaxis in high-risk paediatric patients. It is important to focus on these patients because this patient population has a longer-term EFS of no more than 20%, despite our conventional allo-SCT and our chemotherapy protocols known to be efficacious (Einsiedel *et al.*, 2005; Gaynon *et al.*, 2006). The transplant regimen detailed in this study decreased the incidence of relapse, despite the patients' advanced-stage leukaemia.

The outcome for patients who undergo haplo-SCT after myeloablative conditioning and standard GVHD prophylaxis is particularly dismal because they have the highest risk of graft rejection, aGVHD and, therefore, TRM (Powles *et al.*, 1983; Beatty *et al.*, 1985). Among BM transplants from partially HLA-matched donors, the incidence of primary graft failure is 12.3%. For recipients from an HLA-identical sibling, the failure rate is only 2.0% (Anasetti *et al.*, 1989). Several reports (Chang & Huang, 2012; Huang, 2008) detailed procedures for patients undergoing a haploidentical transplant with a more intensive *in vivo* GVHD prophylaxis. In patients receiving a T-cell-depleted graft, the rate of graft failure remains in the range of 0–17%, and the rate ranges of grades II–IV aGVHD, cGVHD and TRM are 8–48%, 0–35% and 28–65%, respectively. The graft failure rate among those receiving T-cell-replete grafts is in the range of 0–17%, and the incidence ranges of grades II–IV aGVHD, cGVHD and TRM are 16–66%, 13–53% and 9–35%, respectively.

Table 2. Stem cell transplantation and clinical outcome

Patient	Conditioning regimen	Engraftment Neut	Plt	Acute GVHD grade and stage (skin, liver, gut)	Chronic GVHD (affected organ)	Complications within 100 days	Outcome	Survival after SCT (days)	Cause of death	PS (%)
1	TBI + CA + Mel	15	33	III (2, 0, 2)	Moderate (skin, lung, gut)	TMA	CR	4783+	–	90
2	TBI + CA + Mel	15	21	I (1, 0, 0)	Mild (skin)	*Aspergillus*	CR	3964+	–	90
3	TBI + CY + CA* + ATG	15	28	II (3, 0, 1)	Mild (gut)	CMV antigenemia, *candida* sepsis	CR	1457+	–	90
4	Bu2 + Flu + Mel + ATG	15	34	0	Moderate (skin, mouth, gut)	NO	Death	439	EBV-LPD	–
5	TBI + VP16 + CY + ATG	12	22	II (3, 0, 0)	Mild (gut)	CMV antigenemia	CR	1393+	–	90
6	TBI + VP16 + CY + ATG	11	93	II (3, 0, 0)	Mild (skin, gut)	CMV antigenemia	Relapse on day 670	1198+ (CR survival)	–	90
7	TBI + VP16 + CY + ATG	14	28	II (3, 0, 0)	Mild (gut)	CMV antigenemia, *candida* sepsis	CR	1134+	–	90
8	Bu4 + Mel + ATG	13	18	I (1, 0, 0)	Mild (mucosa)	CMV antigenemia, BKV-HC, zoster	CR	1120+	–	90
9	Bu4 + CA + Mel + ATG	13	35	II (3, 0, 0)	NE	NO	Relapse on day 117	549	Relapse	–
10	Bu4 + Flu + Mel + ATG	12	21	II (3, 0, 0)	NO	HC (RRT)	Relapse on day 600	1050+ (CR survival)	–	100
11	TBI + VP16 + CY + ATG	15	27	II (3, 0, 0)	Mild (eye)	EBV-LPD, PRES, HHV6	Relapse on day 405	993+ (CR survival)	–	90
12	TBI + CY + CA* + ATG	15	NE	0	NE	NO	Relapse on day 45	132	Relapse	–
13	TBI + VP16 + CY + ATG	13	25	II (3, 0, 0)	Mild (gut)	*Klebsiella* sepsis, CMV antigenemia	Relapse on day 159	952+ (CR survival)	–	90
14	Bu4 + Flu + Mel + ATG	11	75	III (2, 0, 3)	Mild (skin)	BKV-HC, EBV-LPD, zoster	CR	913+	–	90

ATG, anti-human thymocyte immunoglobulin at 2.5 mg kg^{-1}; Bu2, busulfan at 8 mg kg^{-1}; Bu4, busulfan at 12–16 mg kg^{-1}; CA, cytarabine at 12 g m^{-2}; CA*, cytarabine at 12 g m^{-2} combined with G-CSF; CR, complete remission; CMV, cytomegalovirus; CY, cyclophosphamide at 120 mg kg^{-1}; Flu, fludarabine at 150 mg m^{-2}; HC, hemorrhagic cystitis; Mel, melphalan at 140 mg m^{-2}; Neut, days to reach neutrophil count $>0.5 \times 10^9$ μL^{-1}; NO, not observed; NE, not evaluated; PRES, posterior reversible encephalopathy syndrome; PS, performance status; Plt, days to reach platelet count $>20 \times 10^9$ μL^{-1}, RRT, regimen-related toxicity; TBI, total-body-irradiation (12 Gy); TMA; thrombotic microangiopathy; VP16, VP16 at 1800 mg m^{-2}.

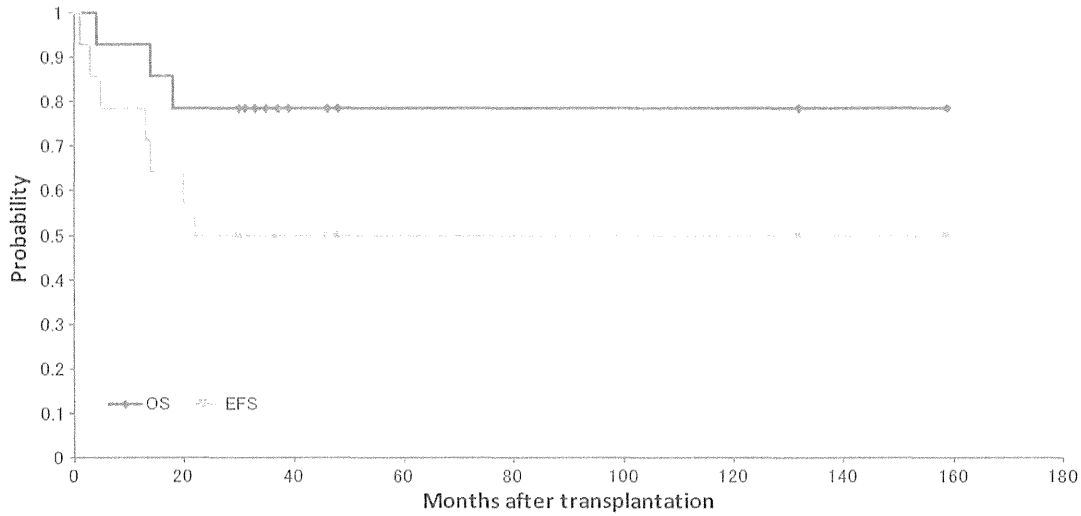


Fig. 1. Probability of OS and EFS. Kaplan–Meier estimates of OS and EFS in 14 patients who underwent T-cell-replete HLA-haploidentical SCT for advanced haematologic malignancies.

The conditioning treatment outlined in this present study, which includes low-dose ATG, demonstrated a satisfactory immunosuppressive effect. All the recipients achieved donor-type engraftments. On the basis of the data from the 14 patients we studied, we consider that the use of PBSCs containing a large number of T-cells (median: 4.36×10^8 $CD3^+$ cells kg^{-1} body weight) is also beneficial to obtain a high engraftment rate. In our study, 7% of patients (one patient) experienced TRM; this was associated with a viral infection. This risk seems to be acceptable, given the patient's poor condition. However, severe immunosuppression resulted in the high risk of infectious complications. We treated these complications through early detection and treatment of infection, including pre-emptive treatment for cytomegalovirus viraemia. We can decrease these infectious complications by using oral administration of BDP for gut GVHD patients instead of increasing general steroid administration. Frequent monitoring of EBV viral load by PCR and pre-emptive rituximab therapy may also reduce the risk of EBV-related lymphoproliferative disease (LPD) (Peric *et al.*, 2011).

The first two patients we treated did not receive ATG, and one of them developed grade III GVHD. All subsequent patients received ATG, and one of them developed grade III GVHD. Neudorf *et al.* (2004) reported on the beneficial effects of grades I–II aGVHD, but also noted the detrimental effects of grades III–IV aGVHD in children with AML. The role of aGVHD in ALL has been less clearly defined than the generally accepted notion that the GVL effect plays an important role in the treatment of AML. Many recent publications (Zecca *et al.*, 2002; Gustafsson Jernberg *et al.*, 2003; Nordlander *et al.*, 2004) have reported the effects of GVHD and GVL on remission rates and OS for acute leukaemic relapse after SCT in both adult and paediatric populations. This indicates that the GVL effect may also improve the ALL survival rate.

To achieve a partial T-cell-depletion of the graft, we used low-dose ATG (2.5 mg kg^{-1} body weight). Although this low-dose ATG can result in high incidences of grade II aGVHD and cGVHD, these GVHDs are manageable with steroids. Temporary augmentation with PSL and the oral administration of BDP are effective treatments. This may be one of the possible reasons for our favourable outcomes.

Haploidentical transplantation is now possible with high sustained engraftment rates, low early post-transplantation mortality rates and low rates of severe GVHD, owing to recent advances in effective T-cell depletion techniques. Despite these successes, poor immune recovery and associated infectious complications leading to mortality and relapse remain major obstacles to overcome. Participants in our study had generally very poor prognoses, yet, with this new treatment protocol, the estimated probability of EFS at 2 years is about 50%. Despite the relatively high incidences of acute and chronic GVHD, our data are encouraging in terms of survival and TRM.

In conclusion, we have shown that T-cell-replete myeloablative haplo-SCT using a GVHD prophylactic regimen consisting of low-dose ATG, tacrolimus, MTX and PSL can reconstitute long-term haematopoiesis with acceptable incidences of treatment-resistant GVHD while preserving GVL effects in high-risk paediatric patients. A large-scale study, however, is needed to confirm these results more definitively.

ACKNOWLEDGMENTS

We wish to thank all the paediatricians and nurses who participated in the treatment and follow up of the patients in this study. A. K., M. I., H. S. and K. M. designed the research; A. K., M. I.,

H. S., K. M., M. A., S. K. T. W. and Y. O. collected the data; A. K. and S. K. analysed the data, interpreted the results and wrote the manuscript; M. H. and H. O. co-designed the experiments and discussed analyses and interpretation. All the authors discussed the results and commented on the manuscript.

CONFLICT OF INTEREST

The authors declare that there are no competing financial interests regarding this article.

REFERENCES

Anasetti, C., Amos, D., Beatty, P.G. *et al.* (1989) Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. *The New England Journal of Medicine*, **320**, 197–204.

Aversa, F., Tabilio, A., Velardi, A. *et al.* (1998) Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. *The New England Journal of Medicine*, **339**, 1186–1193.

Aversa, F., Terenzi, A., Tabilio, A. *et al.* (2005) Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. *Journal of Clinical Oncology*, **23**, 3447–3454.

Beatty, P.G., Clift, R.A., Mickelson, E.M. *et al.* (1985) Marrow transplantation from related donors other than HLA-identical siblings. *The New England Journal of Medicine*, **313**, 765–771.

Chang, Y.J. & Huang, X.J. (2012) Haploidentical hematopoietic stem cell transplantation with unmanipulated granulocyte colony stimulating factor mobilized marrow and blood grafts. *Current Opinion in Hematology*, **19**, 454–461.

Einsiedel, H.G., von, Stackelberg, A., Hartmann, R. *et al.* (2005) Long-term outcome in children with relapsed ALL by risk-stratified salvage therapy: results of trial acute lymphoblastic leukemia-relapse study of the Berlin-Frankfurt-Munster Group 87. *Journal of Clinical Oncology*, **23**, 7942–7950.

Gaynon, P.S. (2005) Childhood acute lymphoblastic leukaemia and relapse. *British Journal of Haematology*, **131**, 579–587.

Gaynon, P.S., Harris, R.E., Altman, A.J. *et al.* (2006) Bone marrow transplantation versus prolonged intensive chemotherapy for children with acute lymphoblastic leukemia and an initial bone marrow relapse within 12 months of the completion of primary therapy: Children's Oncology Group study CCG-1941. *Journal of Clinical Oncology*, **24**, 3150–3156.

Gustafsson Jernberg, A., Remberger, M., Ringdén, O. & Winiarski, J. (2003) Graft-versus-leukaemia effect in children: chronic GVHD has a significant impact on relapse and survival. *Bone Marrow Transplantation*, **31**, 175–181.

Henslee-Downey, P.J., Abhyankar, S.H., Parrish, R.S. *et al.* (1997) Use of partially mismatched related donors extends access to allogeneic marrow transplant. *Blood*, **89**, 3864–3872.

Hijiya, N., Gajjar, A., Zhang, Z. *et al.* (2004) Low-dose oral etoposide-based induction regimen for children with acute lymphoblastic leukemia in first bone marrow relapse. *Leukemia*, **18**, 1581–1586.

Huang, X.J. (2008) Current status of haploidentical stem cell transplantation for leukemia. *Journal of Hematology & Oncology*, **31**, 1–27.

Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T. & Thun, M.J. (2008) Cancer statistics, 2008. *CA: A Cancer Journal for Clinicians*, **58**, 71–96.

Kolb, H.J. (2008) Graft-versus-leukemia effects of transplantation and donor lymphocytes. *Blood*, **112**, 4371–4383.

McNeil, D.E., Cote, T.R., Clegg, L. & Mauer, A. (2002) SEER update of incidence and trends in pediatric malignancies: acute lymphoblastic leukemia. *Medical and Pediatric Oncology*, **39**, 554–557.

Mehta, J., Singhal, S., Gee, A.P. *et al.* (2004) Bone marrow transplantation from partially HLA-mismatched family donors for acute leukemia: single-center experience of 201 patients. *Bone Marrow Transplantation*, **33**, 389–396.

Mochizuki, K., Kikuta, A., Ito, M., Sano, H., Akaihata, M., Kobayashi, S., Ohto, H. & Hosoya, M. (2011) Feasibility of tacrolimus, methotrexate, and prednisolone as a graft-versus-host disease prophylaxis in non-T-cell-depleted haploidentical hematopoietic stem cell transplantation for children. *Clinical Transplantation*, **25**, 892–897.

Neudorf, S., Sanders, J., Kobrinsky, N. *et al.* (2004) Allogeneic bone marrow transplantation for children with acute myelocytic leukemia in first remission demonstrates a role for graft versus leukemia in the maintenance of disease-free survival. *Blood*, **103**, 3655–3661.

Nordlander, A., Mattsson, J., Ringdén, O. *et al.* (2004) Graft-versus-host disease is associated with a lower relapse incidence after hematopoietic stem cell transplantation in patients with acute lymphoblastic leukemia. *Biology of Blood and Marrow Transplantation*, **10**, 195–203.

Parker, C., Waters, R., Leighton, C. *et al.* (2010) Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukaemia (ALL R3): an open-label randomised trial. *The Lancet*, **376**, 2009–2017.

Peric, Z., Cahu, X., Chevallier, P. *et al.* (2011) Features of Epstein-Barr Virus (EBV) reactivation after reduced intensity conditioning allogeneic hematopoietic stem cell transplantation. *Leukemia*, **25**, 932–938.

Powles, R.L., Morgenstern, G.R., Kay, H.E. *et al.* (1983) Mismatched family donors for bone-marrow transplantation as treatment for acute leukaemia. *The Lancet*, **1**, 612–615.

Raetz, E.A., Borowitz, M.J., Devidas, M. *et al.* (2008) Reinduction platform for children with first marrow relapse of acute lymphoblastic Leukemia: A Children's Oncology Group Study [corrected]. *Journal of Clinical Oncology*, **26**, 3971–3978.

Sengsayadeth, S.M., Jagasia, M., Engelhardt, B.G. *et al.* (2012) Allo-SCT for high-risk AML-CR1 in the molecular era: impact of FLT3/ITD outweighs the conventional markers. *Bone Marrow Transplantation*, **47**, 1535–1537.

Suzuki, R., Suzumiya, J., Nakamura, S. *et al.* (2006) Hematopoietic stem cell transplantation for natural killer-cell lineage neoplasms. *Bone Marrow Transplantation*, **37**, 425–431.

Tallen, G., Ratei, R., Mann, G. *et al.* (2010) Long-term outcome in children with relapsed acute lymphoblastic leukemia after time-point and site-of-relapse stratification and intensified short-course multidrug chemotherapy: results of trial ALL-REZ BFM 90. *Journal of Clinical Oncology*, **28**, 2339–2347.

Zecca, M., Prete, A., Rondelli, R. *et al.* (2002) Chronic graft-versus-host disease in children: incidence, risk factors, and impact on outcome. *Blood*, **100**, 1192–1200.

Malignant peritoneal mesothelioma in a child: chemotherapy with gemcitabine and platinum was effective for the disease unresponsive to other treatments

Shogo Kobayashi[a], Tomoko Waragai[a], Hideki Sano[a], Kazuhiro Mochizuki[a], Mitsuko Akaihata[a], Yoshihiro Ohara[a], Mitsuaki Hosoya[a] and Atsushi Kikuta[b]

Malignant peritoneal mesothelioma in children is a very rare disease and has a poor prognosis. Unlike malignant mesothelioma in adults, there is no clear causal association between this very rare malignancy in children and asbestos exposure. We report a case of peritoneal mesothelioma in an 11-year-old boy who presented with ascites. He was diagnosed with malignant mesothelioma on the basis of histopathological findings. His disease showed resistance to pemetrexed, but was treated successfully with platinum-based therapy with gemcitabine. He has achieved long-term survival in partial remission with stable disease. *Anti-Cancer Drugs* 25:1102–1105 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2014, 25:1102–1105

Keywords: chemotherapy, malignant mesothelioma, pediatric oncology, rare tumors, solid tumors

[a]Department of Pediatrics, Fukushima Medical University School of Medicine and [b]Division of Pediatric Oncology, Fukushima Medical University Hospital Cancer Center, Fukushima, Japan

Correspondence to Shogo Kobayashi, MD, PhD, Department of Pediatrics, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima City, Fukushima 960-1295, Japan
Tel: +81 24 547 1295; fax: +81 24 548 6578; e-mail: shogo@fmu.ac.jp

Received 19 March 2014 Revised form accepted 23 May 2014

Introduction

Malignant mesothelioma (MM) can arise in the mesothelial lining of serous cavities [1]. Childhood peritoneal mesothelioma is very rare and generally has a poor prognosis; the incidence is estimated to be around 0.5–1.0 case per 10 000 000 inhabitants per year [2] and the estimated median survival is 6–12 months [3–5]. There are very few pediatric cases published in the literature. So far, only one series of case studies has presented four pediatric peritoneal mesothelioma patients [6] and reported favorable prognosis with a cisplatin-based doublet regimen. Deciding on an optimal treatment for individual patients remains a great challenge for pediatric oncologists because of the lack of clinical experience with and scientific knowledge of this exceedingly rare subset of mesothelioma. Here, we describe the diagnosis and course of a pediatric malignant peritoneal mesothelioma that showed resistance to pemetrexed, but was treated successfully with platinum-based therapy with gemcitabine.

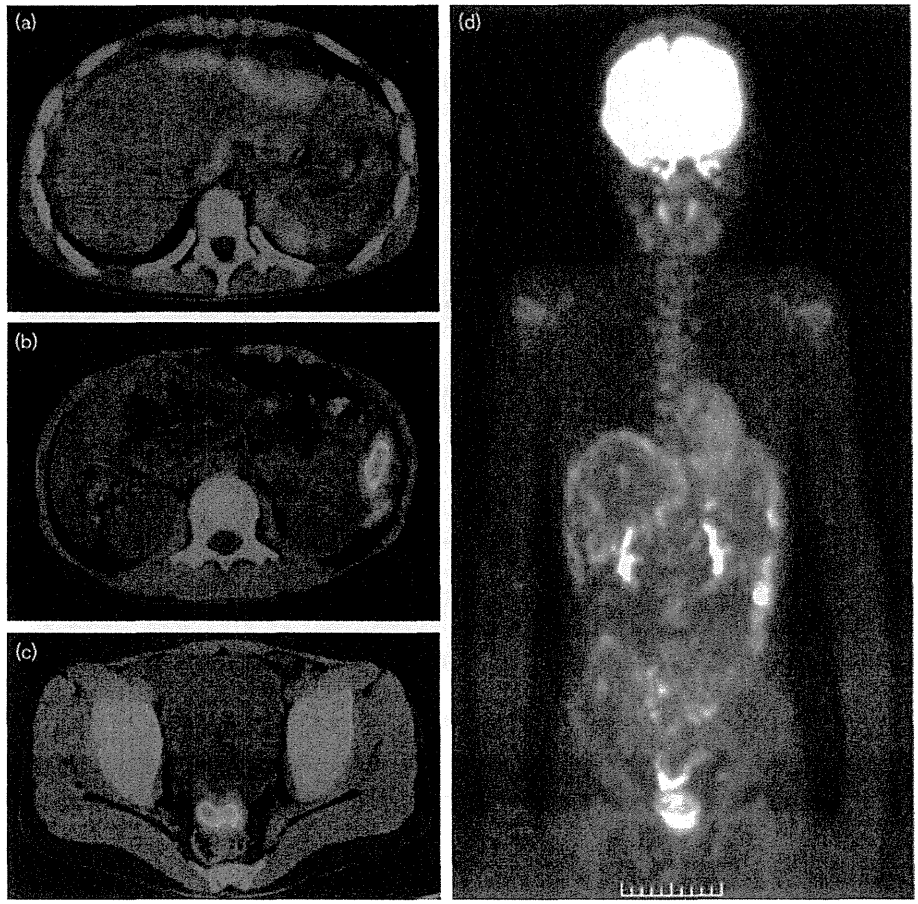
Case report

A previously healthy 11-year-old boy presented with fever, abdominal pain, and decreased appetite, which had begun 2 weeks previously. Results of the clinical examination suggested massive ascites, but not the presence of an abdominal mass. There had been no previous or family history of asbestos exposure. The hemoglobin level was 8.9 g/dl; the leukocyte and platelet counts were normal. The results of renal and liver function tests were within normal limits, except for the albumin level, which was low (1.7 g/dl). Tumor markers were evaluated. The carcinoembryonic antigen, CA19-9, and α-fetoprotein levels were within the normal ranges. The CA125 level was increased at 1712 U/ml (normal range, <35 U/ml). Computed tomography (CT) of the abdomen showed the presence of lobulated masses near the tail of the pancreas and in the rectobladder space with multiple peritoneal implants and ascites. The mass near the tail of the pancreas did not infiltrate the pancreas. ^{18}F-Fluorodeoxyglucose (^{18}F-FDG) PET/CT (PET) showed dense areas of ^{18}F-FDG uptake (Fig. 1) and the disease was localized to the peritoneal cavity. Laparotomy was performed. The peritoneal cavity contained 800 ml of straw-colored, slightly blood-tinged ascitic fluid, as well as numerous deposits of firm white tissue, which formed nodules and plaques on both visceral and parietal surfaces. The omentum contained a number of firm white nodules surrounded by adipose tissue. Biopsy of one of the peritoneal nodules and the omentum showed MM (Fig. 2). Despite the disease spreading widely in the abdominal cavity, no organ metastases were found.

The patient initially received systemic chemotherapy with pemetrexed and cisplatin plus folic acid and vitamin B_{12} substitution. The dosing of chemotherapy followed the weight-based standards used in adult patients (75 mg/m^2 cisplatin plus 500 mg/m^2 pemetrexed on day 1 of a 21-day cycle). The first three cycles of chemotherapy were well tolerated, with a partial

0959-4973 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0000000000000143

Fig. 1



^{18}F-Fluorodeoxyglucose (^{18}F-FDG) PET/computed tomography (PET) shows intense ^{18}F-FDG uptake involving the entire peritoneal lining of the abdomen and pelvis (a). Transverse PET image shows dense areas of ^{18}F-FDG uptake within the surface of the liver, the falciform ligament (b), and the masses in the caudal pancreatic space (c), and rectobladder pouch (d).

radiographic response. CT and PET showed regression and areas of decreased ^{18}F-FDG uptake within the masses in the caudal pancreatic space and rectobladder space. However, progressions and areas of increased ^{18}F-FDG uptake within the masses were seen on CT and PET after six cycles.

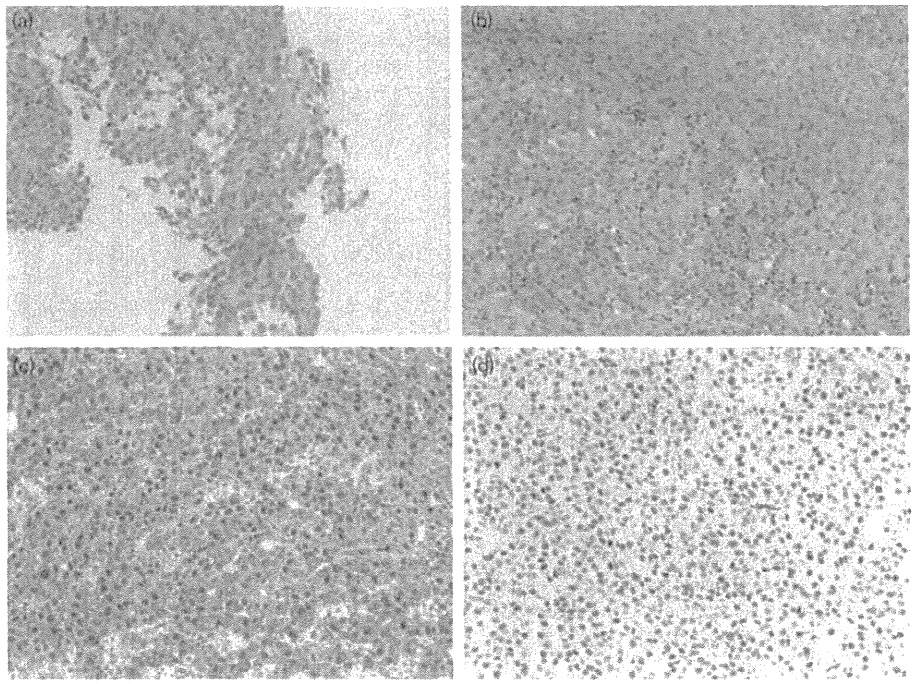
Therefore, a new combination chemotherapy described previously [7] was started with ICE (1.5 g/m^2 ifosfamide + MESNA, days 1–3; 500 mg/m^2 carboplatin, day 3; 100 mg/m^2 etoposide, days 1–3) alternating with VAC (2 mg/m^2 vincristine; 60 mg/m^2 adriamycin; 600 mg/m^2 cyclophosphamide on day 1) at 3-week intervals. However, the tumor progressed after two cycles of the treatment.

Then, the patient began treatment with 28-day cycles of 75 mg/m^2 cisplatin on day 1 and 1250 mg/m^2 gemcitabine on days 1 and 8. The patient's tolerance and clinical response were good after the third course, with PET showing areas of decreased ^{18}F-FDG uptake.

A second-look exploratory laparotomy was performed to determine the treatment strategy on whether to continue chemotherapies or to extend surgical resection. The laparotomy indicated a greater omental mass near the tail of the pancreas, with a moderate amount of turbid ascites. Multiple, variable-sized, nodular lesions were also found scattered diffusely on the surface of the peritoneum, mesentery, diaphragm, and pelvic cavity wall. Partial omentectomy and excisional biopsy of nodules were performed. Histopathologic analysis of the biopsied peritoneum with a normal gross appearance also showed MM and found the disease to be active. Partial but complete cytoreduction of the mass was carried out as the disease was still very diffuse and advanced at this time. Continuation of systemic chemotherapy was considered to be necessary.

The patient then continued to receive the gemcitabine/platinum chemotherapy regimen. Cisplatin was changed to carboplatin because of his renal insufficiency

Fig. 2



(a) Microscopic sections show a tumor with a solid and papillary growth pattern. (b) The papillary areas contain round or cuboidal cells. Neoplastic cells show nuclear atypia and occasional mitotic figures. Immunohistochemical studies show the positivity for calretinin (c), WT1 (d), D2–40, and CAM 5.2 support the diagnosis of malignant mesothelioma.

(C_{cr} < 80 ml/min). Although cisplatin could be administered for the renal failure of this level, we chose carboplatin to be able to continue gemcitabine/platinum chemotherapy regimen because of less nephrotoxicity of carboplatin. Carboplatin was delivered to a targeted area under the concentration–time curve of 5 on day 1, and gemcitabine at 1250 mg/m^2 on days 1 and 8. During treatment, no major toxicity was observed, and the tumor did not progress, which was confirmed by PET.

At present, 25 months after the diagnosis, the patient is still alive with stable residual disease and has a good performance status. He remains ambulatory and attends a school.

Discussion

MM is uncommon in patients of all age groups, and is very rare both in adolescents and in children. Only an estimated 2–5% of all cases present in the first two decades of life [1]. Overall, peritoneal mesothelioma represents ~20% of all MM cases [8,9]. Patients with MM are reported to have a very poor survival rate because of the advanced stage of the disease at presentation and its chemoresistance [10–12]. The median survival time for adults with peritoneal mesothelioma has historically been less than 12 months [3–5], although a recent series of aggressive multimodality therapies along with perioperative hyperthermic intraperitoneal chemotherapy has shown survival times of 50–60 months [13].

No standard treatment strategy is available owing to the rarity of this tumor, which is even rarer in young children. The present case was nonresectable, and the first chemotherapy we administered was pemetrexed together with cisplatin, because its efficacy had been reported in recent studies on treatment of adult peritoneal mesothelioma [14,15], and pemetrexed was started to be covered by health insurance for pleural MM in 2007 in Japan. The child's tolerance was good and three pemetrexed courses resulted in partial regression of tumors. However, the disease was resistant to the treatment after another three courses. The lack of efficacy led us to consider the ICE and VAC regimen, followed by the administration of gemcitabine because of the reported efficacy in this pathology [7,16,17]. The platinum-based therapy with gemcitabine treated and stabilized the disease successfully. Two previously reported cases of patients treated with gemcitabine [6] were alive at 45 and 57 months after diagnosis, and the response observed in our patient provides evidence justifying platinum/gemcitabine therapy for MM. When the pemetrexed plus cisplatin regimen administered to a malignant peritoneal mesothelioma patient as the first-line treatment is not effective, the gemcitabine plus cisplatin regimen should

be considered as a second-line treatment. Otherwise, it may be worth considering platinum/gemcitabine in the first-line treatment and platinum/pemetrexed in the second-line treatment. Moreover, carboplatin may be effective as an alternative to cisplatin against renal disorder, although cisplatin is more effective.

Two years after the initial diagnosis and 14 months after starting gemcitabine, the child is in partial remission, with stable disease. The combination of platinum and gemcitabine showed efficacy for this often fatal neoplasm, of which the median survival time is less than 1 year [18].

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

1 Kelsey A. Mesothelioma in childhood. *Pediatr Hematol Oncol* 1994; **11**:461–462.
2 Kashanskiy SV, André N. Paediatric mesothelioma: is there the such a disease? *Bull Cancer* 2010; **97**:571–576.
3 Deraco M, Bartlett D, Kusamura S, Baratti D. Consensus statement on peritoneal mesothelioma. *J Surg Oncol* 2008; **98**:268–272.
4 Eltabbakh GH, Piver MS, Hempling RE, Recio FO, Intengen ME. Clinical picture, response to therapy, and survival of women with diffuse malignant peritoneal mesothelioma. *J Surg Oncol* 1999; **70**:6–12.
5 Sugarbaker PH, Yan TD, Stuart OA, Yoo D. Comprehensive management of diffuse malignant peritoneal mesothelioma. *Eur J Surg Oncol* 2006; **32**:686–691.
6 Cioffredi LA, Janne PA, Jackman DM. Treatment of peritoneal mesothelioma in pediatric patients. *Pediatr Blood Cancer* 2009; **52**:127–129.
7 Mutafoğlu-Uysal K, Kargi A, Sarialioğlu F, Olgun N, Kovanlikaya A. Malignant pleural mesothelioma in a child: long-term survival with ICE-WAC chemotherapy regimen. *Turk J Pediatr* 2002; **44**:244–247.
8 Antman K, Shemin R, Ryan L, Klegar K, Osteen R, Herman T, *et al.* Malignant mesothelioma: prognostic variables in a registry of 180 patients, the Dana-Farber Cancer Institute and Brigham and Women's Hospital experience over two decades, 1965–1985. *J Clin Oncol* 1988; **6**:147–153.
9 Sridhar KS, Doria R, Raub WA Jr, Thurer RJ, Saldana M. New strategies are needed in diffuse malignant mesothelioma. *Cancer* 1992; **70**:2969–2979.
10 Pollock RE, Karnell LH, Menck HR, Winchester DP. The National Cancer Data Base report on soft tissue sarcoma. *Cancer* 1996; **78**:2247–2257.
11 Chahinian AP, Antman K, Goutsou M, Corson JM, Suzuki Y, Modeas C, *et al.* Randomized phase II trial of cisplatin with mitomycin or doxorubicin for malignant mesothelioma by the Cancer and Leukemia Group B. *J Clin Oncol* 1993; **11**:1559–1565.
12 Ruffie P, Feld R, Minkin S, Cormier Y, Boutan-Laroze A, Ginsberg R, *et al.* Diffuse malignant mesothelioma of the pleura in Ontario and Quebec: a retrospective study of 332 patients. *J Clin Oncol* 1989; **7**:1157–1168.
13 Yan TD, Edwards G, Alderman R, Marquardt CE, Sugarbaker PH. Morbidity and mortality assessment of cytoreductive surgery and perioperative intraperitoneal chemotherapy for diffuse malignant peritoneal mesothelioma – a prospective study of 70 consecutive cases. *Ann Surg Oncol* 2007; **14**:515–525.
14 Jänne PA, Wozniak AJ, Belani CP, Keohan ML, Ross HJ, Polikoff JA, *et al.* Open-label study of pemetrexed alone or in combination with cisplatin for the treatment of patients with peritoneal mesothelioma: outcomes of an expanded access program. *Clin Lung Cancer* 2005; **7**:40–46.
15 Garcia-Carbonero R, Paz-Ares L. Systemic chemotherapy in the management of malignant peritoneal mesothelioma. *Eur J Surg Oncol* 2006; **32**:676–681.
16 Kindler HL, van Meerbeeck JP. The role of gemcitabine in the treatment of malignant mesothelioma. *Semin Oncol* 2002; **29**:70–76.
17 Kindler HL, Millard F, Herndon JE II, Vogelzang NJ, Suzuki Y, Green MR. Gemcitabine for malignant mesothelioma: a phase II trial by the Cancer and Leukemia Group B. *Lung Cancer* 2001; **31**:311–317.
18 Mohamed F, Sugarbaker PH. Peritoneal mesothelioma. *Curr Treat Options Oncol* 2002; **3**:375–386.

BRIEF REPORT

Loss of Mismatched HLA in Myeloid/NK Cell Precursor Acute Leukemia Relapse After T Cell-Replete Haploidentical Hematopoietic Stem Cell Transplantation

Shogo Kobayashi, MD, PhD,[1] Atsushi Kikuta, MD, PhD,[2] Masaki Ito, MD, PhD,[1] Hideki Sano, MD, PhD,[1] Kazuhiro Mochizuki, MD, PhD,[1] Mitsuko Akaihata, MD,[1] Tomoko Waragai, MD,[1] Yoshihiro Ohara, MD,[1] Chitose Ogawa, MD,[2] Satoshi Ono, AS,[3] Hitoshi Ohto, MD, PhD,[3] and Mitsuaki Hosoya, MD, PhD[1]

Myeloid/natural killer cell precursor acute leukemia (MNKL) is an aggressive disease with a high relapse rate even after allogeneic hematopoietic stem cell transplantation (SCT). We report a patient with MNKL who had a donor lymphocyte infusion (DLI) for relapse after T cell-replete human leukocyte antigen (HLA)-haploidentical SCT, but relapsed again 20 months later with loss of mismatched HLA. This case suggests that a strong graft-versus-leukemia effect of haploidentical SCT can be expected in MNKL patients. In the haploidentical setting, DLI should be considered for patients with relapsed leukemia whose leukemic cells have not lost HLA cell surface expression. Pediatr Blood Cancer

Key words: acute; BMT; graft versus host disease; hematology/oncology; leukemias; rare tumors; stem cell transplantation

INTRODUCTION

The efficacy of donor leukocyte infusions (DLI) is excellent in chronic-phase chronic myeloid leukemia (CML) [1–3] but limited in other disorders [1,2,4–8]. We present a male patient who had a leukemic relapse 5 months after T cell-replete human leukocyte antigen (HLA)-haploidentical stem cell transplantation (SCT) for myeloid/natural killer cell precursor acute leukemia (MNKL). He achieved complete remission (CR) with full whole-blood chimerism after DLI without additional chemotherapy; however, he relapsed 20 months after the DLI with loss of mismatched HLA cell surface expression.

CASE REPORT

A 17-year-old male was admitted to his local hospital with headache and stomatorrhagia. Blood count analysis revealed anemia (Hb 4.9 g/dl) and thrombocytopenia (9,000/μl). His white blood cell (WBC) count was 9,600/μl, and 64% of the cells were lymphoblasts. Bone marrow (BM) aspiration revealed a hypercellular marrow with 92.6% myeloperoxidase negative lymphoblasts. Immunophenotypic analysis showed the leukemic blasts to be positive for CD56, CD7, CD33, CD34, CD117, and HLA-DR, but negative for surface CD3 and CD13. Cytogenetic analysis of the bone marrow cells demonstrated complex abnormalities, defined as 46, XY, t(10;11) (p12;q14), add(12)(p13), add(22)(p11.2).

The diagnosis of MNKL was determined and the patient was subsequently treated with induction chemotherapy for acute myeloid leukemia (AML). However, he did not achieve CR and received treatment for acute lymphoblastic leukemia. However, the normal counterpart of peripheral blood increased, 21% leukemic cells persisted in the BM. Thus, a third chemotherapy regimen consisting of high dose cytosine arabinoside (2 g/m^2) for 3 days and VP16 (100 mg/m^2) for 3 days in combination with dexamethasone was administered. After the course of chemotherapy, the patient achieved CR. The patient was transferred to our hospital to receive an allogeneic SCT.

Although the BM aspiration just before starting conditioning regimen showed 20% blasts, the patient underwent haploidentical transplantation using T cell-replete peripheral blood stem cells from his brother with four HLA allele mismatches in the graft-versus-host direction. The conditioning regimen consisted of fractionated total body irradiation (12 Gy total dose), VP16 (1,800 mg/m^2), cyclophosphamide (60 mg/kg × 2) and antithymocyte globulin (1.25 mg/kg × 2). The graft contained 12.7×10^6 CD34$^+$ cells/kg, and 3.7×10^8 CD3$^+$ cells/kg. Graft-versus-host disease (GVHD) prophylaxis consisted of prednisolone (PSL initial dose of 1 mg/kg/day from day +1), tacrolimus and a short course of methotrexate (10 mg/m^2 at day ±1, 7 mg/m^2 at days ±3, ±6). Hematological reconstitution was prompt: a neutrophil count of $>0.5 \times 10^9$/L, and a platelet count of $>50 \times 10^9$/L were observed on day 13 and 24 after transplantation, respectively. The patient achieved CR, and chimerism analysis demonstrated that all BM cells were donor derived on day +33. Grade II acute GVHD (skin rash) observed on day +39 during the tapering process required prolonged immunosuppression, consisting of PSL 0.7 mg/kg and tacrolimus 7 mg daily but subsided with no increase in the dose of immunosuppressive agents which were gradually tapered. PSL was discontinued on day +70, and tacrolimus was tapered to 4 mg/day without the development of GVHD on day +83.

However, 5 months after transplantation, he had a BM relapse of the leukemia. Surface marker analysis showed that the leukemic cells had the same phenotype as the previously relapsed tumor cells. Tacrolimus was discontinued, and 7 days later the patient received DLI at a dose of 1×10^6 CD3$^+$ cells/kg without any prior therapy

[1]Department of Pediatrics, Fukushima Medical University School of Medicine, Fukushima, Japan; [2]Division of Pediatric Oncology, Fukushima Medical University Hospital Cancer Center, Fukushima, Japan; [3]Division of Blood Transfusion and Transplantation Immunology, Fukushima Medical University School of Medicine, Fukushima, Japan

Conflict of interest: Nothing to declare.

*Correspondence to: Shogo Kobayashi, Department of Pediatrics, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima City, Fukushima 960-1295, Japan.
E-mail: shogo@fmu.ac.jp

Received 29 August 2013; Accepted 6 January 2014

DOI 10.1002/pbc.24962
Published online in Wiley Online Library (wileyonlinelibrary.com).