With the exception of neuroblastoma (IVa), the survival of many diagnostic groups in Osaka was lower than that in England and Wales and in the USA, and the report suggested that the reason for this was insufficient introduction and practice of chemotherapy (22). Our study suggested that the low centralization of patients is also related to the lower survival. Previous studies also suggested that the lower survival will also related to the treatment volume in the field of surgery or radiotherapy, although these subjects were adult cancers (23,24). Therefore, to centralize childhood cancer to specific hospitals and to perform a higher volume of procedures are important to ensure better survival. For the centralization of treatment, however, the burden children and their families must deal within their daily lives would increase. A social support system would be needed to achieve and maintain centralization.

In our study, although the identification of treating hospitals was the point, 997 cases did not have treating hospital codes so that the diagnosing hospital code was alternatively adopted. The bias derived from this substitution is assumed minor, because the proportion of cases that the diagnosing hospital code was same as the treating hospital one was 91.8% (3435 cases).

These data included newly diagnosed patients only, so that the specialists might feel that the small degree of centralization would not reflect the realization for childhood cancer treatment. Further study would be needed to investigate the centralization taking into account the succession of treatment.

We confirmed that the hospitals that treated childhood cancers decreased approximately by half during the 1990s, because childhood cancer decreased because of a lower birth rate. The degree of centralization seemed almost constant from 1975 to 2002. The annual average number of cases per hospital marginally increased, although it still was much lower compared with European countries and the USA.

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Conflict of interest statement

None declared.

References

1. Dickstein RJ, Barone JG, Liao JG, Burd RS. The effect of surgeon volume and hospital characteristics on in-hospital outcome after ureteral reimplantation in children. *Pediatr Surg Int* 2006;22:417–21.
2. Killeen SD, O'sullivan MJ, Coffey JC, Kirwan WO, Redmond HP. Provider volume and outcomes for oncological procedures. *Br J Surg* 2005;92:389–402.
3. Ioka A, Tsukuma H, Ajiki W, Oshima A. Hospital procedure volume and survival of cancer patients in Osaka, Japan: a population-based study with latest cases. *Jpn J Clin Oncol* 2007;37:544–53.
4. Stiller CA. Centralisation of treatment and survival rates for cancer. *Arch Dis Child* 1988;63:23–30.
5. Stiller CA, Draper GJ. Treatment centre size, entry to trials, and survival in acute lymphoblastic leukaemia. *Arch Dis Child* 1989;64:657–61.
6. Osaka Prefectural Department of Public Health and Welfare, Osaka Medical Association, Osaka Medical Centre for Cancer and Cardiovascular Diseases. Annual Report of Osaka Cancer Registry No. 71 - Cancer Incidence and Medical Care in Osaka in 2004 and the Survival in 2000: OPDPHW; 2008.
7. Children's Cancer and Leukaemia Group [homepage on the Internet]. Leicester: Children's cancer, Inc.; c2006-08 [cited 2008 Aug 27]. Available from: http://www.ukccsg.org/.
8. Ablett S, Pinkerton CR. Recruiting children into cancer trials–role of the United Kingdom Children's Cancer Study Group (UKCCSG). *Br J Cancer* 2003;88:1661–5.
9. Howell DL, Ward KC, Austin HD, Young JL, Woods WG. Access to pediatric cancer care by age, race, and diagnosis, and outcomes of cancer treatment in pediatric and adolescent patients in the state of Georgia. *J Clin Oncol* 2007;25:4610–5.
10. Kaatsch P, Spix C. German Childhood Cancer Registry, Annual Report 2003. Mainz: German Childhood Cancer Registry; 2004.
11. Ajiki W, Tsukuma H, Oshima A. Step toward the realization of a population-based national childhood cancer registry in Japan: recommendation from population-based cancer registry. *Jpn J Pediatr Oncol.* 2002;39:150–8. (in Japanese).
12. Kobayashi H. Risk-management in Pediatric wards. *Juntendo Med J* 2005;51:490–500. (in Japanese).
13. Makimoto A, Kawamoto H. Future strategy for developing childhood cancer treatment (Featuring article Recent outcomes and problems in childhood cancer). *Pediatr Jpn* 2005;46:1995–2002 (in Japanese).
14. Ajiki W, Hanai A, Tsukuma H, Fujimoto I, Oshima A, Parkin DM. International Incidence of childhood cancer, vol.II (IACR scientific publications; 144). Lyon, the International Agency for Research on Cancer 1998,189–91.
15. Kramárová E, Stiller CA. The international classification of childhood cancer. *Int J Cancer* 1996;68:759–65.
16. Gini C. Sulla misura della concentrazione e della variabilità dei caratteri. Atti del Reale Istituto Veneto di Scienze, Lettere ed Arti, A.A. 1913–1914. 1914;73:1203–48.
17. Makimoto A. Infrastructure development for establishing the clinical traials for child solid tumors in Japan (Featuring article New treatment of solid tumors). *J Pediatr Pract* 2004;67:577–82. (in Japanese).
18. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labor and Welfare. Survey of Medical Care Institutions 2002. Health, Labor and Welfare Statistics Association. Tokyo (Japan): Health, Labor and Welfare Statistics Association; 2004 (in Japanese).
19. Lerman RI, Yitzhaki S. Improving the accuracy of estimates of Gini coefficients. *J Econ* 1989;42:43–7.
20. George D. The small-sample bias of the Gini coefficient: results and implications for empirical research. *Rev Econ Stat* 2003;85: 226–34.
21. Ajiki W, Tsukuma H, Oshima A, Kawa K. Effects of mass screening for neuroblastoma on incidence, mortality, and survival rates in Osaka, Japan. *Cancer Causes Control* 1998;9:631–6.
22. Ajiki W, Tsukuma H, Oshima A. Survival rates of childhood cancer patients in Osaka, Japan. *Jpn J Clin Oncol* 2004;34:50–4.
23. Tanisada K, Teshima T, Ohno Y, Inoue T, Abe M, Ikeda H, et al. Patterns of Care Study quantitative evaluation of the quality of radiotherapy in Japan. *Cancer* 2002;95:164–71.
24. Saika K, Ohno Y, Tanaka H, Hasegawa T, Tsukuma H, Oshima A. The trend of the effect of surgical volume for stomach and lung cancer patients. *Jpn J Appl IT Healthc* 2007;1:42–9.

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ORIGINAL ARTICLE

Low-dose MTX for the treatment of acute and chronic graft-versus-host disease in children

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We report the results of a retrospective analysis in 27 pediatric patients who received low-dose MTX as the second-line treatment for steroid-refractory or -dependent acute and chronic GVHD. Between July 2000 and May 2006, 10 patients with aGVHD and 17 with cGVHD were treated with MTX at a dose of 3–10 mg/m^2 weekly. Seven of ten patients (70%) with aGVHD responded well to MTX, thus resulting in the achievement of either a complete response (CR) or a partial response (PR). The dose of prednisone could be reduced to equal to or lower than 1 mg/kg in the responding patients at the end of MTX therapy. The median number of MTX administrations was five (range, 1–7). Ten (58.8%) of seventeen patients with cGVHD achieved CR or PR. The dose of prednisone could be reduced to lower than 0.4 mg/kg in 16 of 17 patients and seven patients could discontinue prednisone. The median duration of MTX administration was 18 months (range, 1–68). The toxicities of grade III to IV occurred in only six patients presenting cytopenias or elevated levels of serum transaminases. Low-dose MTX was tolerable and effective for the steroid-refractory or -dependent GVHD in reducing the dose of steroid without increasing the risk of opportunistic infection.
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Keywords: low-dose MTX; acute GVHD; chronic GVHD; children

Introduction

GVHD is the most common complication after allogeneic hematopoietic stem cell transplantation (HSCT).[1] Methylprednisolone or prednisone have been mainly used as an initial treatment for acute GVHD (aGVHD) and prednisone and CsA are considered as the first-line therapy for chronic GVHD (cGVHD).[2,3] Against steroid-refractory or -dependent GVHD, for which mortality is reported to be very high,[4,5] the standard second-line treatment remains to be established.

MTX has been widely used as the anti-inflammatory and immunomodulatory agent for the treatment of patients with rheumatoid arthritis and other inflammatory disorders by weekly low-dose (7.5–25 mg) administration and its efficacy and safety have been documented in the literature.[6–10] In addition, low-dose MTX has been employed for a long time as part of the prophylaxis for aGVHD after HSCT.[11–14] On the other hand, MTX had also been used as a part of a combined regimen for the treatment of acute and chronic GVHD, however, there are a few published data or prospective trials to evaluate the feasibility of MTX in the treatment of GVHD.[15,16]

MTX is an antifolate and has been used as the high-dose therapy for the treatment of malignancies since 1947. During the 1980s, the use of low-dose MTX began for the treatment of rheumatoid arthritis.[6,10] One of the mechanisms of the immunomodulatory effect of low-dose MTX is mediated by increasing the release of adenosine which inhibits production of tumor necrosis factor-α, IL-6 and IL-8, in addition to promoting the secretion of IL-10 and IL-4 *in vitro*.[6,17] These cytokines are considered to play an important role in the initiation, propagation or regulation of GVHD,[18–21] thus low-dose MTX should be expected to control GVHD by regulating the cytokine network.

Since 2000, we have employed low-dose MTX treatment for steroid-refractory or -dependent acute and chronic GVHD including that of post donor lymphocyte infusion (DLI). In this report, we show the results of retrospective analysis in 27 patients receiving low-dose MTX as second-line treatment for GVHD.

Patients and methods

Patients

Between July 2000 and May 2006, 27 pediatric patients including 10 with aGVHD and 17 with cGVHD were treated with low-dose MTX. One patient received MTX for his acute and chronic GVHD, which occurred after DLI. The reasons for the administration of MTX were that (1) the clinical manifestations of GVHD did not improve, or

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deteriorated despite the treatment with steroids with or without calcineurin inhibitor; (2) the improvement was observed with first-line therapies but the dose-reduction of prednisone was difficult because of the recurrence of GVHD. Characteristics of aGVHD and cGVHD patients are shown in Tables 1 and 2, respectively.

Low-dose MTX administration

MTX was administered either intravenously or orally at a dose of 3–10 mg/m^2 weekly. The dose of MTX was determined at each physician's discretion for the first few patients, and the majority of patients received MTX at a dose of 5–10 mg/m^2. Because only a few adverse events related to the agent had been observed during this period, we thereafter standardized the initial dose at MTX 10 mg/m^2 from January 2002. Leucovorin was not administered to all patients. Unless a toxicity of higher than grade III due to MTX was observed, then the dose of the agent was neither discontinued nor reduced.

Transplantation procedure

All 10 patients with aGVHD received BMT including one from a matched related donor and nine from mismatched related or matched unrelated donors. Seven were HLA-matched while three were mismatched (one with one locus mismatch and two with two locus mismatch) serologically. Thirteen of seventeen patients with cGVHD received BMT and three received peripheral blood stem cell transplantation, and another one received umbilical cord blood stem cell transplantation. Fourteen were HLA-matched while three were one locus mismatched serologically. The pre-transplant conditioning regimen varied according to diagnosis, disease status, stem cell source and HLA disparities. All but one patient received a myeloablative-conditioning regimen. The remaining one patient with CML received a reduced-intensity conditioning regimen. In case of HSCT from a matched unrelated or mismatched related donor, all patients except three received tacrolimus (FK506) plus short-term MTX 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, 11 as the prophylaxis for aGVHD. The remaining three patients received CsA plus short-term MTX. In case of matched related HSCT, CsA alone was used.

Diagnosis and grading of GVHD

Diagnosis and grading of aGVHD were based on established clinical criteria.[22] Four patients with grade II developed skin stage 3 only. Another six patients suffered grade III including GVHD of the gut that were stage 2–4.

The onset forms and the types of cGVHD were differentiated according to the published classifications.[23] Fourteen of seventeen cases occurred as the quiescent form, and the remaining three as the progressive form. There was no patient with the *de novo* form of cGVHD in this series. The types of cGVHD included two limited and 15 extensive. The most frequently involved organs were the oral mucosa, skin and liver.

Histological diagnoses were made in four of ten patients with acute GVHD of the gut and in one with chronic GVHD of the upper gastrointestinal tract. No other organs

Table 1 Characteristics and outcomes in acute GVHD patients

UPN	*Sex*	*Age*	*Disease*	*Donor*	*HLA disparity*	*Prophylaxis for GVHD*	*At the start of MTX*					*No. of cycles of MTX*	*At the end of MTX*		*Outcome (no. of days follow-up) and disease status*
							aGVHD						*Overall response*	*PSL (mg/kg)*	
							Skin	*Liver*	*Gut*	*Grade*	*PSL (mg/kg)*	*Other agent*			
240	F	4	CML	UBM	1	FK506/sMTX	3	—	—	II	2	FK	6	CR	1	Alive (1347), in CR
245	F	7	ALL	UBM	0	FK506/sMTX	3	—	—	II	2	FK/Pulse	6	CR	0.3	Alive (1237), in CR
257	M	4	WAS	UBM	0	FK506/sMTX	3	—	—	II	2	FK/Pulse	6	CR	1	Alive (1063)
263	F	6	BDA	RBM	0	CsA	3	—	—	II	2	CsA/Pulse	7	PR	1	Alive (982), in CR
180	F	4	NHL	RBM	2	FK506/sMTX	2	—	2	III	2	FK/Pulse	4	CR	0	Died (576) of relapse
214	M	15	AML	UBM/DLI	0	FK506/sMTX	2	—	3	III	2	None	5	CR	0.2	Alive (1760), in CR
291	M	13	AML	UBM	0	FK506/sMTX	—	3	3	III	2	FK/Pulse	1	PR	0.4	Alive (447), in CR
251	M	13	CML	RBM	2	CsA/sMTX	1	3	4	III	2	CsA/Pulse	4	NR	2	Dead (77)
267	F	2	JMML	RBM	1	CsA/sMTX	3	2	3	III	2	FK/Pulse	1	NR	2	Dead (52)
206	M	15	SAA	UBM	0	FK506/sMTX	—	3	4	III	2	CsA/Pulse	5	NR	1	Dead (145)

Abbreviations: SAA = severe aplastic anemia; aGVHD = acute GVHD; BDA = Blackfan–Diamond anemia; CML = chronic myelogenous leukemia; CR = complete response; DLI = donor lymphocyte infusion; F = female; FK506 = tacrolimus; JMML = juvenile myclomonocytic leukemia; M = male; NR = no response; PR = partial response.; PSL = prednisone: NHL = non-Hodgkin's lymphoma; Pulse = steroid pulse therapy; RBM = related bone marrow; sMTX = short-term MTX; UBM = unrelated bone marrow; UPN = unique patient number; Was = Wiskott–Aldrich syndrome.

Table 2 Characteristics and outcomes in chronic GVHD patients

UPN	*Sex*	*Age*	*Disease*	*Donor*	*HLA disparity*	*Prophylaxis for GVHD*	*At the start of MTX*				*Duration of MTX (months)*	*Overall response*	*At the time of last contact*		
							cGVHD		*PSL (mg/kg)*	*Other agent*			*PSL (mg/kg)*	*MTX or other agent*	*Outcome (no. of days follow-up) and disease status*
							From	*Type*							
187	M	4	WAS	UBM	0	FK506/sMTX	Quie	Lim	1.6	FK506	10	CR	0	—	Alive (2163)
205	F	9	ALL	UBM	0	FK506/sMTX	Quie	Lim	0.5	CsA	27	CR	0.17	MMF	Alive (1922) in CR
241	F	6	ALL	RBM	0	CsA	Prog	Ex	1	FK506	31	CR	0	MTX/FK506	Alive (1307) in CR
322	F	2	JMML	RBM	0	CsA	Quie	Ex	0.7	CsA	3	CR	0.13	MTX/CsA	Alive (183) in CR
169	F	12	HLH	RBM/DLI	0	CsA	Quie	Ex	1	—	5	PR	0	—	Alive (2507) in CR
176	F	10	ALL	RBM	1	CsA/sMTX	Quie	Ex	0.8	—	63	PR	0	MTX	Alive (2390) in CR
188	F	11	ALL	RPB	0	CsA	Prog	Ex	2	CsA	41	PR	0	—	Alive (2150) in CR
201	F	5	ALL	RPB	0	CsA	Quie	Ex	1	CsA	53	PR	0	MTX	Alive (1984) in CR
273	M	16	ALL	UBM	1	FK506/sMTX	Quie	Ex	0.2	—	16	PR	0.13	MTX/CsA	Alive (794) in CR
274	F	6	AML	UBM	2	FK506/sMTX	Quie	Ex	0.55	CsA	11	PR	0.15	MTX/CsA	Alive (774) in CR
223	M	16	ALL	RPB	0	CsA	Quie	Ex	0.52	CsA	18	MR	0.07	MMF	Alive (1583) in CR
284	M	6	ALL	UCB	1	FK506/sMTX	Quie	Ex	0.16	—	8	MR	0.08	MTX	Alive (618) in CR
179	M	9	ALL	RBM	2	FK506/sMTX	Quie	Ex	0.5	FK506	68	NR	0	MTX	Alive (2280) in CR
185	F	10	ALL	UBM	0	FK506/sMTX	Prog	Ex	0.3	—	57	NR	0.1	MTX	Alive (2181) in CR
214	M	15	AML	UBM/ DLI	0	FK506/sMTX	Quie	Ex	0.16	—	30	NR	0.4	MTX	Alive (1760) in CR
260	M	16	ALL	UBM	0	FK506/sMTX	Quie	Ex	2	CsA	0	NR	1.7	CsA	Died (272)
287	F	8	CML	UBM	0	FK506/sMTX	Quie	Ex	0.17	—	14	NR	0.28	MTX/MMF	Alive (523) in CR

Abbreviations: cGVHD = chronic GVHD; CML = chronic myelogenous leukemia; CR = complete response; DLI = donor lymphocyte infusion; F = female; FK506 = tacrolimus; HLH = hemophagocytic lymphohistiocytosis; JMML = juvenile myelomonocytic leukemia; M = male; NR = no response = PR = partial response = MR = mixed response = MMF = mycophenolate mofetil; prog = progressive; PSL = prednisone; Quie = quiescent; RBM = related bone marrow; RPB = related peripheral blood; sMTX = short-term MTX; UBM = unrelated bone marrow; UCB = unrelated cord blood; UPN = unique patient number; WAS = Wiskott–Aldrich syndrome.

of GVHD were histologically confirmed in the diagnosis of either acute or chronic GVHD.

Treatment prior to low-dose MTX

All but one of the 10 patients with aGVHD were treated with calcineurin inhibitor and 2 mg/kg prednisone at the start of the low-dose MTX administration. Eight of these patients received high-dose methylprednisolone (pulse) therapies resulting in poor response prior to the low-dose MTX. In one patient, aGVHD of grade III occurred after DLI and prednisone of 2 mg/kg was administered prior to low-dose MTX.

Ten of seventeen patients with cGVHD received a calcineurin inhibitor in combination with various doses of prednisone and remaining seven patients including two patients post DLI were with prednisone alone at the start of low-dose MTX. No other immunosuppressive agents were used prior to MTX for the treatment of acute or chronic GVHD.

Evaluation of response and toxicity

We evaluated overall response in patients with acute and chronic GVHD treated with low-dose MTX as follows; a complete response (CR) was defined as the resolution of all clinical manifestations of aGVHD. Partial response (PR) was defined as an improvement in at least one involved organ without deterioration in others nor the emergence of other organs. Mixed response (MR) was defined as improvement in at least one organ with deterioration in another organ or the emergence of involvement of other organs. No response (NR) was defined as no improvement or deterioration of all affected organs. The manifestation of each organ in acute and chronic GVHD after low-dose MTX was estimated as resolution, improvement, stable or progression.

The toxicity of low-dose MTX was graded based on the Common Terminology Criteria for Adverse Events version 3.0, according to physical examination of patients and laboratory findings including complete blood counts, liver function tests and renal function tests.

Results

Responses

Responses and outcomes in patients with aGVHD are shown in Table 1. The median number of low-dose MTX administrations was five (range, 1–7). Seven of the ten (70%) patients responded well to low-dose MTX and achieved CR or PR. The dose of prednisone was successfully reduced to equal to or lower than 1 mg/kg in these seven responding patients at the end of the MTX therapy. CR was achieved by three of the four patients with aGVHD grade II with stage 3 cutaneous involvement without any other involved organs, and two of six patients with grade III with skin and the gut involvement (one had skin stage 2, gut stage 2 and the other skin stage 2, gut stage 3). In CR and PR patients, signs of improvement of GVHD-related symptoms appeared within median 4 (range, 2–7) days after low-dose MTX administration without additional agents. No other MR patient was identified. Three NR patients had aGVHD of grade III including gut involvement of equal to or more severe than stage 3 and liver disease of equal to or more severe than stage 2, either with or without skin disease. They all died of infectious complications following the progression of aGVHD.

The responses and outcomes of the patients with cGVHD are shown in Table 2. The median duration of low-dose MTX administration was 18 (range, 0–68) months. CR was achieved in four patients, PR in six, MR in two and NR in five patients, respectively. Two of four CR cases had the limited disease and the other 2 cases had the extensive disease, including cutaneous and oral mucosal involvement. In these four CR patients, three began low-dose MTX soon after the diagnosis of cGVHD and the remaining patient began 25 days after the diagnosis. At the time of the last contact, seven patients discontinued prednisone and three of these patients were free from any other immunosuppressive agent. In all except one patient could the dose of prednisone be reduced to lower than 0.4 mg/kg, but four patients were treated with an additional agent such as CsA or MMF. Although the efficacy of low-dose MTX was not observed in NR patients, in two (UPN 179 and 185) of five NR patients, the dose of prednisone could be reduced to lower than 50% of baseline dose after a long duration of MTX administration. Three patients developed the progressive forms of cGVHD with skin involvement of more than 50% of the body surface area. In two of these three patients, cGVHD positively responded to MTX therapy, thus resulting in CR and PR, respectively and prednisone was therefore eventually discontinued. The other patient did not respond to MTX, however, the dose of prednisone could be reduced to 0.1 mg/kg at the time of last contact, namely, 57 months after the start of MTX administration.

The responses in each of the involved organs are shown in Table 3. In the case of aGVHD, seven (88%) of the eight patients with skin diseases showed favorable response, resulting in resolution or improvement. On the other hand, in the cases with aGVHD of the liver, only one (25%) of four cases responded. In the cases with aGVHD of the gut, responses were observed in three (50%) of six cases. In cGVHD, resolution or improvement was observed in 8 (57%) of 14 cases with the oral mucosa, 6 (55%) of 11 cases with the liver and 5 (42%) of 12 cases with the skin.

Table 3 Response of involved organs

Site	*Acute GVHD*			*Chronic GVHD*				
	Skin	*Liver*	*Gut*	*Skin*	*Oral mucosa*	*Liver*	*GI*	*Eyes*
n	8	4	6	12	14	11	1	2
Resolution	5	0	2	3	7	2	1	1
Improvement	2	1	1	2	1	4	0	1
Stable	1	1	1	5	4	5	0	0
Progression	0	2	2	2	2	0	0	0

Abbreviation: GI = gastrointestine.

Table 4 Toxicity related to low-dose MTX

	Hematological toxicity			*Liver toxicity*	
	ANC	*Hb*	*PLT*	*AST*	*ALT*
Grade III	0	0	1	1	1
Grade IV	1	0	2	0	0

Abbreviations: ALT = alanine aminotransferase; ANC = absolute neutrophil count; AST = aspartate aminotransferase; Hb = hemoglobin; PLT = platelet count.

Toxicity

Severe toxicity greater than grade III was observed in six patients (Table 4). As for any hematological adverse effect, one patient developed grade IV neutropenia and three patients developed grade III to IV thrombocytopenia. Both neutropenia and thrombocytopenia occurred in one patient (UPN 267) soon after the first dose of MTX. She suffered from refractory aGVHD of grade III and her general condition rapidly deteriorated with progressive pancytopenia before MTX.

Two patients had elevated levels of serum transaminase up to grade III toxicity and improved after interruption of MTX. Although the liver enzyme levels elevated in other many patients, the levels were limited to grade I to II. No other adverse effects of grade III or more were observed. All instances of grade I and II toxicity, including the liver and marrow, later resolved without any treatment.

One patient (UPN 263) with aGVHD developed hemorrhagic cystitis with BK virus occurring 6 weeks after the beginning of MTX. Another patient (UPN 260) died of pulmonary aspergillosis. He suffered from cGVHD of the skin, liver and lung for about 3 months. No other infectious complications evidently related to MTX therapy occurred.

Overall, the low-dose MTX therapy was well tolerated and median duration of this therapy for cGVHD was 18 months (range, 0–68).

Survival

Overall, 4 of 10 patients with aGVHD died and the probability of survival was 58.3% (95% CI 26.7–89.9%). Three patients with aGVHD grade III died of infectious complications following severe aGVHD and one died of relapsed underlying disease. One of 17 patients with cGVHD died of invasive pulmonary aspergillosis which overlapped with steroid-refractory cGVHD and the probability of survival was 93.8% (95% CI 81.8–105.8%).

Discussion

Acute GVHD is one of the major causes of morbidity and mortality after allogeneic HSCT. It has been reported that only less than 50% of patients showed complete response to initial treatment with steroid for aGVHD.[24,25] A salvage treatment is often required for patients who fail to respond to steroid therapy but high mortality is reported in these steroid-refractory cases.[4,5] To date, many investigators have demonstrated the poor outcome of patients treated with ATG for steroid-refractory aGVHD.[5,26–28] In these reports, the major causes of death were progressive aGVHD, infectious complication or post transplant lymphoproliferative disorder. Recently, efficacies of monoclonal antibodies to some inflammatory cytokines against aGVHD were reported in many literatures. One of these reports demonstrated that 13 (62%) of 21 patients who received infliximab for steroid-refractory aGVHD experienced complete response.[29] However, high rates of infections were also observed and the overall survival rate was 38%. Others have shown similar associations between the use of monoclonal antibodies and infections in aGVHD patients.[30,31] Similarly, the use of MMF for steroid-refractory aGVHD was reported to have relations to high incidence of infectious complications.[32] Pentostatin for the treatment of 23 patients in steroid-refractory aGVHD as phase I dose escalation study was reported in 2005.[33] Although the response rate in that report was 78%, only five patients were alive. Most patients died of refractory GVHD, relapse of disease, or infectious complications. These results showed that one of the most important factors contributing to the lower survival rate after the salvage therapy was the intensification of immunosuppression leading to the opportunistic infection.

Chronic GVHD is also the major cause of non-relapse mortality in patients surviving more than 2 years after allogeneic transplantation.[3] One prospective cohort study demonstrated that response rates to combination therapy with steroid, CsA and azathioprine were 61, 53 and 50% at 6 months, 1 year and 2 years, respectively and the overall survival rate was 39% at 10 years.[34] In addition, cGVHD is associated with substantial deficits of the quality of life such as decreased physical and functional status, sexual inactivity and frequent infection.[35] The long-term treatment with prednisone, which is one of the agents in the first-line treatments for cGVHD, may enhance this compromised quality of life in patients with cGVHD. As a result, mortality in chronic GVHD is largely attributable to infection.[2,3,34] As the secondary or salvage treatment for cGVHD, a number of trials have been published. Most of them reported a success rate of 25 to 50% using a variety of agents such as MMF, a monoclonal antibody to the inflammatory cytokines and rituximab with possibility of increasing risk of infection.[3,36,37]

Considering the circumstances mentioned above, one of the most important strategies for the treatment of acute and chronic GVHD is to lessen the risk of infection. Because it is considered that low-dose MTX has little impact for immunosuppression leading to the risk of infectious complications, the agent may be beneficial for patients with lower immune function by prior intensive immunosuppressive treatment. In the present study, many patients with acute and chronic GVHD was able to reduce the dose of prednisone after the initiation of low-dose MTX without increasing the risk of opportunistic infection and other complications caused by long-term prednisone treatment. In addition, only 1 of 27 patients demonstrated a relapse of an underlying disease, thus suggesting that low-dose MTX does not increase the risk of relapse.

The results of the present study indicate that low-dose MTX might be ineffective in cases of aGVHD with stage 4

disease of the gut or multiple organ involvement. For these patients, a more immunosuppressive agent might thus be needed. In seven patients with aGVHD who responded to this treatment regimen, an improvement of GVHD-related symptoms appeared within median 4 (range, 2–7) days after the initiation of low-dose MTX without any additional agents. It seems that if a sign of the improvement of aGVHD is observed within a week after the first dose of MTX, then a good response may be expected in aGVHD. Low-dose MTX seemed to be effective for cGVHD of the liver, oral mucosa and skin involvement. Depending on the responding organs, the manifestations of the oral mucosa and skin tended to improve within a few weeks after the initiation of MTX treatment while it might take a long time for the findings of liver function tests to normalize. In fact, most of the observed responses in cGVHD of the liver demonstrated either an improvement or stable disease. Many other medications or complications after HSCT might also influence the resolution process of liver disease. Sixteen of seventeen patients were alive at last follow-up either with or without a small dose of prednisone. Given the long-term usage of prednisone influence to morbidity and mortality, this result suggested that low-dose MTX might have a potential to lessen mortality, thus possibly improving the quality of life of patients with cGVHD. The number of cases investigated in this study was too small to draw any definitive conclusions, and therefore further large-scale analyses are required.

Severe toxicities occurred only in a few patients presenting cytopenias or elevated levels of the serum transaminases. Most of these adverse events developed in advanced aGVHD patients. Because these patients were extremely ill and were on many medications that have myelo- and/or hepatotoxicity, it was difficult to evaluate whether these toxicities were related to MTX alone. There were no episodes of severe infectious complications obviously related to MTX. No other well-described adverse event such as gastrointestinal symptoms, immune-mediated pneumonitis, renal impairment and secondary malignancies were seen even in the patients with long-term follow up. These results suggested that low-dose MTX might therefore be safe and well tolerated for long-term use over a period of several years, even in the post transplant setting.

There are two published reports, which evaluated the feasibility of MTX in the treatment of GVHD. Giaccone *et al.*[15] demonstrated the possible steroid-sparing effect of low-dose MTX for patients with long-standing, severe chronic GVHD. Despite the fact that 12 of 14 patients in this retrospective study had at least one high-risk feature such as scleroderma, fasciitis or thrombocytopenia, the disease could be successfully controlled in 10 patients with prednisone at doses below 1 mg/kg every other day without the addition of other agents. On the other hand, Huang *et al.*[16] reported the result of a clinical study using low-dose MTX to treat patients with acute and chronic GVHD. Most patients in this study were not in the advanced stages of GVHD and the response rate was very high for both acute and chronic GVHD including post DLI GVHD. In addition to the efficacy of low-dose MTX, these two reports showed the safety and tolerability of this agent and our present study supported and confirmed the more long-term use of MTX over a period of several years even for children.

In conclusion, low-dose MTX is less toxic, easy to administer and effective to the steroid-refractory or -dependent GVHD, in addition, reducing the dosage and duration of steroid therapy. Low-dose MTX is worthy of further evaluation as second-line treatment of severe GVHD.

References

1 Armitage JO. Bone marrow transplantation. *N Engl J Med* 1994; **330**: 827–838.
2 Vogelsang GB. How I treat chronic graft-versus-host disease. *Blood* 2001; **97**: 1196–1201.
3 Lee SJ, Vogelsang G, Flowers MED. Chronic graft-versus-host disease. *Biol Blood Marrow Transplant* 2003; **9**: 215–233.
4 Martin PJ, Schoch G, Fisher L, Byers V, Appelbaum FR, McDonald GB *et al.* A retrospective analysis of therapy for acute graft-versus-host disease: secondary treatment. *Blood* 1991; **77**: 1821–1828.
5 Arai S, Margolis J, Zaburak M, Anders V, Vogelsang GB. Poor outcome in steroid-refractory graft-versus-host disease with antithymocyte globulin treatment. *Biol Blood Marrow Transplant* 2002; **8**: 155–160.
6 Grim J, Chladek J, Martinkova J. Pharmacokinetics and pharmacodynamics of methotrexate in non-neoplastic disease. *Clin Pharmacokinet* 2003; **42**: 139–151.
7 Kozarek RA, Patterson DJ, Gelfand MD, Botoman VA, Ball TJ, Wilske KR. Methotrexate induces clinical and histologic remission in patients with refractory inflammatory bowel disease. *Ann Intern Med* 1989; **110**: 353–356.
8 Mack DR, Young R, Kaufman SS, Ramey L, Vanderhoof JA. Methotrexate in patients with Crohn's disease after 6-mercaptopurine. *J Pediatr* 1998; **132**: 830–835.
9 Boehm IB, Boehm GA, Bauer R. Management of cutaneous lupus erythematosus with low-dose methotrexate: indication for modulation of inflammatory mechanism. *Rheumatol Int* 1998; **18**: 59–62.
10 van Ede AE, Laan RF, Blom HJ, De Abreu RA, van de Putte LB. Methotrexate in rheumatoid arthritis: an update with focus on mechanisms involved toxicity. *Semin Arthritis Rheum* 1998; **27**: 277–292.
11 Chao NJ, Schmidt GM, Niland JC, Amylon MD, Dagis AC, Long DG *et al.* Cyclosporine, methotrexate, and prednisone compared with cyclosporine and prednisone for prophylaxis of acute graft-versus-host disease. *N Engl J Med* 1993; **329**: 1225–1230.
12 Storb R, Deeg HJ, Whitehead J, Appelbaum F, Beatty P, Bensinger W *et al.* Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med* 1986; **314**: 729–735.
13 Nash RA, Antin JH, Karanes C, Fay JW, Avalos BR, Yeager AM *et al.* Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood* 2000; **96**: 2062–2068.
14 Przepiorka D, Ippoliti C, Khouri I, Woo M, Mehra R, Bherz DL *et al.* Tacrolimus and minidose methotrexate for prevention of acute graft-versus-host disease after matched unrelated donor marrow transplantation. *Blood* 1996; **88**: 4383–4389.
15 Giaccone L, Martin P, Carpenter P, Moravec C, Hooper H, Funke VAM *et al.* Safety and potential efficacy of low-dose

methotrexate for treatment of chronic graft-versus-host disease. *Bone Marrow Transplant* 2005; **36**: 337–341.
16 Huang XJ, Jiang Q, Chen H, Xu L, Liu D, Chen Y *et al.* Low-dose methotrexate for the treatment of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2005; **36**: 343–348.
17 Seitz M. Molecular and cellular effects of methotrexate. *Curr Opin Rheumatol* 1999; **11**: 226–232.
18 Imamura M, Hashino S, Kobayashi H, Kubayashi S, Hirano S, Minagawa T *et al.* Serum cytokine levels in bone marrow transplantation: synergistic interaction of interleukin-6, interferon-γ, and tumor necrosis factor-α in graft-versus-host disease. *Bone Marrow Transplant* 1994; **13**: 745–751.
19 Jacobsohn DA, Vogelsang GB. Anti-cytokine therapy for the treatment of graft-versus-host disease. *Curr Pharm Des* 2004; **10**: 1195–1205.
20 Ferrara JLM. Paradigm shift for graft-versus-host disease. *Bone Marrow Transplant* 1994; **14**: 183–184.
21 Ferrara JLM, Reddy P. Pathophysiology of graft-versus-host disease. *Semin Hematol* 2006; **43**: 3–10.
22 Przepiorka D, Weisdorf D, Martin P, Klingemann H-G, Beatty P, Hows J *et al.* 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
23 Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE *et al.* Chronic graft-versus-host disease syndrome in man. A long-term clinico-pathologic study of 20 Seattle patients. *Am J Med* 1980; **69**: 204–217.
24 Weisdorf D, Haake R, Blazar B, Miller W, McGlave P, Ramsay N *et al.* Treatment of moderate/severe acute graft-versus-host disease after allogeneic bone marrow transplantation: an analysis of clinical risk features and outcome. *Blood* 1990; **75**: 1024–1030.
25 Roy J, McGlave PB, Filipovich AH, Miller WJ, Blazar BR, Ramsay NK *et al.* Acute graft-versus-host disease following unrelated donor marrow transplantation: failure of conventional therapy. *Bone Marrow Transplant* 1992; **10**: 77–82.
26 Khoury H, kashyap A, Adkins DR, Brown RA, Miller G, Vij R *et al.* Treatment of steroid-resistant acute graft-versus-host disease with anti-thymocyte globulin. *Bone Marrow Transplant* 2001; **27**: 1059–1064.
27 Remberger M, Aschan J, Barkholt L, Tollemar J, Ringden O. Treatment of severe acute graft-versus-host disease with anti-thymocyte globulin. *Clin Transplant* 2001; **15**: 147–153.
28 McCaul KG, Nevill TJ, Barnett MJ, Toze CL, Currie CJ, Sutherland HJ *et al.* Treatment of steroid-resistant acute graft-versus-host disease with rabbit antithymocyte globulin. *J Hematother Stem Cell Res* 2000; **9**: 367–374.
29 Couriel D, Saliba R, Hicks K, Ippoliti C, De Lima M, Hosing C *et al.* Tumor necrosis factor-α blockade for the treatment of acute GVHD. *Blood* 2004; **104**: 649–654.
30 Marty FM, Lee SJ, Fahey MM, Alyea EP, Soiffer RJ, Antin JH *et al.* Infliximab use in patients with severe graft-versus-host disease and other emerging risk factors of non-Candida invasive fungal infections in allogeneic hematopoietic stem cell transplant recipients: a cohort study. *Blood* 2003; **102**: 2768–2776.
31 Wolff D, Roessler V, Steiner B, Wilhelm S, Weirich V, Brenmoehl J *et al.* Treatment of steroid-resistant acute graft-versus-host disease with daclizumab and etanercept. *Bone Marrow Transplant* 2005; **35**: 1003–1010.
32 Krejci M, Doubek M, Buchler T, Brychtova Y, Vorlicek J, Mayer J. Mycophenolate mofetil for the treatment of acute and chronic steroid-refractory graft-versus-host disease. *Ann Hematol* 2005; **84**: 681–685.
33 Bolanos-Meade J, Jacobsohn DA, Margolis J, Ogden A, Wientjes MG, Byrd JC *et al.* Pentostatin in steroid-refractory acute graft-versus-host disease. *J Clin Oncol* 2005; **23**: 2661–2668.
34 Arora M, Burns LJ, Davies SM, MacMillan ML, Defor TE, Miller WJ *et al.* Chronic graft-versus-host disease: a prospective cohort study. *Biol Blood Marrow Transplant* 2003; **9**: 38–45.
35 Akpek G, Lee SJ, Flowers ME, Pavletic SZ, Arora M, Lee S *et al.* Performance of a new clinical grading system for chronic graft-versus-host disease: a multicenter study. *Blood* 2003; **102**: 802–809.
36 Lopez F, Parker P, Nademanee A, Rodriguez R, Al-Kadhimi Z, Bhatia R *et al.* Efficacy of mycophenolate mofetil in the treatment of chronic graft-versus-host disease. *Biol Blood Marrow Transplant* 2005; **11**: 307–313.
37 Cutler C, Miklos D, Kim HT, Treister N, Woo SB, Bienfang D *et al.* Rituximab for steroid-refractory chronic graft-versus-host disease. *Blood* 2006; **108**: 756–762.

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A study of rasburicase for the management of hyperuricemia in pediatric patients with newly diagnosed hematologic malignancies at high risk for tumor lysis syndrome

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Abstract Tumor lysis syndrome (TLS), including hyperuricemia, is a frequent serious complication in patients with hematologic malignancies. This study in Japanese patients evaluated the efficacy, safety, and pharmacokinetic profile of rasburicase in pediatric patients with hematologic malignancies. Patients aged <18 years at high risk for TLS, with newly diagnosed hematologic malignancies, were randomized to intravenous rasburicase 0.15 mg/kg/day ($n = 15$) or 0.20 mg/kg/day ($n = 15$) for 5 days. Chemotherapy was started 4–24 h after the first rasburicase dose. Response was defined as a reduction in plasma uric acid to ≤6.5 mg/dL (patients <13 years) or ≤7.5 mg/dL (patients ≥13 years) by 48 h after the first administration, lasting until 24 h after the final administration. Response rates were 93.3 and 100% with rasburicase 0.15 and 0.20 mg/kg/day, respectively. Uric acid levels declined rapidly within 4 h of starting rasburicase administration in both groups. Most adverse events were related to the underlying chemotherapy regimens. Two hypersensitivity reactions, including grade 1/2 pruritus, were considered to be related to rasburicase. Rasburicase is effective and well tolerated for the management of hyperuricemia in Japanese pediatric patients at high risk of developing TLS.

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1 Introduction

Patients with hematologic malignancies are usually treated with aggressive chemotherapy regimens that result in the rapid destruction of tumor cells and the release of purine metabolites into the circulation [1]. This may lead to the development of tumor lysis syndrome (TLS), which is characterized by severe hyperuricemia, hyperphosphatemia, hyperkalemia, and hypocalcemia [2, 3]. Moreover, as a consequence of hyperuricemia, crystals of uric acid may form in the renal tubules and distal collecting system, leading to renal insufficiency and acute renal failure [4]. Patients with malignancies that have a high proliferation rate or a large tumor burden, such as acute lymphoblastic leukemia or Burkitt's lymphoma, have a particularly high risk of developing TLS. The metabolic disturbances resulting from TLS may lead to acute renal failure and rapidly become life threatening in pediatric patients. Appropriate management for metabolic abnormalities in these patients is therefore essential in order to reduce the risk of developing acute renal failure [5–7].

The current treatment of hyperuricemia in Japan includes urinary alkalinization, hydration, and allopurinol. Allopurinol inhibits xanthine oxidase and thus prevents the formation of uric acid and controls plasma uric acid levels during purine catabolism [4]. Allopurinol, however, cannot reduce the level of pre-existing uric acid and causes increases in serum levels of xanthine and hypoxanthine, which may lead to xanthine nephropathy [8, 9]. In addition, urinary alkalinization can cause renal precipitation of calcium phosphate [4].

Rasburicase is a recombinant form of the endogenous enzyme urate oxidase. It is produced following the proteolytic hydrolysis of *Aspergillus flavus* urate oxidase, which permits the formation of oligodeoxynucleotide probes that are used to obtain DNA fragments from *Aspergillus flavus* cDNA and genomic libraries [10]. Rasburicase is approved for the prevention and treatment of hyperuricemia in children with leukemia or lymphoma in the USA and the EU. This agent oxidizes uric acid, converting it to allantoin, a substance that is approximately 5–10 times more soluble than uric acid and is easily excreted in the urine [8]. Rasburicase is administered intravenously, making it more convenient to administer to patients with chemotherapy-associated gastrointestinal toxicities than the oral drug allopurinol. Moreover, rasburicase can reduce pre-existing uric acid levels [11].

In a US open-label, randomized study in 52 children with leukemia or lymphoma at high risk for TLS, administration of rasburicase (0.20 mg/kg/day) for 5–7 days during induction chemotherapy achieved significantly more rapid control of uric acid and lower levels of plasma uric acid than allopurinol (300 mg/m^2/day) for 5–7 days [12]. This led the investigators to conclude that rasburicase is a safe and an effective alternative to allopurinol during initial chemotherapy in pediatric patients.

The aim of this study was to investigate the efficacy, safety, and pharmacokinetic profile of rasburicase as a single agent in Japanese pediatric patients with hematologic malignancies at high risk for TLS. In particular, the safety of rasburicase administered before chemotherapy was evaluated in this patient population.

2 Materials and methods

2.1 Study design and patients

This was a multicenter, open-label, randomized, parallel-group study of repeated doses of rasburicase in Japanese pediatric patients with newly diagnosed hematologic malignancies at high risk of developing TLS.

The study protocol was approved by the institutional review boards of all participating centers. Written informed consent was obtained from the legally authorized representative of each patient before randomization to one of two doses of rasburicase (0.15 or 0.20 mg/kg).

Japanese pediatric patients (aged <18 years) were eligible for study entry if they had newly diagnosed hematologic malignancies with hyperuricemia (uric acid >7.5 mg/dL for patients aged ≥13 years; uric acid >6.5 mg/dL for patients aged <13 years) or newly diagnosed hematologic malignancies presenting with a high tumor burden, regardless of uric acid level [defined as non-Hodgkin's lymphoma (NHL) stage IV; NHL stage III with at least one lymph node or mass >5 cm in diameter or lactate dehydrogenase (LDH) three or more times the upper limit of normal (ULN)]; or acute leukemia with a white blood cell (WBC) count ≥50,000/mm^3 and LDH three or more times ULN. Patients were required to have a performance status of 3 or less on the Eastern Cooperative Oncology Group (ECOG) scale (or 30 or more on the Lansky score) and a minimum life expectancy of 45 days. Patients received induction chemotherapy between 4 and 24 h after the first administration of rasburicase.

Exclusion criteria included the administration of allopurinol within 72 h before the start of rasburicase administration; known history of severe allergy and/or severe asthma; low birth weight (<2,500 g) or gestational age (<37 weeks); previous therapy with urate oxidase; known positive tests for hepatitis B surface antigen, hepatitis C virus antibodies, or HIV-1 or HIV-2 antibodies; severe

disorders of the liver or kidney [alanine aminotransferase (ALT) levels more than five times ULN, total bilirubin more than three times ULN, creatinine more than three times ULN]; or uncontrollable infection (including viral infection). The enzymatic conversion of uric acid to allantoin by rasburicase produces hydrogen peroxide [13]. This can lead to methemoglobinemia and hemolysis in certain "at-risk" populations such as those with glucose-6-phosphate dehydrogenase (G6PD) deficiency, and hence G6PD deficiency contraindicates the use of rasburicase. Therefore, patients with a known family history of G6PD deficiency, and known history of methemoglobinemia and hemolysis, were also excluded.

Randomization was performed centrally, and patients were stratified by baseline weight (<10 or ≥10 kg) until 15 patients had been enrolled in each dose group. To ensure the exact evaluation of pharmacokinetics, at least 10 patients weighing ≥10 kg were included in each dose group. The protocol did not require that a minimum number of patients weighing <10 kg should be enrolled.

2.2 Treatment

Rasburicase (SR29142) was supplied by sanofi-aventis (Tokyo, Japan). Patients were randomized to one of two dose groups of rasburicase (0.15 or 0.20 mg/kg). Rasburicase 0.20 mg/kg has been approved in 50 countries worldwide, but in the USA, the doses of rasburicase 0.15 and 0.20 mg/kg have been approved. Given that the efficacy of rasburicase 0.15 and 0.20 mg/kg was recently demonstrated in a previous study in adult Japanese patients [14], both these doses were selected for use in this pediatric trial. Rasburicase was administered intravenously for 30 min once daily for 5 consecutive days.

Chemotherapy, including cytoreductive corticosteroids, was started 4–24 h after the first dose of rasburicase. Separate lines were used for administration of chemotherapy and infusion of rasburicase to prevent drug–drug interactions. When this was not possible, the line was flushed with isotonic saline (≥15 mL) before and after infusion of rasburicase. Other anti-hyperuricemic agents (e.g. allopurinol) or treatment with sodium bicarbonate for urine alkalization were not permitted until the final blood sampling for plasma uric acid was completed on day 6.

2.3 Efficacy assessments

The primary efficacy endpoint was response rate (RR), as determined by assays of plasma uric acid concentration. Treatment was considered to be successful and the patient considered to be a responder if the plasma uric acid level decreased to ≤7.5 mg/dL in patients aged ≥13 years or ≤6.5 mg/dL in patients aged <13 years by 48 h after the start of the first rasburicase administration, and lasting until 24 h after the start of the final rasburicase administration (day 5).

Secondary endpoints included plasma uric acid concentration and change in concentration from baseline. The rate of plasma uric acid inhibition over time versus baseline was also evaluated at 4 and 48 h after the first rasburicase administration and at 24 h after the last rasburicase administration. The rate of uric acid inhibition (%) was calculated as the concentration of plasma uric acid at baseline minus the concentration of plasma uric acid at each timepoint divided by the concentration at baseline multiplied by 100. Blood samples were collected for the plasma uric acid levels ≤10 min before and 4 h (±10 min) after the first rasburicase administration on day 1; samples were also collected before rasburicase administration (±10 min) on days 2–5, and 24 h (±10 min) after the last rasburicase administration on day 6.

2.4 Safety assessments

Safety was assessed by clinical observations (including vital signs), standard laboratory tests, and the occurrence of adverse events (AEs). AEs were summarized by type of event and toxicity grade according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (translated into Japanese by the Japan Clinical Oncology Group/Japan Society of Clinical Oncology). These events were classified by each investigator as either rasburicase-related or other (related to underlying hematologic malignancies or chemotherapy). Rasburicase-related AEs were defined as all events excluding events due to the underlying disease or chemotherapy. Renal function (creatinine, potassium, phosphorus, and calcium levels) was also assessed at baseline (7 days before starting the first administration of rasburicase), and on day 3, 5, 8, 15, 22, 29, and 36.

2.5 Evaluation of anti-rasburicase antibodies

To evaluate the relationship between hypersensitivity reactions and the appearance of anti-rasburicase antibodies, the antibodies were assessed by qualitative enzyme-linked immunosorbent assay (ELISA) at baseline and on day 29. The results were expressed qualitatively due to the lack of immunopurified reference human antibody directed against rasburicase, with the conventional properties of antibodies utilized to detect antibodies directed against rasuburicase. The range of anti-human immunoglobulin calibration was between 0 and 1,000 ng/mL and plasma samples from healthy volunteers were used as reference controls. Plasma collected from healthy volunteers was assayed by ELISA

to determine background interference in the detection of anti-rasburicase immunoglobulin. If the samples were positive for anti-rasburicase antibodies on day 29, then further blood samples were collected from the patient at 6 months (±2 weeks) and every 6 months (±2 weeks) thereafter until the sample was negative. Samples that were antibody positive were analyzed for inhibition of rasburicase uricolytic activity. Anti-*S. cerevisiae* protein (SCP) antibodies were also assessed by ELISA at baseline.

2.6 Pharmacokinetics

The pharmacokinetic assay was performed in 10 patients (weight ≥10 kg) in each dose group at the following 10 timepoints: Day 1, before rasburicase administration (within 10 min of the start of administration), at the end of the first administration (within 10 min following completion of administration), 4 and 8 h (±10 min) after the start of administration; day 2, before rasburicase administration (within 10 min of the start of administration); and day 5, before rasburicase administration (within 10 min of the start of administration), at the end of administration, 4, 8, and 24 h (±10 min) after the last administration. A total sample of 10 mL from each patient was immediately centrifuged at <4°C and then frozen until assay. Frozen samples were sent to the laboratories (SBI-BIO, Paris, France), where they were assayed concomitantly.

The following pharmacokinetic parameters were determined: area under the rasburicase plasma concentration–time curve from 0 to 24 h (AUC_{0-24}) on days 1 and 5; minimum rasburicase plasma concentration observed before treatment administration during repeated dosing (C_{min}) on days 1 and 5; plasma concentration of rasburicase at the end of infusion (C_{eoi}) on days 1 and 5; terminal half-life ($t_{1/2z}$) on day 5; and accumulation ratio for AUC_{0-24} and C_{eoi} (defined as the ratio of day 5 to day 1 for AUC_{0-24} and C_{eoi}). Rasburicase plasma concentrations were determined by ELISA.

2.7 Statistical analysis

All patients who received at least one dose of rasburicase were evaluated for efficacy and safety. The RR, with 95% exact binomial confidence intervals (CIs), was calculated as the number of responding patients divided by the number of evaluable patients multiplied by 100. Patients who failed to complete days 1–5 of treatment, for reasons other than hyperuricemia, were considered as nonevaluable for RR. Descriptive statistics were used to summarize uric acid concentrations, and change from baseline and the rate of plasma uric acid concentration decline over time.

For the purposes of the statistical analysis, the study had a planned sample size of 30 patients, i.e. 15 in each dose group (0.15 and 0.20 mg/kg). Assuming that the true RR would be 95% in each dose group, the probability of at least one failure among 15 patients treated with each dose of rasburicase would be 79%, with an expected lower 95% CI of 71%. Based on a sample size of 15 patients per dose group, it could therefore be concluded with 95% confidence that the true RR would be at least 71%.

Pharmacokinetic parameters for rasburicase were determined using WinNonlin Professional Edition software (version 3.3 Pharsight Corp, Mountain View, CA, USA) using a noncompartmental method. To assess drug accumulation from day 1 to day 5, the parameters AUC_{0-24} and C_{eoi} were analyzed using the linear mixed-effects model:

$$\text{Log (parameter)} = \text{dose} + \text{day} + \text{dose} \times \text{day} + \text{patient (dose)} + \text{error}$$

Fixed-effect terms included dose (0.15 and 0.20 mg/kg), day (1 and 5), and the interaction term dose by day. The random-effect term was patient within dose. The model was estimated using generalized least squares (GLS) with restricted maximum likelihood (REML) estimates of random effects, using SAS® PROC MIXED.

The 95% CIs for the variance estimates were computed using the simple chi-squared method for within-patient variance, the Modified Large Sample procedure for between-patient variance, and the Graybill–Wang procedure for the total-patient variance [15].

3 Results

3.1 Patients

Between June 2005 and April 2006, 31 patients were enrolled and 30 patients were subsequently randomized and treated (rasburicase 0.15 mg/kg, $n = 15$; rasburicase 0.20 mg/kg, $n = 15$). One enrolled patient was not randomized to treatment because of an ineligible ALT level. This patient was excluded from the efficacy and safety analyses.

Patient baseline characteristics were similar between the rasburicase dose groups (Table 1). The median age was 8.8 years, and over half of the patients (53.3%) were aged between 6 and 12 years. Overall median weight was 30.8 kg, and one patient weighed <10 kg. Most patients had an ECOG performance status of 0 (40%) or 1 (40%). A total of 43.3% of patients were hyperuricemic, and 73.3% of patients had acute leukemia. All patients were classified as high risk for TLS in accordance with the inclusion criteria (i.e. had a newly diagnosed hematologic malignancy with hyperuricemia or a high tumor burden).

Table 1 Baseline characteristics of randomized patients

	Rasburicase		Total (n = 30)
	0.15 mg/kg (n = 15)	0.20 mg/kg (n = 15)	
Age (years)			
Median	11	7	9
Range	1–17	0–16	0–17
Age group, n (%)			
<2 years	1 (6.7)	1 (6.7)	2 (6.7)
2–5 years	2 (13.3)	4 (26.7)	6 (20.0)
6–12 years	7 (46.7)	9 (60.0)	16 (53.3)
13–17 years	5 (33.3)	1 (6.7)	6 (20.0)
Male/female, n (%)	9/6 (60.0/40.0)	10/5 (66.7/33.3)	19/11 (63.3/36.7)
Weight (kg)			
Median	44.7	25.5	30.8
Range	10.2–70.3	5.7–49.7	5.7–70.3
ECOG performance status, n (%)			
0	7 (46.7)	5 (33.3)	12 (40.0)
1	5 (33.3)	7 (46.7)	12 (40.0)
2	0 (0.0)	3 (20.0)	3 (10.0)
3	3 (20.0)	0 (0.0)	3 (10.0)
Hyperuricemic, n (%)[a]			
Yes	8 (53.3)	5 (33.3)	13 (43.3)
No	7 (46.7)	10 (66.7)	17 (56.7)
Diagnosis, n (%)			
Malignant lymphoma	6 (40.0)	2 (13.3)	8 (26.7)
Acute leukemia	9 (60.0)	13 (86.7)	22 (73.3)

ECOG Eastern Cooperative Oncology Group

[a] Defined as uric acid >7.5 mg/dL in patients ≥13 years or uric acid >6.5 mg/dL in patients <13 years at baseline

3.2 Administration of rasburicase

Twenty-nine of 30 patients completed days 1–5 of treatment; one patient in the rasburicase 0.20 mg/kg group withdrew from the study on day 1 after the first administration of rasburicase due to the lack of WBC count at baseline. Another patient (0.15 mg/kg group) completed 5 days of treatment but withdrew from the study on day 8 to avoid life-threatening complications due to three concomitant grade 4 AEs (cerebral hemorrhage, brain edema, and brain herniation). Fifteen patients in the 0.15 mg/kg group and 14 patients in the 0.20 mg/kg group were therefore evaluable for response.

3.3 Efficacy

The overall RR for all patients was 96.6% (95% CI 82.2–99.9%) (Table 2). The RR was slightly higher in the rasburicase 0.20 mg/kg group than in the 0.15 mg/kg group (100% [95% CI 76.8–100.0%] vs. 93.3% [95% CI 68.1–99.8%]).

RRs for patients with hyperuricemia at baseline were 87.5 and 100.0% in the rasburicase 0.15 and 0.20 mg/kg groups, respectively (Table 2).

Mean plasma uric acid concentrations by dose of rasburicase over time are presented in Fig. 1. Rasburicase produced a rapid decrease in plasma uric acid concentrations in both dose groups. Uric acid levels remained low throughout treatment in all patients except one non-responder in the 0.15 mg/kg group. Uric acid levels declined rapidly within 4 h of the first rasburicase dose in both dose groups and remained low up to 120 h after the first administration of rasburicase (Fig. 1). Mean plasma uric acid concentrations were reduced by 84.8% (95% CI 76.7–92.9%) and 92.9% (95% CI 88.7–97.0%) compared with baseline at 4 h after the first rasburicase administration for the 0.15 and 0.20 mg/kg groups, respectively. Reductions in the level of plasma uric acid were similar between the two groups at 24 h after the last administration of rasburicase on day 6 (approximately 88%).

3.4 Safety

Owing to the severity of the underlying disease, all patients experienced at least one grade 3/4 AE, regardless of rasburicase treatment. The most common grade 3/4 AEs were leukopenia (86.7% of patients), neutropenia (83.3%), lymphocytopenia (80.0%), and increased ALT levels

Table 2 Summary of efficacy

	Rasburicase dose		Total
	0.15 mg/kg	0.20 mg/kg	
Number of evaluable patients	15	14	29
Responders, *n* (%) [95% CI]	14 (93.3) [68.1–99.8%]	14 (100) [76.8–100%]	28 (96.6) [82.2–99.9%]
Hyperuricemic			
Evaluable patients, *n*	8	5	13
Responders, *n* (%)	7 (87.5)	5 (100)	12 (92.3)
Nonhyperuricemic			
Evaluable patients, *n*	7	9	16
Responders, *n* (%)	7 (100)	9 (100)	16 (100)
Inhibitory rate (%)[a]			
Evaluable patients, *n*	15	14	29
Mean [95% CI]	84.8 [76.7–92.9]	92.9 [88.7–97.0]	88.7 [84.1–93.3]

CI confidence interval

[a] Measured on day 1, 4 h after administration of rasburicase. The rate of uric acid inhibition (%) was calculated as follows: (plasma uric acid concentration at baseline – plasma uric acid concentration at each timepoint) divided by (plasma uric acid concentration at baseline) multiplied by 100

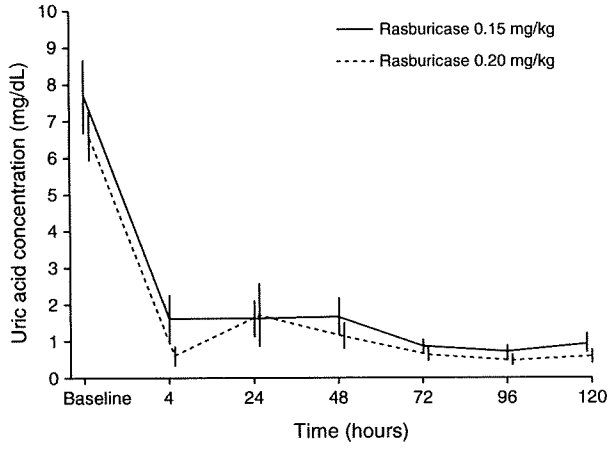

Fig. 1 Mean plasma uric acid concentration by dose over time. Patients aged <18 years with newly diagnosed hematologic malignancies with hyperuricemia, or newly diagnosed hematologic malignancies presenting with a high tumor burden regardless of uric acid level, were randomly allocated (based on stratification by weight [<10 or ≥10 kg]) to receive rasburicase (SR29142) administered at either 0.15 or 0.20 mg/kg/day for 5 days, followed by chemotherapy starting from 4 to 24 h after the first infusion of rasburicase

(70.0%). All of these AEs occurred with a similar frequency in both dose groups, with the exception of neutropenia (rasburicase 0.15 mg/kg, 93.3%; rasburicase 0.20 mg/kg, 73.3%). The majority of AEs reported during the study period were judged by the investigators to be related to the underlying malignancies and/or chemotherapy. There was no difference in the safety profiles of rasburicase 0.15 mg/kg/day for 5 days and rasburicase 0.20 mg/kg/day for 5 days.

The three grade 4 AEs (cerebral hemorrhage, brain edema, and brain herniation) experienced by one patient in the rasburicase 0.15 mg/kg group who withdrew from the study on day 8, were judged by the investigators to be unrelated to rasburicase treatment. The patient died after withdrawal from the study. No other deaths were reported during the study.

Drug-related AEs occurred in six patients ($n = 4$ in the rasburicase 0.15 mg/kg group; $n = 2$ in the 0.20 mg/kg group). One patient in the rasburicase 0.20 mg/kg group experienced grade 3/4 hemolysis; the patient did not have G6PD deficiency. The risk of rasburicase-induced hemolysis, possibly leading to hemolytic anemia and methemoglobinemia, is greater in patients with G6PD deficiency because of the accumulation of hydrogen peroxide [13]. However, rare cases of rasburicase-induced methemoglobinemia have been reported in patients without G6PD deficiency [12, 16]. Two rasburicase-related AEs occurred after the administration of rasburicase but before the start of chemotherapy (grade 1/2 hypersensitivity in the rasburicase 0.15 mg/kg group and grade 3/4 anemia in the rasburicase 0.15 mg/kg group).

Hypersensitivity reactions (all grades) were reported in 8 patients (53.3%) in the 0.15 mg/kg group and 12 patients (80.0%) in the 0.20 mg/kg group. Grade 3/4 events were only reported in two patients in the 0.15 mg/kg group: one patient completely recovered from the hypersensitivity reaction by day 6, although the other patient experienced persistent drug hypersensitivity until day 32. However, these grade 3/4 hypersensitivity reactions were judged to be unrelated to rasburicase. Only two AEs (hypersensitivity [grade 1/2] and hemoglobin decreased [grade 1/2]), observed before chemotherapy, were considered to be related to rasburicase. Anti-rasburicase antibodies or anti-SCP antibodies were not observed in any patients with hypersensitivity reactions.

A slight decrease in serum creatinine levels from baseline was observed. The mean values (±standard deviation) of serum creatinine were 52.3 μmol (±22.6) at baseline, 43.6 μmol (±16.3) on day 3, and 33.5 μmol (±11.5) on

day 36 for the 0.15 mg/kg rasburicase group; and 44.4 μmol (±19.1) at baseline, 36.7 μmol (±11.8) on day 3, and 27.1 μmol (±7.1) on day 36 for the 0.20 mg/kg group. No clinically significant changes were observed for the other renal function parameters (potassium, phosphorous, and calcium) during the study period.

3.5 Antibodies

Anti-SCP antibodies were detected in one patient before administration of rasburicase 0.20 mg/kg. Anti-rasburicase antibodies were detected in another patient in this group on day 29 and the patient tested negative for antibodies 6 months after the first administration. Neither patient experienced a hypersensitivity reaction during the study.

3.6 Pharmacokinetics

Blood samples to determine plasma concentrations of rasburicase were collected from 20 patients, 10 in each dose group. One patient in the 0.20 mg/kg dose group was excluded due to only two samples having been collected on day 1 of the study. Therefore, 19 patients were evaluable for AUC_{0-24} on day 1 and C_{min}, C_{eoi}, AUC_{0-24}, and $t_{1/2z}$ on day 5.

The pharmacokinetic profile of rasburicase is summarized in Table 3. Increase in exposure to rasburicase over days 1–5, as measured by AUC_{0-24} and C_{eoi}, was dose proportional. For the 1.33-fold increase in dose from 0.15 to 0.20 mg/kg, AUC_{0-24} increased 1.13-fold and 1.30-fold on days 1 and 5, respectively, while C_{eoi} increased 1.21-fold and 1.23-fold on days 1 and 5, respectively.

Rasburicase accumulated slightly on day 5, as assessed by AUC_{0-24} and C_{eoi}. The accumulation ratios of AUC_{0-24} and C_{eoi} (defined as the ratio of day 5 to day 1 for AUC_{0-24} and C_{eoi}) were 1.13 (95% CI 1.02–1.25) and 1.17 (95% CI 1.09–1.27), respectively, indicating slight accumulation. Mean $t_{1/2z}$ was comparable for both dose groups.

4 Discussion

The data from this study show that administration of rasburicase 0.15 or 0.20 mg/kg before the start of chemotherapy is well tolerated in Japanese pediatric patients with acute leukemia or non-Hodgkin's lymphoma. A rapid reduction in plasma uric acid levels to ≤7.5 mg/dL in patients ≥13 years or ≤6.5 mg/dL in patients <13 years within 48 h after the start of the first rasburicase administration occurred and lasted until 24 h after the last rasburicase administration on day 5 in 28 of 29 patients (96.6%). Moreover, 12 of 13 patients with hyperuricemia at baseline responded to treatment. A high overall RR of 96.6% was observed, indicating the efficacy of rasburicase for both the prophylaxis and treatment of hyperuricemia in pediatric patients receiving chemotherapy.

Notably, all evaluable patients in the rasburicase 0.20 mg/kg group achieved a response and only one evaluable patient in the 0.15 mg/kg group did not respond. In addition, there was a greater reduction in plasma uric acid concentrations from baseline at 4 h with the higher dose of rasburicase (92.9 vs. 84.8%), further demonstrating the greater efficacy of the rasburicase 0.20 mg/kg dose.

These findings add further credence to the results of the randomized US study conducted by Goldman et al. [12],

Table 3 Pharmacokinetic parameters after once-daily intravenous administration of rasburicase over 30 min (5-day treatment)

Rasburicase dose group (mg/kg)	Day 1		Day 5			
	AUC_{0-24} (ng h/mL)	C_{eoi} (ng/mL)	AUC_{0-24} (ng h/mL)	C_{eoi} (ng/mL)	$t_{½z}$ (h)	C_{min} (ng/mL)
0.15						
n	10	10	10	10	10	10
Mean (SD)	28,200 (7,270)	2,160 (512)	29,700 (6,460)	2,490 (373)	11.6 (5.0)	536 (218)
CV (%)	26	24	22	15	43	41
0.20[a]						
n	9[a]	10	9[a]	9[a]	9[a]	9[a]
Mean (SD)	31,500 (4,540)	2,580 (432)	38,100 (5,640)	3,050 (383)	11.2 (3.1)	780 (335)
CV (%)	14	17	15	13	27	43

[a] One patient in the rasburicase 0.20 mg/kg dose group had only two pharmacokinetic samples taken on day 1 because the patient withdrew from the study due to a low white blood cell count on day 1 after the first administration of rasburicase

AUC_{0-24} area under the rasburicase plasma concentration–time curve from 0 to 24 h, *C_{eoi}* plasma concentration of rasburicase at the end of infusion, *C_{min}* minimum rasburicase plasma concentration, *CV* coefficients of variation, *GM* geometric mean, *SD* standard deviation, *$t_{1/2z}$*, terminal half-life

which demonstrated more rapid control of uric acid and a lower plasma uric acid concentration during the first 96 h of therapy with rasburicase 0.20 mg/kg/day compared with 5–7 days of treatment with allopurinol in pediatric patients with high risk for TLS. In addition, several single-arm studies conducted in Europe, North America, Australia, and Asia have evaluated the 0.20 mg/kg dose of rasburicase for up to 7 days in pediatric and adult patients with high risk for TLS [17–21]. In line with our findings and those of Goldman et al. [12], these studies also reported numerically greater response rates (based on normalization of uric acid concentration) of 97–100% with rasburicase 0.20 mg/kg.

This is the first report to comprehensively assess rasburicase-related AEs occurring before the start of chemotherapy in pediatric patients. The majority of AEs reported during the treatment period were judged to be related to the underlying malignancies or chemotherapy by the investigators, with a low incidence of rasburicase-related AEs. Only two rasburicase-related AEs, including one hypersensitivity reaction, were observed before the start of chemotherapy in the rasburicase 0.15 mg/kg group. Most rasburicase-related AEs observed after the start of chemotherapy had a similar profile to those related to the underlying malignancies or chemotherapy. Patients who receive chemotherapy for hematologic malignancies are often exposed to risk of renal dysfunction. In the present study, renal parameters such as serum creatinine were not aggravated until completion of chemotherapy, suggesting that rasburicase might preserve renal function during induction chemotherapy.

Interestingly, new guidelines regarding the management of patients at risk of developing TLS and its prevention have recently been published [22]. Prevention strategies, including hydration and prophylactic rasburicase in high-risk patients, hydration plus allopurinol or rasburicase for intermediate-risk patients, and close monitoring for low-risk patients, are advised [22]. In addition, the guidelines advise aggressive hydration and diuresis plus allopurinol or rasburicase for hyperuricemia as primary management of established TLS.

An observational study has shown that treatment with rasburicase according to this new guideline is effective in preventing and controlling hyperuricemia and TLS in children with hematologic malignancies [23]. The study reported that the duration of rasburicase treatment should be tailored to the duration and intensity of tumor cell lysis in the patient by closely monitoring clinical chemistry. The superiority of rasburicase in comparison with allopurinol for the prophylaxis and treatment of hyperuricemia in children with leukemia and lymphoma has been demonstrated [24]. Rasburicase, administered at a dose of 0.20 mg/kg for 5 consecutive days, resulted in a rapid and significant decrease in uric acid levels after 4 h [24], in line with the findings reported in the current study. Rasburicase was also a more potent and more rapid uricolytic agent than allopurinol.

As rasburicase is a recombinant protein, antibodies can be produced against this agent. However, the clinical implication of such anti-rasburicase antibodies is unknown. In this trial, anti-rasburicase antibody production was reported in one patient on day 29, however, this patient did not experience a hypersensitivity reaction during the study. None of the patients had any anti-rasburicase antibodies on day 8. In previous studies in which rasburicase was administered to patients with cancer, although a small number of patients were shown to have anti-rasburicase antibodies, production of the antibody was not associated with the clinical status of the patients or the occurrence of AEs, including hypersensitivity reactions [25]. Goldman et al. [12] reported no cases of rasburicase antibody production in the US pediatric study using rasburicase 0.20 mg/kg, whereas Pui et al. [26] reported antibody production in 17 of 121 children and young adults treated with rasburicase 0.15 or 0.20 mg/kg. Other studies evaluating rasburicase did not assess rasburicase antibody production [18–21]. The production of anti-SCP antibody was also reported in one patient before the first administration of rasburicase. In this patient, a hypersensitivity reaction was not experienced during this study. This suggests that there was no correlation between the presence of anti-rasburicase or anti-SCP antibodies and the occurrence of hypersensitivity reactions in this study. However, because of the limited number of patients with antibody production in the current study, further studies are required in order to confirm this finding.

The pharmacokinetic data obtained in this study support the premise of dose proportionality of rasburicase, with only slight drug accumulation during 5 days of treatment. These data are consistent with the known pharmacokinetic profile of rasburicase in Western populations [26], suggesting that there is no ethnic variation in terms of the pharmacokinetic profile of rasburicase. Based on the results presented here, a daily rasburicase dose of 0.20 mg/kg might be recommended, particularly for patients who are more seriously ill and at high risk of developing TLS. However, as only a small sample size was studied in the present study and no comparator or placebo arm was included for comparison, further studies are needed to confirm the optimal dose of rasburicase for patients in different risk categories.

In conclusion, this study provides further evidence that rasburicase is highly effective in the control of hyperuricemia, a component of TLS, in pediatric patients undergoing chemotherapy for non-Hodgkin's lymphoma or acute leukemia. The study also demonstrates that rasburicase is safe and well tolerated when administered before the start of chemotherapy in this group of patients.

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References

1. Ronco C, Inguaggiato P, Bordonia V, De Cal M, Bonello M, Andrikos E, et al. Rasburicase therapy in acute hyperuricemia and renal dysfunction. Contrib Nephrol. 2005;147:115–23.
2. Jones DP, Stapleton FB, Kalwinsky D, McKay CP, Kellie SJ, Pui CH. Renal dysfunction and hyperuricemia at presentation and relapse of acute lymphoblastic leukemia. Med Pediatr Oncol. 1990;18:283–6.
3. Arrambide K, Toto RD. Tumor lysis syndrome. Semin Nephrol. 1993;13:273–80.
4. Davidson MB, Thakkar S, Hix JK, Bhandarkar ND, Wong A, Schreiber MJ. Pathophysiology, clinical consequences, and treatment of tumor lysis syndrome. Am J Med. 2004;116:546–54.
5. Cohen LF, Balow JE, Magrath IT, Poplack DG, Ziegler JL. Acute tumor lysis syndrome. A review of 37 patients with Burkitt's lymphoma. Am J Med. 1980;68:486–91.
6. Stapleton FB, Strother DR, Roy Ş, Wyatt RJ, McKay CP, Murphy SB. Acute renal failure at onset of therapy for advanced stage Burkitt lymphoma and B cell acute lymphoblastic lymphoma. Pediatrics. 1988;82:863–9.
7. Bowman WP, Shuster JJ, Cook B, Griffin T, Behm F, Pullen J, et al. Improved survival for children with B-cell acute lymphoblastic leukemia and stage IV small noncleaved-cell lymphoma: a pediatric oncology group study. J Clin Oncol. 1996;14:1252–61.
8. Cheson BD, Dutcher BS. Managing malignancy-associated hyperuricemia with rasburicase. J Support Oncol. 2005;3:117–24.
9. Sood AR, Burry LD, Cheng DK. Clarifying the role of rasburicase in tumor lysis syndrome. Pharmacotherapy. 2007;27:111–21.
10. Legoux R, Delpech B, Dumont X, Guillemot J-C, Ramond P, Shire D, et al. Cloning and expression in *Escherichia coli* of the gene encoding *Aspergillus flavus* urate oxidase. J Biol Chem. 1992;267:8565–70.
11. Oldfield V, Perry CM. Spotlight on rasburicase in anticancer therapy-induced hyperuricemia. BioDrugs. 2006;20:197–9.
12. Goldman SC, Holcenberg JS, Finklestein JZ, Hutchinson R, Kreissman S, Johnson FL, et al. A randomized comparison between rasburicase and allopurinol in children with lymphoma or leukemia at high risk for tumor lysis. Blood. 2001;97:2998–3003.
13. Bessmertny O, Robitaille LM, Cairo MS. Rasburicase: a new approach for preventing and/or treating tumor lysis syndrome. Curr Pharm Des. 2005;11:4177–85.
14. Ishizawa K, Ogura M, Hamaguchi M, Hotta T, Ohnishi K, Sasaki T, et al. Safety and efficacy of rasburicase (SR29142) in a Japanese phase II study. Cancer Sci. 2008 Dec 14. [Epub ahead of print].
15. Graybill FA, Wang C. Confidence intervals on nonnegative linear combinations of variances. J Am Stat Assoc. 1980;75:869–73.
16. Kizer N, Martinez E, Powell M. Report of two cases of rasburicase-induced methemoglobinemia. Leuk Lymphoma. 2006;47:2648–50.
17. Pui CH, Jeha S, Irwin D, Camitta B. Recombinant urate oxidase (rasburicase) in the prevention and treatment of malignancy-associated hyperuricemia in pediatric and adult patients: results of a compassionate-use trial. Leukemia. 2001;15:1505–9.
18. Bosly A, Sonet A, Pinkerton CR, McCowage G, Bron D, Sanz MA, et al. Rasburicase (recombinant urate oxidase) for the management of hyperuricemia in patients with cancer: report of an international compassionate use study. Cancer. 2003;98:1048–54.
19. Jeha S, Kantarjian H, Irwin D, Shen V, Shenoy S, Blaney S, et al. Efficacy and safety of rasburicase, a recombinant urate oxidase (Elitek™), in the management of malignancy-associated hyperuricemia in pediatric and adult patients: final results of a multicenter compassionate use trial. Leukemia. 2005;19:34–8.
20. Shin HY, Kang HJ, Park ES, Choi HS, Ahn HS, Kim SY, et al. Recombinant urate oxidase (rasburicase) for the treatment of hyperuricemia in pediatric patients with hematologic malignancies: results of a compassionate prospective multicenter study in Korea. Pediatr Blood Cancer. 2006;46:439–45.
21. Wang L-Y, Shih LY, Chang H, Jou ST, Lin KH, Yeh TC, et al. Recombinant urate oxidase (rasburicase) for the prevention and treatment of tumor lysis syndrome in patients with hematologic malignancies. Acta Haematol. 2006;115:35–8.
22. Coiffier B, Altman A, Pui CH, Younes A, Cairo MS. Guidelines for the management of pediatric and adult tumor lysis syndrome: an evidence-based review. J Clin Oncol. 2008;26:2767–78.
23. Bertrand Y, Mechinaud F, Brethon B, Mialou V, Auvrignon A, Nelken B, et al. for SFCE (Société Française de Lutte contre les Cancers et Leucémies de l'Enfant et de l'Adolescent) recommendations for the management of tumor lysis syndrome (TLS) with rasburicase: an observational survey. J Pediatr Hematol Oncol. 2008;30:267–71.
24. Rényi I, Bárdi E, Udvardi E, Kovács G, Bartyik K, Kajtár P, et al. Prevention and treatment of hyperuricemia with rasburicase in children with leukemia and non-Hodgkin's lymphoma. Pathol Oncol Res. 2007;13:57–62.
25. Pui CH. Rasburicase: a potent uricolytic agent. Expert Opin Pharmacother. 2002;3:433–42.
26. Pui CH, Mahmoud HH, Wiley JM, Woods GM, Leverger G, Camitta B, et al. Recombinant urate oxidase for the prophylaxis or treatment of hyperuricemia in patients with leukemia or lymphoma. J Clin Oncol. 2001;19:697–704.