研究成果刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者	書 籍 名	出版社名	出版地	出版年	ページ
奥山 虎之	小児希少疾病における医薬品開発の課題		レギュラトリーサイエンス	医薬品医療機器レギュラトリーサイエンス財団		2014	49-53
中村秀文, 大澤真木子, 横山輝路, 吉田克己, 鈴木淳	日本人小児部分てんかんに対するレベチラセタム併用療法の有効性と安全性の検討 多施設共同非盲検試験（N01223）14週間での評価		BRAIN and NERVE	医学書院			

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Go Tajima, Nobuo Sakura, Motomichi Kosuga, Torayuki Okuyama, Masao Kobayashi	Effects of idursulfase enzyme replacement therapy for Mucopolysaccharidosis type II when started in early infancy:comparison in two siblings.	Mol Genet Metab	108	172-177	2013
Iijima K, Sako M, Saito Oba M, Ito S, Hataya H, Tanaka R, Ohwada Y, Kamei K, Ishikura K, Yata N, Nozu K, Honda M, Nakamura H, Nagata M, Ohashi Y, Nakanishi K, and Yoshikawa N,	Study Group of Kidney Disease in Children: Cyclosporine C2 Monitoring for the Treatment of Frequently Relapsing Nephrotic Syndrome in Children:A Multicenter Randomized Phase II Trial.	Clinical Journal of the American Society of Nephrology	9(2)	1-8	2014

Tahara T, Asano Y, Mitamura K, Nakamura H, Itoh S:	Safty of oseltamivir in infants less than one year old: Prospective surveillance during the 2004-2005 influenza season in Japan.	Journal of Pediatric Infectious Diseases	8(2)	71-81	2013
中村秀文, 大澤真木子, 横山輝路, 吉田克己, 鈴木淳	日本人小児部分てんかんに対するレベチラセタム併用療法の有効性と安全性の検討 多施設共同非盲検試験（N01223）14 週間での評価	BRAIN and NERVE	65：9	1083-1092	2013
中村秀文：	小児用薬開発を巡る国際的現状とわが国の課題	医薬品医療機器レギュラトリーサイエンス	44：5	00-403	2013
中村秀文	小児の特徴と現場における小児用量の考え方	調剤と情報	20：2	12-17	2014
Ueno H, Okita H, Akimoto S, Kobayashi K, Nakabayashi K, Hata K, Fujimoto J, Hata J, Fukuzawa M, Kiyokawa N.	DNA methylation profile distinguishes clear cell sarcoma of the kidney from other pediatric renal tumors.	PLoS One	Apr 26;8(4):e62233.		2013
Kiyokawa N, Iijima A, Tomita O, Miharu M, Hasegawa D, Kobayashi K, Okita H, Kajiwara M, Shimada H, Inukai T, Makimoto A, Fukushima T, Nanmoku T, Koh K, Manabe A, Kikuchi A, Sugita K, Fujimoto J, Hayashi Y and Ohara A.	Significance of CD66c expression in childhood acute lymphoblastic leukemia	Leukemia Res	in press.		

Enosawa S; Horikawa R; Yamamoto A, Sakamoto S, Shigeta T, Nosaka S, Fujimoto J, Tanoue A, Nakamura K, Umezawa A, Matsubara Y, Matsui A and Kasahara M.	Hepatocyte transplantation using the living donor reduced-graft in a baby with ornithine transcarbamylase deficiency:	a novel source for hepatocytes. Liver Transplant.	in press.		
Tsurusawa M, Mori T, Kikuchi A, Mitsui T, Sunami S, Kobayashi R, Takimoto T, Saito A, Watanabe T, Fujimoto J, Nakazawa A, Ohshima K, and Horibe K	for the lymphoma committee of the Japanese Pediatric Leukemia/Lymphoma Study Group. Improved treatment results of children with B-cell non-Hodgkin lymphoma: A report from the Japanese Pediatric Leukemia/Lymphoma Study Group B-NHL03 study.	Pediatr Blood Cancer	Feb 13.	doi: 10.1002/ pbc.2497 5. [Epub ahead of print]	

研究成果の刊行物・別刷り

Molecular Genetics and Metabolism 108 (2013) 172–177

Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Effects of idursulfase enzyme replacement therapy for Mucopolysaccharidosis type II when started in early infancy: Comparison in two siblings

Go Tajima [a], Nobuo Sakura [a], Motomichi Kosuga [b], Torayuki Okuyama [b,*], Masao Kobayashi [a]

[a] Department of Pediatrics, Hiroshima University Hospital, Hiroshima, Japan
[b] Center for Lysosomal Storage Diseases, National Center for Child Health and Development, Tokyo, Japan

ARTICLE INFO

Article history:
Received 26 December 2012
Accepted 26 December 2012
Available online 9 January 2013

Keywords:
Mucopolysaccharidosis type II
Enzyme replacement therapy

ABSTRACT

Mucopolysaccharidosis type II (MPS II) is a lysosomal storage disorder that is progressive and involves multiple organs and tissues. While enzyme replacement therapy (ERT) with idursulfase has been shown to improve many somatic features of the disease, some such as dysostosis multiplex and cardiac valve disease appear irreversible once established, and little is known about the preventative effects of ERT in pre-symptomatic patients. We report on two siblings with severe MPS II caused by an inversion mutation with recombination breakpoints located within the *IDS* gene and its adjacent pseudogene, *IDS-2*. The siblings initiated treatment with idursulfase at 3.0 years (older brother) and 4 months (younger brother) of age, and we compared their outcomes following 2 years of treatment. At the start of treatment, the older brother showed typical features of MPS II, including intellectual disability. After 34 months of ERT, his somatic disease was stable or improved, but he continued to decline cognitively. By comparison, after 32 months of ERT his younger brother remained free from most of the somatic features that had already appeared in his brother at the same age, manifesting only exudative otitis media. Skeletal X-rays revealed characteristic signs of dysostosis multiplex in the older brother at the initiation of treatment that were unchanged two years later, whereas the younger brother showed only slight findings of dysostosis multiplex throughout the treatment period. The younger brother's developmental quotient trended downward over time to just below the normal range. These findings suggest that pre-symptomatic initiation of ERT may prevent or attenuate progression of the somatic features of MPS II. Follow-up in a larger number of patients is required to confirm the additive long-term benefits of ERT in pre-symptomatic patients.

1. Introduction

Mucopolysaccharidosis (MPS) type II (Hunter syndrome, OMIM #309900), is an inborn error of glycosaminoglycan (GAG) metabolism caused by deficient activity of lysosomal iduronate 2-sulfatase (IDS, EC 3.1.6.13). The responsible gene, IDS, is located on chromosome Xq28, and the disease shows classic X-linked recessive inheritance. Rarely, females may be affected as a result of biallelic mutations, skewed X-inactivation, uniparental isodisomy, or X-autosome translocations [1,2]. Dermatan sulfate and heparan sulfate, the substrates for IDS, accumulate in the lysosomes of various tissues and organs of affected patients, leading to the development of characteristic signs and symptoms of MPS II after the first year of life. (HOS reference). Somatic features include coarse facies, straw-like hair, rough and thickened skin, macrocephaly, disproportionate short stature due to dysostosis multiplex, decreased joint mobility, cardiac valve disease and left ventricular hypertrophy, hepatosplenomegaly, obstructive sleep apnea, and restrictive lung disease. Frequent otitis media and hernias (inguinal and umbilical) may be the earliest presenting signs, but are non-specific. Patients with little to no IDS activity (severe form) exhibit progressive somatic disease, cognitive decline, and death during adolescence (HOS). Patients with some residual IDS activity (mild form) have largely somatic disease with normal intellectual development [3].

In recent years, enzyme replacement therapy (ERT) with recombinant human iduronate-2sulfatase (idursulfase, Elaprase®, Genzyme, a Sanofi Company and Shire Human Genetic Therapies, Cambridge, MA) has been available for the treatment of MPS II. Weekly infusions of idursulfase have been shown to improve walking capacity, hepatosplenomegaly, and urinary GAG levels [4]. However, ERT appears to be less effective in correcting disease manifestations once developed in the skeletal system and heart valves [5,6]. Intravenously administered ERT has not been shown to slow or prevent the deterioration of the central nervous system in patients with the severe phenotype, most likely because it does not cross blood–brain barrier at the labeled dose [7]. Although idursulfase is approved for use only in patients who are at least 5 years of age, a recent report from the Hunter Outcome Survey (HOS) suggests that it can safely reduce urinary GAG levels and hepatomegaly in young children, some

* Corresponding author. Fax: +81 3 3417 2238.
E-mail address: tora@nch.go.jp (T. Okuyama).

1096-7192/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ymgme.2012.12.010

of whom were below 1 year of age [8]. Recognizing that MPS II is a progressive disease that has some irreversible features, a panel of MPS II experts has recommended starting ERT as early as possible to achieve the best outcomes [9].

In this report, we describe our treatment experience in two Japanese brothers with the severe form of MPS II who started ERT at 4 months of age (pre-symptomatic) and 3 years of age (symptomatic). Our findings suggest that early, pre-symptomatic treatment is associated with a better clinical outcome as evidenced by the amelioration or prevention of certain somatic manifestations, e.g. dysostosis multiplex and cardiac valve disease, which once established, appear to be irreversible.

1.1. Case report

1.1.1. Patient 1 (older brother)

A 2 year 7 month old boy presented to our metabolism clinic with dysmorphic features, cardiac and skeletal disease, and severe developmental delay. He was the first child born to non-consanguineous Japanese parents. Following an uneventful pregnancy and neonatal period, he was noted to have a small ventricular septal defect during a febrile illness at 3 months of age. At 9 months of age, the ventricular septal defect had closed but mild mitral valve regurgitation was present. His parents noticed a gibbus deformity at approximately 1 year of age, and by age 2 he had developed stiffness in his elbow and fingers. His psychomotor development was moderately delayed: he walked at 1.5 years and was still non-verbal. Other past medical history was notable for a febrile seizure, umbilical hernia, enlarged adenoids, and bilateral otitis media. On physical examination, the boy had a coarse facies and disproportionately short limbs. His was above average in height (92.2 cm, +0.6 SD), overweight (17.0 kg, +3.2 SD), and had macrocephaly (50 cm, +0.5 SD). He had marked hepatomegaly and a nonpalpable spleen. Urinary GAG analysis revealed an elevated uronic acid level of 254 mg/g creatinine (normal mean ± SD, 30.0 ± 12.8) with increased amounts of dermatan sulfate (63%) and heparan sulfate (12%) relative to chondroitin sulfate (25%), consistent with MPS I or II. The diagnosis of MPS II was confirmed by the absence of detectable I2S activity in leukocytes.

No potential disease-causing mutation was found by sequencing all 9 exons of the IDS gene and their intron-exon junctions by conventional PCR-based methods [10]. To detect a recombination mutation between IDS and its adjacent putative pseudogene, IDS-2, that leads to an inversion and non-functional *IDS* gene, we performed a simple and rapid assay involving two PCR reactions. The first reaction selectively amplifies a 2.8 kb DNA fragment from the recombinant gene but not the wild type IDS gene, while the second reaction selectively amplifies a 3.5 kb DNA fragment from the wild type IDS gene but not the recombinant gene (Fig 1a) [11]. Genetic testing of the patient revealed an abnormal banding pattern indicative of recombination between the IDS gene and the IDS-2 pseudogene (Fig 1b).

1.1.2. Patient 2 (younger brother)

The younger brother was born just after his older brother was diagnosed with MPS II. Birth weight (2.966 kg) and length (47 cm) were normal for his gestational age of 39 weeks. There were no abnormal findings on initial physical examination, but the urinary uronic acid level was elevated at 423 mg/g creatinine (normal mean ± SD, 43.4 ± 12.9), and urinary GAG analysis showed increased amounts of dermatan sulfate (55%) and heparan sulfate (11%) relative to chondroitin sulfate (34%). IDS activity in leukocytes was below the detectable limit. As expected, Patient 2 had the same recombination mutation as his older brother.

1.1.2.1. Enzyme replacement therapy. Treatment with intravenous recombinant idursulfase was started at 3.0 years of age for Patient 1 and 4 months of age for Patient 2. Although the recommended dose of idursulfase is 0.5 mg/kg/week, Patient 1 received only 0.3–0.4 mg/kg/week for the first 1.5 years until his weight reached 20 kg (4.5 years of age) because of a restriction by the health insurance system; subsequently, he received 0.5 mg/kg/week of idursulfase. The dose for Patient 2 was 0.5 mg/kg/week from the start of treatment. As of December 2012, Patients 1 and 2 had received ERT for 34 and 32 months, respectively. Both patients have tolerated ERT well with only mild and intermittant urticaria.

2. Results

2.1. Urinary GAG

The uronic acid in urine was measured at several time points after initiation of ERT using the carbazole reaction method (SRL Medisearch, Tokyo, Japan). Fig. 2 shows the changes observed in both patients over time. In Patient 1, the uronic acid level decreased to approximately half of the baseline level after 3 months and then plateaued at 130–180 mg/g creatinine (29–49% reduction from baseline) (Fig. 2a). The uronic acid level in Patient 2 showed a continuous decrease to below 100 mg/g creatinine (76% reduction from baseline), but remains above the normal range (Fig. 2b).

2.2. Liver and spleen size

The liver edge of Patient 1 extended 4 cm below the right costal margin at baseline, and it rapidly became non-palpable after the initiation of ERT. The spleen was not palpable at any time, and by ultrasound, it was at the upper limit of normal size for age and remained stable during the first 28 months of ERT. Patient 2's liver and spleen were normal in size before and during ERT.

2.3. Cardiac function

At baseline, Patient 1's echocardiogram revealed moderate mitral valve regurgitation and a mildly distorted left ventricular wall, although the ejection fraction was normal at 69 %. These findings showed little change after 22 months of ERT. In Patient 2, no abnormalities were detected by echocardiography before and after 11 months of ERT.

2.4. Respiratory and Hearing

Patient 1 had bilateral exudative otitis media and adenoid hypertrophy at baseline that did not respond well to ERT. Although an adenoidectomy was performed at 3.5 years of age, exudative otitis media and hearing impairment persisted. Patient 2 also had exudative otitis media during the ERT period. Neither patient developed sleep apnea.

2.5. Skeletal X-rays

At baseline, dysostosis multiplex was already apparent in Patient 1. The most prominent findings were hypoplastic changes of the vertebral bodies giving rise to a characteristic protrusion of the antero-inferior surface, the so-called inferior tongue. Other mild signs of dysostosis multiplex included oar-like ribs, bullet-shaped phalanges, and iliac flaring. After 27 months of ERT, these findings showed little change. Similar, but milder findings of oar-like ribs and bullet-shaped phalanges were present in Patient 2 at 3 months of age. After 25 months of ERT, "inferior tongue" had become notable and oar-like ribs had progressed (data not shown).

2.6. Joints

Patient 1 had stiffness in multiple joints of his extremities at baseline. There was no obvious change with ERT, although accurate

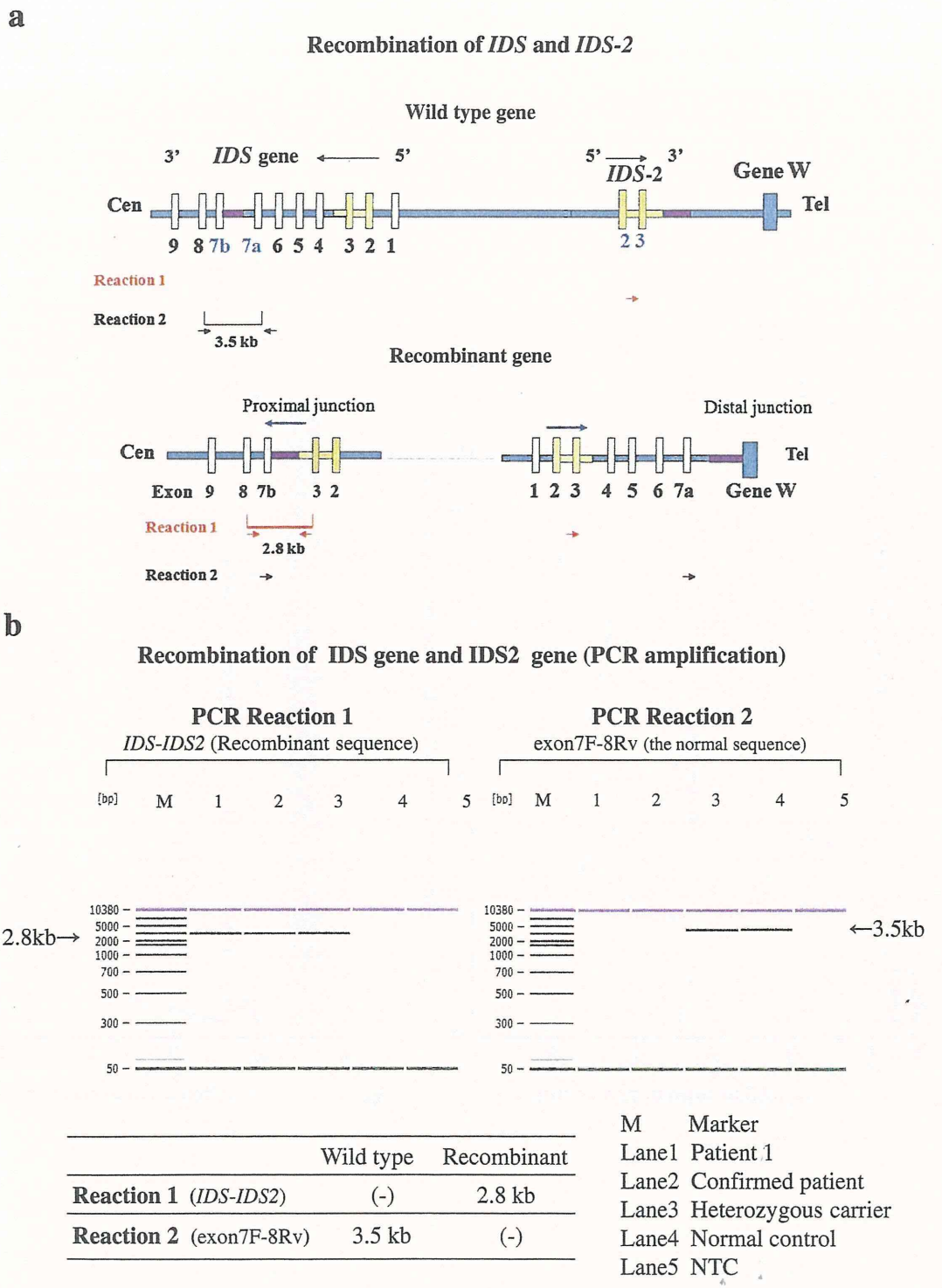
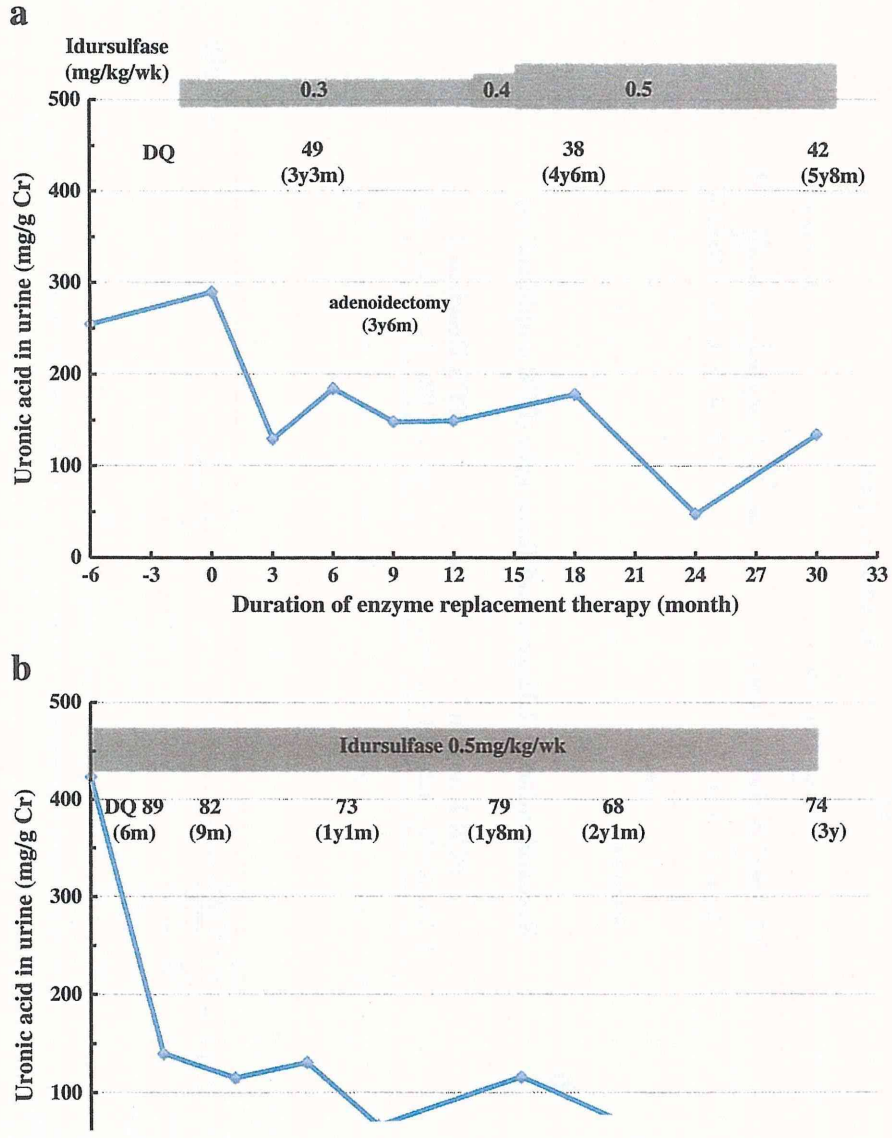


Fig. 1. Genetic diagnosis of MPS II by detecting recombination of the IDS and IDS-2 genes. The pseudogene IDS-2, which consists of sequences that are homologous to exons 2 and 3 and intron 7 of the IDS gene, is located ~20 kb telomeric to *IDS* in Xq27.3–q28. In the recombinant gene, exons 1, 4, 5, 6, 7a are translocated to the IDS-2 locus, thereby grossly altering the structure of the IDS gene. PCR reaction 1 amplified a 2.8 kb fragment of the recombinant gene in Patients 1 and 2 and the heterozygous carrier, but not in the normal control. PCR reaction 2 amplified a 3.5 kb fragment of the wild-type *IDS* gene in the heterozygous carrier and normal control, but not in the two patients.

measurement was difficult. Patient 2 had normal joint mobility that was maintained during ERT.

3. Magnetic resonance imaging of the central nervous system

By MRI, Patient 1 had dilated perivascular spaces in the cerebral white matter both at baseline and after 22 months of ERT, and at the latter timepoint, mild dilatation of the lateral ventricles also was apparent. At baseline, Patient 2's MRI showed only subtle changes in the corpus callosum that were suggestive of dilated perivascular spaces. After 14 months ERT, the dilated perivascular spaces became more typical and resembled those of his brother. Patient 2 did not show any evidence of hydrocephalus or cerebral atrophy (Data not shown).


a
Idursulfase
(mg/kg/wk)
0.3
0.4
0.5
DQ
49
(3y3m)
38
(4y6m)
42
(5y8m)
adenoidectomy
(3y6m)
Uronic acid in urine (mg/g Cr)
500
400
300
200
100
0
-6
-3
0
3
6
9
12
15
18
21
24
27
30
33
Duration of enzyme replacement therapy (month)
b
Idursulfase 0.5mg/kg/wk
DQ 89
(6m)
82
(9m)
73
(1y1m)
79
(1y8m)
68
(2y1m)
74
(3y)
Uronic acid in urine (mg/g Cr)
500
400
300
200
100

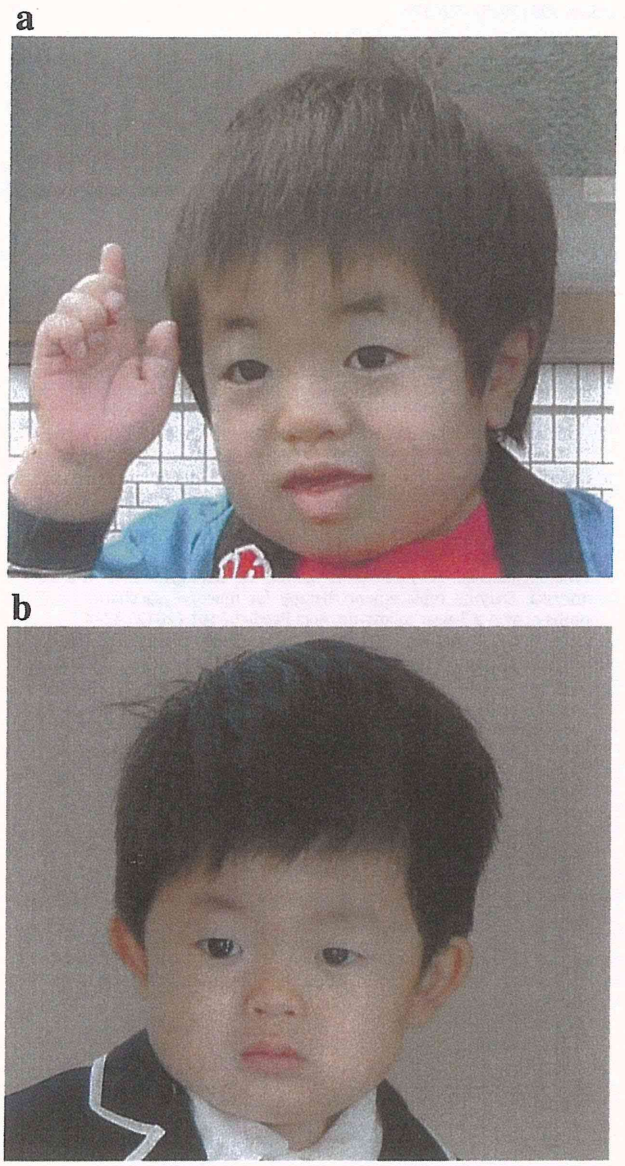


Fig. 3. Facial appearances of the brothers. (a) Patient 1 at the age of 2 years and 9 months, before initiation of ERT. (b) Patient 2 at the age of 2 years and 10 months, after 31 months of ERT.

Patient 1 (Fig. 3a; 2 years and 9 months old). Patient 1 had coarse features affecting his nose, lips, and tongue, whereas Patient 2 did not show any MPS II-related facial features.

4. Discussion

Since idursulfase ERT for MPS II became commercially available (2006 in the US; 2007 in the European Union and Japan), there has been an increasing number of reports on its clinical effects. Idursulfase has been shown to improve walking capacity while reducing hepatosplenomegaly and urinary GAG levels. The most common adverse events have been infusion-related reactions, including some reports of anaphylactic reactions [5]. However, most of the treatment effects described to date have been in patients above age 5 who manifested typical symptoms of MPS II before initiation of ERT [12,13]. The results suggest that once established, pathological changes in certain organs and tissues, e.g. the bones, joints, heart valves, and central nervous system are difficult to correct [9]. A recent analysis of the effects of ERT in patients younger than 6 years old enrolled in the Hunter Outcome Survey (HOS) has shown a similar safety profile and reduction in hepatomegaly as in older patients [8]. Of these 124 children treated ERT, 11 initiated treatment during the first year of life, and the youngest treated was 1 month of age. However, no individual outcome data have been reported.

There is limited information on the ability of idursulfase to prevent the occurrence of disease manifestations in pre-symptomatic MPS II patients. MPS II is difficult to diagnose in early infancy before the development of typical signs and symptoms due to the insidious progression of disease [14,15]. The few patients that have been diagnosed early usually had a previously affected relative that prompted pre-symptomatic testing, as was the case for our siblings. Only one recent case report has described the effects of idursulfase ERT initiated in an asymptomatic infant with MPS II [16]. This boy was diagnosed at 14 days of life on the basis of an older affected sister, who interestingly, was found to have low IDS activity and be heterozygous for a missense mutation, p.Tyr523Cys/c.1568A>G in exon 9, with almost totally skewed X-inactivation of the normal *IDS* gene. Idursulfase (0.5 mg/kg/wk) was initiated at 3 months of life and 3-year follow-up was provided. The affected boy did not develop coarse facial features, joint disease, or organomegaly, and his cardiac function remained normal; the only abnormal finding was a mild deformity of one vertebrae. In contrast, the older sister showed typical clinical features of MPS II when she was diagnosed at age 3, including severe intellectual disability (IQ=50) that worsened over time (IQ=24 at age 10) despite 5 years of ERT. Considering her severe phenotype, it is surprising that her affected brother has maintained a normal IQ of 98 at 3 years of age. An earlier report had described this mutation as mild [17]. It is possible that the sister had other unknown central nervous system complications or effects of skewed X-inactivation that affected her cognitive status, or that the original assignment as a mild mutation was incorrect. Another possibility is that ERT started in early infancy had a protective effect on the central nervous system, but animal data suggest that intravenously administered idursulfase is unable to cross the blood–brain barrier at this dose.

Our experience has been similar to this recent case report, with a better outcome observed when treatment was initiated at 4 months of age instead of at 3 years of age, a difference of 2.7 years. The reduced dose that the older brother received for the initial 15 months of treatment may have contributed to some of the differences in outcomes. Nevertheless, somatic symptoms were present in Patient 1 before 2 years of age, but none were seen in Patient 2 at the same age except for possibly exudative otitis media. The only other somatic finding has been slight signs of dysotosis multiplex by X-ray. The prognosis for his mental development seems less promising, given the gradual decline in DQ from normal to slightly below normal. Although hearing problems due to chronic exudative otitis media may have contributed to the apparent decline in DQ, it has been reported that speech development is less affected in patients with mild compared to severe MPS II despite similar otological findings [18]. The inversion mutation is predicted to be a severe mutation that leads to a non-functional *IDS* gene, and in one series it was present in 13% of boys with MPS II [19]. Treatment options to prevent further deterioration of his intellectual abilities appear limited at this time. Previous reports on the therapeutic effects of hematopoietic stem cell transplantation (HSCT) in MPS II patients have generally been negative, but most patients had pre-existing CNS disease and little clinical data exists on the use of this procedure as a preventative measure in patients with normal cognitive function [20]. According to a recent report, donor-derived cells were detected in the brain of a transplanted MPS II patient [21]. To determine whether HSCT may be beneficial to MPS II patients at risk for CNS involvement, additional data must be collected on cases in which HSCT is performed as early as possible. Intrathecal delivery of ERT to treat MPS II-related CNS disease is currently being investigated in an ongoing Phase 1 clinical trial, and the results have not yet been published.

In summary, this is the second detailed case report of idursulfase ERT started in early infancy in a patient with MPS II. In contrast to

the older brother who had typical features of MPS II at the initiation of ERT that did not completely resolve after 2 years of treatment, we believe that the near absence of somatic findings in the younger brother after 2 years of treatment is attributable to early ERT administered in the pre-symptomatic state. The effect of early ERT on the younger brother's intellectual development is less clear. Long-term observation of these and other similar cases should help to clarify the extent of the preventative effects of ERT on the somatic and CNS aspects of MPS II as well as to define the optimal timing of treatment to achieve the best possible outcomes.

References

[1] K. Tuschl, A. Gal, E. Paschke, S. Kircher, O.A. Bodamer, Mucopolysaccharidosis type II in females: case report and review of literature, Pediatr. Neurol. 32 (2005) 270–272.

[2] L.L. Pinto, T.A. Vieira, R. Giugliani, I.V. Schwartz, Expression of the disease on female carriers of X-linked lysosomal disorders: a brief review, Orphanet J. Rare Dis. 5 (2010) 14–23.

[3] J.B. Holt, M.D. Poe, M.L. Escolar, Natural progression of neurological disease in mucopolysaccharidosis type II, Pediatrics 127 (2011) 1258–1265.

[4] J. Muenzer, J.E. Wraith, M. Beck, R. Giugliani, P. Harmatz, C.M. Eng, A. Vellodi, R. Martin, U. Ramaswami, M. Gucsavas-Calikoglu, S. Vijayaraghavan, S. Wendt, A.C. Puga, B. Ulbrich, M. Shinawi, M. Cleary, D. Piper, A.M. Conway, A. Kimura, A phase II/III clinical study of enzyme replacement therapy with idursulfase in mucopolysaccharidosis II (Hunter syndrome), Genet. Med. 8 (2006) 465–473.

[5] L.A. Clarke, Idursulfase for the treatment of mucopolysaccharidosis II, Expert. Opin. Pharmacother. 9 (2008) 311–317.

[6] J. Muenzer, M. Beck, C.M. Eng, R. Giugliani, P. Harmatz, R. Martin, U. Ramaswami, A. Vellodi, J.E. Wraith, M. Cleary, M. Gucsavas- Calikoglu, A.C. Puga, M. Shinawi, B. Ulbrich, S. Vijayaraghavan, S. Wendt, A.M. Conway, A. Rossi, D.A. Whiteman, A. Kimura, Long-term, open-labeled extension study of idursulfase in the treatment of Hunter syndrome, Genet. Med. 13 (2011) 95–101.

[7] S.A. Sawaf, E. Mayatepek, B. Hoffmann, Neurological findings in Hunter disease: pathology and possible therapeutic effects reviewed, J. Inherit. Metab. Dis. 31 (2008) 473–480.

[8] J. Muenzer, M. Beck, R. Giugliani, Y. Suzuki, A. Tylki-Szymanska, V. Valayannopoulos, A. Vellodi, J.E. Wraith, Idursulfase treatment of Hunter syndrome in children younger than 6 years: results from the Hunter Outcome Survey, Genet Med 13 (2011) 102–109.

[9] J.E. Wraith, M. Scarpa, M. Beck, O.A. Bodamer, L. De Meirleir, N. Guffon, A. Meldgaard Lund, G. Malm, A.T. Van der Ploeg, J. Zeman, Mucopolysaccharidosis type II (Hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy, Eur. J. Pediatr. 167 (2008) 267–277.

[10] J.J. Hopwood, S. Bunge, C.P. Morris, P.J. Wilson, C. Steglich, M. Beck, E. Schwinger, A. Gal, Molecular basis of mucopolysaccharidosis type II: mutation in the iduronate-2-sulfatase gene, Hum. Mutat. 2 (1993) 435–442.

[11] K. Lagerstedt, S.L. Karsten, B.M. Carlberg, W.J. Kleijer, T. Tönnesen, U. Pettersson, M.L. Bondeson, Double-strand breaks may initiate the inversion mutation causing the Hunter syndrome, Hum. Mol. Genet. 6 (1997) 627–633.

[12] J. Muenzer, M. Gucsavas-Calikoglu, S.E. McCandless, T.J. Schuetz, A. Kimura, A phase I/II clinical trial of enzyme replacement therapy in mucopolysaccharidosis II (Hunter syndrome), Mol. Genet. Metab. 90 (2007) 329–337.

[13] T. Okuyama, A. Tanaka, Y. Suzuki, H. Ida, T. Tanaka, G.F. Cox, Y. Eto, T. Orii, Japan Elaprase® Treatment (JET) study: idursulfase enzyme replacement therapy in adult patients with attenuated Hunter syndrome (Mucopolysaccharidosis II, MPS II), Mol. Genet. Metab. 99 (2010) 18–25.

[14] E. Glamuzina, Fettes e, Bainbridge K, Crook V, Finnegan N, Abulhoul L, Vellodi A: Treatment of mucopolysaccharidosis type II (Hunter syndrome) with idursulfase: the relevance of clinical trial end points, J. Inherit. Metab. Dis. 34 (2011) 749–754.

[15] C. Alcalde-Martín, J.M. Muro-Tudelilla, R. Cancho-Candela, L.G. Gutiérrez-Solana, G. Pintos-Morell, M. Martí-Herrero, P. Munguira-Aguado, E. Galán-Gómez, First experience of enzyme replacement therapy with idursulfase in Spanish patients with Hunter syndrome under 5 years of age: Case observations from the Hunter Outcome Survey (HOS), Eur. J. Med. Genet. 53 (2010) 371–377.

[16] A. Tylki-Szymanska, A. Jurecka, Z. Zuber, A. Rozdzynska, J. Marucha, B. Czartoryska, Enzyme replacement therapy for mucopolysaccharidosis II from 3 months of age: a 3-year follow-up, Acta Paediatr. 101 (2012) 42–47.

[17] J.J. Jonsson, E.L. Aronovich, S.E. Braun, C.B. Whitley, Molecular diagnosis of mucopolysaccharidosis type II (Hunter syndrome) by automated sequencing and computer-assisted interpretation: toward mutation mapping of the iduronate-2-sulfatase gene, Am. J. Hum. Genet. 56 (1995) 597–607.

[18] Y.S. Cho, J.H. Kim, T.W. Kim, S.C. Chung, S.A. Chang, D.K. Jin, Otologic manifestations of Hunter syndrome and their relationship with speech development, Audiol. Neurotool. 13 (2008) 206–212.

[19] M.L. Bondeson, N. Dahl, H. Malmgren, W.J. Kleijer, T. Tönnesen, B.M. Carlberg, U. Pettersson, Inversion of the IDS gene resulting from recombination with IDS-related sequences is a common cause of the Hunter syndrome, Hum. Mol. Genet. 4 (1995) 615–621.

[20] N. Guffon, Y. Bertrand, I. Forest, A. Fouilhoux, R. Froissart, Bone marrow transplantation in children with Hunter syndrome: Outcome after 7 to 17 years, J. Pediatr. 154 (2009) 733–737.

[21] K. Araya, N. Sakai, I. Mohri, K. Kagitani-Shimono, T. Okinaga, Y. Hashii, H. Ohta, I. Nakamichi, K. Aozasa, M. Taniike, K. Ozono, Localized donor cells in brain of a Hunter disease patient after cord blood stem cell transplantation, Mol. Genet. Metab. 98 (2009) 255–263.

RSMP vol.4 no.1, 49-53, Jan 2014

特集（希少疾病用医薬品・医療機器の開発促進）

小児希少疾病における医薬品開発の課題

New Drug Development for Pediatric Ultra-are Diseases

奥山　虎之
Torayuki OKUYAMA

Abstract

In general, we call a disease having less than fifty thousand patients in Japan as a rare disease. However, in pediatric field, there are lots of diseases having less than several hundred patients. We call such kind of a disease as an ultra-rare disease. In Europe and USA, many drugs have been developed for several ultra-rare diseases. Since it was not easy to participate in the international clinical trial, we had a serious problem of drug lag in Japan. But recently we have successfully joined in the clinical trial of the drug for the enzyme replacement therapy of mucopolysaccharidosis type IV. To avoid the problem of drug lag, it is essential to participate in the international clinical trial. Data base of clinical information of ultra-rare diseases is also very important.

抄　録

希少疾患とは，おおむね罹患者が５万人以下の疾患のことである．しかし，小児科領域においては，患者数が10人から100人程度の疾患，すなわち ultra-rare disease（超希少疾患）が少なくない．近年，超希少疾患の治療医薬品の開発が欧米を中心に進んでいる．我が国においては，このような薬剤の開発スキームに乏しく，それが深刻なドラッグラグの原因となっていた．しかし，ムコ多糖症Ⅳ型酵素製剤の国際共同治験に日本が参加しえたことを契機に，今後，多数の超希少疾患の臨床開発が国際共同治験で行われることが期待される．そのためには，患者登録と超希少疾患のデータベース作成も併せて進める必要がある．

Key words: ultra-rare disease, mucopolysaccharidosis, enzyme replacement therapy, international clinical trial

はじめに

一般に，希少疾患というと，国内患者数がおおむね５万人以下の疾患をさす．しかし，小児科領域においては，全国患者数が数100人程度の疾患も少なくない．場合によっては，10人以下という疾患もある．これらの疾患は ultra-rare disease（超希少疾患）と呼ばれることもある．

超希少疾患については，疾患の認知度が医療関係者の間でも極めて低い状況にある．そのため，「何人くらいの患者がどこでどのような生活をしているか」という基本的な疫学的情報が十分に把握されていない．また，医師の認識不足から，診断されずに放置されている患者が少なからずいる

国立成育医療研究センター　臨床検査部
〒157-8535　東京都世田谷区大蔵2-10-1

と考えられる.

先天代謝異常症は，糖，脂質，アミノ酸などの代謝経路に存在する酵素やトランスポーターの先天的な欠損により，過剰な中間代謝産物が非生理的に蓄積することが原因で発症する遺伝性疾患である．比較的頻度の高い疾患であるファブリー病やムコ多糖症Ⅱ型においても国内で確認されている患者は数100人程度である．また，ムコ多糖症Ⅵ型患者は８人，アデノシンデアミナーゼ欠損症は３人が確認されているにすぎない．一方，近年の分子生物学，細胞生物学などの発展に伴い，先天代謝異常症に対する病態解明が飛躍的に進展し，酵素補充療法製剤をはじめとして多くの治療用医薬品が開発されている．これらの薬剤は，欧米諸国では薬事承認され広く使用されている.

超希少疾患治療医薬品の開発･承認については，数年前まで欧米に比べてわが国の状況はかなり深刻な状況であったが，最近ようやく解決の糸口が見えてきた感がある．本稿においては，筆者が日本先天代謝異常学会や患者家族会とともに行ってきた経験をもとに，超希少疾病用治療薬開発を推進するために必要な諸課題ついて言及する.

ドラッグラグ解消に未承認薬問題検討会議が果たした役割

超希少疾患の治療薬開発においては，候補薬の治験は，国際共同治験として実施される場合が多い．これまで，日本は，この国際共同治験に参加することができなかった．そのため，欧米での治験の推移を傍観するしかなかった．欧米での承認後も，日本での臨床開発が積極的にできる環境が整っていないことから，いわゆるドラッグラグの状況が長く続いていたが，この問題を解決するために，厚生労働省の未承認薬使用問題検討会議が重要な役割を果たした．以下，ムコ多糖症Ⅱ型（ハンター病）の酵素補充療法製剤であるイデュロサルファーゼの国内承認までの過程をもとに，ドラッグラグ解消のための規制当局の取り組みを検証する.

ハンター病は，イズロネート２-サルファターゼという酵素の先天的な欠損により，分解できないムコ多糖が全身に蓄積し，関節拘縮，骨の変形，水頭症，精神運動発達障害，難聴，中耳炎，心臓弁膜症，閉塞性呼吸障害，肝脾腫大などの全身的な症状を呈する先天代謝異常症である．イデュロサルファーゼは，遺伝子組み換え技術を用いて生合成されたイズロネート２-サルファターゼであり，シャイア社という米国企業により開発された．欧米を中心とした国際共同治験が行われ，2006年７月に米国で，2007年１月に欧州でそれぞれ承認された．日本は，この国際共同治験に参加できなかったが，米国の治験に日本人４名が参加した．2007年の米国での承認前後から，同疾患の患者会である日本ムコ多糖症親の会と日本先天代謝異常学会が中心となり，規制当局へ同医薬品の早期承認に関する要望書を提出した．これを受けて，2006年10月の厚生労働省未承認薬使用問題検討会議で国内開発スキームを検討した（**図１**）．その結果,

１）イデュロサルファーゼの承認申請のために，国内で新たな治験を行わない

２）海外の治験データに基づく承認申請を認める

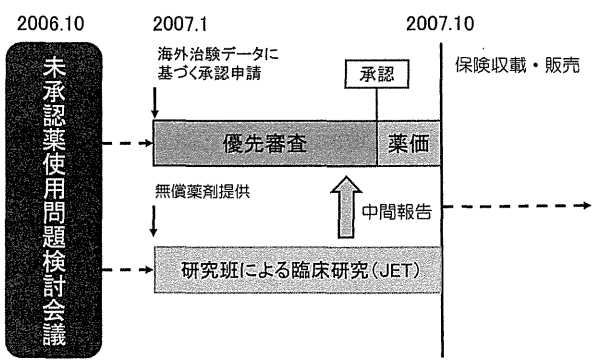


図１　ムコ多糖症Ⅱ型酵素製剤イデュロサルファーゼの国内臨床開発のスキーム

未承認薬使用問題検討会議での検討結果に基づき，海外の治験データに基づいた承認申請を製薬企業が行った．審査と同時並行で，研究班による日本人患者を対象とした投与試験を実施し，その中間報告を審査の参考資料とした.

3）審査期間中に，専門医が研究班を組織し，数名の日本人患者を対象とした投与試験を臨床研究として実施し，その中間報告を審査の対象とする．

という3点が確認された．製薬企業は，2007年1月に承認申請を行った．また，筆者は研究代表者として研究班を組織し，日本人10名に対する投与試験を米国での治験プロトコールに準じて作成したプロトコールで実施した[1]．審査は，順調に行われ，2007年10月に薬事承認・保険収載された．現在，約150名の患者がイデュロサルファーゼの治療を継続している．

未承認薬使用問題検討会議の検討によって国内での新たな治験が回避されたことにより，日本での承認が早まったことは患者家族にとって大きな朗報となった．しかし，この結果，ドラッグラグは大幅に改善されたことは確かであるが，完全に解消にされたわけではなく，欧米での承認から日本での承認までは，約1年を要した．

国際共同治験の必要性

ドラッグラグを完全に解消するためには，日本が国際共同治験に参加して，治験と審査の時期を諸外国と同様にすることが必要である（**図2**）．イデュロサルファーゼの教訓をもとに，ムコ多糖症Ⅳ A 型に対する酵素補充療法製剤の国内臨床開発においては，国際共同治験 MOR004に参加することを推進し，これを実現させた．これは希少疾患としては，国内初の国際共同治験である．同国際共同治験の概要を**図3**に示す．国際共同治験に参加するために，著者らはまず国内患者の実態調査を行い，国内患者数，生活状況などを調査した．同研究は，希少疾患の疫学的調査の一環として行いその結果は厚生労働科学研究の研究報告書として発表した[2]．なお，この調査は，日本ムコ多糖症親の会の全面的な協力で行われた．また，MOR004に日本が参加できた大きな要因には，上記の調査結果により，日本が同製剤のマーケットとして成り立つことを示しえたことも指摘できる．その他，韓国・台湾などのアジア諸国との連携をはかったことも重要であった．希少疾患の国際共同治験に参加できない状況は，韓国，台湾も同様であり，今回東アジアの3国の専門医が一致協力して，この治験参加を要望したことも一因である．今回，MOR004という国際共同治験に参加できたことにより，今後同様の国際共同治験に日本が参加できる基礎ができたと考えられる．

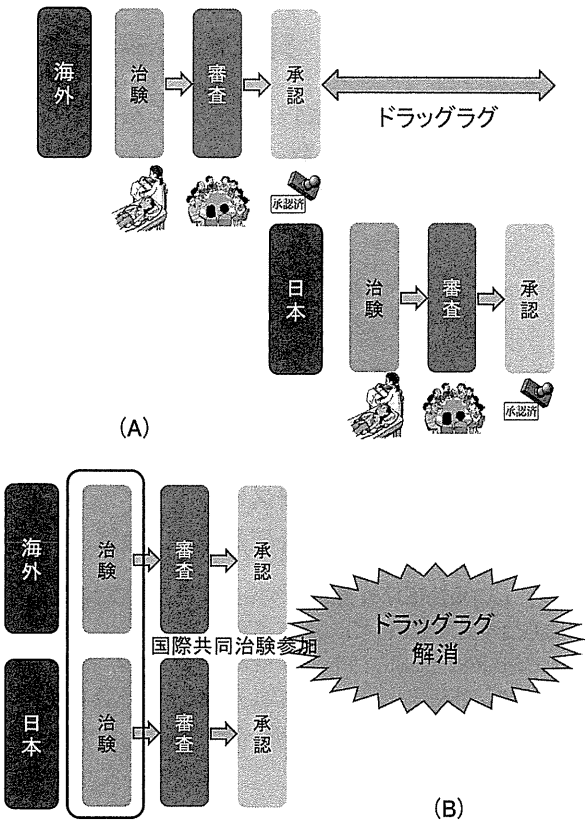


図2　ドラッグラグと国際共同治験
日本が海外で実施される国際共同治験に参加せず，海外で承認された後に国内臨床開発を行うことにより，ドラッグラグを生じる（A）．ドラッグラグを解消するためには，日本も国際共同治験に参加して，審査の時期を国外と同時期にする必要がある（B）．

患者登録制度の必要性について

国際共同治験を行うにあたって，企業側からは，治験に参加できる患者の人数と，実際に承認された後にこの薬剤を使用すると予想される患者

MOR-004 第3相試験

- BMN110の安全性と有効性の評価
- ムコ多糖症ⅣA型(モルキオA症候群)
- 3群で検討
  プラセボ投与群
  BMN110 2mg/kg 毎週投与群
  BMN110 2mg/kg 隔週投与群
- 主要評価項目
  6分間歩行距離の増加
- 副次的評価項目
  3分間昇段テスト
  尿中ケラタン硫酸(KS)の減少

図3 ムコ多糖症Ⅳ A型に対する酵素補充療法製剤 BMN 110の国際共同治験 MOR004の概要
MOR004は，BMN11の第3相試験で，日本，韓国，台湾などアジア諸国を加えた国際共同治験として行われた．小児希少疾患治療薬の国際共同治験に参加した例としては，国内初の治験である．

数とその年齢分布等の疫学的情報を強く求められた．これは，製薬企業として必要なマーケットリサーチに相当する貴重な情報であるが，製薬企業のみでは入手することが困難な情報でもある．MOR004の治験参加が実現したことは，治療薬開発を推進するうえで，超希少疾患の臨床情報データベースを体系的に整備することの重要性を示唆している．日本先天代謝異常学会では，21の患者家族会の協力の下に，患者登録と超希少疾患のデータベース作成を進めている（図4）．

治験デザインの検討

超希少疾患の治験は，「対象患者数が十分に得られない」状況で行われる．その中で，臨床的にも明らかな有効性を示すことは，困難な場合が少なくない．特に，精神運動発達遅滞の予防につながるような薬剤においては，発達評価を指標とする治験が必要になるが，短期間で発達について有

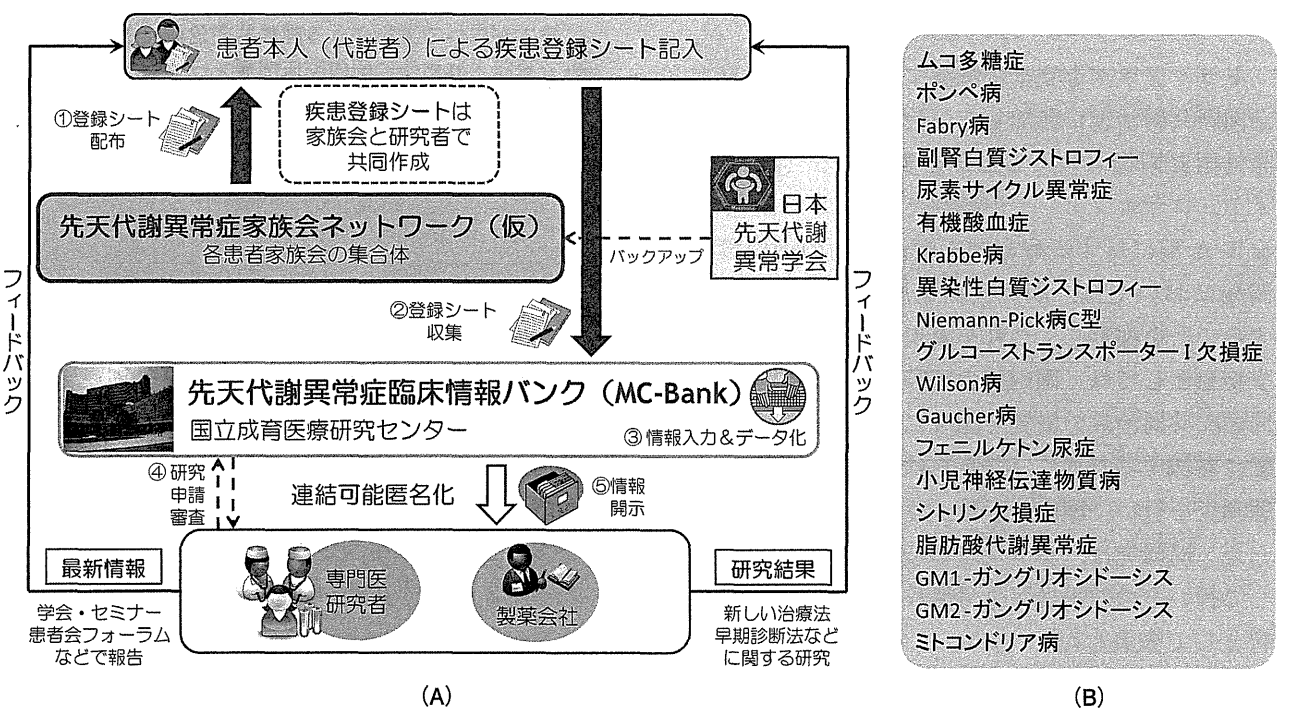


図4 先天代謝異常症患者登録フォローアップシステムの概要
患者家族会を主体とした先天代謝異常症の臨床情報データベースの構築とその臨床研究や治験への活用法を示す（A）．対象疾患は，現在19疾患におよぶ（B）．

効性を評価することは困難である．そのような場合，適切なバイオマーカーの推移を主要評価項目とするなど，サロゲートエンドポイントの活用も必要となろう

結　　語

小児希少疾患治療薬の臨床開発の諸問題について検討した．特に，ドラッグラグ解消のためには，国際共同治験に日本が積極的に参加することが不可欠であること，患者登録と超希少疾患のデータベース作成の必要性について述べた．

文　　献

1）Okuyama T, Tanaka A, Suzuki Y, Ida H, Tanaka T, Cox GF, Eto Y and Orii T. Japan Elaprase® Treatment (JET) study: Idursulfase enzyme replacement therapy in adult patients with attenuated Hunter syndrome (Mucopolysaccharidosis II, MPS II). Mol Genet Metab. 2010; 99: 18-25.

2）鈴木康之．ムコ多糖症Ⅳ型に対する疫学調査：厚生労働科学研究補助金（医療技術実用化総合研究事業）「新規治療法が開発された小児希少難病の疫学調査と長期フォローアップ体制の確立に関する研究」（研究代表者：奥山虎之）平成22年研究報告書11-13.

CJASN ePress. **Published on December 5, 2013 as doi: 10.2215/CJN.13071212**

Article

Cyclosporine C_2 Monitoring for the Treatment of Frequently Relapsing Nephrotic Syndrome in Children: A Multicenter Randomized Phase II Trial

Kazumoto Iijima, Mayumi Sako, Mari Saito Oba, Shuichi Ito, Hiroshi Hataya, Ryojiro Tanaka, Yoko Ohwada, Koichi Kamei, Kenji Ishikura, Nahoko Yata, Kandai Nozu, Masataka Honda, Hidefumi Nakamura, Michio Nagata, Yasuo Ohashi, Koichi Nakanishi, and Norishige Yoshikawa, Japanese Study Group of Kidney Disease in Children

Due to the number of contributing authors, the affiliations are provided in the Supplemental Material.

Correspondence: Prof. Kazumoto Iijima, Department of Pediatrics, Kobe University Graduate School of Medicine, 5-1 Kusunoki-cho 7 chome, Chuo-ku, Kobe, 650-0017 Japan. Email: iijima@med.kobe-u.ac.jp

Summary

Background and objectives An open-label, multicenter, randomized phase II trial was conducted from July 1, 2005 to March 29, 2011 to compare two protocols for treating children with frequently relapsing nephrotic syndrome using microemulsified cyclosporine.

Design, setting, participants, & measurements Ninety-three children with frequently relapsing nephrotic syndrome were randomly assigned to group A (n=46) or group B (n=47). In both groups, the 2-hour postdose cyclosporine level was monitored. For group A, the cyclosporine target was set to 600–700 ng/ml for the first 6 months and 450–550 ng/ml for the next 18 months; for group B, it was set to 450–550 ng/ml for the first 6 months and 300–400 ng/ml for the next 18 months. The primary end point was the sustained remission rate. At the end of the study, if there was no difference in safety profile between the two groups and the sustained remission rate in group A was superior to group B with a decision threshold of 8%, then the regimen for group A would be determined the better treatment.

Results Eight children from an ineligible institution, where cyclosporine levels were not measured, were excluded from all analyses. At 24 months, the sustained remission rate was nonsignificantly higher in group A (n=43) than group B (n=42; 64.4% versus 50.0%; hazard ratio, 0.57; 95% confidence interval, 0.29 to 1.11; P=0.09), and the progression-free survival rate was significantly higher (88.1% versus 68.4%; hazard ratio, 0.33; 95% confidence interval, 0.12 to 0.94; P=0.03). The relapse rate was significantly lower in group A than group B (0.41 versus 0.95 times/person-year; hazard ratio, 0.43; 95% confidence interval, 0.19 to 0.84; P=0.02). The rate and severity of adverse events were similar in both treatment groups.

Conclusion The sustained remission rate was not significantly different between the two treatment groups, but the regimen with the higher 2-hour postdose cyclosporine level target improved progression-free survival and reduced the relapse rate.

Clin J Am Soc Nephrol 9: ●●●–●●●, 2014. doi: 10.2215/CJN.13071212

Introduction

Cyclosporine has been found to be effective for the treatment of frequently relapsing nephrotic syndrome (FRNS) or steroid-dependent nephrotic syndrome (SDNS) in children (1–6). Kidney Disease Improving Global Outcomes Clinical Practice Guideline for Glomerulonephritis recommends that cyclosporine or tacrolimus be given as corticosteroid-sparing agents for FRNS children (7). However, tacrolimus is still off label for FRNS in Japan. Therefore, the development of more effective and safer regimens with cyclosporine for FRNS children is important.

A protocol for treating children with FRNS using Sandimmune, an older formulation of cyclosporine, was previously established in Japan (8). In patients who received Sandimmune in a dose that maintained the whole-blood trough level (C_0) at 80–100 ng/ml for the first 6 months and 60–80 ng/ml for the next 18 months, the estimated sustained remission rate (SRR) was 57% at month 24, and mild chronic cyclosporine nephrotoxicity was found in 20% of patients who underwent renal biopsy after 24 months of treatment.

In 2000, a newer formulation of microemulsified cyclosporine (mCyA; Neoral Novartis, Basel, Switzerland) was introduced in Japan. We previously examined whether treatment with mCyA, titrated by C_0 monitoring with the C_0 target set to the same concentrations mentioned above, was effective and safe in children with FRNS the Japanese Study Group of Renal Disease in Children 07 (the JSRDC07) trial (9). In the JSRDC07 trial, the estimated SRR at month 24 was 58.1%, and mild chronic cyclosporine nephrotoxicity was detected in only 8.6% of patients. Based on these results, the Japanese Society for Pediatric Nephrology

(JSPN) recommended mCyA titrated by C_0 monitoring, in which the C_0 target was set to 80–100 ng/ml for the first 6 months and 60–80 ng/ml for the next 18 months, as the standard treatment with mCyA for children with FRNS.

Because cyclosporine is stably absorbed after administration of mCyA, the dose of mCyA can be titrated based on the area under the concentration time curve during the first 4 hours after treatment (AUC_{0-4}) in children who receive kidney transplants (10). It has been reported that the best single-point predictor of AUC_{0-4} is the 2-hour postdose cyclosporine level (C_2) and that C_2 management of mCyA treatment is effective and safe in pediatric kidney transplant recipients (11). One of the clinical benefits of C_2 monitoring, shown in the majority of studies on transplantation, is a reduction in mean cyclosporine dose, which may reduce the rate of adverse effects of cyclosporine, including chronic cyclosporine nephrotoxicity (12). Several reports described the efficacy and/or safety of mCyA treatment with C_2 monitoring, mainly with single daily dose, in children with FRNS (13–20). However, there were few prospective studies to determine appropriate C_2 target with two divided oral doses of mCyA in children with FRNS. In addition, it is not known whether C_2 monitoring or C_0 monitoring is better in children with FRNS.

To address these questions, we first needed to decide on an appropriate treatment protocol for C_2 monitoring in children with FRNS. Therefore, we conducted an open-label, multicenter, randomized phase II controlled trial designed to select a better treatment for FRNS in children by comparing two target cyclosporine C_2 levels (the Japanese Study Group of Kidney Disease in Children 03 [JSKDC03] trial; University Hospital Medical Information Network–Clinical Trials Registry: C000000008).

Materials and Methods

Patients

The study was approved by the institutional review board at each center and complied with the Declaration of Helsinki. Written assent was obtained from patients when they were old enough to understand, and written informed consent was obtained from all of their parents.

Patients were registered from 14 centers in Japan (Supplemental Table 1) and randomized to the higher (group A) or lower target C_2 group (group B) between July 1, 2005 and January 9, 2009. To be included in the study, patients needed to (*1*) have FRNS, (*2*) be 1–18 years old, and (*3*) have renal biopsy findings showing minor glomerular abnormalities, diffuse mesangial proliferation, or FSGS within 12 months before enrollment. Patients were excluded from the study if they had been treated with cyclosporine, were pregnant, or had (*1*) a history of steroid resistance, (*2*) a creatinine clearance rate of ≤60 ml/min per 1.73 m^2, (*3*) active infections, (*4*) secondary nephrotic syndrome, (*5*) poorly controlled hypertension, or (*6*) severe liver dysfunction. The last patient visit was on March 29, 2011.

The definitions of nephrotic syndrome (21,22) are as follows. Nephrotic syndrome was defined as urine protein-to-creatinine ratio≥1.8 or above and serum albumin≤2.5g/dl. Remission was defined as negative protein on urine dipstick test or urine protein-to-creatinine ratio<0.2 for 3 consecutive days. Relapse was defined as protein≥2+ on urine dipstick test for 3 consecutive days. FRNS was defined as two or more relapses within 6 months after initial remission or four or more relapses within any 12-month period. SDNS was defined as relapse occurring two times consecutively during the reduction of the prednisolone dosage or within 2 weeks after its discontinuation. Steroid-resistant nephrotic syndrome (SRNS) was defined as the daily administration of prednisolone at 60 mg/m^2 per day that does not lead to remission within 4 weeks.

Trial Design

The JSKDC03 was an open-label, multicenter, prospective, randomized phase II controlled trial. We adopted the selection design proposed by Simon *et al.* (23) and generalized by Sargent *et al.* (24), which is frequently used for the development of antibacterial and anticancer agents, for the comparison of the C_2 monitoring of mCyA in phase II trial setting. The selection design has been used to choose which regimen should be further tested in a phase III trial, typically in limited number of patients. Randomized phase II design does not bring a confirmatory result; however, it has the advantage of being able to evaluate with a uniform evaluation criteria.

The purpose of this trial was to select a better treatment for FRNS in children by comparing two target cyclosporine C_2 levels: a higher target C_2 (group A) and a lower target C_2 (group B). A statistically significant difference in primary end point between the two groups was not required in this trial. The criteria for selection were as follows: when there was no difference in safety profile between the two groups and the SRR at 24 months in group A was superior to the SRR in group B with a decision threshold of 8%, the regimen for group A was selected as the better treatment for FRNS. Otherwise, the regimen for group B was selected. The decision threshold of 8% was set before the start of the study based on a consensus reached by pediatric nephrologists in the JSKDC.

The total sample size was determined as 100. Randomization of the patients into two groups was performed in a 1:1 ratio with a dynamic balancing method. A prestudy calculation of sample size and the method of randomization are described in detail in Supplemental Appendix.

Experimental Intervention

Within 7 days after randomization, treatment with mCyA commenced. mCyA was administered orally at least 15 minutes before meals and started at a dose of 3–4 mg/kg body wt divided into two equal doses. We adjusted each dose of mCyA to the target C_2 ranges by increasing or decreasing it by 20%–30%.

The total duration of mCyA treatment was 24 months. Group A received mCyA in a dose producing a whole-blood C_2 level between 600 and 700 ng/ml for the first 6 months and between 450 and 550 ng/ml for the next 18 months. Group B received mCyA in a dose producing a whole-blood C_2 level between 450 and 550 ng/ml for the first 6 months and between 300 and 400 ng/ml for the next 18 months.

How to determine the target C_2 levels and corticosteroid treatment at the relapse during the study is described in Supplemental Appendix. No patients received corticosteroids as a maintenance therapy. Measurement of cyclosporine

concentrations and other variables is also described in Supplemental Appendix.

After 24 months of treatment, the dose of mCyA was tapered off within 3 months, and all patients were scheduled to undergo renal biopsies.

The use of immunosuppressive agents, except for prednisolone and mCyA, was prohibited during the trial. The experimental intervention was stopped if (*1*) patients developed FRNS, SDNS, or SRNS after the start of mCyA treatment, (*2*) patients and/or their parents required the intervention to be stopped, (*3*) patients developed severe adverse events that required intervention to be stopped, (*4*) the primary investigator or the institutional review board at each center decided to stop the trial, or (*5*) patients were not followed up.

End Points

The primary end point was relapse-free survival based on the period of time until the first relapse. There were two secondary end points. One end point was the probability of progression-free survival based on the time until the progression to FRNS, SDNS, or SRNS. The other end point was the relapse rate, which was calculated by dividing the total number of relapses by the total duration of observations for all patients combined.

We also evaluated the rate and severity of development of chronic cyclosporine nephrotoxicity and other adverse events that occurred during the trial. A pathologist on our team (M.N.) evaluated the development of chronic cyclosporine nephrotoxicity, which was defined as cyclosporine-associated arteriolopathy and/or cyclosporine-induced tubulointerstitial lesions showing characteristic striped tubulointerstitial lesions.

Statistical Analyses

Statistical analyses were performed on an intention-to-treat basis. Individuals that did not complete 24 months of the study were still included in the analysis and counted as events. The Kaplan–Meier method was used to estimate the SRR at 24 months after randomization based on the relapse-free survival. The Cox proportional hazard model was used to estimate the hazard ratio and its 95% confidence interval (95% CI) between the groups. These methods and the log-rank test were also used to analyze progression-free survival. The unequal variance *t* test was used to compare the distributions of the average of C_2 and AUC_{0-4}. Fisher's exact test was used to assess the statistical significance of comparisons at the patient level. All statistical analyses were conducted using SAS 9.1 software (SAS Institute, Cary, NC).

Adverse events corresponding to defined classes were tabulated first for 2 years.

Results

Patients

Between April of 2005 and March of 2009, 94 children with minimal change nephrotic syndrome, diagnosed based on pathologic analysis, were registered. One patient was later found to be ineligible because of not meeting the definition of FRNS; therefore, 93 patients were randomly assigned to two treatment groups (group A, n=46; group B, n=47). However, eight patients (three patients in group A; five patients in group B) were from an institution deemed ineligible, because C_2 levels were not measured; thus, these patients were excluded from all analyses. Twenty-five patients discontinued the treatment regimen before the end

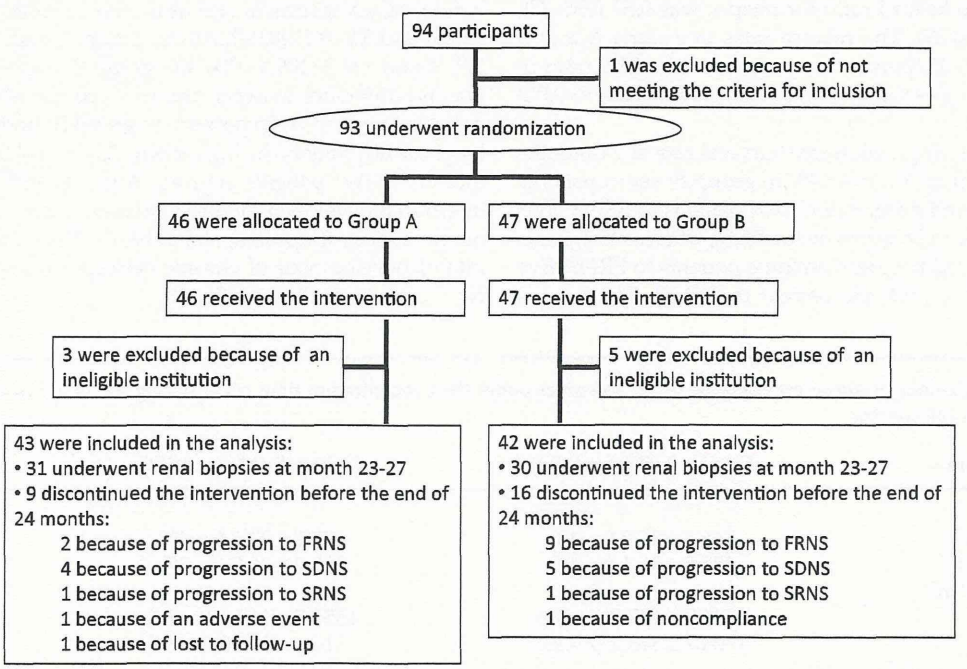


Figure 1. | Flow diagram of the patients. FRNS, frequently relapsing nephrotic syndrome; SDNS, steroid-dependent nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome.

of the 2-year study period but were included in the analysis for their time in the study. Eleven patients (two patients in group A; nine patients in group B) discontinued treatment because of progression to FRNS. Nine patients (four patients in group A; five patients in group B) discontinued treatment because of progression to SDNS. Two patients (one patient in group A; one patient in group B) discontinued treatment because of progression to SRNS. One patient (group A) discontinued treatment because of an adverse event, one patient (group A) discontinued treatment because of loss to follow-up, and one patient (group B) discontinued treatment because of noncompliance (Figure 1).

Characteristics of the patients are shown in Table 1. There was no clinically important difference between the two treatment groups.

Table 1. Characteristics of the patients

Variables	Group A (n=43)	Group B (n=42)
Men	32 (74.4)	31 (73.8)
Age at entry (yr)	7.0±4.3	7.1±3.7
1–5	25 (59.5)	19 (45.2)
6–10	8 (19.1)	14 (33.3)
11–13	6 (14.3)	5 (11.9)
14–18	4 (9.3)	4 (9.5)
Minimal change subtype of NS	43 (100.0)	42 (100.0)
Duration of NS (mo)	18.9±35.5	12.7±15.9
History of SDNS	26 (60.5)	26 (61.9)
Previous treatment with immunosuppressive agent(s)	8 (18.6)	10 (23.8)
Mizoribine	6 (14.0)	9 (21.4)
Cyclophosphamide	1 (2.3)	1 (2.4)
Chlorambucil	1 (2.3)	0 (0)
Total protein (g/dl)	5.9±0.6	5.8±0.7
Albumin (g/dl)	3.4±0.7	3.3±0.7
BUN (mg/dl)	11.5±4.0	12.8±3.4
Creatinine (mg/dl)	0.3±0.1	0.4±0.1
Study baseline eGFR (ml/min per 1.73 m^2)	122.3±30.6	116.5±21.4

Values are n (%) or mean±SD. NS, nephrotic syndrome; SDNS, steroid-dependent nephrotic syndrome; eGFR, estimated GFR.

C_2 and AUC_{0-4} Levels of Cyclosporine

The mean C_2 levels during the first 6 months, the mean C_2 levels during the next 18 months, and the AUC_{0-4} levels at 3 and 9 months after randomization were all significantly higher in group A than group B ($P<0.001$ in all cases) (Table 2). The distribution of exact mean C_2 levels and actual doses of mCyA received by patients in the two groups are shown in Supplemental Tables 2 and 3, respectively.

Efficacy

The primary end point, relapse-free survival, is shown in Figure 2. The estimated SRR 24 months after randomization was 64.4% (95% CI, 48.0% to 76.8%) in group A and 50.0% (95% CI, 34.2% to 63.9%) in group B. The SRR in group A was 14.4% higher than the SRR in group B, which was larger than the decision threshold of 8%; 27 of 43 patients in group A and 21 of 42 patients in group B had not experienced any relapse by the end of 24 months after randomization. The hazard ratio for relapse was 0.57 (95% CI, 0.29 to 1.11; P=0.09). The relapse rates in groups A and B were 0.41 and 0.95/person-year, respectively. The ratio of the two relapse rates was 0.43 (95% CI, 0.19 to 0.84; P=0.02) (Table 3).

The estimated progression-free survival rate at 24 months was 88.1% in group A and 68.4% in group B; seven patients in group A showed progression (two patients to FRNS, four patients to SDNS, and one patient to SRNS), whereas 15 patients in group B showed progression (nine patients to FRNS, five patients to SDNS, and one patient to SRNS). The hazard ratio for progression was 0.33 (95% CI, 0.12 to 0.94; P=0.03) (Figure 3).

Safety

The medians (25th and 75th percentiles) of estimated GFRs before mCyA treatment and at month 24 were 119.0 (106.4–130.9) and 116.0 (106.9–129.0) in group A and 114.0 (102.4–125.0) and 121.3 (109.9–134.3) in group B, respectively. There was no difference between the two groups; 61 patients (31 patients in group A; 30 patients in group B) underwent renal biopsies: 60 patients during months 23–27 and one patient at month 31. Two patients in group A (6.5%) and zero patients in group B developed mild to moderate chronic cyclosporine nephrotoxicity (Supplemental Table 4). This difference in the rate of development of chronic cyclosporine nephrotoxicity was not statistically significant.

Table 2. Mean 2-hour postdose cyclosporine levels and areas under the concentration time curve during the first 4 hours after treatment with cyclosporine

Cyclosporine	Group A (Mean±SD)	Group B (Mean±SD)	P Value
C_2 (ng/ml)			
Months 1–6	566.4±86.9 (n=43)	472.7±73.7 (n=42)	<0.001
Months 7–24	489.5±56.4 (n=40)	382.2±86.8 (n=37)	<0.001
AUC_{0-4} (ng·h/ml)			
Month 3	1944.7±487.9 (n=39)	1554.7±462.8 (n=40)	<0.001
Month 9	1704.7±545.2 (n=36)	1316.6±366.0 (n=34)	<0.001

C_2, 2-hour postdose cyclosporine level; AUC_{0-4}, area under the concentration time curve during the first 4 hours after treatment with cyclosporine.