SUPPLEMENTARY APPENDIX

SUPPLEMENTRY TEXT

Pre-study calculation of sample size

The sustained remission rate (SRR) at 24 months in Group B was assumed to be approximately 60%, and the difference in SRR between the two groups was expected to be 15%. If the true difference in SRR between the two groups was indeed at least 15%, then there was a 75% chance of selecting Group A, given the total sample size of 100. The probability that the SRR in Group A was higher than that in Group B was over 90%. The statistical power of this study to reach the significant difference between the groups was 36% under the assumption described above.

Method of randomization

Randomization of the patients into two groups was performed in a 1:1 ratio with a dynamic balancing method, with stratification by site, sex, renal biopsy findings, and duration of disease, to minimize differences in the distribution of baseline variables between the two groups. Dynamic allocation is otherwise known as covariate-adaptive randomization or minimization [S1]. The probability of being assigned to a group varies in order to minimize covariate imbalance.

How to determine the target C_2 levels

We reported an effective and safe treatment protocol for mCyA titrated by monitoring the whole-blood trough level (C_0) in children with FRNS. [9 in the text] In this study, patients received mCyA in a dose that maintained C_0 between 80-100 ng/ml of cyclosporine during the first 6 months, and the dose was adjusted to maintain a level between 60-80 ng/ml for the next 18 months. The probability of relapse-free survival at month 24 was 58.1 %, and mild chronic cyclosporine nephrotoxicity was detected in only 8.6% of patients who underwent renal biopsy after 24 months of treatment. Therefore, we concluded that this regimen is effective and safe. In this trial, mean C_2

levels at month 1 was 486.0±203.9 ng/ml, and there was a tendency for patients with higher C_2 levels at month 1 to have lower relapse rates during the treatment. Also, an international consensus statement on patient management by mCyA C_2 monitoring described that the C_2 target used for maintenance phase adult kidney transplantation was 800 ng/ml. Based on these previous results, we consider that 24 months of treatment for children with FRNS by mCyA C_2 monitoring with a C_2 target between 300 and 700 ng/ml should be effective and safe. However, it is still unclear whether a higher C_2 target or a lower C_2 target within this range is more effective and safer. Therefore, the C_2 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 A, and it was set to 450-550 ng/ml for the first 6 months and 300-400 ng/ml for the next 18 months for Group B.

Corticosteroid treatment

When patients had relapses of nephrotic syndrome prior to the start of mCyA treatment, they received 2 mg/kg/day of prednisolone, divided into 3 doses (maximum dose of 80 mg/day), until 3 days after obtaining complete remission, or for 4 weeks. This was followed by a single dose of 2 mg/kg (maximum dose of 80 mg/day) of prednisolone in the morning on alternate days for 2 weeks, then 1 mg/kg (maximum dose of 40 mg/day) on alternate days for 2 weeks, and then 0.5 mg/kg (maximum dose of 20 mg/day) on alternate days for 2 weeks. When patients had relapses during mCyA treatment, they received 2 mg/kg/day of prednisolone, divided into 3 doses (maximum dose of 80 mg/day), until 3 days after obtaining complete remission, followed by the same tapering method as described above. No patients received corticosteroids as a maintenance therapy.

Measurement of cyclosporine concentrations and other variables

At week 2 and months 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, and 24 after the start of treatment, we measured the height, weight, and blood pressure of each patient, and collected urine and blood samples from each patient. We measured blood levels of cyclosporine C_2 and the following variables: urinary levels of protein, creatinine, and beta 2 microglobulin; red blood count and white blood

count; blood hemoglobin and urea nitrogen; and serum levels of total protein, albumin, creatinine, sodium, potassium, magnesium, amylase, glutamic oxaloacetic transaminase, and glutamic pyruvic.

At the same time points, estimated glomerular filtration rates were calculated by the Schwartz method. [S2] At months 3 and 9, cyclosporine C_0, C_1, C_3, and C_4 concentrations, as well as C_2 concentrations, were measured by radioimmunoassay using a monoclonal antibody specific for cyclosporine [S3], and AUC_{0-4} values were calculated by the trapezoid method. [S4]

The AUC_{0-4} values and C_2 levels but no other CyA concentrations including C_0 levels at months 3 and 9 were recorded in the case report form.

Discussion on the reason why the prevalence of CyA nephrotoxicity in the present study was lower than that in previous studies

Kengne-Wafo et al. reported that 31 % of steroid-dependent nephrotic syndrome children treated with mCyA with mean C_2 levels of 466 ± 134 ng/ml showed chronic cyclosporine nephrotoxicity.[15 in the Text] The reason why the prevalence of cyclosporine nephrotoxicity in the present study was lower than that in Kengne-Wafo's study is unclear. However, the mean duration of treatment was 4.7 ± 2.0 years before biopsy in Kengne-Walo's study, which was much longer than that in the present study (24 months treatment). Therefore, it is possible the shorter duration of cyclosporine treatment may be due to the lower cyclosporine nephrotoxicity in the present study.

Discussion on the target C_2 levels for phase III trials

As seen in Table S2 and mentioned in "DISCUSSION", it was difficult to control C_2 levels in children, especially when the C_2 target is relatively high. That is probably because 1) As the minimum dose of mCyA capsule is 10 mg, and the concentration of mCyA liquid is 100 mg/ml in Japan, the minimum unit of change in mCyA dose is 10 mg, 2) A slight difference in dose of mCyA induce a relatively large difference in C_2 levels in children when the C_2 target is relatively high.

The mean C_2 levels during the first 6 months in Group A did not reach the target range in our study. However, in approximately 60% of patients in Group A, the mean C_2 levels during the first 6

months were higher than the upper limit of the target C_2 level of Group B (550 ng/ml). We are afraid that true mean C_2 levels will be lower than the target C_2 level if the target C_2 level is decreased (for example, between 550 and 650 ng/ml).

Collectively, we recommend the C_2 monitoring regimen for Group A (C_2 target level: between 600 and 700 ng/ml for the first 6 months, and between 450 and 550 ng/ml for the next 18 months) for phase III trials to compare the efficacy and safety of the regimen those of the JSPN-recommended C_0 monitoring protocol (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).

Physicians who participated in JSKDC03

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S3. Wolf BA, Daft MC, Koenig JW, Flye MW, Turk JW, Scott MG. Measurement of cyclosporine

concentrations in whole blood: HPLC and radioimmunoassay with a specific monoclonal antibody and 3H- or 125I-labeled ligand compared. Clin Chem 35:120-124, 1989

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Table S1. Participating centers.

1. Ashikaga Red Cross Hospital, Ashikaga, Japan
2. Dokkyo Medical University School of Medicine, Tochigi, Japan
3. Fukuoka Children's Hospital and Medical Center for Infectious Diseases, Fukuoka, Japan
4. Hyogo Prefectural Kobe Children's Hospital, Kobe, Japan
5. Japanese Red Cross Society Fukuoka Hospital, Fukuoka, Japan
6. Japanese Red Cross Society Himeji Hospital, Himeji, Japan
7. Kobe University Graduate School of Medicine, Kobe, Japan
8. Kumamoto Chuo Hospital, Kumamoto, Japan
9. National Center for Child Health and Development, Tokyo, Japan
10. National Hospital Organization Saitama National Hospital, Saitama, Japan
11. Tokyo Metropolitan Children's Medical Center, Fuchu, Japan
12. Tokyo Women's Medical University, Tokyo, Japan
13. Wakayama Medical University, Wakayama, Japan
14. Yokohama City University Medical Center, Yokohama, Japan

Table S2. Distribution of exact mean C_2 levels.

	Group A (n=43)		Group B (n=42)	
Months 1-6	n	%	n	%
<300 ng/ml	0	0	0	0
300-400 ng/ml	3	7.0	8	19.1
400<-<450 ng/ml	1	2.3	8	19.1
450-550 ng/ml	14	32.6	19	45.2
550<-<600 ng/ml	10	23.3	4	9.5
600-<700 ng/ml	12	27.8	3	7.1
over 700 ng/mL	3	7.0	0	0

	Group A (n=40)		Group B (n=37)	
Months 7-24	n	%	n	%
<300 ng/ml	0	0	4	10.8
300-400 ng/ml	2	5.0	18	48.7
400<-<450 ng/ml	9	22.5	9	24.3
450-550 ng/ml	25	62.5	4	10.8
550<-<600 ng/ml	3	7.5	1	2.7
600-<700 ng/ml	1	2.5	1	2.7
over 700 ng/ml	0	0	0	0

Table S3. Actual dosage of mCyA in the 2 groups.

	Group A				
	n	Mean ± SD (mg/kg/day)	Minimum (mg/kg/day)	Median (mg/kg/day)	Maximum (mg/kg/day)
The first dosage	43	3.1 ± 0.8	1.8	3.0	5.3
Months 1 - 3	43	5.0 ± 1.2	2.7	4.9	7.6
Month 6	43	4.9 ± 1.2	2.9	4.7	7.7
Month 9	40	4.7 ± 1.2	2.5	4.6	7.1
Months 12 - 24	40	4.9 ± 1.4	2.4	4.6	7.7

	Group B				
	n	Mean ± SD (mg/kg/day)	Minimum (mg/kg/day)	Median (mg/kg/day)	Maximum (mg/kg/day)
The first dosage	42	3.1 ± 0.8	1.7	2.9	5.2
Months 1 - 3	42	4.2 ± 1.1	1.7	4.1	6.9
Month 6	42	4.1 ± 1.1	1.3	4.1	6.9
Month 9	37	3.8 ± 1.0	1.3	3.9	6.1
Months 12 - 24	37	3.8 ± 1.2	1.5	3.7	6.7

Table S4. Chronic cyclosporine nephrotoxicity

Group	Age/sex	Relapses during the study	Progression to FRNS	AUC_{0-4} at month 3 (ng·h/ml)	AUC_{0-4} at month 9 (ng·h/ml)	Renal pathology	
						Arteriolar hyalinosis	Striped fibrosis
A	15/male	No	No	1904	2690	Mild to moderate	Mild
A	12/female	No	No	1934	2003	No	Mild

FRNS, frequently relapsing nephrotic syndrome; AUC_{0-4}, area under the concentration-time curve during the first 4 h after treatment with cyclosporine