Supplemental Information

Supplemental Table 1. Daily dosage in the remission and maintenance phases of the study and at each visit

Phase/Visit	Tacrolimus/Low-Dose Steroid			High-Dose Steroid	
	n	Mean (SD) Tacrolimus Dose, mg/day	Mean (SD) Steroid Dose, mg/day	*n*	Mean (SD) Steroid Dose, mg/day
Remission phase	67	4.72 (1.81)	25.45 (9.16)	67	52.41 (14.15)
Maintenance phase	49	4.07 (1.72)	5.84 (1.91)	50	15.16 (8.07)
Visit 3 (Week 2)	67	5.93 (1.53)	32.13 (7.75)	66	59.87 (12.65)
Visit 4 (Week 4)	58	5.46 (1.59)	32.80 (5.22)	63	60.12 (12.21)
Visit 5 (Week 6)	56	5.02 (1.42)	28.60 (7.30)	62	56.14 (12.25)
Visit 6 (Week 8)	55	4.91 (1.49)	21.14 (8.38)	59	47.89 (13.50)
Visit 7 (Week 12)	49	4.67 (1.52)	8.87 (4.88)	50	34.81 (14.57)
Visit 8 (Week 16)	46	4.46 (1.55)	5.23 (1.22)	47	16.23 (12.55)
Visit 9 (Week 20)	45	4.14 (1.68)	5.42 (1.71)	43	7.29 (6.34)
Visit 10 (Week 24)	42	4.24 (1.69)	5.38 (1.58)	40	5.4 (2.24)

SD, standard deviation.

Supplemental Table 2: Serious treatment-emergent adverse events

System Organ Class	Tacrolimus and Low-Dose Steroid (*n*=67)	High-Dose Steroid (*n*=69)
Overall	6 (9.0), 10	4 (5.8), 5
Gastrointestinal disorders	2 (3.0), 2	1 (1.5), 1
Abdominal pain	1 (1.5), 1	0
Anal fistula	0	1 (1.5), 1
Gastrointestinal disorder	1 (1.5), 1	0
General disorders and administration site conditions	2 (3.0), 2	2 (2.9), 2
Chest pain	0	1 (1.5), 1
Generalized edema	1 (1.5), 1	0
Edema	1 (1.5), 1	1 (1.5), 1
Infections and infestations	2 (3.0), 2	1 (1.5), 1
Herpes zoster	0	1 (1.5), 1
Pneumonia	1 (1.5), 1	0
Tuberculosis	1 (1.5), 1	0
Injury, poisoning and procedural complications	0	1 (1.5), 1
Ankle fracture	0	1 (1.5), 1
Renal and urinary disorders	3 (4.5), 3	0
Oliguria	2 (3.0), 2	0
Renal failure acute	1 (1.5), 1	0
Skin and subcutaneous tissue disorders	1 (1.5), 1	0
Pruritus generalized	1 (1.5), 1	0

Values expressed as number of patients (%), number of cases.

Supplemental Table 3. Laboratory test changes from baseline showing a significant between-group difference at Week 8 and Week 24 after study drug initiation

Laboratory Evaluation Change from Baseline	Tacrolimus and Low-Dose Steroid (*n*=67)		High-Dose Steroid (*n*=69)		*P* value
	n	Mean (SD)	*n*	Mean (SD)	
Hematology					
Week 8					
Neutrophil (% WBC)	55	0.7 (11.2)	58	8.5 (16.5)	0.005[a]
Lymphocyte (% WBC)	55	2.1 (9.7)	58	–4.1 (13.7)	0.006[b]
Monocyte (% WBC)	55	0.01 (2.4)	58	–1.4 (2.9)	0.005[b]
Week 24					
Hemoglobin (g/dL)	43	–0.7 (1.0)	40	–0.04 (1.2)	0.006[b]
Red blood cell (10^6/μL)	43	–0.2 (0.3)	39	–0.03 (0.4)	0.016[b]
Hematocrit (%)	42	–1.6 (2.5)	40	0.09 (3.5)	0.012[b]
Biochemistry					
Week 8					
HDL-cholesterol (mg/dL)	55	4.0 (21.4)	56	13.7 (27.5)	0.040[b]
Total cholesterol (mg/dL)[c]	56	–179.5 (139.5)	59	–114.8 (124.1)	0.005[a]
Triglyceride (mg/dL)[c]	55	–130.0 (128.9)	56	–82.2 (167.7)	0.017[a]
AST (IU/L)	56	–8.3 (8.9)	59	–5.2 (9.4)	0.031[a]
ALT (IU/L)	56	0.1 (13.6)	59	8.5 (16.4)	0.001[a]
Chloride (mMol/L)	52	1.1 (5.4)	57	–1.1 (4.9)	0.016[a]
Bilirubin (mg/dL)	55	0.4 (0.3)	57	0.3 (0.2)	0.036[†]
Week 24					
Total cholesterol (mg/dL)[c]	43	–210.2 (133.3)	40	–126.0 (95.2)	0.004[a]
LDL cholesterol (mg/dL)[c]	40	–153.7 (131.3)	37	–87.5 (66.8)	0.025[a]
Triglyceride (mg/dL)[c]	42	–150.3 (129.5)	38	–61.9 (140.8)	0.004[a]
Bilirubin (mg/dL)	42	0.4 (0.3)	39	0.2 (0.3)	<0.001[a]
AST (GOT) (IU/L)	43	–8.3 (11.9)	40	–1.9 (20.8)	0.016[a]
Creatinine (mg/dL)	43	0.0 (0.3)	40	–0.07 (0.3)	0.039[a]

Total protein (g/dL)	43	2.2 (1.0)	40	1.7 (0.8)	0.020[a]
Albumin (g/dL)	43	2.1 (0.8)	40	1.6 (0.8)	0.003[a]
Urinalysis					
Week 8					
pH	56	–0.6 (1.0)	59	–0.3 (0.9)	0.024[a]
Vital signs					
Week 8					
Body weight (kg)	56	–4.0 (4.2)	59	–1.7 (3.8)	0.003[a]
Week 24					
Body weight (kg)	43	–4.0 (4.7)	40	–1.0 (2.4)	0.002[a]

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GOT, glutamic-oxaloacetic transaminase; HDL, high density lipoprotein; LDL, low density lipoprotein; SD, standard deviation; WBC, whole blood count.

[a]*P* value by Wilcoxon rank sum test.

[b]*P* value by *t* test.

[c]Dyslipidemia, including raised triglycerides and cholesterol, is a complication of persistent nephrotic syndrome and directly contributes to podocyte injury in minimal change nephrotic syndrome. Hyperlipidemia is associated with increased risk of accelerated cardiovascular disease and progressive kidney disease. Since the extent of dyslipidemia correlates with the magnitude of proteinuria, large reductions in cholesterol and triglyceride are expected to accompany improved proteinuria.[1, 2]

1. Macé C, Chugh SS: Nephrotic syndrome: Components, connections, and angiopoietin-like 4-related therapeutics. *J Am Soc Nephrol* 25:2393–2398, 2014

2. Agrawal S, Zaritsky JJ, Fornoni A, Smoyer WE: Dyslipidaemia in nephrotic syndrome: Mechanisms and treatment. *Nat Rev Nephrol* 14:57–70, 2018

Clinical Study Protocol

Protocol No.: PRGNS-11-02-KOR

Subtitle: T_OPTIMUM Study

Open-Label, Randomized, Comparative, Multi-Center Clinical Trial on the Therapeutic Effect of Tacrolimus (Prograf Cap.®) in Combination with Low-Dose Corticosteroid Compared with High-Dose Corticosteroid alone in Patients with Minimal-Change Nephrotic Syndrome (MCNS)

Protocol Version: Draft Version 5.5 (25 OCT 2017)

Sponsor: Astellas Pharma Korea, Inc.

Table of Contents

Study Protocol Summary

Study Title *English*	Open-Label, Randomized, Comparative, Multi-Center Clinical Trial on the Therapeutic Effect of Tacrolimus (Prograf Cap.®) in Combination with Low- Dose Corticosteroid compared with High-Dose Corticosteroid alone in Patients with Minimal-Change Nephrotic Syndrome (MCNS)
Study Centers	Seoul National University Hospital/Seoul St. Mary's Hospital/ Samsung Medical Center/ Asan Medical Center/ Kyunghee University Medical Center/ Seoul National University Bundang Hospital/ St. Vincent Hospital/ Severance Hospital/ Inje University Seoul Paik Hospital/ Chungnam National University Hospital/ Dong-A University Hospital/ Kyungpook National University Hospital/ Hallym University Sacred Heart Hospital/ Konkuk University Medical Center/ Kangdong Sacred Heart Hospital
Sponsor	Astellas Pharma Korea Ltd.
Principal Investigators	[redacted]
Study Period	[redacted]iod: 24 weeks; subject enrollment period: about 5 years)
Study Design	Open-label, randomized, comparative, multi-center clinical trial
Study Phase	Phase III
Study Subjects	Patients with minimal-change nephrotic syndrome
Study Objective	To compare the therapeutic effect of tacrolimus (Prograf Cap.®) in combination with low-dose corticosteroid with high-dose corticosteroid alone in patients with minimal-change nephrotic syndrome
Study Background or Expected Effect	Among the patients whose renal tissues were tested in South Korea, minimal-change nephrotic syndrome accounts for 15.5-26.3% of the primary glomerulonephritis cases and is the most common cause of such. The standard therapy for glomerulonephritis is the administration of high-dose corticosteroid. The response from the medication is slow in adults, however, unlike in pediatric patients, many patients have shown frequent relapse or tolerance (resistance) against steroid. In addition, the side effects of high-dose steroid give the patients many problems. Tacrolimus (Prograf Cap.®) is a calcineurin inhibitor whose therapeutic effectiveness when added to the standard therapy, high-dose

	steroid, has been reported in steroid-resistant patients with minimal-change nephrotic syndrome. As there has been no study on the effectiveness of tacrolimus in combination with low-dose steroid, a comparative study with the standard therapy is required to prove its effect. It is expected that this new regime will show a therapeutic effect similar to that of the standard therapy, which can lead to a decrease in the dose of steroid that has to be administered, as well as in the side effects of such.
Inclusion/ Exclusion Criteria	**<u>Inclusion criteria: Patients who meet the following criteria can be enrolled in the study:</u>** (1) male or female patients who are 16 years old or more and less than 80 years old; (2) patients who have been diagnosed with initial or relapsed primary minimal-change nephrotic syndrome; (3) patients whose urine protein-creatinine ratio (UPCR) is more than 3.0 at the screening visit (spot urine); and (4) patients who voluntarily consented to participate in the study by signing the consent form (patients who are 19 or older than 19 can sign on the consent form by themselves; for 16- to 18-year-old patients, they and their parents have to sign the consent forms).
	<u>Exclusion criteria: Patients who fall under at least one of the following criteria should not be enrolled in the study:</u> (1) patients whose MDRD eGFR is less than 30 ml/min/1.73 m^2; (2) patients who were treated with immunosuppressants, such as more than 1mg/day of tacrolimus, more than 50mg/day of cyclosporine, cyclophosphamide (Cytoxan), mizoribine (Bredinin), levamisole, azathioprine, mycophenolate mofetil, or rituximab, within two weeks before the study; (3) patients to whom more than 10 mg prednisolone or an equivalent dose of steroid was administered daily within two weeks before the study; (4) patients who are pregnant, breastfeeding, or planning to be pregnant or to breastfeed within six months after the study completion, or who cannot or do not want to use any contraceptive method; (5) patients who are hypersensitive to the investigational drug or to macrolide, such as azithromycin, clarithromycin, or roxithromycin; (6) patients who are currently taking bosentan (Tracleer Tab.); (7) patients who are currently taking potassium-sparing diuretics; (8) patients who were treated with a live vaccine within four weeks before their Visit 1; (9) patients whose liver panel laboratory test result is 3 x Upper Limit of Normal or more, or acute hepatitis patients whose serum billirubin has been clinically significantly higher than 3.6 mg/dL for more than 1 month; (10) patients who have a significant general disease that makes it inappropriate for them to participate in this study as adjudged by the investigator (e.g., cardiovascular–acute myocardial infarction, heart failure [classified as more than New York Heart Association {NYHA} class III], hepatic/gastrointestinal/neurologic disease, blood disorder, cancer, infection, renal disorder other than minimal-change nephrotic syndrome, rheumatic arthritis with pneumonia interstitialis); (11) patients who have genetic problems such as galactose intolerance, Lapp lactose deficiency, or glucose-galactose malabsorption;

	(12) patients to whom another investigational drug was administered within 30 days from the enrollment in the study; and (13) patients who participated in the past phases of this study.
Discontinuation Criteria	**Discontinuation Criteria** • Subjects whose participation in this study is inappropriate as they fall under one or more of the exclusion criteria • Subjects who are hypersensitive to the investigational or control drug • Subjects who show significant adverse events from the administration of the investigational drug • Subjects who no longer want to participate in the study • Subjects who do not follow the guidelines given by the investigators • Subjects whose continued participation in the study will be harmful for them as adjudged by the investigators • Subjects whose blood tacrolimus concentration is found to be exceeded 10 ng/ml for three consecutive times • Female subjects who become pregnant in the course of the study • Subjects who are treated with a live vaccine in the course of the study
Ingredients & Quantity of the Investigational Drug	The investigational drug, Prograf Cap.®, marketed by Astellas Ltd., is a 0.5 or 1 mg capsule that is to be orally taken. This investigational drug is the same as the marketed drug in terms of its ingredients and formation. (Prograf Cap.® contains tacrolimus [FK506], a calcineurin phosphatase inhibitor, and its structure consists of macrolide lactone produced by *streptomyces tsukubaensis*.) For steroid, only prednisolone oral drug will be used in this study.
Test Group	Steroid 0.5 mg/kg qd + tacrolimus 0.05 mg/kg bid
Control Group	Steroid 1 mg/kg qd
Study Design	**Summary of the study method:** The study subjects will be randomized at visit 1 (screening visit, week 0) and will then be made to take the investigational drug for 24 weeks. The subjects will visit the study center for a total of 10 visits to include week 0 (weeks 0, 1, 2, 4, 6, 8, 12, 16, 20, and 24). As for randomization, block randomization will be used. (The strata factor for minimal-change nephrotic syndrome has not been determined; the strata factor with regard to the drug indications will not be considered and will be done only by the center.) The following definitions will apply in this study: 1. complete remission induction: UPCR is less than 0.2; and 2. relapse criteria: among the patients who have complete remission, proteinuria is observed (UPCR>3.0). **Administration dose and route:** The subjects in the test group will take steroid 0.5 mg/kg once a day and Prograf Cap.® twice a day on an empty stomach one hour before or 2-3 hours after meals for maximum absorption, and the subjects in the control group will take steroid 1 mg/kg once a day (However, the prescribed dose of prednisolone cannot exceed 80mg/day. If daily dose of prednisolone calculated based on the subject's weight exceeds 80mg a day, the subject

	will be given 80mg.). Dose adjustment will be done in two different phases (remission and tapering/maintenance phases). In the remission phase, the subjects in the test group will be treated with 0.5 mg/kg steroid qd and 0.05 mg/kg Prograf Cap.® bid for an additional two weeks from the complete remission of the initial proteinuria (UPCR<0.2), and the subjects in the control group will be treated with 1 mg/kg qd for the phase. The dose of the steroid will be determined after rounding off. (If the dose is between 32.5mg and 37.4mg, 35 mg steroid will be given and if the dose is between 27.5mg and 32.4mg, 30mg steroid will be given, in tablet form, in the study.) The subjects who do not show complete remission within 8 weeks after the administration of the investigational drug should be discontinued from the study. In the test group, the level of tacrolimus will be maintained at 5-10 ng/ml in the remission phase, and at 3-8 ng/ml in the tapering and maintenance phase. In the tapering and maintenance phase, the dose of the steroid will be decreased by 5 mg per week in both groups three weeks after complete remission. The maintenance dose of the steroid after being decreased will be 7.5 mg/day for the subjects who weigh over 80 kg, and 5 mg/day for the subjects who weigh under 80 kg. Prograf Cap.® will be administered in the closest dose that the subjects can take, using a 0.5 mg tablet, twice a day (12 hours apart). A week after the administration of the first dose, the 12-hour trough level of tacrolimus after the last dose will be checked, and the dose for the next two to four weeks will be adjusted to make the blood level 5-10 ng/ml. If the 12-hour trough level of tacrolimus is more than 10 and less than 15 ng/ml, the dose will be decreased by 30%. If the 12-hour trough level is 15 or more than 15 ng/ml, the administration of the drug will be temporarily stopped for one week, and a 30%-decreased dose from the initial dose will be administered when the retest shows 10 or less than 10 ng/ml 12-hour trough level. If the 12-hour trough level is still over 10 ng/ml, the drug level in the blood will be checked every week, and a 30%-decreased dose will be re-administered when the 12-hour trough level is shown to be 10 or less than 10 ng/ml. If a subject shows a exceeded 10 ng/ml 12-hour trough level of tacrolimus for three consecutive weeks, the subject should be withdrawn from the study.
Compliance Assessment	The prescribed and returned investigational drug will be confirmed to calculate the drug compliance of the subjects. The proper drug compliance rate on the part of each subject is 80-110%.
Efficacy & Safety Assessment	**<u>Concomitant medication</u>:** All immunosuppressant drugs will be prohibited during the study period (e.g., tacrolimus, cyclosporin, cyclophosphamide, levamisole, azathioprine, mycophenolate mofetil, rituximab). Steroids should not be used to treat kidney syndrome, and the use of steroids will be based on the investigator's judgment. If a subject is taking steroid-based oral contraception, extra care should be taken by the subject. A live vaccine should not be administered to the subjects; in particular, the subjects taking high-dose corticoids should not be given any other vaccination. Any medications that have high affinity with serum protein such as anticoagulants, oral medication for diabetes should be carefully administered. If a subject uses any prohibited medication, he/she can be withdrawn from the study as adjudged by the investigator. **<u>Study flowchart</u>:** Please find the attached flowchart.

	Efficacy assessment: **Primary efficacy endpoint:** - The percentage of subjects who show a decreased UPCR of less than 0.2 up to eight weeks in both the test and control groups **Secondary efficacy endpoint:** - The period until the UPCR is decreased below 0.2 in both the test and control groups. - The percentage of subjects who show relapse after the remission up to 24 weeks in both the test and control groups - The period until the relapse happens from the complete remission up to 24 weeks in both the test and control groups. **Safety assessment:** All adverse events will be assessed based on the symptoms that the subjects will complain of, the measured vital signs, the results of the physical examination to be conducted by the investigators, and the results of the electrocardiogram, chest X-ray, hematology, chemistry, and urinalysis. The percentage of adverse events up to 24 weeks will be evaluated after the administration of the investigational drug.
Criteria for Non-inferiority	ä=-20%, the difference in the maximum successful treatment rate between the new therapy and the standard therapy As the current therapeutic effect of steroid for MCNS shows a 40-60% remission rate until eight weeks and 70-90% until 12 weeks, a 20% non-inferiority margin is reasonable.
Sample Size Determination	The purpose of this study is to compare the effect of the administration of a low-dose steroid with tacrolimus for eight weeks with the standard therapy, high-dose steroid alone, in patients with minimal-change nephrotic syndrome. In the previous study[7], 68 subjects (84%) showed complete remission among the 81 subjects who were treated with high-dose steroid. Forty-seven subjects showed complete remission within four weeks, and 16 subjects showed complete remission between four and eight weeks. A total of 63 subjects (78%) thus showed complete remission within eight weeks. Based on the study results, the complete remission ratio within eight weeks was found to be 0.8(80%). Moreover, the current therapeutic effect of steroid in patients with minimal-change nephrotic syndrome shows a 40-60% remission rate until eight weeks, and 70-90% until 12 weeks, based on the results of the previous study.[16,17] The range of those rates is about 20%. Based on those results, the non-inferiority margin was determined 0.2 and the sample size was calculated. To determine the sample size needed to achieve the study objective, the following assumptions were made: 1) significance level: a=0.025; 2) ratio of subject number in both groups (test group:control group) = 1:1; and 3) second error (p) = 0.20; statistical power = 80%.

4) The study hypotheses (one-tailed test) are as follows:
H0: difference of complete remission by group ≥ 0.20;
H1: difference of complete remission by group < 0.20; and
5) drop-out rate: 15%.

The complete-remission rate was set at 0.8 in both the tacrolimus

and low-dose steroid group and the tacrolimus and high-dose steroid group, and the non-inferiority margin was set at 0.2. The sample size was calculated using the Power Analysis and Sample Size (PASS) software, considering a 0.025 significance level and 0.8 statistical power (1-â).

	Subject no./group	**Subject no./group considering the drop-out rate**	**Total subject no.**
Mean	64	76	152

The required number of subjects for each group shall be 64 for the analysis of the non-inferiority of tacrolimus in combination with low-dose steroid compared with that in combination with high-dose steroid, with 80% statistical power at a 2.5% significance level (one-tailed). Considering the 15% drop-out rate, the number of subjects in each group will be 76, totaling 152.

Statistical Analysis Method

Efficacy assessment:

Primary efficacy endpoint:

1) The percentage of subjects who show a less-than-0.2-decreased UPCR up to eight weeks in both the test and control groups (this study is a non-inferiority test with a 95% confidence level upper limit)

Secondary efficacy endpoint:

1) The period until the UPCR is decreased to below 0.2 in both the test and control groups (the median time will be presented, and logrant test will be carried out to compare the test and control drugs)
2) The percentage of subjects who show relapse after remission up to 24 weeks in both the test and control groups (Chi-square test or Fisher's exact test will be carried out to obtain this value)
3) The period until relapse occurs from the complete remission up to 24 weeks in both the test and control groups (the median time will be presented, and logrant test will be carried out to compare the test and control drugs)

	<u>Safety assessment</u>: All the subjects who had been treated with the investigational drug more than once will be included in the safety assessment. All adverse events based on CTCAE ver. 4.0 will be presented with the severity level (mild, moderate, or severe based on Spilker's three-level severity). After analyzing the occurrence of adverse events, the occurrence of adverse events leading to subject withdrawal, the occurrence of serious adverse events, and the relationship with the investigational drug, and after coding these using MedDRA, the number and rate of subjects who showed adverse events will be summarized and presented after being classified by site (organ). The percentage of adverse events up to 24 weeks after the administration of the investigational drug compared to the percentage of subjects by group will be tested via Chi-square test or Fisher's exact test. The abnormality of the laboratory test will be determined after analysis using T-test or Wilcoxon rank sum test.

<Study Flowchart>[10]	V1[14]	V2	V3	V4	V5	V6/ EOS	V7	V8	V9	V10/ EOS	UV[11]
Criteria for investigational-drug administration →	**0w -7~1d**	**1w ±3d**	**2w ±3d**	**4w ±3d**	**6w ±3d**	**8w ±3d**	**12w ±7d**	**16w ±7d**	**20w ±7d**	**24w ±7d**	**Xw ±1d**
Consent form	O										
Eligibility check	O										
Randomization	O										
Patient history/demographic data	O										
Vital signs[1]	O		O	O	O	O	O	O	O	O	
Height, weight[2]	O		O	O	O	O	O	O	O	O	
Hematology[3]	O		O	O	O	O	O	O	O	O	
Chest X-ray[4]	O		O		O					O	
ECG[5]	O									O [5-1]	O
Cardiac symptoms[6]	O	O	O	O	O	O	O	O	O	O	
Chemistry[7]	O		O	O	O	O	O	O	O	O	
Lipid panel[8]	O		O	O	O	O	O	O	O	O	
C-reactive protein	O		O	O	O	O	O	O	O	O	
Urinalysis	O	O	O	O	O	O	O	O	O	O	
Pregnancy test	O									O	
HbA1C	O			O		O		O		O	
UPCR, urine Na, K	O	O	O	O	O	O	O	O	O	O	
PRG trough level[9]		O	O	O	O	O	O	O	O	O	O [12,13]
Investigational-drug distribution	O		O	O	O	O	O	O	O		O [13]
Concomitant-medication check	O		O	O	O	O	O	O	O	O	O
Drug compliance check			O	O	O	O	O	O	O	O	O [13]
Adverse-events check	O	O	O	O	O	O	O	O	O	O	O

1) Measure the blood pressure using an automatic blood pressure manometer approved by FDA. The mean value is obtained after measurement in a sitting position two times, with a 5-minute interval.
2) The height is checked only at visit 1. For other visits, only the weight is checked.
3) CBC with differential count
4) Check the chest PA and confirm the cardiothoracic ratio.
5) Perform ECG and check if there is any tall T-wave, ST-elevation/depression, or T-wave inversion, and confirm the PR interval.
5-1) ECG will be performed at Visit 1 and either Visit 10 or last visit.
6) Check for cardiac symptoms (e.g., chest pain, chest discomfort, palpitation, breathing difficulty when exercising or standing up). If a subject shows cardiac symptoms, chest X-ray, ECG, and troponin T should be performed regardless of the visit.
7) Protein, albumin, cholesterol, bilirubin, ALP, AST, ALT, BUN, Cr, uric acid, glucose, Na, K, Cl
8) LDL-cholesterol, HDL-cholesterol. The subjects should fast for at least eight hours before the test is carried out.
9) The minimum tacrolimus level in the blood should be checked 12 hours after the drug administration.
10) End of study visit should be performed for subjects: who do not show complete remission within eight weeks (at V6), who show relapse after complete remission within 24 weeks (at V10), or whose participation in the study is discontinued. The reason for the discontinuation of a subject's participation in the study will be obtained from the

concerned subject on a separate CRF at the end of the study visit, and follow-up will no longer be made after the last study visit.

11) Unscheduled visit (UV): The subjects can make an unscheduled visit to the study center in the following cases: for tacrolimus blood level retest, for prescription of the investigational drug in the tapering and maintenance phase at 10 weeks for a subject who shows complete remission at eight weeks, or due to the occurrence of an adverse event.

12) If the tacrolimus level in the blood is more than 15 ng/ml, the investigational drug should be temporarily stopped, and retest should be done after one week. The subject should visit the center for retest up to three weeks, until the tacrolimus level in the blood is already less than 10 ng/ml. The retest for visit 2 can be done at visit 3, UV (three weeks), or visit 4; the retest for visit 3 at UV (three weeks), visit 4, or UV (five weeks); the retest for visit 4 at UV (five weeks), visit 5, or UV (seven weeks); the retest for visit 5 at UV (seven weeks), visit 6, or UV (nine weeks); the retest for visit 6 at UV (9, 10, 11 weeks); the retest for visit 7 at UV (13, 14, 15 weeks); the retest for visit 8 at UV (17, 18, 19 weeks); and the retest for visit 9 at UV (21, 22, 23 weeks), based on the subject's condition.

13) Prescribe the investigational drug with a reduced dose at 3 weeks for the subjects who showed remission at week 1; and at 10 weeks, for the subjects who showed remission at eight weeks.

14) Visit 1 is up to eight days from the test and until day 1 (first drug administration) after the randomization.

1. Study Name and Phase

1.1 Study title

Korean: Open-Label, Randomized, Comparative, Multi-Center Clinical Trial on the Therapeutic Effect of Tacrolimus (Prograf Cap.®) in Combination with Low-Dose Corticosteroid Compared with High-Dose Corticosteroid alone in Patients with Minimal-Change Nephrotic Syndrome (MCNS)

English: Open-Label, Randomized, Comparative, Multi-Center Clinical Trial on the Therapeutic Effect of Tacrolimus (Prograf Cap.®) in Combination with Low-Dose Corticosteroid Compared with High-Dose Corticosteroid alone in Patients with Minimal-Change Nephrotic Syndrome (MCNS)

1.2 Study design and phase

Study design: Randomized, open-label, comparative clinical trial

Study phase: Phase III

2. Name and Address of the Study Center

3.

4. Study Objective and Background

4.1 Study objective

To evaluate the efficacy and safety of tacrolimus (Prograf Cap.®) in treating patients who have been initially diagnosed with or who have relapsed minimal-change nephrotic syndrome (MCNS) through renal biopsy

- Primary objective: The primary objective of this study is to compare the percentage of subjects who show complete remission based on the urine protein-creatinine ratio (UPC**R) in the test (new-treatment) and control (standard-treatment) groups.**
- Secondary objective: The secondary objective of this study is to compare the periods until the UPCR has decreased to below 0.2, the percentages of subjects who show relapse after remission up to 24 weeks, and the periods until relapse occurs from complete remission up to 24 weeks in the test and control groups. To evaluate the aforementioned values, the UPCR, serum creatinine, and blood tacrolimus concentration will be obtained.

4.2 Study medical background

Among the patients whose renal tissues were tested in South Korea, MCNS **accounts** for 15.5-26.3% of the primary glomerulonephritis cases and is the most common cause of such.[1-3] The standard therapy for MCNS has been known to be the administration of high-dose corticosteroid (1 mg/kg/day) in adults, which shows better effects, with a complete remission rate of at least 80% compared to other primary glomerulonephritis diseases.[4,5] The response to the medication is slow in adults, however, unlike in pediatric patients, and many patients have shown frequent relapse or tolerance against or resistance to steroid. For some patients, resistance to steroid is observed even in the early stage.[6,7] In addition, the side effects of the administration of high-dose steroid for a long time give the patients many problems. To avoid these side effects of steroid, cytotoxic agents such as cyclophosphamide or chlorambucil can be used, but their use must be limited due to their serious side effects, such as pancytopenia from a bone marrow suppressant. Recently, the synaptopodine-stabilizing effect of calcineurine inhibitor showed good results in comparative studies with cyclosporine.[8-10] It is effective for steroid-resistant nephritis syndrome showing resistance to or dependence on cyclosporine.[11,12] Tacrolimus (Prograf Cap.®) is a calcineurin inhibitor with less side effects than cyclosporine in terms of aesthetics[13], and its therapeutic effectiveness when used alone has been reported in steroid-resistant (or dependent) patients with MCNS. The sample size of those studies, however, was small, and comparative studies have so far been conducted only with cyclophosphamide.[15] As there has been no study on the effectiveness of tacrolimus in combination with a low-dose steroid, a comparative study with the standard therapy (high-dose steroid) is required to prove its effect. It is expected that this new regime will show a therapeutic effect similar to that of the standard therapy, which can lead to a decrease in the dose of steroid that has to be administered, as well as in the side effects of such.

5. Information regarding the Investigational Drug

5.1 Generic name, ingredient, quantity, and formation of the investigational drug

Prograf Cap.® contains tacrolimus [FK506], a calcineurin phosphatase inhibitor, and its structure consists of macrolide lactone produced by *streptomyces tsukubaensis*.

Brand name	Prograf Cap.®	Solondo Tab.
Generic name	Tacrolimus	Prednisolone

Quantity	0.5 mg, 1 mg/capsule	5 mg/tablet
Formation	Soft capsule	Tablet

5.2 Packing and labeling of the investigational drug

The investigational drug for this study was manufactured by Astellas Irand, Inc. and by Astellas Pharma Tech Co., Ltd. and was imported by Astellas Pharma Korea, Inc. The investigational drug will be provided to the study pharmacist of each study center. The container, packaging, or labeling of the investigational drug will follow the pharmaceutical laws of South Korea, and the relevant details of such laws will be indicated on the packaging, label, or container of the investigational drug. The delivery of the investigational drug will follow the SOP of Astellas Pharma Korea, Inc., and the relevant information are as follows:

- "Clinical Use" mark;
- product code and generic name of the main ingredient;
- manufacturing number, shelf life, or date of retesting;
- storage condition;
- name and address of the party that obtained approval for the clinical trial; and
- "This cannot be used for any purpose other than for clinical trial" mark.

5.3 Dispensing, storage, handling, and recording of the investigational drug

The study pharmacist should sign on the drug receipt form after confirming the quantity of the investigational drug received from the sponsor. The study pharmacist is responsible for handling, storing, and maintaining the investigational drug according to the SOP of the pharmacy. The dispensing of the investigational drug (test or control drug) should be made based only on a prescription that reflects the signature of the principal investigator or the sub-investigators.

The study pharmacist should record the relevant information (English initials of the subjects, randomization number, date dispensed, and quantity dispensed) on the investigational-drug accountability log, and should store and maintain the record.

After visit 1, the subjects will be given the investigational drug based on the bodyweight of the subject taken at of visit 1 (screening visit). For the test group, 0.5 mg/kg qd steroid and 0.05 mg/kg bid tacrolimus will be administered. The closest dose that will be prescribed as the smallest tablet dose is 0.5 mg. (e.g., If a subject should be given 5.7 mg, 5.5 mg bid will be prescribed. If a subject should be given 5.8 mg, 6.0 mg bid will be prescribed.) For the control group, only 1 mg/kg qd steroid will be prescribed. The dose of the steroid will be determined after rounding off the required dose. (If the dose is between 32.5mg and 37.4mg, 35 mg steroid will be given and if the dose is between 27.5mg and 32.4mg, 30mg steroid will be given, in tablet form, in the study)

6. Indication

Primary MCNS diagnosed in the present or past through renal biopsy

7. Expected Study Period

The enrollment of study subjects will start on the IRB approval date, and the expected last subject enrollment is March 2017 The last dose of the last subject is expected to be administered in August 2017. The study will be over by December 2017. (The enrollment period is about5 years from the IRB approval date.)

8. Study Method

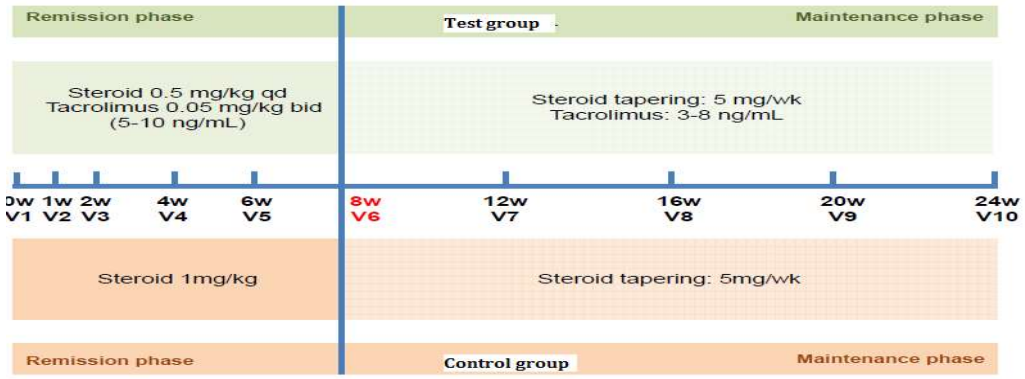
8.1 Summary of the study method

The patients who are eligible to participate in this study based on the inclusion/exclusion criteria will be randomized at visit 1 using a Web-based program. The test group will be treated with steroid 0.5 mg/kg qd and tacrolimus 0.05 mg/kg bid for 24 weeks, which will include the remission and tapering/maintenance phases. The enrollment date of a study subject is defined as the date of the subject's visit 1. The primary endpoint is the difference in the percentage of subjects who showed a decreased UPCR value of less than 0.2 up to eight weeks.

8.1.1 Definition of the terms used in this study

1) Complete remission induction: less than 0.2 UPCR
2) Relapse: Among the patients with complete remission, proteinuria is observed (UPCR>3.0).
3) Remission phase: The period until UPCR<0.2 is reached after the start of the treatment (up to eight weeks). 0.5 mg/kg steroid will be administered in the test group, and 1 mg/kg in the control group. As for the test group, the tacrolimus concentration in the blood should be maintained at 5-10 ng/ml.
4) Tapering and maintenance phase: The period from the remission phase up to 24 weeks. After the remission induction, 0.5 mg/kg steroid will be administered in the test group, and 1 mg/kg in the control group, for two weeks. After that, the dose of the steroid will be decreased by 5 mg every week in both groups, and the tacrolimus concentration should be maintained at 3-8 ng/ml. The subjects will be instructed by the study coordinator regarding the schedule for decreasing the steroid dose at the outpatient visits and through weekly phone calls.

8.1.2 Study schedule



8.1.3 Summary of visit schedule

1) Visit 1 (0 week): remission phase
   - Informed consent: Refer to the informed-consent form section for the details.
   - Demographics, detailed patient history (including renal biopsy and diagnosis) collection (past medical history that occurred within 1 year before the administration of the investigational drug or existing disease will be collected)
   - Concomitant medications that had been taken within 30 days before the administration of the investigational drug or currently being taken during participation in the study
   - Vital signs, height, and weight
   - Blood test (hematology, chemistry, lipid panel, CRP, HbA1C) and urine test (urinalysis, UPCR, urine Na, urine K): The test results taken within two weeks from visit 1 can be used.
   - Urine pregnancy test
   - Chest X-ray (chest PA): Check the cardiothoracic ratio.
   - ECG: Check if there is any tall T-wave, ST elevation/depression, or T-wave inversion, and confirm the PR interval.
   - Cardiac-symptoms check (e.g., chest pain, chest discomfort, palpitation, breathing difficulty when exercising or standing up)
   - Inclusion/exclusion criteria check
   - Randomization: The study coordinator performs randomization according to the Web-based randomization procedure.
   - Dispensing of the investigational drug
     - Test group: steroid 0.5 mg/kg; tacrolimus 0.05 mg/kg bid
     - Control group: steroid 1 mg/kg
   - Adverse-event check

2) Visit 2 (week 1): remission phase
   - **Test group:** The blood tacrolimus concentration will be obtained. After the investigator confirms the blood tacrolimus concentration, the dose will be adjusted by the study coordinator with the subject by phone call, based on the investigator's opinion. If the blood level is 5-10 ng/ml, the dose will be maintained. If it is more than 10 ng/ml and less than 15 ng/ml, the dose will be decreased by 30%, and the administration should be stopped for one week until it is found to have decreased to less than 10 ng/ml when being retested to see if it is 15 ng/ml or more. In that case, the re-administration dose is 30% less than the initial dose. If the level is still higher than 10 ng/ml after one week,

the administration should be stopped, and the level should be retested every week until it is found to have decreased to less than 10 ng/ml. In that case, the re-administration dose should be 30% less than the initial dose. If the level exceeds 10 ng/ml for three consecutive weeks, the subject should be withdrawn from the study.

- Urine test (urinalysis, UPCR, urine Na, urine K)
- Adverse-event check
- Cardiac-symptoms check (e.g., chest pain, chest discomfort, palpitation, breathing difficulty when exercising or standing up)

3) Visits 3, 4, 5, and 6 (weeks 2, 4, 6, and 8): remission phase
   - Vital signs and weight
   - Adverse-event check
   - Concomitant-medication check
   - Drug compliance check
   - Blood test (hematology, chemistry, lipid panel, CRP, HbA1C) and urine test (urinalysis, UPCR, urine Na, urine K): Refer to the study flowchart for the details.
   - If a subject shows UPCR≥0.2, he/she will be made to complete the study.
   - Chest X-ray (chest PA): Check the cardiothoracic ratio (only at visits 3 and 5 – weeks 2 and 6).
   - Cardiac-symptoms check (e.g., chest pain, chest discomfort, palpitation, breathing difficulty when exercising or standing up)
   - Test group: blood tacrolimus concentration check and dispensing of the investigational drug
     - Tacrolimus: The blood tacrolimus level should be maintained at 5-10 ng/ml. To ensure the subjects' safety, the dose should be adjusted as follows:
       - ✓ **10< blood tacrolimus level <15 ng/ml**: 30% decreased dose from the previous dose (decreased by 0.5 mg)
       - ✓ **≥ 15 ng/ml**: Temporary discontinuation of drug administration. If the level is found to have decreased to **10 ng/ml or less** at the retest, a 30% decreased dose from the initial dose can be administered. If the level is still higher than 10 ng/ml after one week, the administration should be stopped, and the level should be retested every week until it is found to have decreased to 10 ng/ml or less. In that case, the re-administration dose should be 30% less than the initial dose. If the level exceeds10 ng/ml for three consecutive weeks, the subject should be withdrawn from the study.

     (1) Steroid: Maintain at 0.5 mg/kg.
   - Control group: Dispense of the investigational drug as follows:
     - Steroid: Maintain at 1 mg/kg.

4) Visits 7, 8, 9, and 10 (weeks 12, 16, 20, and 24): Tapering and maintenance phase
   - Vital signs and weight
   - Adverse-event check
   - Concomitant-medication check
   - Drug compliance check
   - Blood test (hematology, chemistry, lipid panel, CRP, HbA1C) and urine test (urinalysis, UPCR, urine Na, urine K): Refer to the study flowchart for the details.
   - Urine pregnancy test (only at visit 10)

- Chest X-ray (chest PA): Check the cardiothoracic ratio (only at visits 3 and 5 – weeks 2 and 6).
- ECG: Check if there is any tall T-wave, ST elevation/depression, or T-wave inversion, and confirm the PR interval (only at visit 10 – weeks 24).
- Cardiac-symptoms check (e.g., chest pain, chest discomfort, palpitation, breathing difficulty when exercising or standing up)
- Test group: blood tacrolimus concentration check and dispensing of the investigational drug
    - Tacrolimus: The blood tacrolimus level should be maintained at 3-8 ng/ml. The dose will be determined after the blood tacrolimus level is checked through the blood test. To ensure the subjects' safety, the dose should be adjusted as follows:
        - ✓ **8<blood tacrolimus level<15 ng/ml**: 30% decreased dose from the previous dose (decreased by 0.5 mg)
        - ✓ **≥15 ng/ml**: Temporary discontinuation of drug administration. If the level is found to have decreased to **8 ng/ml or less** at the retest, a 30% decreased dose from the initial dose can be administered. If the level is still higher than **8 ng/ml** after one week, the drug administration should be stopped, and the level should be retested every week until it is found to have decreased to 8 ng/ml or less. In that case, the re-administration dose should be 30% less than the initial dose. If the level exceeds 10 ng/ml for three consecutive weeks, the subject should be withdrawn from the study.
    - Steroid: After the remission induction, the dose will be maintained for **two weeks**. From **three** to 24 weeks from the remission induction, the dose will be decreased by **5 mg every week**. For the subjects who weigh 80 kg or more, the dose will be decreased to 7.5 mg, and for the subjects who weigh under 80 kg, the dose will be decreased to 5 mg. The subjects will be instructed by the study coordinator regarding the schedule for decreasing the steroid dose at the outpatient visits and through weekly phone calls.
- Control group: dispensing of the investigational drug:
    - Steroid: After the remission induction, the dose will be maintained for **two weeks**. From **three** to 24 weeks from the remission induction, the dose will be decreased by **5 mg every week**. For the subjects who weigh 80 kg or more, the dose will be decreased to 7.5 mg, and for the subjects who weigh under 80 kg, the dose will be decreased to 5 mg. The subjects will be instructed by the study coordinator regarding the schedule for decreasing the steroid dose at the outpatient visits and through weekly phone calls.

5) Unscheduled visit (UV): UV can be performed in the following cases. The concomitant medications and adverse events (AEs) will be checked at every visit.

- If the tacrolimus level in the blood is more than 15 ng/ml, the investigational drug should be temporarily stopped, and retesting should be done after one week. The subject should visit the center for retesting up to three weeks, until the tacrolimus level in the blood is already less than 10 ng/ml.
  The retesting for visit 2 (week 1) can be done at visit 3, UV (week 3) at visit 4;
  that for visit 3 at UV (week 3), visit 4, or UV (week 5);
  that for visit 4 at UV (week 5), visit 5, or UV (week 7);
  that for visit 5 at UV (week 7), visit 6, or UV (week 9);
  that for visit 6 at UV (weeks 9, 10, and 11);
  that for visit 7 at UV (weeks 13, 14, and 15);
  that for visit 8 at UV (weeks 17, 18, and 19);

and that for visit 9 at UV (weeks 21, 22, and 23), based on the subject's condition.

- For the subjects who showed complete remission at week 8, the subjects should return to the site for blood tacrolimus level check, drug compliance check, and dispensing of the investigational drug for the tapering/ maintenance phase.
- When a subject complains of an AE, he/she should return to the center and should undergo the necessary tests.

6) End of study visits: The subjects who do not show complete remission within eight weeks, who show relapse after complete remission within 24 weeks, or whose participation in the study is discontinued must carry out the procedure of end of study visits (e.g., the last study visit of a subject who does not show complete remission within eight weeks will be visit 6 {week 8}, and exactly the same procedure as that in visit 6 will be performed in the case of a subject who reaches visit 10 {week 24}). The reason for the discontinuation of a subject's participation in the study will be obtained from the concerned subject on a separate CRF at the end of study visits, and follow-up will no longer be made after the last study visit. The period of temporary discontinuation of the drug administration should not be included in the eight-week study period.

8.1.4 Dose adjustment due to an AE

After the administration of the investigational drug, all AEs that are classified more than 'moderate' based on Spilker's three-level severity, including those related to the investigational drug, will be taken into consideration for dose adjustment by the investigators. The dose will be decreased to maintain the compliance criteria (within the range of 80~110%) and the investigators can administer additional medications to improve the symptoms based on conventional method. If a subject, however, can no longer manage the AE, the administration of the investigational drug can be discontinued in consultation with the investigator, and the concerned subject can be withdrawn early from the study.

8.1.5 Subject discontinuation and consent withdrawal

The subjects can be withdrawn from the study at any point during the study period. If a subject wants to withdraw his/her consent and does not provide any reason for such, the principal investigator or sub investigators should be notified of such. The investigators can decide to withdraw the following subjects:

- those whose participation in this study is inappropriate as they fall under one or more of the exclusion criteria;
- those who are hypersensitive to the investigational or control drug;
- those who show significant AEs from the administration of the investigational drug;
- those who no longer want to participate in the study;
- those who do not follow the guidelines given by the investigators;
- those whose continued participation in the study will be harmful for them as adjudged by the investigators;
- those whose blood tacrolimus concentration is found to exceed 10 ng/ml three consecutive times;
- female subjects who become pregnant in the course of the study; and
- those who are treated with a live vaccine in the course of the study.

For every discontinuation, the reason and date of withdrawal should be recorded in the source document and CRF. If a subject is withdrawn from the study due to an AE, the investigators should follow up the subject until the AE is resolved, until the subject's condition is as it was before the investigational drug was administered, until the abnormal result is brought back to the normal range, and until the investigators adjudge the AE to have already been resolved or that no more follow-up is required.

Even if the subject could not finish the whole study procedure, all the study subjects should complete all the tests for end of study visits, as much as possible. The investigators should do their best to contact the subjects with whom contact has been lost, to obtain from them the detailed information regarding the reason for their withdrawal from the study.

If a subject did not meet the aforementioned inclusion/exclusion criteria and the investigators are concerned about his/her temporary decreased renal function, the investigators should consider early termination after investigating the reason for the acute renal failure.

A subject who was withdrawn from the study must not be enrolled again therein. A new subject should be assigned a new randomization number.

8.2 Inclusion/exclusion criteria

<u>Inclusion criteria</u>: The patients who meet the following criteria can be enrolled in the study:

1) male or female patients who are 16 years old or more and less than 80 years old;
2) patients who have been diagnosed with initial or relapsed primary MCNS;
3) patients whose UPCR is more than 3.0 at visit 1 (spot urine); and
4) patients who voluntarily consented to participate in the study by signing the informed-consent form (patients who are19 or older can sign the informed-consent form by themselves; for 16- to 18-year-old patients, they and their parents have to sign the consent forms).

<u>Exclusion criteria</u>: Patients who fall under at least one of the following criteria should not be enrolled in the study:

1) patients whose PMDRD eGFR is less than 30 ml/min/1.73 m^2;
2) patients who were treated with immunosuppressants, such as tacrolimuscyclosporine, cyclophosphamide (Cytoxan), mizoribine (Bredinin), levamisole, azathioprine, mycophenolate mofetil, or rituximab, within two weeks before the study;
3) patients to whom more than 10 mg prednisolone or an equivalent dose of steroid was administered daily within two weeks before the study;
4) patients who are pregnant, breastfeeding, or planning to become pregnant or to breastfeed within six months after the study completion, or who cannot or do not want to use any contraceptive method;
5) patients who are hypersensitive to the investigational drug or to macrolide, such as azithromycin, clarithromycin, or roxithromycin;
6) patients who are currently taking bosentan (Tracleer Tab.);
7) patients who are currently taking potassium-sparing diuretics;
8) patients who were treated with a live vaccine within four weeks before their visit 1;
9) patients whose liver panel laboratory test result is three times the normal range, or acute-hepatitis patients whose serum bilirubin has been clinically significantly higher than 3.6 mg/dL for more than one month;
10) patients who have a significant general disease that makes it inappropriate for them to participate in this study, as adjudged by the investigator (e.g., cardiovascular-acute

myocardial infarction, heart failure {classified as more than NYHA class III}, hepatic/gastrointestinal/neurologic disease, blood disorder, cancer, infection, renal disorder other than MCNS, rheumatic arthritis with pneumonia interstitialis);
11) patients who have genetic problems such as galactose intolerance, Lapp lactose deficiency, or glucose-galactose malabsorption;
12) patients to whom another investigational drug was administered within 30 days from their enrollment in the study; and
13) patients who have participated in the previous phases of this study.

8.3 Targeted number of subjects, and rationale

The purpose of this study is to compare the effect of the administration of a low-dose steroid with tacrolimus for eight weeks with the standard therapy, high-dose steroid, in patients with MCNS.

In the previous study[7], 68 subjects (84%) showed complete remission among the 81 subjects who were treated with high-dose steroid. Forty-seven subjects showed complete remission within four weeks, and 16 subjects showed complete remission between four and eight weeks. A total of 63 subjects (78%) thus showed complete remission within eight weeks. Based on the study results, the complete remission ratio within eight weeks was found to be 0.8 (80%).

Moreover, the current therapeutic effect of steroid in patients with MCNS shows a 40-60% remission rate until eight weeks, and 70-90% until 12 weeks, based on the results of the previous study.[16,17] The range of such rates is about 20%. Based on such results, the non-inferiority margin was determined to be 0.2, and the sample size was calculated.

To determine the sample size needed to achieve the study objective, the following assumptions were made:

1) significance level: a = 0.025;
2) ratio of number of subjects in both groups (test group:control group) = 1:1; and
3) second error (p) = 0.20; statistical power = 80%.
4) study hypotheses (one-tailed test):
    - H0: difference in complete remission by group ≥ 0.20;
    - H1: difference in complete remission by group < 0.20; and
5) drop-out rate: 15%.

The complete-remission rate was set at 0.8 in both the tacrolimus and low-dose steroid group and the high-dose steroid group, and the non-inferiority margin was set at 0.2. The sample size was calculated using the Power Analysis and Sample Size (PASS) software, considering a 0.025 significance level and 0.8 statistical power (1-â) (refer to Attachment 10. Targeted Number of Subjects and Its Calculation PASS Output).

	Subject No./Group	Subject No./Group Considering the Drop-out Rate	Total No. of Subjects
Mean	64	76	152

The required number of subjects for each group shall be 64 for the analysis of the non-inferiority of tacrolimus in combination with low-dose steroid compared with that in combination with high-dose steroid, with 80% statistical power at a 2.5% significance level (one-tailed). Considering the 15% drop-out rate, the number of subjects in each group will be 76, totaling 152.

8.4 Rationale of setting the control group

The purpose of this study is to prove the antiproteinuric effect of tacrolimus, the test drug, in patients with MCNS. A control group was set for the study, and the selection criteria for the control group are the same as those for the test group demographically and medically. The patients with MCNS shall be randomized into either the test or control group. (Strata randomization will not be used as the strata factor for MCNS has not been determined.) The control and test groups shall show similar distributions in all the other parts, except for the administration of the test drug. As mentioned in the study background section, the subjects of this study can also be treated with high-dose steroid, but the use of high-dose steroid can have a number of side effects. Tacrolimus shows its antiproteinuric effect through synaptopodine stabilization, and it is expected to reduce the side effects of the use of high-dose steroid. During the study period, the use of the investigational drug is not expected to put the participant in any additional risk, such as severe or irreversible damage. If there is any risk factor or evidence of severe or irreversible damage, immediate study termination shall be considered.

8.5 Randomization

All the eligible subjects will be stratified based on the center at visit 1 (screening visit, week 0). The randomization table will be prepared using SAS 9.1, based on the block randomization method, which involves mixing block sizes 4 and 6 in each stratum. The strata factor for MCNS has not been determined, and the strata factor with regard to the drug indications will not be considered and will be determined only by the center. The subjects will be randomized into either the test group (low-dose steroid + Prograf) or the control group (high-dose steroid) at a 1:1 ratio. The Web-based randomization of Medical Research Collaborating Center (MRCC) of Seoul National University Medical School/Seoul National University Hospital will be utilized. As such, the maintenance and utilization will be done by the study designee at MRCC. Access to and maintenance of the randomization information will be separately handled by the study investigators and sponsor.

8.6 Dose, method, and period of investigational-drug administration

The patients who signed the informed-consent form and who are determined to be eligible to participate in this study based on the inclusion/exclusion criteria presented at visit 1 will start taking the investigational drug. In the previous study, BMC Research Note (2009) 2:144, which was conducted using cyclosporine for MCNS, it was indicated that the dose of cyclosporine was 2 mg/kg, and in another study, Transplantation Proceedings (2002) 34:1951-1952, the dosage of cyclosporine and tacrolimus was determined to be 50:1 (cyclosporine:tacrolimus). As clinical nephrology (2006) 65(4):276-279 and lupus (2007) 16:46-51 studies also used 0.05 mg/kg bid tacrolimus, the dose of tacrolimus for this study was determined to be 0.05 mg/kg bid.

The administration of the investigational drug will be done separately, in two phases: the remission and tapering/maintenance phases. In the remission phase, the test group will be treated with 0.5 mg/kg qd steroid and 0.05 mg/kg bid Prograf Cap.® on an empty stomach. The control group, on the other hand, will be treated only with 1 mg/kg qd steroid. (However, the prescribed dose of prednisolone cannot exceed 80mg/day. If daily dose of prednisolone calculated based on the subject's weight exceeds 80mg a day, the subject will be given 80mg.) The blood tacrolimus level in the remission phase should be maintained within the range of 5-10 ng/ml in the test group. The tapering/maintenance phase is defined as the phase after the remission phase and up to 24 weeks. When a subject shows complete remission (UPCR<0.2),

the dose of the steroid will be decreased by 5 mg every week in both groups after the same dose is maintained for two weeks. (The dose of the steroid will be maintained at 0.5 mg/kg in the test group and at 1 mg/kg in the control group for two weeks before the tapering). The dose of the steroid for maintenance is 7.5 mg/day for the subjects who weigh 80 kg or more, and 5 mg/day for the subjects who weigh under 80 kg. The blood tacrolimus level in the tapering/maintenance phase should be maintained within the range of 3-8 ng/ml in the test group. Prograf Cap.® is recommended to be taken on an empty stomach at least 1 hour before or 2-3 hours after a meal for maximum absorption. Also, as fatty foods or grapefruit juice can affect the body's absorption of the investigational drug, the investigators should instruct the subjects to limit their intake of these.

8.7 Prohibited concomitant medications

The subjects will be prohibited from taking any immunosuppressant drug (e.g., tacrolimus, cyclosporine, cyclophosphamide, levamisole, azathioprine, mycophenolate mofetil, rituximab) during the study period. Steroids should not be used to treat kidney syndrome, and the use of steroids will be based on the investigator's judgment. If a subject is taking steroid-based oral contraception, extra care should be taken by the subject in doing so. A live vaccine should not be administered to the subjects; in particular, the subjects taking high-dose corticoids should not be given any other vaccination. Any medications that have high affinity with serum protein such as anticoagulants, oral medication for diabetes should be carefully administered. If a subject uses any prohibited medication, he/she can be withdrawn from the study, as adjudged by the investigator. As for prohibited concomitant medications or those required to be used cautiously, refer to section 9. Expected side effects and use precautions or package insert.

8.8 Observation lists, methods and clinical laboratory test lists

Refer to the following study flowchart:

<Study Flowchart>[10]	**V1[14]**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**UV[11]**
Criteria for investigational-drug administration	**0w -7~1d**	**1w ±3d**	**2w ±3d**	**4w ±3d**	**6w ±3d**	**8w ±3d**	**12w ±7d**	**16w ±7d**	**20w ±7d**	**24w ±7d**	**Xw ±1d**
Consent form	O										
Eligibility check	O										
Randomization	O										
Patient history/ demographic data	O										
Vital signs[1]	O		O	O	O	O	O	O	O	O	
Height, weight[2]	O		O	O	O	O	O	O	O	O	
Hematology[3]	O		O	O	O	O	O	O	O	O	
Chest X-ray[4]	O		O		O					O	
ECG[5]	O									O 5-1)	○
Cardiac symptoms[6]	O	O	O	O	O	O	O	O	O	O	
Chemistry[7]	O		O	O	O	O	O	O	O	O	

Lipid panel[8]	O		O	O	O	O	O	O	O	O	
C-reactive protein	O		O	O	O	O	O	O	O	O	
Urinalysis	O	O	O	O	O	O	O	O	O	O	
Pregnancy test	O									O	
HbA1C	O			O		O		O		O	
UPCR, urine Na, K	O	O	O	O	O	O	O	O	O	O	
PRG trough level[9]		O	O	O	O	O	O	O	O	O	O [12,13]
Investigational-drug distribution	O		O	O	O	O	O	O	O		O [13]
Concomitant-medication check	O		O	O	O	O	O	O	O	O	O
Drug compliance check			O	O	O	O	O	O	O	O	O [13]
Adverse-event check	O	O	O	O	O	O	O	O	O	O	O

1) Measure the blood pressure using an automatic blood pressure manometer approved by FDA. The mean value is obtained after measurement in a sitting position two times, with a 5-minute interval.
2) The height is checked only at visit 1. For other visits, only the weight is checked.
3) CBC with differential count
4) Check the chest PA and confirm the cardiothoracic ratio.
5) Perform ECG and check if there is any tall T-wave, ST-elevation/depression, or T-wave inversion, and confirm the PR interval.
5-1) ECG will be performed at Visit 1 and either Visit 10 or last visit.
6) Check for cardiac symptoms (e.g., chest pain, chest discomfort, palpitation, breathing difficulty when exercising or standing up). If a subject shows cardiac symptoms, chest X-ray, ECG, and troponin T should be performed regardless of the visit.
7) Protein, albumin, cholesterol, bilirubin, ALP, AST, ALT, BUN, Cr, uric acid, glucose, Na, K, Cl
8) LDL-cholesterol, HDL-cholesterol. The subjects should fast for at least eight hours before the test is carried out.
9) he minimum tacrolimus level in the blood should be checked 12 hours after the drug administration.
10) The subjects who do not show complete remission until eight weeks, who show relapse after complete remission within 24 weeks, or whose participation in the study is discontinued must carry out the procedure of End of study visits (e.g., the last study visit of a subject who does not show complete remission until eight weeks will be visit 6 (eight weeks), and exactly the same procedure as that of visit 6, as for a subject who reaches visit 10 (24 weeks), will be performed. The reason for the discontinuation of a subject's participation in the study will be obtained from the concerned subject on a separate CRF at the end of the study visit, and follow-up will no longer be made after the last study visit.
11) Unscheduled visit (UV): The subjects can make an unscheduled visit to the study center in the following cases: for tacrolimus blood level retest, for prescription of the investigational drug in the tapering and maintenance phase at 10 weeks for a subject who shows complete remission at eight weeks, or due to the occurrence of an adverse event.
12) If the tacrolimus level in the blood is more than 15 ng/ml, the investigational drug should be temporarily stopped, and retest should be done after one week. The subject should visit the center for retest up to three weeks, until the tacrolimus level in the blood is already less than 10 ng/ml. The retest for visit 2 can be done at visit 3, UV (three weeks), or visit 4; the retest for visit 3 at UV (three weeks), visit 4, or UV (five weeks); the retest for visit 4 at UV (five weeks), visit 5, or UV (seven weeks); the retest for visit 5 at UV (seven weeks), visit 6, or UV (nine weeks); the retest for visit 6 at UV (9, 10, 11 weeks); the retest for visit 7 at UV (13, 14, 15 weeks); the retest for visit 8 at UV (17, 18, 19 weeks); and the retest for visit 9 at UV (21, 22, 23 weeks), based on the subject's condition.
13) Prescribe the investigational drug with a reduced dose from 3 weeks for the subjects who showed remission at week 1 and from 10 weeks, for the subjects who showed remission after eight weeks.
14) Visit 1 is up to eight days from the test and until day 1 (first drug administration) after the randomization.

8.9 Compliance assessment

The prescribed and returned investigational drug will be confirmed to calculate the subjects' drug compliance. The proper drug compliance rate on the part of each subject is 80-110%.

8.10 Efficacy endpoint, evaluation, and reporting method

8.10.1 Efficacy assessment

Primary efficacy endpoint:

- The percentage of subjects who show a decreased UPCR of less than 0.2 up to eight weeks in both the test and control groups

Secondary efficacy endpoint:

- The period until the UPCR has decreased to below 0.2 in both the test and control groups
- The percentage of subjects who show relapse after remission up to 24 weeks in both the test and control groups
- The period until relapse happens from complete remission up to 24 weeks in both the test and control groups

Safety assessment:

- All AEs will be assessed based on the symptoms that the subjects will complain of, the measured vital signs, the results of the physical examination to be conducted by the investigators, and the results of the electrocardiogram, chest X-ray, hematology, chemistry, and urinalysis. The percentage of AEs up to 24 weeks will be evaluated after the administration of the investigational drug.

8.10.2 Evaluation of efficacy

Evaluation of the primary efficacy will be conducted through a one-tailed test at a 2.5% significance level, and evaluation of the secondary efficacy will be conducted through a two-tailed test at a 5% significance level. The efficacy analysis will be additionally performed mainly in the intention-to-treat (IIT) and per-protocol (PP) groups. If non-inferiority is proven in both analysis groups, non-inferiority can be established. Safety analysis, on the other hand, will be performed in the Safety Analysis Set.. The IIT group will include all the subjects who were randomized and treated with the investigational drug (low-dose steroid + Prograf Cap.®, or high-dose steroid) at least once while the PP group will include the subjects who meet the following criteria

The Per-Protocol analysis set for the primary efficacy assessment will include the subjects in the ITT analysis set who meet the following criteria:
1) those who meet all the inclusion and exclusion criteria;
2) those who showed 80-110 % drug compliance at every visit until the primary endpoint assessment (Visit 6 (8 weeks))
3) those who followed the procedure of the study protocol until the primary endpoint assessment (Visit 6 (8 weeks))

The Per-Protocol analysis set for the secondary efficacy assessment will include the subjects who meet the following criteria:
1) those who are eligible to participate in this study based on the study's inclusion/exclusion criteria;
2) those who showed 80-110% drug compliance; and
3) those who followed the procedure of the study protocol and who completed the study.

Safety analyses will include all subjects who take investigational products at least one or more.

Assessment of demographics prior to the administration of the investigational drug:

To assess if there is any significant difference in demographics between the groups prior to the administration of the investigational drug, t-test or Wilcoxon rank sum test will be performed for the continuous variables, and chi-square test or Fisher's exact test for the non-continuous variables.

Primary efficacy endpoint:

1) The percentage of subjects who show a decreased UPCR of less than 0.2 up to eight weeks in both the test and control groups
   * This study is a non-inferiority test with a 95% confidence level upper limit.
   If the complete remission rates of the control and test groups are defined as δ, a one-tailed test will be performed using a 95% lateral confidence level upper limit of δ at a 2.5% significance level. If the 95% lateral confidence level upper limit is less than 0.2, it means

that the effect of the test drug is inferior to that of the control drug. If it is 0.2 or more, it means that the test drug is not inferior to the test drug.

Secondary efficacy endpoint:

1) The period until the UPCR decreases to below 0.2 in both the test and control groups: The median time will be presented, and the log rank test will be carried out to compare the test and control drugs.

2) The percentage of subjects who show relapse after remission up to 24 weeks in both the test and control groups: Chi-square test or Fisher's exact test will be carried out to obtain this value.
3) The period until relapse occurs from complete remission up to 24 weeks in both the test and control groups: The median time will be presented, and the logrant test will be carried out to compare the test and control drugs.

8.10.3 Safety assessment

1) All the subjects to whom the investigational drug was administered more than once will be included in the safety assessment.
2) An adverse event (AE) is defined as any untoward sign (e.g., abnormal lab result), symptom, or disease. AE will be based on the Common-Terminology Criteria for Adverse Events (CTCAE) Ver. 4.0, and it does not necessarily have a causal relation with the treatment in this study.
3) The severity of all the AEs will be assessed based on Spilker's three-level severity scheme: mild, moderate, or severe.

Mild	AEs that do not disrupt normal daily activities or require any treatment
Moderate	AEs that disrupt normal daily activities, may require treatment, and may be resolved after the treatment
Severe	Severe AEs that require intensive treatment and that are life-threatening or give sequela to the subjects even after their resolution

4) After analyzing the occurrence of AEs, the occurrence of AEs leading to subject withdrawal, the occurrence of serious adverse events (SAEs), and the relationship with the investigational drug, and after coding these using MedDRA, the number and rate of subjects who showed AEs will be summarized and presented after being classified by site (organ).

5) Causality assessment
   a. Definitely related
      - There is evidence of the administration of the investigational drug.
      - The AE occurs within a reasonable time after the administration of the investigational drug.
      - The AE is unlikely to be attributed to any reason other than the administration of the investigational drug.
      - The result of the rechallenge (if possible) is positive.
      - The AE is considered related to the investigational drug based on the reported information regarding the AEs of the drug or of the other drugs in the same class.
   b. Probably related
      - There is evidence of the administration of the investigational drug.
      - The AE occurs within a reasonable time after the administration of the investigational drug.
      - The AE is unlikely to be attributed to any reason other than the administration of the investigational drug.
      - The AE disappears after the discontinuation of the investigational drug.

c. Possibly related
   - There is evidence of the administration of the investigational drug.
   - The AE occurs within a reasonable time after the administration of the investigational drug.
   - The AE is likely to be attributed to the administration of the investigational drug at the same level as any other reason.
   - The AE disappears after the discontinuation of the investigational drug (if applicable).

d. Probably not related
   - There is evidence of the administration of the investigational drug.
   - There are other reasons that are more likely to generate the AE than the administration of the investigational drug.
   - The result of the discontinuation of the investigational drug (if applicable) is negative or not clear.
   - The result of the rechallenge is negative or not clear.

e. Definitely not related
   - When a subject was not treated with the investigational drug, or when the sequence of the occurrence of the AE and the administration of the investigational drug is not reasonable.
   - There is another definite reason for the AE.

6) The percentage of AEs up to 24 weeks after the administration of the investigational drug compared to the percentage of subjects by group will be tested via chi-square test or Fisher's exact test.
7) As for the failure of the treatment or withdrawal due to an AE related to the investigational drug, chi-square test or Fisher's exact test will be used.
8) As for the laboratory test results, the results of weeks 2, 4, 6, 8, 10, 12, 16, 20, and 24 will be compared to those of visit 1 in both groups. The changes in value will be analyzed using t-test and Wilcoxon rank sum test.
9) As for the values of the vital signs and the other biological test results, the results will be analyzed and presented as laboratory results.

8.10.4 Handling of missing data

The missing data for efficacy analysis will be basically compensated for using the last observation carried forward (LOCF) method. The most conservative method will be used depending on the case, and the reason will be recorded in the report. The missing values of the efficacy and safety variables at visit 1 (baseline, week 0) will not be compensated for.

9. Expected Side Effects and Use Precautions

9.1 Expected Side Effects and Use Precautions of Tacrolimus (Prograf Cap.®):

1) SAES

a) heart failure, angina pectoris, arrhythmia, myocardial infarction, pericardial effusion, and myocardial damage: a myocardial abnormality (e.g., st-t change, decreased cardial function, cardiac enlargement, ventricular hypertropy), heart failure, ventricular arrhythmia, superventricular arrhythmia, angina pectoris, myocardial infarction, or pericardial effusion can occur due to the administration of the investigational drug. the investigator should thoroughly observe the subjects through ecocardiogram, ecg, chest x-ray, or other tests. if a subject shows abnormality, the investigator should take appropriate action, such as dose decrease of the investigational drug.

b) acute renal failure or nephrotic syndrome: acute renal failure or nephrotic syndrome can occur due to the administration of the investigational drug. the investigator should perform clinical tests (e.g., creatinine, bun, creatinine clearance, urine protein, urine nag, urine β**-microglobuline**) or other tests frequently, and should thoroughly observe the subject. if a subject shows abnormality, the investigator should take appropriate action, such as dose decrease of the investigational drug.

c) thrombotic microangiopathy: thrombotic microangiopathy such as hemolytic uremic syndrome (hus) or thrombotic thrombocytopenic purpura (less than 5%) can occur due to the administration of the investigational drug. the investigators should perform tests regularly or use other methods to observe the subjects thoroughly. if a subject shows abnormality, the investigator should take appropriate action, such as dose decrease of the investigational drug.

d) Blood: pensytopenia and thrombotic thrombocytopenic purpura may occasionally occur and pure red-cell aplasia (PRCA), agranulocytosis and hemolytic anemia (occurrence unknown) can occur due to the administration of the investigational drug. the investigators should perform tests regularly or use other methods to observe the subjects thoroughly. if a subject shows abnormality, the investigator should take appropriate action, such as dose decrease of the investigational drug.

e) convulsion: convulsion can sometimes occur due to the administration of the investigational drug. in this case, the investigators should take appropriate action, such as dose decrease or temporary discontinuation of the investigational drug.

f) ileus: convulsion from intestinal obstruction (ileus) can sometimes occur due to the administration of the investigational drug. in this case, the investigators should take appropriate action, such as dose decrease or temporary discontinuation of the investigational drug.

g) infections: bacterial, virus, fungal, or protozoal infection can occur, or an existing infection can worsen, due to to the administration of the investigational drug. general or local infection can occur. cmv infection from this investigational drug is less frequent, however, than infection from cyclosporine. if a subject shows infection, the investigator should take appropriate action, such as dose decrease of the investigational drug or administration of antibiotics.

h) lymphoma and other malignancies: a lymphoproliferative disorder related to e**pstein-barr virus (ebv) infection or lymphoma can occur** due to to the administration of the investigational drug. in this case, the investigators should take appropriate action, such as dose decrease or temporary discontinuation of the investigational drug. this especially occurs more frequently in babies (less than 2 years old), infants, or patients who are taking antilymphocyte antibodies at the same time. as an overly suppressed immune system can increase the possibility of the development of a malignant tumor, the investigator should observe the subjects thoroughly and should take appropriate action, such as dose decrease or temporary discontinuation of the investigational drug, if a subject shows abnormality.

i) central nervous system: cases of generalized convulsion, **altered mental status, alienation, speech disorder, visual disorder, and hemiplegia have been reported overseas. if these aes occur, the investigator should perform neurologic tests, ct, and mri, and should take appropriate action, such as** dose decrease or temporary discontinuation of the investigational drug.

j) cerebral vascular system: cerebral diseases such as cerebral infarction or stroke rarely occur due to the investigational drug (incidence: less than 5%). if they do occur, however, the investigators should take **appropriate action, such as** dose decrease or temporary discontinuation of the investigational drug.

k) interstitial pneumonitis: interstitial pneumonitis can occur in patients with rheumatic arthritis. the investigators should observe the subjects carefully, and if a subject shows any respiratory symptom, such as fever, cough, or breathing difficulty, the administration of the investigational drug should be discontinued, and chest x-ray, ct, and blood test should be performed immediately. the investigator should also consider the possibility of infection and should take appropriate action, such as administration of steroids.

l) diabetes, hyperglycemia: diabetes or its worsening (incidence: less than 5%), and hyperglycemia (incidence: more than 15%), can occur. the investigators should observe the subjects thoroughly and should take appropriate action, such as dose decrease or temporary discontinuation of the investigational drug, if such aes occur.

m) pancreatitis: pancreatitis (incidence: less than 5%) can occur. the investigators should thus perform regular tests to observe the subjects, and should **take appropriate action, such as** dose decrease or temporary discontinuation of the investigational drug.

n) mucocutaneous ocular syndrome (stevens-johnson syndrome): steven-johnson syndrome can occur (incidence: less than 5%). the investigaors should discontinue the administration of the investigational drug and should take **appropriate action** if this happens.

o) breathing difficulty: breathing difficulty and acute respiratory distress syndrome (incidence: less than 5%, respectively) can occur. the investigator should thus observe the subjects thoroughly and should **take appropriate action, such as** dose decrease or temporary discontinuation of the investigational drug, if such aes occur.

2) OTHER AEs

a) Kidney: Nephropathy (increased BUN, creatinine, decreased creatinine clearance, and proteinuria), decreased urine amount, polyuria, urinary frequency, urinary urgency, and a feeling of residual urine after voiding can be observed less frequently.

b) Metabolic abnormality: Urine glucose, hyperkalaemia, hyperuricemia, and hypomagnesaemia can be observed, and hypercholesterolemia, hypertriglyceridemia, hyperchloremia, and hypercalcemia can be observed less frequently.

c) Circulatory system: Increased blood pressure, edema, tachycardia, palpitation, abnormality in ECG, and bradycardia can be observed less frequently.

d) Psychoneurosystem: Delirium, headache, tremor of the eyelids or muscles around the eyes, orthocolosis, paralysis, sensory disorder, insomnia, seizure, altered mental status, loss of local sense, or excitement can be observed less frequently.

e) Gastrointestinal system: Nausea, vomiting, bloating, intestinal-duct movement abnormality, loss of appetite, diarrhea, abdominal pain, gastric ulcer, duodenal ulcer, or melena can be observed less frequently and gastrointestinal perforation (Fregnancy of occurance unkown) can occur.

f) Pancreas: Increased amylases can sometimes be observed.

g) Liver: Increased GOT, GPT, LDH, or γ-GT can sometimes be observed.

h) Blood: Anemia, increased or decreased platelet, or decreased lymphocytes can sometimes be observed.

i) Skin: Itchy sense, alopesia, or erythema can sometimes be observed.

j) Others: Tubular necrosis, hypertropic cardiomyopathy (especially in children), gynecomastia, vasodilation, decreased concentration of phosphoric acid in the blood, burning sense, rashes, fatigue, muscle pain, abnormal taste sense, and heavy menstrual period can be observed less frequently. Aplastic anemia, colitis, toxic epidermal necrolysis (Lyell syndrome), glaucoma, ketosis, nystagmus, mania, meningitis, macrorhinia, thrombophlebitis, thrombosis, vasculitis, pleural effusion, foreign-body sensation in the nose or throat, ocular pain, weight loss, and pains can hardly be observed.

3) Warnings and precautions

a) As the use of immunosuppressants may increase the sensitivity to infections and the possibility of generating lymphoma, only physicians experienced in immunosuppressive therapy and management of organ transplant patients should use the investigational drug. Patients receiving the drug should be managed in facilities with adequate laboratory equipment and staff, and the physicians responsible for maintenance therapy should have all the necessary information for patient follow-up.

b) The investigational drug, particularly when used in high doses, can cause neurotoxicity or nephrotoxicity. Nephrotoxicity was reported in 40 and 36% of the liver transplantation patients in U.S. and European randomized trials, respectively. Nephrotoxicity presenting increased creatinine and decreased urine output was clearly observed in the early period after the transplantation. Patients with renal disease should thus be monitored carefully, and the dose given to them should be decreased. If a patient shows continuously increased creatinine even after the dose decrease, the patient should be treated with other immunosuppressants. Extra care should be taken when administering drugs that may be associated with nephrotoxicity. In particular, cyclosporine should not be used concurrently to prevent excessive nephrotoxicity. If a subject needs to be treated with cyclosporine after this drug, or vice versa, the next drug should be administered 24 hours after the last dose of the previous drug is administered. If the concentration of the investigational drug or cyclosporine is high, the administration should be delayed.

c) Mild to severe hyperkalemia was reported in 44 and 10% of the liver transplantation patients in U.S. and European randomized trials, respectively. As such, the blood potassium level should be assessed frequently, and potassium-sparing diuretics should not be used while receiving the investigational drug.

d) Neurotoxicity such as delirium, headache, change in motor function or mental status, or parethesia was reported in 55% of the liver transplantation patients in a randomized trial. The delirium and headache was related to the high dose of tacrolimus, and was reversed when the administration was discontinued. Posterior reversible encephalopathy syndrome (PRES) was reported in patients treated with tacrolimus. The symptoms indicating PRES include headache, altered mental status, seizures, visual disturbances, and hypertension. Diagnosis may be confirmed through a radiological procedure. If PRES is suspected or diagnosed, blood pressure control should be maintained, and immediate reduction of immunosuppression is advised. This syndrome is characterized by reversal of symptoms upon reduction or discontinuation of immunosuppression.

e) Likewise, patients treated with other types of immunosuppressants and those receiving the investigational drug have an increased risk of developing lymphomas and other malignancies, particularly of the skin. The risk appears to be related to the intensity and duration of immunosuppression rather than to the use of any specific agent. Lymphoproliferative disorder (LPD), which is related to Epstein-Barr virus (EBV) infection, has been reported. The risk of acquiring this disease is especially high in pediatric patients, who are more prone to infection by EBV, or in immunosuppressed organ transplant recipients. As such patients have higher sensitivity to infection due to their overly suppressed immune system, more care should be taken by them when using other immunosuppressants concurrently.

f) Patients receiving immunosuppressants have an increased risk of acquiring opportunistic infections, including latent virus infection. Nephropathy related to BK virus infection

and JC-virus-associated progressive multifocal leukoencephalopathy (PML) have been observed in patients receiving tacrolimus. These infections may have serious and sometimes fatal outcomes.

g) Pure red-cell aplasia (PRCA) has been reported in patients treated with tacrolimus. Most of the patients reported risk factors for PRCA, such as parvovirus B19 infection, an underlying disease, or concomitant medications associated with PRCA.

h) Hypertension is a common adverse effect of the investigational drug and may require antihypertensive therapy. Mild to moderate hypertension occurs more frequently than severe hypertension. Blood pressure control can be accomplished with any of the common antihypertensive agents, although careful consideration should be made prior to the use of antihypertensive agents associated with hyperkalemia (e.g., potassium-sparing diuretics). Calcium-channel-blocking agents may affect the metabolism of tacrolimus and therefore require dosage reduction of the drug.

i) Patients with renal or hepatic impairment: Dose reduction is required for patients with renal dysfunction. Patients who show hepatic dysfunction after liver transplantation have a high possibility of developing renal dysfunction due to high blood tacrolimus concentration. Extreme care should thus be taken by such patients, and dose adjustment should be considered. Based on some studies, these patients require dose reduction.

j) To prevent the occurrence of AEs due to high-dose administration, or rejection reaction due to low-dose administration, the blood tacrolimus trough level should be checked frequently, and the dose should be adjusted accordingly. As tacrolimus can be attached to the container (about 20%) during analysis, a standard substance and sample should be handled in the same manner to minimize the loss due to the attached tacrolimus.

k) As renal impairment can occur frequently, clinical tests (e.g., creatinine, BUN, creatinine clearance, urine protein, urine NAG, urine β-microglobulin) should be performed frequently so that the patient can be thoroughly monitored. In particular, the occurrence of renal dysfunction at an early stage after the drug administration should be watched out for. As the worsening of renal dysfunction can cause other complications, dose reduction or temporary discontinuation should be done if a subject shows abnormality.

l) As the incidence of pancreatic dysfunction such as hyperglycemia and urine glucose is high, clinical tests (e.g., blood test, amylase, urine glucose level) should be performed frequently to monitor the patient thoroughly. In particular, the occurrence of pancreatic dysfunction at an early stage after the drug administration should be watched out for, and dose reduction or temporary discontinuation should be done if a subject shows abnormality.

m) The steroid dose can be reduced due to the administration of the investigational drug, but the AEs from steroids should also be continuously observed.

n) Food: grapefruit juice (Grapefruit juice increases the blood tacrolimus level and may therefore cause AEs.)

o) New-onset infection or worsening of infection should be watched out for.

p) As there is a possibility of increased sensibility to infection due to an overly suppressed immune system and the occurrence of malignant tumors such as lymphoma, enough care should be taken.

q) Heart failure, arrhythmia, myocardial infarction, angina pectoris, and myocardiopathy (including decreased heart function, heart wall enlargement) can occur during the administration of the investigational drug. As such, the patient's condition should be carefully observed via ECG, echocardiogram, or chest X-ray when the investigational drug is administered.

9.2 EXPECTED SIDE EFFECTS AND USE PRECAUTIONS FOR STEROID USE (SOLONDO TAB.):

1) AEs

a) Infection: new-onset infection, worsening of infection

b) Endocrine: secondary adrenocortical unresponsiveness, diabetes, menstrual irregularities, Cushing's syndrome (moon face), buffalo hump

c) Gastrointestinal: peptic ulcer, pancreatitis, diarrhea, vomiting, nausea, gastric pain, heartburn, bloating, thirst, loss of appetite, increased appetite, growth inhibition in childhood

d) Neurological: mental disorder, depression, euphoria, insomnia, headache, dizziness, convulsion, excitement, increased intracranial pressure with papilledema (pseudotumor cerebri) usually after treatment

e) Musculoskeletal: osteoporosis, aseptic necrosis of the femoral and humeral head, myopathy, vertebral compression fracture, loss of muscle mass, pathological fracture of the long bones, muscle pain, joint pain, muscle weakness

f) Protein/lipid metabolism: negative nitrogen balance, fatty liver

g) Fluid and electrolyte disturbance: edema, fluid retention, sodium retention, potassium loss, congestive heart failure in susceptible patients, hypertension, hypokalemic alkalosis

h) Eye: Increased intraocular pressure, glaucoma, posterior subcapsular cataracts (symptom: cloudy eyes), central serous chorioretinopathy, and multiple posterior retinitis pigmentosa (symptoms: decreased visual acuity, distorted vision, distorted smaller or central vision, and blindness). In central serous chorioretinopathy, local retinal detachment is observed, and when it progresses, multiple posterior retinitis pigmentosa occurs, which shows general retinal detachment. As bacteria or virus can cause secondary infection of the eyes, regular tests should be performed. Due to the central serous chorioretinopathy, retinal disorders or ophthalmoptosis can occur.

i) Blood: increased white blood cells, thrombosis

j) Heart: myocardial infarction, cerebral stroke, aneurysm

k) Skin: acne, alopesia, hirsutism, pigmentation, itching, petechiae or ecchymoses, purpura, streak, abnormal sweating, facial erythema, thin and fragile skin, impaired wound healing

l) Hypersensitivity

m) Others: fever, **fatigue, steroidal nephrosis, weight gain, or increased or decreased number/mobility of sperms**

2) Warning and precautions

a) Secondary adrenocortical insufficiency may occur due to the long-term administration of the investigational drug. This may persist for months after the discontinuation of the therapy. As the immediate discontinuation of the investigational drug may cause acute adrenal failure and sometimes fever, headache, loss of appetite, fatigue, muscle pain, joint pain, or shock, gradual dose deduction should be performed. In any situation where withdrawal syndrome occurs after the drug discontinuation, re-administration or dose increase should immediately be done. During the long-term drug administration, if any case of stress (e.g., trauma, operation, infection) occurs during this period, the dose should be increased temporarily, and if this happens during the drug discontinuation period, re-administration should be done temporarily. As the mineralocorticoid secretion may be impaired, salt and/or a mineralocorticoid should be concurrently administered.

b) As there has been a report that the administration of corticoids worsens bronchial asthma, greater care should be taken when the investigational drug is administered to asthma patients, who are more sensitive to drugs, food, or any additive.

c) Pediatric patients who are taking immunosuppressants are more prone to infection than healthy pediatric patients. For example, measles or chicken pox can have severe or fatal outcomes in patients who are taking corticoid, a type of immunosuppressant. Extreme

care should be taken by patients (adults or children) who have never had such diseases. If a patient is exposed to chicken pox or measles, he/she should get varicella zoster immune globulin (VZIG) or immunoglobulin (IG), respectively, within 3- to 10-day exposure. If a patient shows symptoms of chicken pox, the use of antiviral drugs should be considered.

d) Live vaccines should not be administered to a patient who is on corticoid therapy. In particular, no other vaccination should be allowed for a patient who is on high-dose corticoid therapy, considering the possibility of developing neurological complications or deficiency of antibody reaction.

10. Safety assessment, including AE and evaluation criteria/method

10.1 AE management

All the AEs that occur after the subjects sign the informed-consent form should be recorded on the CRF. The investigators should notify the sponsor when they find any information regarding important risk factors, prohibited concomitant medications, AEs, and use precautions related to the investigational drug during the study period.

10.2 SAEs

1) Result in death or are life-threatening
2) Require inpatient hospitalization or lead to prolongation of hospitalization
3) Result in persistent or significant disability/incapacity
4) Result in a congenital anomaly or a birth defect
5) Even if an event is not life-threatening, if it results in death or hospitalization, it can be considered serious, based on the investigator's judgment, if it may jeopardize the subject or if an internal or surgical intervention is required to prevent one of the other outcomes listed in the aforementioned definition from occurring (e.g., allergic bronchospasm requiring intense treatment in an emergency room or at home, blood dyscrasias or convulsion not resulting in hospitalization, or development of drug dependency or abuse).

10.3 AE reporting procedure

The principal investigator must train the subinvestigators, coordinator, and subjects or their legally accepted representatives regarding all the AEs that can occur after the administration of the investigational drug, and must instruct them to report all the AEs that occur. The information regarding all associated general, clinical, or pathologic symptoms after the administration of the investigational drug, such as its type, onset time, severity, action (medication), progress, and causality to the investigational drug, should be recorded on the CRF and stored according to the GCP.

The investigator must notify the Sponsor, Astellas Pharma Korea, Inc., of all SAEs that have occurred through phone calls or fax, after completing the SAE form provided by the sponsor, regardless of the SAE's expectancy or causality to the investigational drug.

Contact	Tel. No.	E-mail Address	Fax No.
[redacted]	[redacted]	[redacted]	[redacted]

The study monitor will help the study investigators obtain the additionally required information or the type of documents to be used, and will ask them to submit AE reports to the IRB. Astellas must immediately report all unexpected, related adverse drug reactions with the SAE report from the investigator to the KFDA director within the following timeline, according to KGCP:

- For an AE resulting in death or that is life-threatening: within seven days from the day the sponsor became aware of the matter. In this case, the detailed information must be reported within eight days from the initial reporting.
- For any other serious and unexpected adverse drug reaction: within 15 days from the day the sponsor became aware of the matter

Pregnancy itself is not an AE or SAE, but if the pregnancy results in an AE or an SAE, it must be reported after completing the relevant form.

10.4 Follow-up of AEs

The principal investigator or sub investigators should follow up the subject until the AE is resolved and until the subject's condition is as it was before the investigational drug was administered, until the abnormal result is brought back to the normal range, and until the investigators adjudge the AE to have been resolved or adjudge that no more follow-up is required. Also, they must do their best to obtain information regarding the reason for the drug discontinuation and regarding the action or progress of the AE. (If a subject does not want to visit the center, the follow-up can be made through e-mail or phone calls.)

10.5 Action for AEs

The study investigators and staffs must exert every possible effort to ensure the subjects' safety. If a subject shows an SAE, the investigators should discontinue his/her participation in the study and should take proper and prompt action to minimize the effects of the SAE. The responsibilities of each study staff when an SAE occurs are as follows:

1) Responsibility of the principal investigator
   If an SAE occurs during the study period, the principal investigator must immediately notify the IRB and the sponsor of such, and must stop the administration of the investigational drug (partially or completely) until a contrary instruction is given by IRB and the sponsor.
2) Responsibility of the study investigators

If an SAE occurs during the study period, the study investigator must notify the principal investigator and sponsor of such.

3) Responsibility of the IRB
   If an SAE occurs during the study period, IRB must take the required action for the principal investigator to stop the administration of the investigational drug (partially or completely).
4) Responsibility of the sponsor
   If the sponsor was notified by the principal investigator or study coordinator of the occurrence of a serious and unexpected AE, it must submit an AE report, and a copy of such report received from the investigators or study coordinator must immediately be given to the KFDA director. If the study is a multi-center study, the remaining centers must also be notified of the AE.

11. Informed-consent form, subjects' compensation, and subjects' treatment or hospital visit after the study

11.1 Subject information sheet and informed-consent form

The study investigators should explain the study to the subjects, including the effects of the investigational drug as well as its AEs and the study procedure, and should obtain the subjects' consent (refer to Attachment 4. Subject Information Sheet; Attachment 5. Informed-Consent Form; and Attachment 6. Subject Information and Approval Sheet for Underage Subjects).

11.2 Subjects' compensation

Refer to Attachment 7. Policy for Subjects' Compensation.

11.3 Guidelines for the subjects' treatment or hospital visit after the study

The investigators should guide the subjects who have been withdrawn from the study or who did not show any response to the study treatment to obtain other proper treatments. Also, the investigators should instruct the subjects who completed the study to obtain treatment at any time following the instruction of the medical staff, to prevent the delayed occurrence of AEs.

12. Plan to ensure the subjects' safety

12.1 Study center

The head of the study center must equip the center with all the facilities needed for the study, and must have qualified professionals for the proper conduct of the study.

12.2 Approval and amendment of the study protocol

To obtain approval or amend the approved study protocol for the conduct of the study, the initial or each amended study protocol must obtain approval from the IRB beforehand. No subject can be enrolled in the study prior to such approval.

12.3 Understanding of the study protocol

This study will be conducted based on the Declaration of Helsinki announced at the 59th World Medical Association Convention in Seoul in 2008, and will protect the well-being and rights of the study subjects. The study investigators and coordinator should understand the study protocol accurately and should take an active role in solving any problem that may be seen in the subjects.

12.4 Subjects' consent to participate in the study

Before the start of the study, the subjects should be given all the information about the study, including the effects of the investigational drug as well as its AEs and safety information. The subjects should voluntarily decide whether to participate in the study or not to, and if they decide to participate in the study, they should sign the informed-consent form.

12.5 Selection of appropriate subjects

The eligibility of the patients to participate in the study will be thoroughly assessed through the pertinent tests and through adequate discussions with the patients prior to the study enrollment.

12.6 Study inspection

The principal investigator should regularly report to the study sponsor the study progress, status, and results as well as the AEs that may occur, and the sponsor should conduct inspection regularly with regard to the study status.

12.7 Study monitoring

Study monitoring will be conducted to check if the CRF is accurate, complete, and reliable compared to the source documents, and if the study is being conducted based on the approved study protocol, KGCP, and Enforcement rule no. 28, and if the rights and well-being of the subjects are appropriately being protected. The monitoring will be conducted through regular visits and phone calls from the staff of CRO. During the visits, the monitor basically checks the original copy of the medical records, the investigational-drug accountability record, and the study file (record retention). The monitor will also check the progress of the study conduct, and will discuss with the investigators any problem that may arise in the course of the study.

The monitor will set the appropriate dates for the monitoring visits after discussing the matter with the investigators. The investigators must allow the monitor to review the source documents (e.g., hospital or personal medical charts, laboratory test results report, records of appointment dates with the doctors) to confirm the data in the CRF as addressed in GCP.

12.8 Subject information confidentiality

All the records where the subjects' identities can be disclosed will be kept confidential, and the subjects' identities will not be disclosed in the publication of the study results. The details of this matter are as follows:

The study sponsor, monitors, and inspectors can have access to the subjects' records for monitoring, inspection, and study maintenance purposes. The investigators must understand that the sponsor or inspector can review or even copy the subjects' medical charts or CRF, based on the contract with the sponsor. All the information regarding the study, however, should be kept confidential. A facility for confidential-document retention, and its maintenance standard, should be established.

All the study-related documents shall reflect only the subjects' identity codes (generally the subjects' initials) and not the subjects' names.

12.9 Handling of the investigational drug

The investigational drug should be stored at room temperature (1-30℃) and cannot be used without prescription by the principal investigators or sub-investigators.

The sponsor should directly give the investigational drug to the study pharmacist after the discussion with the principal investigator, and should keep the receipt of the investigational drug issued by the pharmacy.

The investigational drug should be marked "For clinical trial." The study pharmacist should store and maintain the investigational drug so that it would not be used for purposes other than for the clinical trial. During the study period, the sponsor should check the quantity and storage condition of the investigational drug and should make sure that the study is being conducted properly. If there was discontinuation or completion of the study, or if the investigators did not follow the study protocol, the unused investigational drug should be returned and destroyed by the sponsor. The study pharmacist should keep the return form of the investigational drug after the discussion with the principal investigator.

12.10 Action for handling AEs

If an AE occurs, the investigator should immediately make arrangements for the performance of the necessary tests and treatment by medical professionals. If an SAE occurs, the study must be discontinued, and prompt and proper action should be taken based on section 10.5 (Action for AE).

13. Other information regarding scientific and moral clinical trials

All the study-related documents should be kept in a separate and secure place. The designee should be selected after the study result report submission, and the documents should be retained for 3 years from the study completion date.

13.1 Plan for database establishment and for the handling of the study results

All the data obtained from this study will be recorded only in the e-CRF developed by Medical Research Collaborating Center of Seoul National University Hospital. After the completion of the data inputting, logical database checking will be performed to check if there is any non-logical date or abnormal laboratory test result. The queries will be resolved after the principal investigator, the sub-investigators and the study coordinator discuss these. All the required corrections of the database will be documented as attachments or audit trails. To obtain accurate, reliable data and to ensure the safety of the study subjects, the investigators will retain all the test results, clinical records, and medical records of the subjects as source documents, and direct access to such data will be given only to the principal investigators(and sub-investigators), study coordinator, and the IRB or KFDA personnel.

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Appendix 1. Signature

"We investigators confirm that this study protocol contains all the required information and regulations to conduct this clinical trial. We affix our signatures to this form to indicate that we agree to all the study details and data-recording method. We will conduct the study in accordance with the protocol and ethical principles reflected in the Declaration of Helsinki, KGCP, and relevant regulations. To change this study protocol, the written agreement of both the investigators and the sponsor is required."

Sponsor

Signature: _____

Date: [redacted]

Name: _____

Company: Korea, Inc. [redacted]

Investigator

Signature: _____

Date: [redacted]

Name: _____

Company: Koreal, Inc. [redacted]

Appendix 2. Contact Details of the Study Sponsor

24-hour contact number for SAE reporting	Astellas Pharma Korea, Inc. [redacted]
	Astellas Pharma Korea, Inc. [redacted]
	Astellas Pharma Korea, Inc. [redacted]

Appendix 3. List of Drug Interactions with Other Medications

Drug interactions with other medications or other forms of drug interaction of tacrolimus – extracted from the package insert of Prograf Cap.®

1) Prohibited concomitant medications

a) When the investigational drug was concomitantly treated with cyclosporine at the early stage of the clinical trial, elevated/additive nephrotoxicity was reported. If a subject needs to be treated with cyclosporine after the investigational drug, or vice versa, the next drug should be administered 24 hours after the last dose of the previous drug is administered. If the concentration of the investigational drug or cyclosporine is high, the administration should be delayed.

b) As hyperkalemia can be observed during the treatment, potassium-sparing diuretics (e.g., spironolacton, triamterene) cannot be concomitantly administered, or excessive potassium intake should be avoided. Frequent blood tests to check the potassium level is recommended. (Hyperkalemia can occur.)

c) Bosentan (Tracleer Tab.): As this drug and bosentan are metabolized by CYP3A4, their concomitant administration may increase the blood concentration of bosentan. Also, as bosentan induces CYP3A4, the blood concentration of the investigational drug may be changed due to the concomitant administration of bosentan.

2) Allowed concomitant medications with precautions

a) No study has been conducted to investigate the drug interaction between tacrolimus and other medications. Due to the drug's synergic or additive effect on renal dysfunction, a concomitant medication should be carefully used. These medications include aminoglycoside antibiotics, amphotericin B, cotrimoxazole, gyrase inhibitors, nonsteroidal anti-inflammatory drugs, vancomycin, ciplatin, sulfamethoxazole/trimethoprim, and ibuprofen.

b) Fluconazole, erythromycin (They increase the blood concentration of tacrolimus, which may lead to AEs such as renal dysfunction.)

c) Any medication that has neurotoxicity: The neurotoxicity may increase when this drug is administered with a medication that has strong neurotoxicity, such as ganciclovir or acyclovir.

d) As tacrolimus strongly binds to serum protein, medications that have high affinity with serum protein (e.g., anticoagulants, oral diabetes medications) may have interaction with it. Thus, it should be carefully administered.

e) HV protease inhibitor: ritonavir, saquinavir, and nelfinavir

f) St. John's Wort: St. John's Wort induces CYP3A enzymes and may decrease the tacrolimus whole blood concentration. Any food containing St. John's Wort is thus not recommended to be taken while tacrolimus is being administered.

g) Medications that have immunosuppressive effects (immunosuppressant: steroids; antirheumatic medications {DMARD}: e.g., methotrexate): As both drugs have immunosuppressive effects, excessive immunologic inhibition can occur.

h) Eplerenone (As the blood potassium level can increase, it should be regularly monitored.)

3) Medications that may affect the blood tacrolimus concentration
As tacrolimus is metabolized mainly by CYP3A4 enzymes, drugs or substances known to inhibit these enzymes may increase the tacrolimus whole blood concentration. Drugs known to induce CYP3A4 enzymes may decrease the tacrolimus whole blood concentration due to the decreased metabolism of tacrolimus. When these drugs are used in combination, a blood level test as well as ECG monitoring of QT prolongation or adequate dose adjustment according to the renal function are strongly recommended. If such drugs are co-administered with tacrolimus, their doses should be adjusted to maintain the appropriate level of tacrolimus in the blood.

4) Medications that increase the blood concentration of tacrolimus
- Calcium channel blocker: nicardipine, verapamil, diltiazem, nifedipine, nilvadipine
- Antifungal agents: clotrimazole, itraconazole, ketoconazole, fluconazole
- Antibiotics: josamycin, clarithromycin, troleandomycin, erythromycin
- Gastroprokinetic agents: cisapride, metoclopramide, lansoprazole
- Amiodarone
- HIV protease inhibitor: ritonavir, saquinavir, nelfinavir
- HCV protease inhibitor: telaprevir, boceprevir
- Others: bromocriptine, cimetidine, cyclosporine, danazole, ethinyl estradiol, methylprednisolone, metoclopramide, cloranfenicol, nefazodone, MgOH-Al, omeprazole, lansoprazole, protease inhibitor

5) Medications that decrease the blood concentration of tacrolimus
- Antiepileptic drugs: carbamazepine, phenobarbital, phenytoin
- Antibiotics: rifabutin, rifampicin, rifampin
- Antifungal agents: caspofungin
- Others: sirolimus

6) **Other medications that may affect the metabolism of tacrolimus:** barbiturate, corticosteroids, cortisone, ergotamine, ethinyl estradiol, gastodene, isoniazid, tamoxifen, triacetylrorendomycin, MgCl-Al. (Tacrolimus inhibits the metabolism of the following drugs: cyclosporine, cortisone, testosterone, phenobarbital, antipyrine, and steroid contraceptive pills. As such, if a patient is taking contraceptive pills, greater care should be taken in administering tacrolimus.)

7) **Other interactions:** Immunosuppressants may decrease the effects of vaccines. In particular, live vaccines should not be used during the administration of tacrolimus. (There has been a case where a patient developed rashes after vaccination with a live vaccine while the patient was taking a type of immunosuppressant; live vaccines include vaccines for measles, mumps, rubella, polio, BCG, yellow fever, and TY 21a typhoid. The HA vaccine for flu is a non-active vaccine, but its effect may be decreased due to the administration of tacrolimus.)