Supplementary (web extra) material

Pharmacokinetic studies

A 4-point abbreviated pharmacokinetic profile for CsA was measured 4 weeks after start of CsA therapy by CEDIA-Cyclosporine Plus Combi Kit, Thermo Fisher Microgenics Corporation, and the CsA-AUC was calculated as described. [1]

A 3-point abbreviated pharmacokinetic profile for MPA was performed during MMF-therapy after three and six months based on MPA plasma concentrations before oral intake of MMF (C_0) and 30 min ($C_{0.5}$) and 2 hours (C_2) thereafter[2]. The area under the time-concentration curve (AUC) was calculated as follows: MPA -AUC = $7.75+(6.49*C_0)+(0.76*C_{0.5})+(2.43*C_2)$[2]. All pharmacokinetic profiles were analyzed in a central laboratory (Department of Clinical Pharmacology, Charité Berlin). MPA was measured by CEDIA-MPA Immunoassay, Thermo Fisher Microgenics Corporation. To determine whether the MPA-AUC could reliably discriminate patients with a recurrence of the nephrotic syndrome from those with no recurrence, receiver operating characteristic (ROC) plots of sensitivity versus 1-specificity were generated using Analyse-It version 1.44 (Analyse-It Software, UK).

Relapse therapy

The standard therapy for relapses of the nephrotic syndrome consisted of prednisone 60 mg/m² BSA per day (maximum 80 mg/day); after three days of absent proteinuria, the prednisone dosage was reduced to 40 mg/m² every other day for four weeks, without a change in the concomitant medication with MMF or CsA, respectively.[3]

References

1. Filler G, Feber J, Lepage N, Weiler G, Mai I. Universal approach to pharmacokinetic monitoring of immunosuppressive agents in children. Pediatr Transplant 2002;6:411-8.
2. Pawinski T, Kunicki PK, Sobieszczanska-Malek M, Gralak B, Szlaska I. A limited sampling strategy for estimating mycophenolic acid area under the curve in adult heart transplant patients treated with concomitant cyclosporine. J Clin Pharm Ther 2009;34:89-101.

3. Cyclophosphamide treatment of steroid dependent nephrotic syndrome: comparison of eight week with 12 week course. Report of Arbeitsgemeinschaft fur Padiatrische Nephrologie. Arch Dis Child 1987;62:1102-6.

Suppl. Table 1

Previous Treatment of FR-SSNS	
Medication	**Patients (n)**
No Treatment	15
Levamisol	1
Levamisol/ MMF	1
Cyclophosphamide (CP)	13
CP / Chlorambucil	1
CP/ Levamisol	3
CP/ CsA	14
CP/ Chlorambucil/ CsA	1
CP/ Levamisol/ CsA	1
CsA	6
CsA/ MMF	2

Immunosuppressive therapy before start of the study. The sequence of medication is listed in chronological order.

Suppl. Table 2

Patient	MMF-Therapy (n=4)	CsA-Therapy (n=6)	Number of Relapses
1		Enalapril	0
2		Enalapril	0
3		Nifedipine	3 (2 CsA/1 MMF)
4	Nifedipine Enalapril	Nifedipine Enalapril	0
5	Enalapril	Enalapril	1 (MMF)
6	Ramipril		1 (MMF)
7	Enalapril		2 (2 MMF)
8		Enalapril	0

Antihypertensive medication and number of relapses in each treatment arm

Suppl. Table 3

	MMF-Therapy		CsA-Therapy	
Parameter	mean	Range	mean	range
Hemoglobin (g/dl)	13.3	11.3-15.3	12.4	9.9-15.3
PCV [l/l]	0.39	0.35-0.5	0.36	0.29-0.45
WBC [n/µl]	7.16	3.2 -13.1	6.58	3.2-11.9
Sodium [mmol/l]	139	131-143	139	133-143
Potassium [mmol/l]	4.10	3.5-4.7	4.58	3.8-5.4
Magnesium [mmol/l]	0.85	0.68-1.04	0.82	0.64-1.66
ASAT [U/l]	19.8	7.8-192	16.8	5-55
ALAT [U/l]	24.3	10-66	26.0	9-42
Cholesterol [mg/dl]	173	104-315	181	120-257
Triglycerides [mg/dl]	87.8	21-427	95.9	20-344
LDL-C [mg/dl]	108	64-232	101	18-164
HDL-C [mg/dl]	58	29-160	63	37-152

Laboratory values determined at the end of each treatment period

Suppl. Table 4

Miscellaneous Adverse Effects (Table 3)

	MMF-Therapy	CsA Therapy
Fractured bone	2	
Insect bite	1	
Bruising trauma	1	
Painful Hydrocele	1	
Amputation of Finger after injury		1
Tic bite		1
Greenstick fracture		1
Total	5	3

Legends to supplementary figures

Suppl. Fig. 1: Study Synopsis

Suppl. Fig. 2: MPA-AUC measured in 26 patients with an unchanged MMF dose at 3 and 6 months.

Suppl. Figure 3: Efficacy of CsA and MMF (in patients with high MPA exposure) in preventing relapses in FR-SSNS patients

Kaplan-Meier survival-analysis: Time without relapse (cumulative sustained remission) during treatment with CsA or MMF (in 21 patients with an MPA-AUC >50 µg*h/ml).

Suppl. Fig. 3A, in the first treatment year (p=0.32, long-rank test).

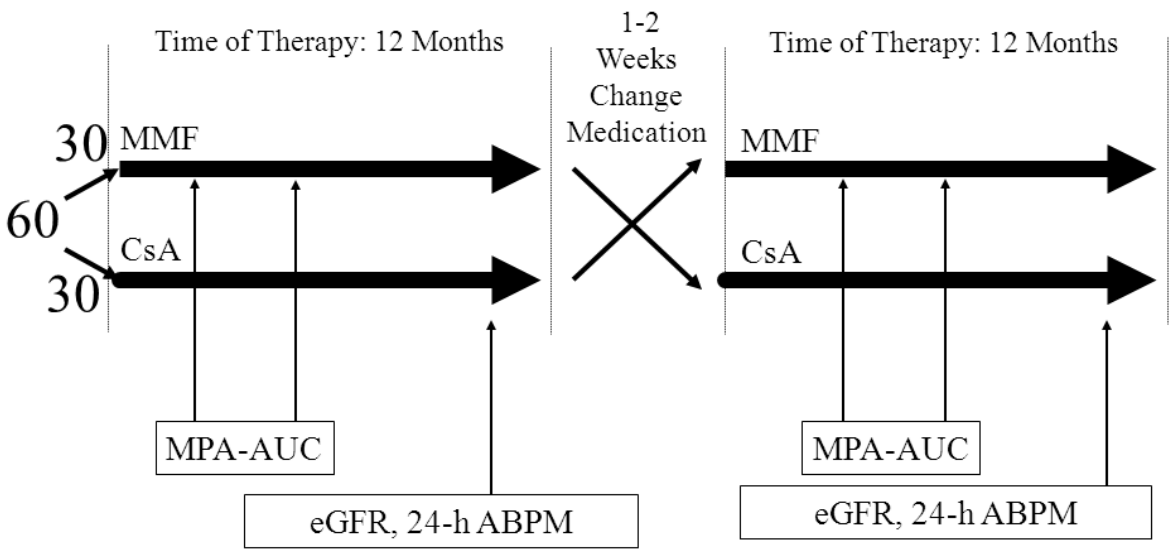
Suppl. Fig. 3B, in the second treatment year (p=0.62, long-rank test).

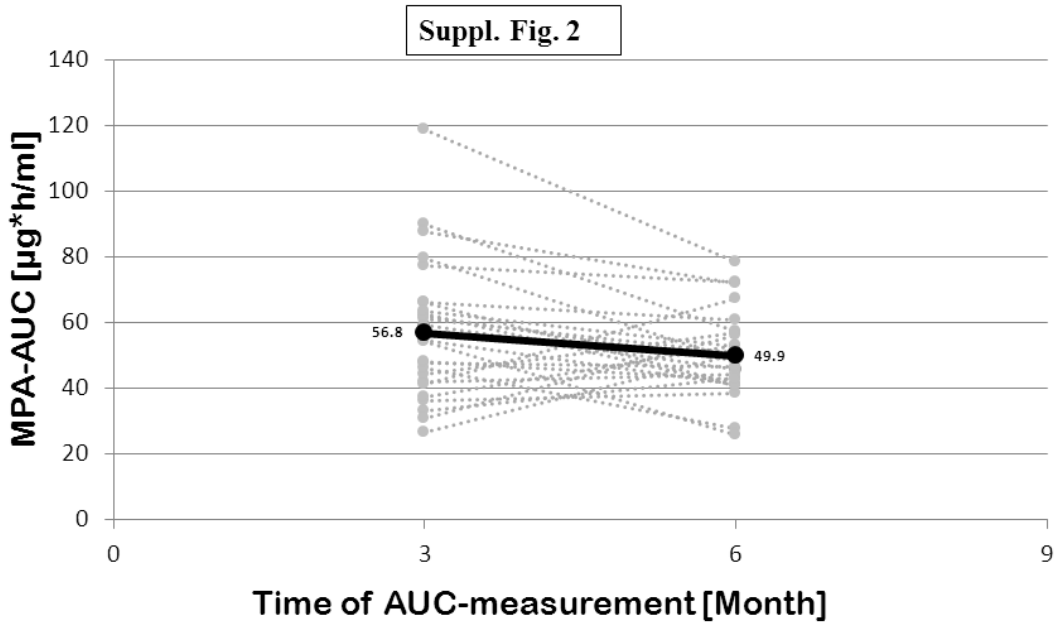
Suppl. Fig. 4: ROC curves computed for 3-month MPA-C-0 values (n=43). Diagnostic sensitivities (true positives) were calculated for each individual AUC-C-0 value as the fraction of patients with a recurrence to have lower values. The corresponding diagnostic specificities (false negatives) were calculated as the fraction of patients with no recurrence to have higher values. MPA-C-0 concentrations had no significant predictive value for relapses (MPA-C0-AUC=0.50; p=0.45)

Suppl. Fig. 5: Renal Function (eGFR) measured at 0.12, and 24 months.

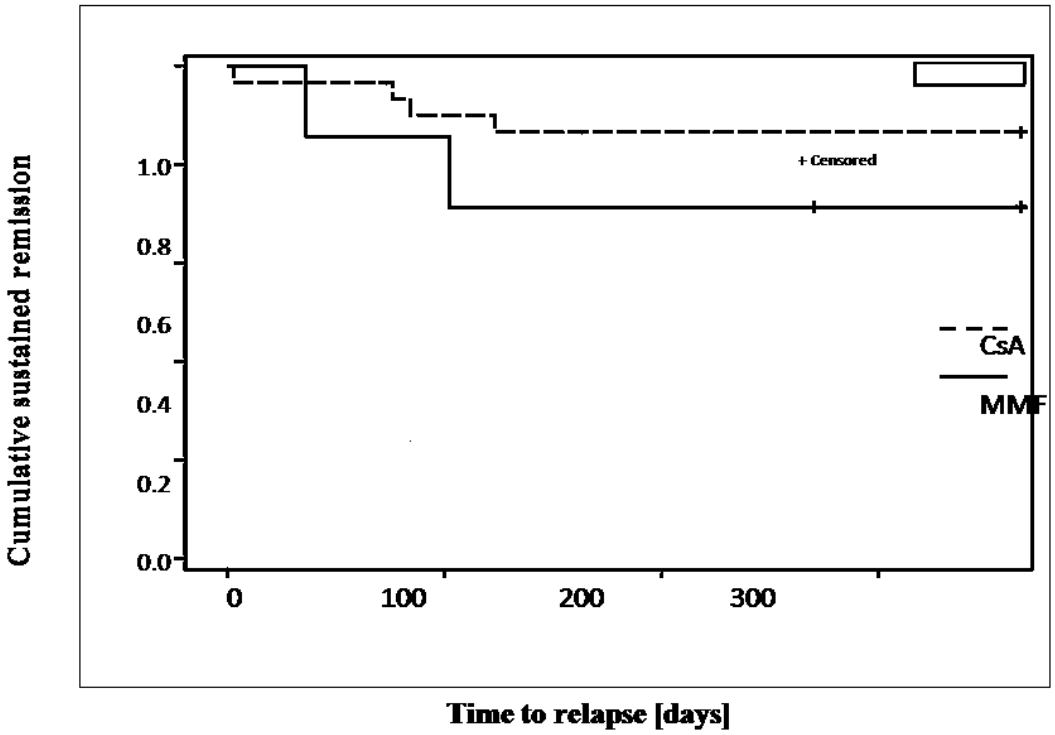
When compared at time points 12 and 24 months, renal function (eGFR) decreased during CsA therapy in the second year in group A (p=0.004) and increased with MMF therapy during the second year in group B (0.006).

Suppl. Fig. 1

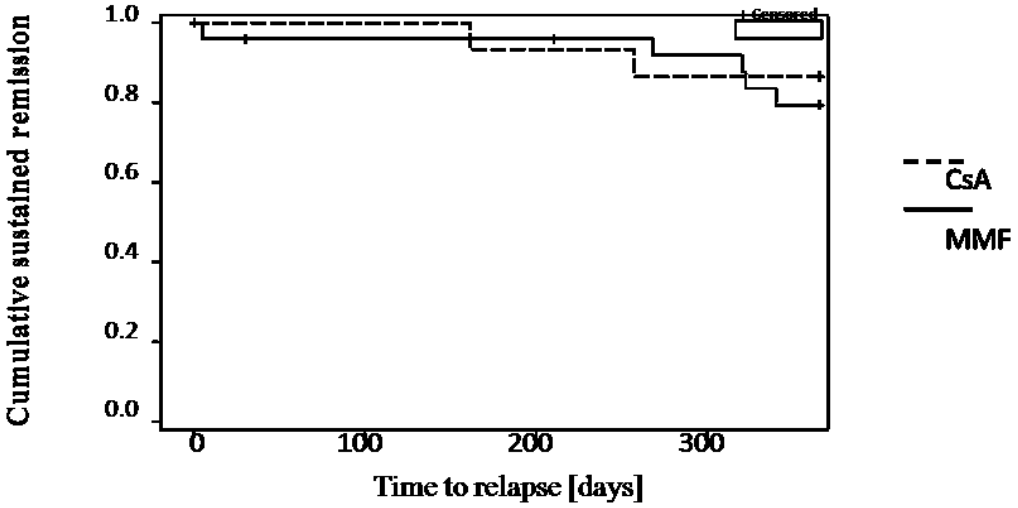

Time of Therapy: 12 Months
1-2 Weeks Change Medication
Time of Therapy: 12 Months
30
MMF
60
CsA
30
MMF
CsA
MPA-AUC
eGFR, 24-h ABPM
MPA-AUC
eGFR, 24-h ABPM


Suppl. Fig. 2
MPA-AUC [µg*h/ml]
140
120
100
80
60
40
20
0
56.8
49.9
0
3
6
9
Time of AUC-measurement [Month]

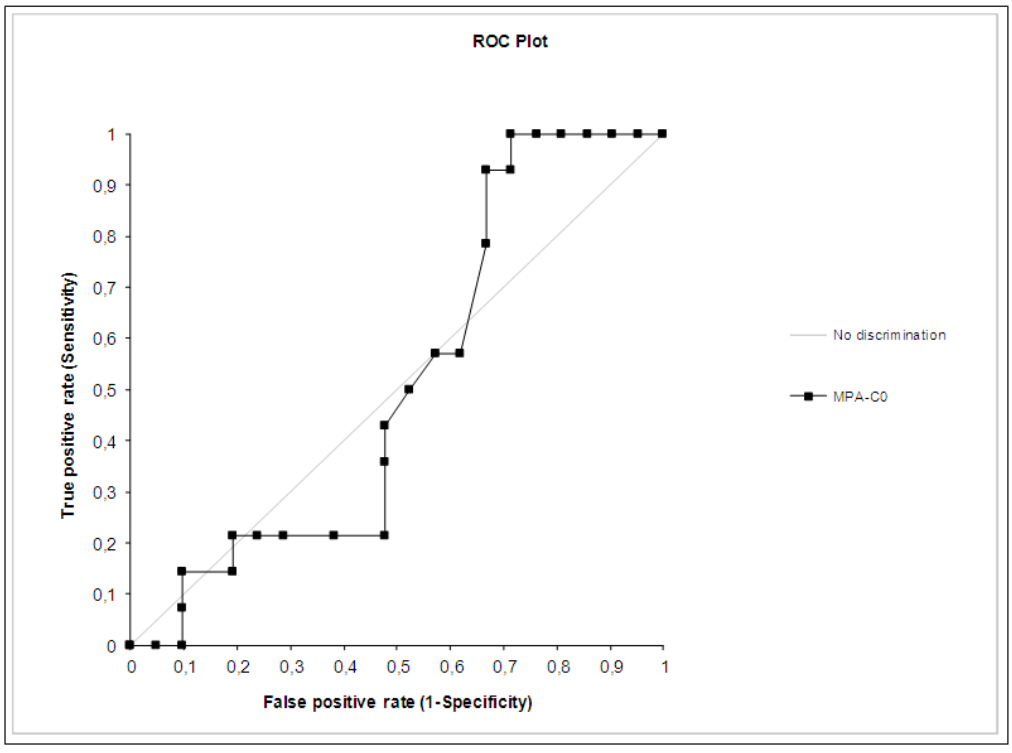
Suppl. Fig. 3A


Cumulative sustained remission
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
+ Censored
CsA
MMF
Time to relapse [days]

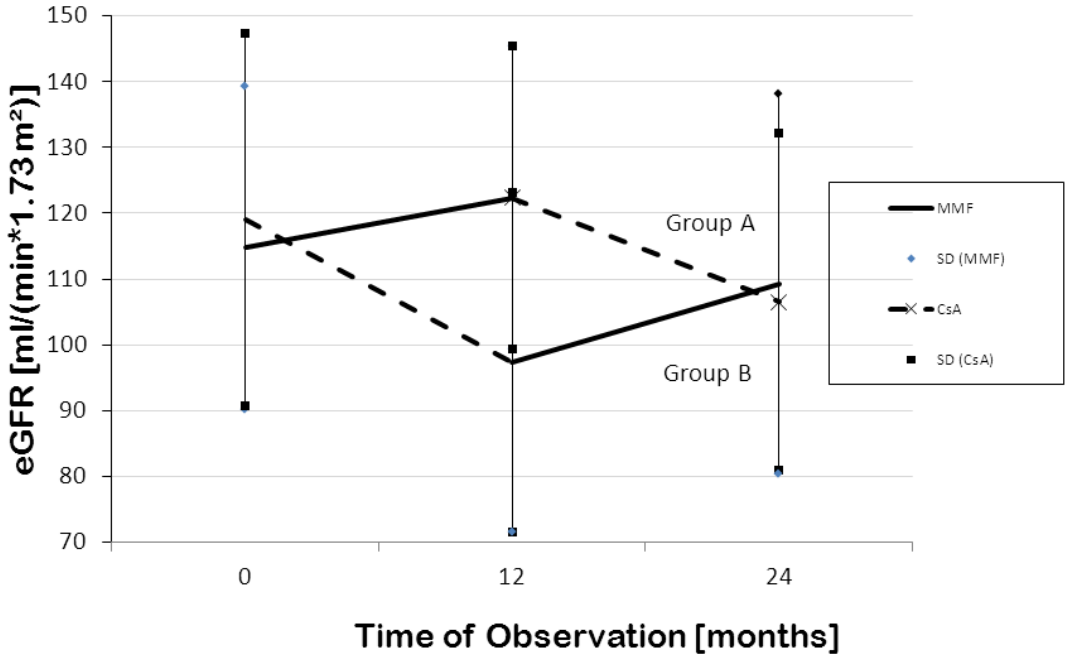
Suppl. Fig. 3B



Suppl. Fig. 4



Suppl. Fig. 5


150
140
130
120
110
100
90
80
70
eGFR [ml/(min*1.73 m²)]
0
12
24
Time of Observation [months]
Group A
Group B
MMF
SD (MMF)
CsA
SD (CsA)