Supplemental Material

Supplemental Methods

Study design and objectives

We performed an international, retrospective case series in patients from 18 kidney transplant centers. Sixteen centers collected data as part of The Post-Transplant Glomerular Disease (TANGO) project,[1] two other centers from the Netherlands (Leiden University Medical Center and Erasmus MC, University Medical Center Rotterdam) contributed their data separately. The primary objective of the study was to give an overview of the clinical course and outcomes of patients who are treated with long-term apheresis after a diagnosis of recurrent FSGS. Our secondary objectives were to determine the incidence of complications and provide an overview of different treatment regimens used for post-transplant FSGS

Out of 18 centers that were approached for participation, 5 were not able to participate because long-term apheresis was not performed in that specific center. In 13 remaining centers, data was collected from 30 patients who received 31 transplants. Subsequently, 4 patients were excluded because apheresis was terminated at 6 months, or cumulative time on apheresis was shorter than 6 months, which excluded another 2 centers from participation. Finally, 27 transplants from 26 patients, treated in 11 transplant centers (6 European, 3 North American and 2 Brazilian centers) fulfilled inclusion criteria and were included in the study (Supplemental Figure 1). Since no comparative analyses that require independence were performed, the two transplants from one patient will be discussed as separate "patients" (patient 22 and patient 9).

Patient selection and data collection

In participating centers, all adult patients (>18 years old) who received a kidney transplant between January 2005 and December 2019 were reviewed for a diagnosis of recurrent FSGS, based on kidney

biopsy and/or clinical symptoms. Patients with post-transplant FSGS who were treated with plasmapheresis or immunoadsorption for a minimum of 6 subsequent months were included in the study. Detailed de-identified patient information was extracted from medical records. Decisions regarding treatment of patients with post-transplant FSGS were made by the treating physician in the participating transplant centers.

Definitions

The majority of recurrent FSGS cases were detected by occurrence of (nephrotic-range) proteinuria post-transplant and was confirmed by kidney biopsy showing either FSGS lesions by light microscopy and/or diffuse foot process effacement by electron microscopy. In 4 patients, post-transplant FSGS was not biopsy-confirmed but was immediately treated due to the high clinical suspicion of recurrent FSGS. Five patients had non-nephrotic proteinuria at manifestation of recurrent FSGS; these patients all had a kidney biopsy that confirmed the diagnosis. Four of these patients had received prophylactic apheresis pre-transplant.

Complete remission was defined as a reduction of proteinuria below 0.3 g/24h (or 0.3 g/g of urinary creatinine) with stable eGFR (*i.e.*, maximum decline of 15%). Partial remission was defined as a reduction of proteinuria below 2.0 g/24h (or 2.0 g/g), or a reduction of proteinuria of at least 50% of the highest value to a level below 3.5g/24h (or 3.5 g/g), both with stable eGFR.[2] eGFR was estimated by the Modification of Diet in Renal Disease (MDRD) Study equation.[3] Donor specific antibody (DSA) was recorded if mean fluorescent intensity of anti-HLA antibodies against donor HLA antigens exceeded the center's threshold for positivity. Proteinuria was checked by spot urine, protein to creatinine ratio, or 24-hour urine collection, depending on transplant center's clinical practice. For patients 8,9, 21, 22 and 24, proteinuria was only available in g/L.

Standard CMV prophylaxis was given to all kidney transplant recipients in the first 3-6 months post kidney transplantation in high risk patients in participating centers, except in one center where PCR surveillance was used. None of the patients received extended CMV prophylaxis while being on chronic apheresis.

Statistical analysis

Data are shown as frequencies (percentages) for categorical variables and as medians (interquartile range (IQR)). Medians and frequencies were calculated by complete case analysis. Descriptive statistical analyses were performed using STATA (v. 15.1, StataCorp LLC). All figures were made with Prism 7.02 software (GraphPad software, Inc), including Kaplan-Meier analysis. Graphs with clinical outcomes and treatment regimen were combined to create one graph per patient with 3 y-axes.

Missing data

Race was not allowed to be reported following IRB regulations in 9 patients. Time to kidney failure and donor age were missing in two cases, pre-transplant DSA and HLA-mismatch were missing in one patient.

Ethical considerations

The overall study protocol was submitted and approved by the ethical committee (Medisch Ethische toetsingscommissie) of the University Medical Center Groningen in the Netherlands (protocol number: 202000444). The overall protocol of TANGO-study was approved by the ethical committee of the Partners Human Research Committee (PHRC) at the Massachusetts General Hospital in Boston (protocol number: 2015P000993), and at each participating TANGO-center.

All protocols are in accordance with International Conference on Harmonization Good Clinical Practice Guidelines and the Declaration of Helsinki. The clinical and research activities being reported are consistent with the principles outlined in the 'Declaration of Istanbul on Organ Trafficking and Transplant Tourism'.[4]

Supplemental Results

Cohort demographics (continued)

Median follow up after kidney transplant was 4.1 (IQR, 3.0-6.3) years, and most patients receiving long-term apheresis were transplanted in recent years (2015-2019, n=16). Median age at transplant was 37 (IQR, 27-49) years and 56% of the cohort was male. Thirteen patients (48%) had received previous transplants: 9 patients (33%) had received one prior kidney transplant, 3 (11%) patients had lost two prior transplants and in one (4%) patient, three prior allografts had failed. Recurrent FSGS was the main cause of previous graft failure (83% of total), except for three allografts, in which graft failure was attributed to thrombotic microangiopathy, interstitial fibrosis, and surgical complications. Fifteen kidney transplants were from living donors (56%). Induction therapy was given to 26 (96%) patients, of whom 16 (59%) were treated with anti-thymocyte globulin (ATG) and 10 (37%) with basiliximab. The main immunosuppressive regimen consisted of a calcineurin inhibitor combined with mycophenolate mofetil (MMF) and glucocorticoids (89%). Early steroid withdrawal (<3 months) was performed in three patients (11%). Six patients received 1 (n=4), 2 (n=1) and 3 (n=1) session(s) of plasmapheresis pre-transplant. In one other patient, two doses of rituximab were given pre-transplant. Other patients did not receive prophylactic treatment.

Substitution fluids

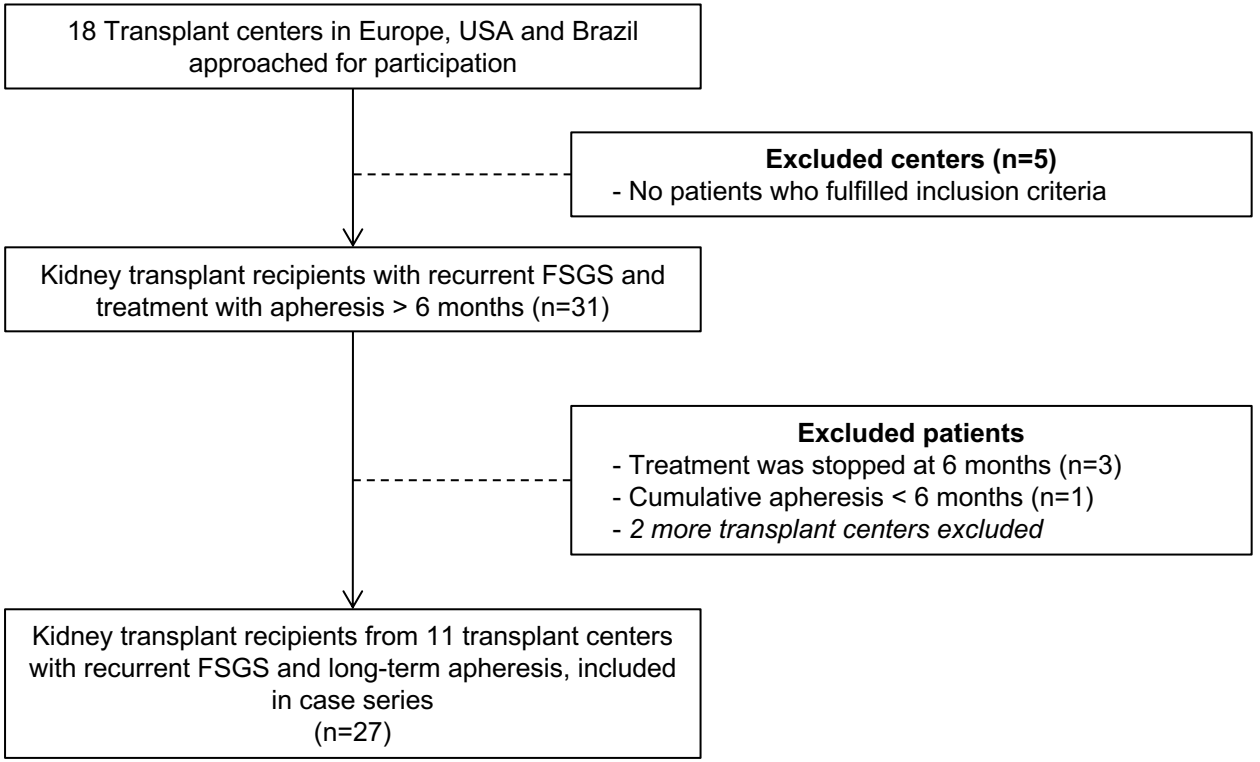
Albumin was used as substitution fluid for all patients on plasmapheresis, except in 5 patients, who received temporary substitution with albumin in combination with fresh frozen plasma to prevent bleeding complications. Citrate was used as anti-coagulant in 24 (89%) patients, two patients received heparin, and one patient received both.

Four patients received intravenous immune globulin (IVIG) to replace immunoglobulins in attempt prevent infectious complications (patient 2, 3, 7 and 13).

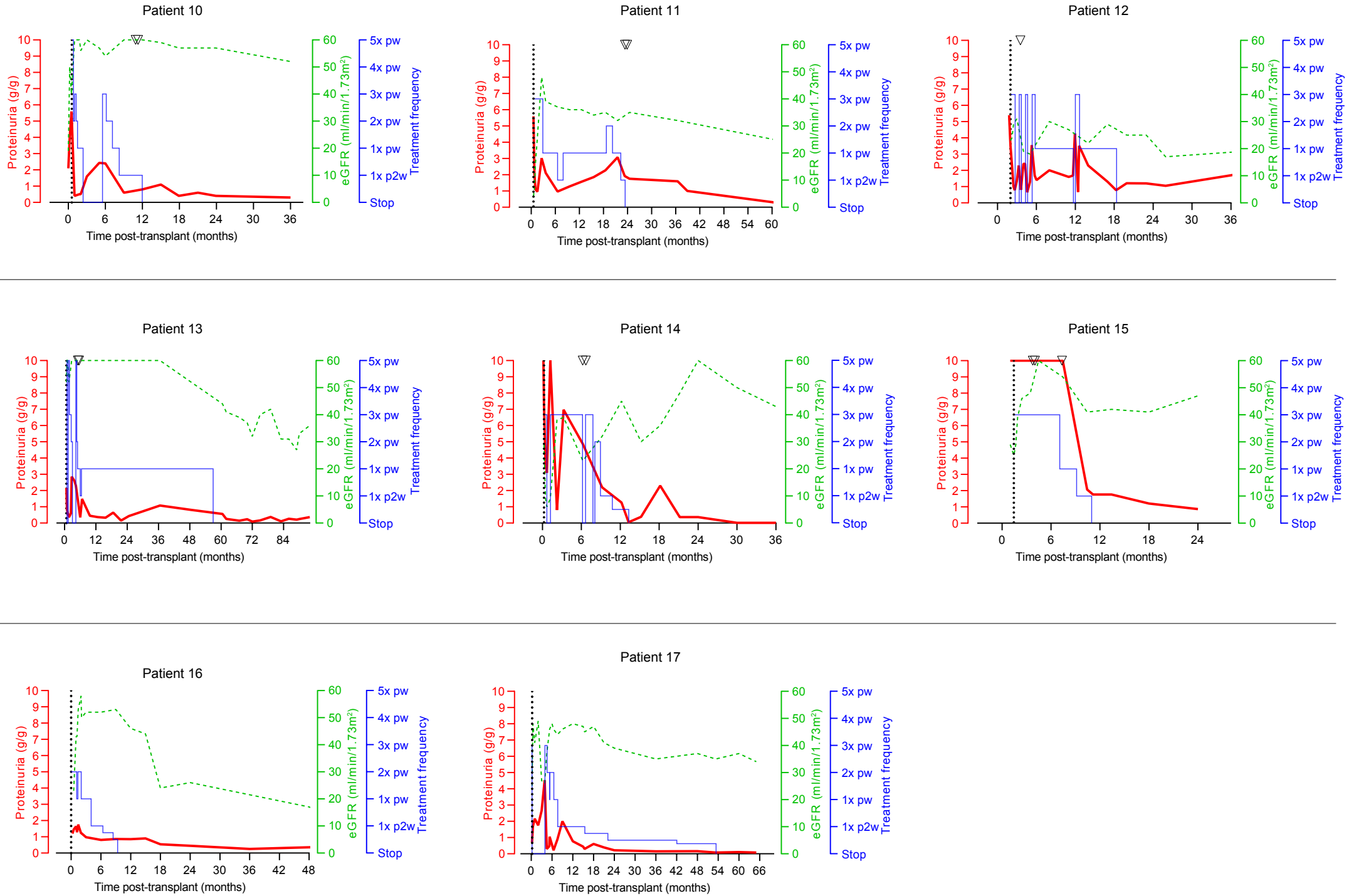
Post-transplant complications

Three patients experienced an acute cellular rejection that was treated with pulse glucocorticoids (n=2) or ATG (n=1). One of these patients developed (histologically-confirmed) antibody-mediated rejection with subsequent graft failure a couple of years after successful cessation of apheresis as treatment for recurrent FSGS. In 5 (21%) patients, apheresis had to be temporarily stopped due to shunt thrombosis.

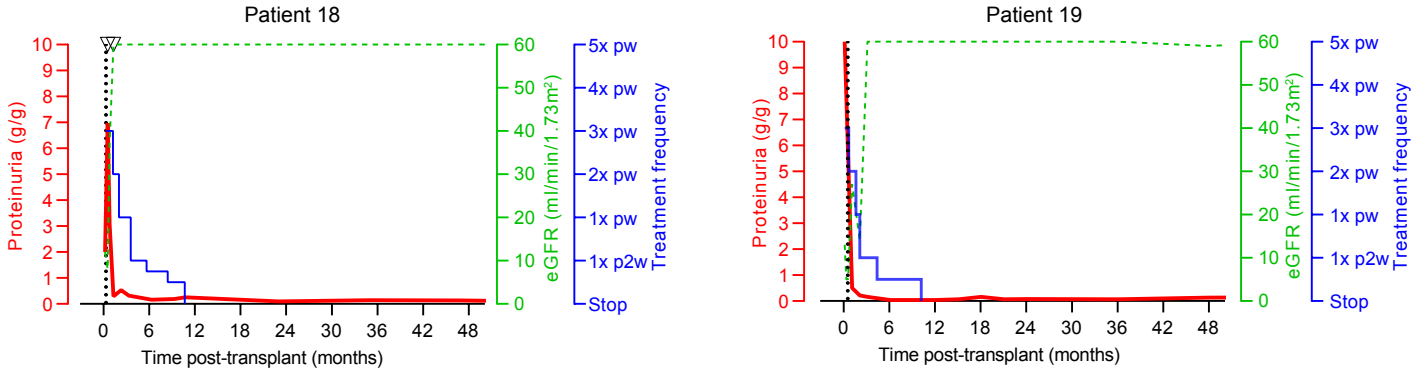
Supplemental Figure 1. Flow chart of the study population



Supplemental Figure 2. Clinical course of patients with continued (partial) remission after cessation of long-term apheresis for post-transplant FSGS. Proteinuria, eGFR and treatment regimen in patients successfully weaned off of apheresis. Each graph represents one patient. Blue lines represent frequency of plasmapheresis, the dashed vertical line indicates start of apheresis. Triangles represent one dose of rituximab. Patients 16 and 17 received prophylactic plasmapheresis pre-transplant. eGFR, estimated glomerular filtration rate calculated by Modification of Diet in Renal Disease Study (MDRD); Pw, per week; p2w, per 2 weeks.

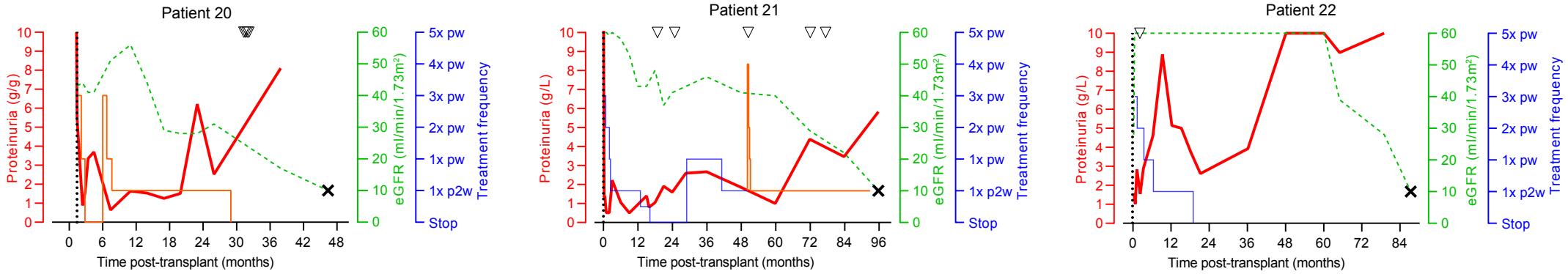


Supplemental Figure 2 (continued)

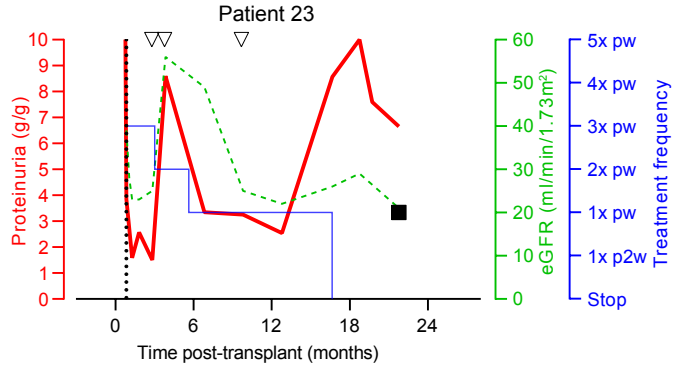


Supplemental Figure 3. Clinical course of patients with termination of long-term apheresis because of refractory FSGS or infection. Proteinuria, eGFR and treatment regimen in patients with termination of long-term apheresis due to increased level of proteinuria on treatment (A) or infection (B). Each graph represents one patient. Blue and orange lines represent plasmapheresis and immunoadsorption, respectively. The dashed vertical line indicates start of apheresis. Triangles represent one dose of rituximab, X represents graft failure, ■ represents patient death. Patients 21 and 22 received prophylactic plasmapheresis pre-transplant. eGFR, estimated glomerular filtration rate calculated by Modification of Diet in Renal Disease Study (MDRD); Pw, per week; p2w, per 2 weeks.

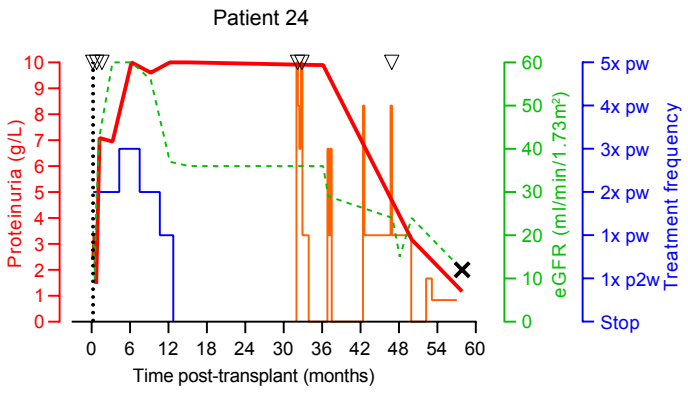
A

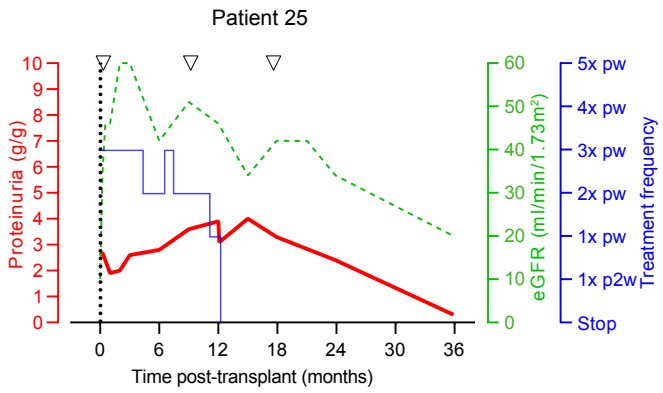


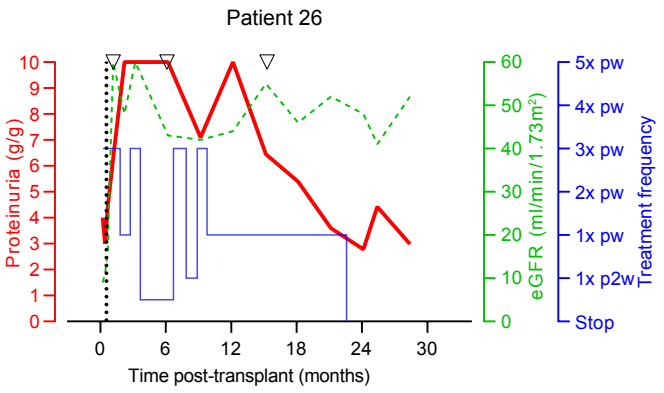
B

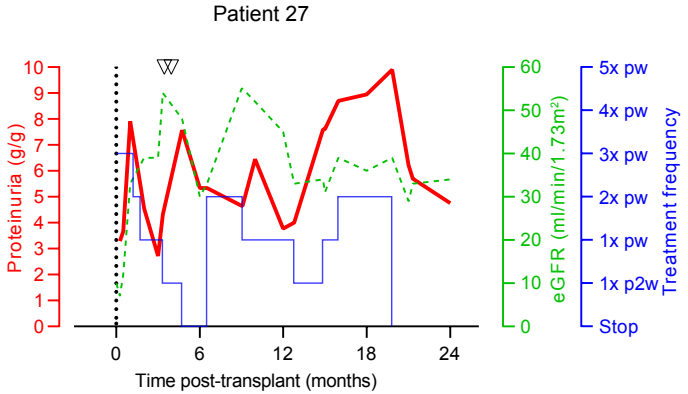


Supplemental Figure 4. Clinical course of patients without response to long-term apheresis for post-transplant FSGS. Proteinuria, eGFR and treatment regimen in patients without remission despite long-term apheresis. Each graph represents one patient. Blue and orange lines represent plasmapheresis and immunoadsorption, respectively. The dotted black line indicates start of apheresis. Triangles represent one dose of rituximab, X represents graft failure. Patient 25 received prophylactic plasmapheresis pre-transplant. eGFR, estimated glomerular filtration rate calculated by Modification of Diet in Renal Disease Study (MDRD); Pw, per week; p2w, per 2 weeks.

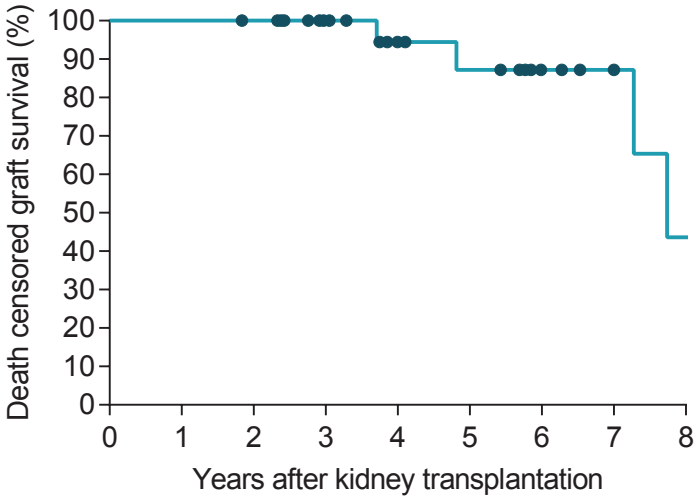








Supplemental Figure 5. Graft survival in patients with long-term apheresis. Kaplan-Meier curve to graft survival in 27 patients who received long-term apheresis for recurrent FSGS



Supplemental Table S1. Treatment and outcomes of long-term apheresis for recurrent FSGS.

Variable	**(n=27)**
Time from transplant to first clinical symptoms, days	5 [1-11]
Time from first clinical symptoms to start treatment, days	4 [1-15]
Type of apheresis	
Plasmapheresis	20 (74)
Immunoadsorption	3 (11)
Both	4 (15)
Cumulative time on apheresis, months	23 [12-48]
Rituximab use	21 (78%)
Number of doses	2 [2-3]
ACE/ARB use	23 (85%)
Outcomes	
Initial remission	23 (85)
Continued remission on chronic apheresis	9 (39)
Continued remission after termination of apheresis	10 (43)
Cessation of apheresis due to refractory FSGS	3 (13)
Cessation of apheresis due to Infection	1 (4)
No remission	4 (15)
Graft failure	5 (19)
Post-transplant FSGS	4 (15)
Antibody mediated rejection	1 (4)
Time from transplantation to graft failure, years	7.3 [4.8- 7.7]
Patient death	1 (4)
Time from transplantation to patient death, years	1.8

Values represent frequency (percentage) or median [interquartile range]. FSGS, focal segmental glomerulosclerosis.

Supplemental references

1. Uffing A, Pérez-Sáez MJ, La Manna G, et al. A large, international study on post-transplant glomerular diseases: the TANGO project. *BMC Nephrol*. 2018;19(1):229. doi:10.1186/s12882-018-1025-z

2. Troyanov S, Wall CA, Miller JA, Scholey JW, Cattran DC, Toronto Glomerulonephritis Registry Group. Focal and segmental glomerulosclerosis: definition and relevance of a partial remission. *J Am Soc Nephrol*. 2005;16(4):1061-1068. doi:10.1681/ASN.2004070593

3. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med*. 1999;130(6):461-470.

4. The Declaration of Istanbul on Organ Trafficking and Transplant Tourism (2018 Edition). *Transplantation*. 2019;103(2):218-219. doi:10.1097/TP.0000000000002540