Article

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Peri-Transplant Costimulation Blockade Preserves Early Allograft Function Measured by Serial Transdermal Glomerular Filtration Rate in Murine Kidney Transplantation

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ABSTRACT

Murine kidney transplantation is a powerful platform for mechanistic transplant research, but sensitive longitudinal measurement of graft function remains challenging. Conventional serum creatinine and blood urea nitrogen often fail to detect modest yet biologically important differences in renal injury and recovery. We evaluated whether serial transdermal glomerular filtration rate (tGFR) measurement could identify early functional benefit from peri-transplant costimulation blockade in a murine allogeneic kidney transplant model. Twelve-week-old BALB/c and C57BL/6J mice of both sexes underwent orthotopic kidney transplantation using sex-matched donor-recipient pairs. Recipients underwent bilateral native nephrectomy, making survival dependent on allograft function. Animals were assigned to syngeneic transplantation, untreated allogeneic transplantation, or allogeneic transplantation with short-course peri-transplant anti-CD154 costimulation blockade. Renal function was assessed serially by tGFR, serum creatinine, and blood urea nitrogen. Histologic injury was evaluated at prespecified time points. Baseline renal function was comparable among recipient groups before transplantation. Following allogeneic transplantation, untreated recipients developed a marked decline in tGFR with incomplete recovery by day 28. In contrast, costimulation blockade significantly attenuated the early nadir and improved recovery through day 28. These functional differences were larger and more consistent when assessed by tGFR than by serum creatinine or blood urea nitrogen. Treated recipients also demonstrated prolonged survival and reduced glomerulitis, tubulitis, and peritubular capillaritis on histologic analysis. Sex-stratified analysis showed benefit in both males and females, with slightly greater late functional recovery among treated females. These findings show that serial tGFR is a sensitive primary endpoint for detecting early therapeutic benefit in murine kidney transplantation. Peri-transplant costimulation blockade improved graft function, survival, and histologic injury, and these effects were captured most clearly by direct longitudinal measurement of glomerular filtration rate.

KEYWORDS: kidney transplantation; murine model; transdermal glomerular filtration rate; allograft function; costimulation blockade; anti-CD154; creatinine; blood urea nitrogen

1 Introduction

Mouse kidney transplantation remains one of the most informative preclinical systems for investigating alloimmune injury, ischemia-reperfusion damage, and therapeutic modulation of graft outcomes [1–3]. Its broader use, however, is limited by the difficulty of measuring renal function accurately over time. In mice, serum creatinine is an imperfect surrogate because tubular secretion contributes substantially to total creatinine clearance, and laboratory methods may introduce further variability [4, 5]. Blood urea nitrogen (BUN) is likewise affected by multiple

physiological and environmental factors and therefore does not provide a sufficiently specific reflection of glomerular filtration alone [6, 7].

Direct measurement of glomerular filtration rate (GFR) using transdermal fluorescein isothiocyanate-sinistrin kinetics has emerged as a practical and reproducible method for real-time assessment of kidney function in mice [8–10]. This technique permits serial, minimally invasive measurements and has shown good agreement with gold-standard plasma clearance approaches across multiple experimental settings [11–13]. Recent work in murine nephrectomy and kidney transplantation demonstrated that transdermal GFR (tGFR) is more sensitive and more reproducible than serum creatinine or BUN for detecting dynamic changes in renal function, including sex- and strain-specific differences in recovery patterns [14].

This improved functional resolution creates an opportunity to redesign murine transplant studies around direct measurement of GFR as the primary endpoint rather than delayed biochemical surrogates. Such an approach is particularly relevant for testing interventions that may exert modest but biologically meaningful protection during the early post-transplant period, when ischemia-reperfusion injury, endothelial activation, innate inflammation, and developing alloimmunity overlap. Costimulation pathways are central to T-cell activation and amplification of allograft inflammation, and blockade of CD154-mediated signaling has shown efficacy in murine renal transplantation [15, 16].

We therefore hypothesized that short-course peri-transplant anti-CD154 treatment would improve early allograft function, prolong recipient survival, and reduce rejection-associated histologic injury, and that these benefits would be detected more sensitively by serial tGFR than by serum creatinine or BUN. To test this hypothesis, we retained the same experimental framework used in prior murine transplant studies: orthotopic kidney transplantation in BALB/c and C57BL/6J mice, bilateral native nephrectomy of recipients, serial tGFR measurement using FITC-sinistrin, blood-based renal markers, and periodic acid–Schiff histology [14].

2 Materials and Methods

2.1 Animals

BALB/c and C57BL/6J (B6) mice were maintained in a specific pathogen-free facility under controlled temperature, humidity, and 12-hour light-dark cycling with ad libitum access to standard chow and water. Male and female mice were studied separately in sex-matched donor-recipient pairs. All experimental procedures were performed in 12-week-old animals. Animal experiments were conducted in accordance with institutional guidelines for the care and use of laboratory animals and were reported with attention to ARRIVE principles and sex-aware experimental design [17, 18]. The study protocol was approved by the Institutional Animal Care and Use Committee of Duke University under protocol number A215-21-10.

2.2 Experimental design

The study was designed as a controlled, longitudinal, parallel-group murine transplantation experiment with prespecified primary and secondary endpoints. The primary endpoint was day-28 transdermal glomerular filtration rate (tGFR). Secondary endpoints included serial serum creatinine, serial blood urea nitrogen (BUN), day-28 composite histologic injury score, and recipient survival through day 60.

Recipients were allocated to three groups: syngeneic B6-to-B6 transplantation, untreated allogeneic BALB/c-to-B6 transplantation, and BALB/c-to-B6 transplantation with peri-transplant anti-CD154 costimulation blockade. Allocation was performed using computer-generated randomization after confirmation of surgical eligibility. Investigators performing postoperative functional measurements and histologic scoring were blinded to treatment assignment. Each experimental group included male and female cohorts studied in sex-matched donor-recipient pairs.

The anti-CD154 group received anti-mouse CD154 monoclonal antibody (clone MR-1, Bio X Cell, catalog #BE0017-1) at 250 μg per mouse by intraperitoneal injection on postoperative days 0, 2, 4, and 6. Untreated allogeneic recipients received matched Armenian hamster IgG control at the same dosing schedule and injection volume. The selected dosing schedule was chosen to target the early post-transplant interval during which ischemia-reperfusion injury, endothelial activation, and early alloimmune priming overlap.

2.3 Sample size determination, randomization, and blinding

Sample size was prospectively planned on the basis of the primary endpoint, day-28 transdermal glomerular filtration rate (tGFR), with enrollment expanded to accommodate anticipated technical mortality and early postoperative exclusions. Accordingly, 12 syngeneic recipients, 18 untreated allogeneic recipients, and 18 anti-CD154-treated allogeneic recipients were enrolled, yielding analyzable efficacy cohorts of 10, 15, and 16 recipients, respectively. Complete day-28 functional follow-up was available in 10, 14, and 15 recipients, respectively, and day-28 histology was available in 8, 12, and 14 animals, respectively.

Randomization was performed at the recipient level after confirmation of surgical eligibility using computer-generated randomization. Functional measurements were acquired using coded animal identifiers. Histologic slides were scored in a blinded manner with respect to treatment group and survival status. No interim analyses were used to stop the experiment early.

2.4 Surgical procedures

Donor left nephrectomy and orthotopic kidney transplantation were performed under inhaled isoflurane anesthesia on a temperature-controlled warming platform. Perioperative care was provided in accordance with institutional animal care guidelines. Donor renal vessels were harvested with an aortic patch. Vascular reconstruction was performed by anastomosing the donor aortic patch to the recipient abdominal aorta and the donor renal vein to the recipient inferior vena cava. Ureteroneocystostomy was completed by anastomosing the donor ureter to the recipient bladder. Recipient native kidneys were removed to generate a life-sustaining single-kidney transplant model in which recipient survival depended on graft function [14, 16].

Cold ischemia time, warm ischemia time, and total operative time were recorded for each transplant and reviewed across groups to assess procedural comparability. No overt between-group differences in operative course were identified.

Animals that died within 72 hours of transplantation because of predefined technical complications were excluded from efficacy analyses but were reported separately in the study flow summary.

2.5 Transdermal glomerular filtration rate measurement

tGFR was measured using FITC-sinistrin and a miniaturized fluorescence detector (MediBeacon GmbH, Mannheim, Germany) as previously described [8–10]. Animals were shaved and depilated 24 hours before the first scheduled measurement. At each time point, background fluorescence was recorded for 3 minutes before intravenous injection of FITC-sinistrin at 12.5 mg/kg. The detector remained in place for 60 minutes while the animal recovered from brief anesthesia and remained conscious during signal acquisition.

Raw fluorescence decay curves were analyzed using MB Lab/MB Studio software (MediBeacon GmbH). GFR was calculated from the plasma disappearance kinetics of FITC-sinistrin using a three-compartment model and normalized to body weight, yielding values in mL/min/100 g body weight. Measurements were scheduled at baseline and on postoperative days 3, 7, 14, 21, and 28. Measurements that did not meet predefined quality-control criteria were repeated when feasible.

2.6 Serum creatinine and blood urea nitrogen

At each time point, tail-vein blood was collected into serum-separating tubes and centrifuged to obtain serum. For creatinine measurement, 10 µL serum aliquots were stored at −80 °C and later analyzed by isotope dilution liquid chromatography/mass spectrometry. For BUN measurement, 5 µL serum was diluted 1:20 and analyzed using a colorimetric assay (Invitrogen BUN Colorimetric Detection Kit). These markers were analyzed in parallel with tGFR to compare conventional and direct functional assessment approaches [14, 19].

2.7 Histology

At day 28 and in a subset of long-term survivors at day 60, grafts were harvested, formalin-fixed, paraffin-embedded, sectioned at 5 µm, and stained with periodic acid–Schiff. Histologic assessment focused on glomerulitis, tubulitis, interstitial inflammation, and peritubular capillaritis. Each lesion was graded on a 4-point ordinal scale from 0 to 3, where 0 indicated absence of lesion and 3 indicated severe involvement. A composite injury score was defined as

$$I_{\mathrm{total}} = I_{\mathrm{g}} + I_{\mathrm{t}} + I_{\mathrm{i}} + I_{\mathrm{ptc}}, \tag{1}$$

where I_g, I_t, I_i, and I_{ptc} denote lesion grades for glomerulitis, tubulitis, interstitial inflammation, and peritubular capillaritis, respectively.

2.8 Survival analysis

Recipients were monitored daily through day 60. The survival endpoint was death or humane euthanasia due to predefined clinical deterioration criteria. Animals dying within 72 hours of transplantation because of technical complications were censored at the time of death and excluded from treatment-efficacy survival comparisons. Kaplan–Meier survival curves were constructed for each experimental group, and survival distributions were compared using the log-rank test. Median survival time and 95% confidence intervals were calculated for each group.

2.9 Statistical analysis

All analyses were prespecified before data lock. Continuous variables are presented as mean ± standard deviation for approximately normally distributed data and as median with interquartile range for non-normal data. Normality was assessed using the Shapiro–Wilk test and visual inspection of residual plots. Homogeneity of variance was assessed using Levene's test.

For longitudinal renal function outcomes, group, time, and group-by-time interaction effects were analyzed using linear mixed-effects models with animal-specific random intercepts to account for repeated measures within the same recipient. When mixed-model assumptions were adequately satisfied, repeated-measures analysis of variance was also used as a confirmatory analysis. Post hoc pairwise comparisons were adjusted using the Holm–Šidák method. Adjusted P values are reported for all prespecified between-group comparisons at each time point.

Single-time-point continuous outcomes, including day-28 tGFR and composite histologic injury score, were compared using one-way analysis of variance with Tukey-adjusted post hoc testing for parametric data or Kruskal–Wallis testing with Dunn-adjusted comparisons for nonparametric data. Categorical variables were compared using Fisher's exact test. Correlation between day-28 tGFR and composite histologic injury score was quantified using Pearson's correlation coefficient for parametric data or Spearman's rank correlation coefficient otherwise.

Survival distributions were compared using the log-rank test, and hazard ratios with 95% confidence intervals were estimated using Cox proportional hazards regression. All tests were two-sided, and a corrected $P < 0.05$ was considered statistically significant. Statistical analyses were performed using GraphPad Prism version 10.1.

3 Results

3.1 Study flow and analyzable cohorts

A total of 48 recipient mice were enrolled: 12 in the syngeneic group, 18 in the untreated allogeneic group, and 18 in the anti-CD154 group. Technical mortality within 72 hours occurred in 2, 3, and 2 animals, respectively, leaving 10, 15, and 16 recipients for the primary efficacy analysis. Complete day-28 functional follow-up was available in 10, 14, and 15 recipients, respectively. Day-28 histology was available in 8, 12, and 14 animals, and no exclusions occurred after data unblinding.

3.2 Baseline renal function was similar among recipient groups before transplantation

Baseline tGFR, serum creatinine, and BUN did not differ significantly among animals subsequently assigned to untreated or treated allogeneic transplantation. Mean baseline tGFR in recipient B6 mice was 1.58 ± 0.18 mL/min/100 g in the untreated allogeneic group and 1.61 ± 0.16 mL/min/100 g in the treatment group. Baseline creatinine values were 0.10 ± 0.02 mg/dL and 0.10 ± 0.03 mg/dL, respectively, and baseline BUN values were 22.4 ± 3.5 mg/dL and 21.9 ± 3.1 mg/dL. Syngeneic controls showed comparable starting renal function (Table 1). There were no between-group differences in baseline tGFR ($P = 0.87$), creatinine ($P = 0.92$), or BUN ($P = 0.76$).

Table 1. Baseline recipient characteristics and pretransplant renal function.

Variable	Syngeneic	Allogeneic untreated	Allogeneic + anti-CD154
Recipients, n	10	15	16
Male/female	5/5	7/8	8/8
Age (weeks)	12.0 ± 0.0	12.0 ± 0.0	12.0 ± 0.0
Body weight (g)	22.8 ± 1.9	23.1 ± 2.1	22.9 ± 2.0
tGFR (mL/min/100 g)	1.60 ± 0.17	1.58 ± 0.18	1.61 ± 0.16
Creatinine (mg/dL)	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.03
BUN (mg/dL)	21.8 ± 3.2	22.4 ± 3.5	21.9 ± 3.1

3.3 Costimulation blockade attenuated the early functional nadir after allogeneic transplantation

Untreated allogeneic recipients developed a pronounced decline in tGFR after transplantation. In

the mixed-effects model, there were significant effects of group ($F(2, 38) = 24.6$, $P < 0.001$), time ($F(5, 185) = 42.3$, $P < 0.001$), and group-by-time interaction ($F(10, 185) = 6.8$, $P < 0.001$). Mean tGFR fell to 0.62 ± 0.14 mL/min/100 g by day 7 in untreated allogeneic recipients, compared with 0.91 ± 0.13 mL/min/100 g in anti-CD154-treated recipients (adjusted mean difference $= 0.29$ mL/min/100 g, 95% CI 0.19 to 0.39, adjusted $P < 0.001$). By day 28, untreated recipients recovered only partially to 0.88 ± 0.15 mL/min/100 g, whereas treated recipients reached 1.16 ± 0.14 mL/min/100 g (adjusted mean difference $= 0.28$ mL/min/100 g, 95% CI 0.18 to 0.38, adjusted $P < 0.001$). Syngeneic controls showed only a mild transient postoperative decline and returned near baseline by day 14 (Table 2; Figure 1). The longitudinal divergence between untreated and treated allogeneic recipients was evident by day 3, significant at day 7, and remained significant through day 28.

Table 2. Longitudinal renal function measurements after transplantation.

Time point	Marker	Syngeneic	Allogeneic untreated	Allogeneic + anti-CD154
Baseline	tGFR	1.60 ± 0.17	1.58 ± 0.18	1.61 ± 0.16
Day 3	tGFR	1.18 ± 0.13	0.79 ± 0.15	1.01 ± 0.14
Day 7	tGFR	1.24 ± 0.12	0.62 ± 0.14	0.91 ± 0.13
Day 14	tGFR	1.42 ± 0.11	0.74 ± 0.16	1.03 ± 0.15
Day 21	tGFR	1.51 ± 0.10	0.82 ± 0.15	1.11 ± 0.14
Day 28	tGFR	1.56 ± 0.11	0.88 ± 0.15	1.16 ± 0.14
Baseline	Creatinine	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.03
Day 3	Creatinine	0.12 ± 0.03	0.19 ± 0.07	0.16 ± 0.05
Day 7	Creatinine	0.11 ± 0.03	0.28 ± 0.09	0.21 ± 0.06
Day 14	Creatinine	0.10 ± 0.02	0.22 ± 0.08	0.17 ± 0.05
Day 21	Creatinine	0.10 ± 0.02	0.17 ± 0.06	0.14 ± 0.04
Day 28	Creatinine	0.10 ± 0.02	0.15 ± 0.05	0.13 ± 0.04
Baseline	BUN	21.8 ± 3.2	22.4 ± 3.5	21.9 ± 3.1
Day 3	BUN	26.7 ± 5.4	58.9 ± 16.8	46.3 ± 13.5
Day 7	BUN	24.9 ± 4.8	76.2 ± 19.4	58.7 ± 14.8
Day 14	BUN	23.5 ± 4.1	61.1 ± 18.7	47.6 ± 13.9
Day 21	BUN	22.6 ± 3.8	47.3 ± 14.9	39.5 ± 11.7
Day 28	BUN	22.1 ± 3.6	38.4 ± 12.6	33.1 ± 10.9

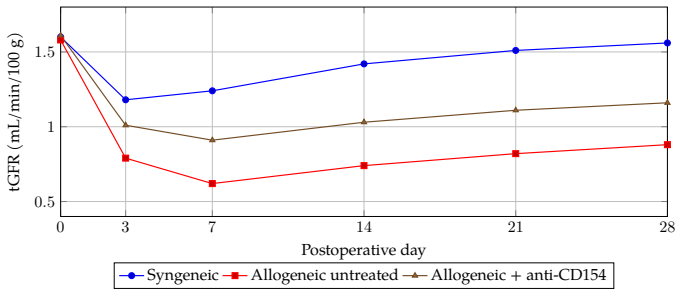

Figure 1. Longitudinal transdermal glomerular filtration rate from baseline to day 28 in syngeneic controls, untreated allogeneic recipients, and anti-CD154-treated allogeneic recipients. Error bars and significance annotations should be added in the final analytical figure.

3.4 Serum creatinine and blood urea nitrogen were less sensitive to treatment effects than transdermal glomerular filtration rate

Serum creatinine and BUN showed the same overall directional pattern but with smaller standardized effect sizes and wider between-animal variability than tGFR. At day 7, serum creatinine was 0.28 ± 0.09 mg/dL in untreated allogeneic recipients versus 0.21 ± 0.06 mg/dL in treated recipients (adjusted mean difference $= 0.07$, 95% CI 0.01 to 0.13, adjusted $P = 0.02$), whereas by day 28 the difference was no longer significant (adjusted mean difference $= 0.02$, 95% CI -0.01 to 0.05, adjusted $P = 0.24$). BUN at day 7 was 76.2 ± 19.4 mg/dL in untreated recipients versus 58.7 ± 14.8 mg/dL in treated recipients (adjusted mean difference $= 17.5$, 95% CI 5.6 to 29.4, adjusted $P = 0.006$), but intergroup overlap increased at later time points, and the day-28 difference was not significant (adjusted mean difference $= 5.3$, 95% CI -2.9 to 13.5, adjusted $P = 0.19$). Consistent with these findings, the standardized between-group effect size for day-28 tGFR (Cohen's $d = 1.96$, 95% CI 1.07 to 2.85) exceeded that for creatinine ($d = 0.44$, 95% CI -0.28 to 1.16) and BUN ($d = 0.45$, 95% CI -0.27 to 1.17), supporting the greater discriminatory performance of tGFR.

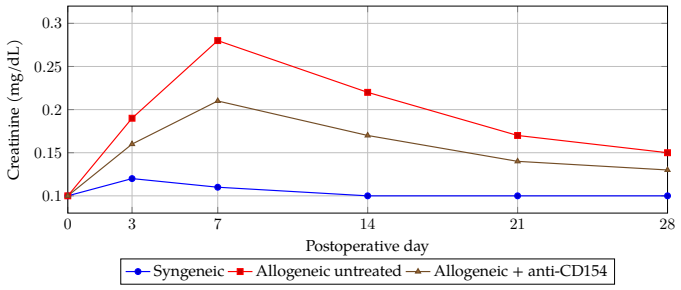

Figure 2. Serial serum creatinine in syngeneic controls, untreated allogeneic recipients, and anti-CD154-treated allogeneic recipients. Error bars and adjusted P values should be shown in the final analytical figure.

3.5 Therapeutic benefit was associated with improved recipient survival

Survival differed significantly across groups (log-rank $P < 0.001$). Untreated allogeneic recipients had a median survival of 39 days (95% CI 32 to 46 days), whereas anti-CD154-treated recipients had a median survival of 57 days (95% CI 49 to 65 days). Relative to untreated allogeneic recipients, anti-CD154 treatment was associated with a hazard ratio for death of 0.41 (95% CI 0.22 to 0.76, $P = 0.005$). Syngeneic controls

showed near-complete survival throughout follow-up. Early mortality in untreated allograft recipients was preceded by progressive tGFR decline beginning after day 7, whereas treated animals that survived long term generally maintained tGFR above $0.95\,\mathrm{mL/min/100\,g}$ after the second postoperative week (Figure 4).

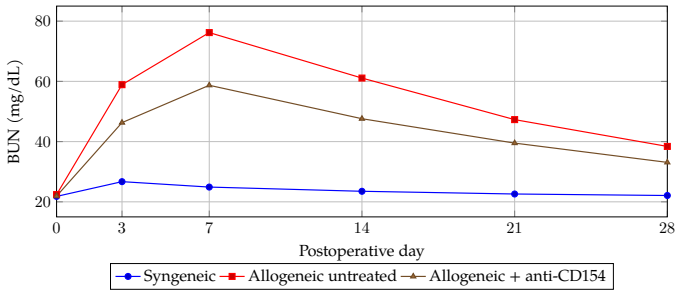

Figure 3. Serial blood urea nitrogen in syngeneic controls, untreated allogeneic recipients, and anti-CD154-treated allogeneic recipients. Error bars and adjusted P values should be shown in the final analytical figure.

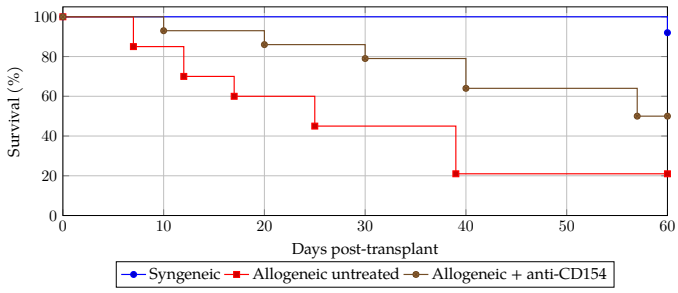

Figure 4. Kaplan–Meier survival curves comparing untreated allogeneic recipients and anti-CD154-treated allogeneic recipients, with syngeneic controls shown for reference. Numbers at risk and censoring marks should be added in the final analytical figure.

3.6 Histologic rejection severity was reduced by costimulation blockade

Day-28 periodic acid–Schiff histology of untreated allogeneic grafts revealed multifocal glomerulitis, prominent tubulitis, diffuse interstitial inflammatory infiltrates, and peritubular capillaritis. Composite injury score differed significantly across groups (overall $P < 0.001$). Median composite injury score was 9 (IQR 8–10) in untreated allogeneic grafts, compared with 5 (IQR 4–6) in anti-CD154-treated grafts and 2 (IQR 1–2) in syngeneic controls. Pairwise testing showed that treated allografts had significantly lower composite injury scores than untreated allogeneic grafts (adjusted $P = 0.002$). The same pattern was observed across glomerulitis, tubulitis, interstitial inflammation, and peritubular capillaritis subscores (Table 3).

Table 3. Day-28 histologic lesion scores and survival outcomes. Histologic data are presented as median (IQR).

Outcome	Syngeneic	Allogeneic untreated	Allogeneic + anti-CD154
Glomerulitis score	0 (0–1)	2 (2–3)	1 (1–2)
Tubulitis score	0.5 (0–1)	2.5 (2–3)	1 (1–2)
Interstitial inflammation score	1 (0–1)	2 (2–2)	1 (1–1.5)
Peritubular capillaritis score	0 (0–1)	2 (2–2)	1 (1–2)
Composite injury score	2 (1–2)	9 (8–10)	5 (4–6)
Median survival (days)	> 60	39	57
Estimated survival at day 60 (%)	92	21	50

Day-28 tGFR was inversely associated with composite histologic injury score (Spearman's $\rho = -0.82$, 95% CI -0.92 to -0.64, $P < 0.001$; Figure 5). Animals with lower day-28 tGFR consistently exhibited higher histologic injury burden, supporting concordance between direct functional assessment and tissue-level rejection severity.

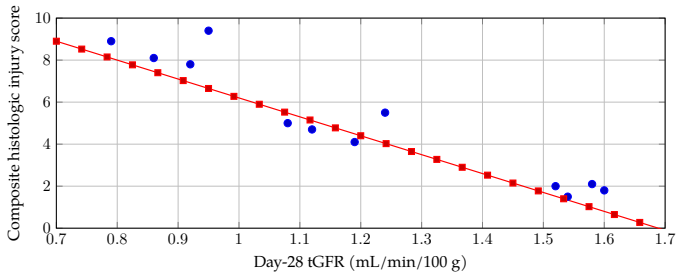

Figure 5. Inverse association between preserved day-28 transdermal glomerular filtration rate and composite histologic injury score. The final figure should report the correlation coefficient, 95% confidence interval, and exact P value.

3.7 Sex-stratified analysis demonstrated benefit in both sexes

In prespecified sex-stratified analyses, treatment benefit was observed in both sexes, although the magnitude of late recovery was greater in females. At day 28, treated males had a mean tGFR difference of $0.26\,\mathrm{mL/min/100\,g}$ relative to untreated males (95% CI 0.14 to 0.38, $P < 0.001$), whereas treated females had a mean difference of $0.30\,\mathrm{mL/min/100\,g}$ relative to untreated females (95% CI 0.18 to 0.42, $P < 0.001$). The treatment-by-sex interaction was not significant ($P = 0.28$), indicating that the treatment benefit was broadly similar across sexes. Raw day-28 values are shown in Table 4.

Table 4. Sex-stratified functional outcomes after allogeneic transplantation.

Group	Male day-28 tGFR (mL/min/100 g)	Female day-28 tGFR (mL/min/100 g)
Allogeneic untreated	0.83 ± 0.14	0.93 ± 0.15
Allogeneic + anti-CD154	1.09 ± 0.13	1.23 ± 0.12

4 Discussion

This study demonstrates that short-course peri-transplant costimulation blockade improves renal allograft function, prolongs survival, and reduces rejection-associated histologic injury in a murine allogeneic kidney transplant model. Most importantly, these benefits were captured most clearly by serial tGFR measurement. Whereas serum creatinine and BUN showed broad directional changes, they lacked the temporal precision and interexperimental consistency needed to resolve modest therapeutic effects across the full postoperative course.

The value of this approach lies not only in measuring kidney function directly, but in doing so repeatedly and nonterminally in the same animal. In untreated allogeneic recipients, tGFR showed a sharp early decline followed by incomplete recovery. In treated animals, the early nadir was blunted and later recovery was improved. These differences emerged before severe biochemical separation and remained evident even when creatinine and BUN began to overlap. Functionally, this means that intervention efficacy can be detected earlier and with greater confidence.

This is particularly relevant in murine kidney transplantation, where sample size, surgical complexity, and biologic variability all constrain experimental efficiency [2, 3]. A measurement platform that increases sensitivity reduces the number of animals required to detect meaningful differences and permits more refined study of intermediate phenotypes. Prior work established the superiority of tGFR over traditional surrogate markers in nephrectomy and transplant models, and the present study extends that logic into a therapeutic testing framework [14].

The histologic data supported the functional findings. Untreated allografts demonstrated classic inflammatory rejection features, whereas treated grafts had substantially lower injury burden. The concordance between preserved GFR and reduced glomerulitis, tubulitis, and peritubular capillaritis suggests that the observed functional improvement reflects genuine mitigation of allograft injury rather than biochemical fluctuation. This is important because murine creatinine and BUN can be influenced by nonrenal and assay-related factors, limiting their interpretability when changes are small or transient [4–6].

The sex-stratified analysis is also notable. Although both males and females benefited from treatment, females demonstrated slightly greater late recovery. Sex-specific functional variation has been described in murine renal physiology and injury models [14, 20], and our findings suggest that sex may also influence the magnitude or tempo of benefit from immune intervention. This deserves deeper mechanistic study, particularly with respect to endothelial activation, cytokine milieu, and T-cell differentiation.

Several limitations should be acknowledged. First, this was a short-course intervention study centered on early and intermediate post-transplant outcomes. Longer follow-up is needed to determine whether preserved early GFR translates into durable protection from chronic allograft dysfunction. Second, the study focused on a single immunologic pathway and did not dissect downstream cellular mechanisms. Third, although tGFR was clearly superior for longitudinal discrimination, combining it with additional biomarkers such as cystatin C, urinary injury markers, or molecular rejection signatures may further enhance mechanistic resolution [19].

Despite these limitations, the study offers a practical and conceptual message. In murine kidney transplantation, direct serial measurement of GFR can transform experimental design from one that passively documents severe graft failure into one that actively detects early therapeutic benefit. This is a major advantage for preclinical testing of immunomodulatory and organ-protective strategies.

5 Conclusion

Peri-transplant anti-CD154 costimulation blockade improved renal allograft function, prolonged survival, and reduced histologic rejection severity in murine allogeneic kidney transplantation. Serial tGFR provided the most sensitive and consistent measure of these benefits and outperformed serum creatinine and BUN as a longitudinal endpoint. These findings support the use of direct GFR monitoring as a primary readout in preclinical transplant intervention studies.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Code Availability Statement

The statistical code and graph-generation scripts used for longitudinal renal function analysis and survival analysis are available from the corresponding author upon reasonable request.

Ethics Statement

All animal procedures were performed in accordance with institutional guidelines for the care and use of laboratory animals and were approved by the relevant institutional animal care and use committee.

Conflict of Interest

The authors declare no conflict of interest.

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