ORIGINAL ARTICLE

Paricalcitol versus placebo for reduction of proteinuria in kidney transplant recipients: a double-blind, randomized controlled trial

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SUMMARY

Proteinuria after kidney transplantation is accompanied by an increased risk of graft failure. In this single-center, placebo-controlled, double-blind trial we studied whether vitamin D receptor activator paricalcitol might reduce proteinuria. Patients with urinary protein-to-creatinine ratio (UPCR) ≥20 mg/mmol despite optimization of the renin angiotensin aldosterone system (RAAS) blockade were randomly assigned to receive 24 weeks' treatment with 2 μg/day paricalcitol or placebo. Primary endpoint was change in UPCR, and main secondary endpoints were change in urinary albumin-to-creatinine ratio (UACR) and 24-h proteinuria. Analysis was by intention to treat. One hundred and sixty-eight patients undergo randomization, and 83 were allocated to paricalcitol, and 85 to placebo. Compared with baseline, UPCR declined in the paricalcitol group (−39%, 95% CI −45 to −31) but not in the placebo group (21%, 95% CI 9 to 35), with a between group difference of −49% (95% CI −57 to −41; $P < 0.001$). UACR and 24-h proteinuria decreased only on paricalcitol therapy and significantly differed between groups at end-of-treatment ($P < 0.001$). Paricalcitol was well tolerated but incidence of mild hypercalcemia was higher than in placebo. In conclusion, addition of 2 μg/day paricalcitol lowers residual proteinuria in kidney transplant recipients. Long-term studies are needed to determine if the reduction in proteinuria improves transplant outcomes (ClinicalTrials.gov, number NCT01436747).

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Key words
angiotensin converting enzyme inhibitor, angiotensin receptor blocker, kidney transplantation, paricalcitol, proteinuria

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Introduction

Over the past decades there has been remarkable improvement in short-term graft survival, whereas long-term graft failure and premature mortality after kidney transplantation remain a challenge [1]. Transplant patients with graft failure are prone to a threefold greater mortality risk than patients with functioning grafts, with a 3.5–5% annual risk of cardiovascular events [2,3]. Identification of modifiable risk factors responsible for kidney graft loss would improve targeted interventions against graft deterioration.

Proteinuria is known to be an independent risk factor for cardiovascular mortality as well as a predictor of graft failure after kidney transplantation [4,5]. Because it is often a marker of graft injury and is a potentially

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modifiable risk factor, it is important to recognize early [6]. Transplant specific diagnoses, specifically antibody-mediated rejection (AMR), transplant glomerulopathy (TG) and interstitial fibrosis and tubular atrophy (IFTA), have been more commonly found on biopsy studies in transplant recipients with proteinuria than native kidney diseases [7]. Strategies to inhibit AMR and non-immunological interventions used to slow the progression of IFTA, such as renin-angiotensin-aldosterone system (RAAS) blockade, have had little success in clinical trials in kidney transplant recipients [8,9]. Therefore, new approaches to stop progressive graft dysfunction are needed.

Epidemiological studies have shown associations between low 25-hydroxyvitamin D levels and proteinuria [10], as well as increased IFTA in kidney grafts [11]. There is evidence to suggest that low levels of vitamin D contribute to a lack of RAAS suppression [12,13]. Consequently, drugs with vitamin D agonist properties have been proposed as potential renoprotective agents. In preclinical models and clinical studies in patients with diabetic kidney disease, a selective vitamin D receptor agonist, paricalcitol, reduced proteinuria and slowed the progression of kidney injury with little effects on mineral metabolism [14,15]. Data from experimental studies have indicated that paricalcitol may be renoprotective by downregulating RAAS [16], and trough effects on inflammatory and fibrotic pathways [17]. Similar effects, although less established, have been suggested in kidney transplant recipients [18–20].

We undertook a randomized, placebo-controlled trial to prospectively test the effectiveness of paricalcitol for the reduction of residual proteinuria in kidney transplant recipients after optimization of the RAAS blockade. Additionally, we studied the effects of paricalcitol on RAAS activity and biomarkers related to pathways of inflammation and fibrosis.

Patients and methods

Study design

This study was an investigator-initiated, single center, randomized, double-blind, placebo-controlled trial. Patients were included between July of 2012 and October of 2014; the last follow-up visit of the last patient took place in July of 2015. The study was conducted according to the principles of the Declaration of Helsinki. The study protocol has been approved by the National Medical Ethics Committee (Nr. 31-06-2011). The Centre for Kidney Transplantation, University Medical Centre Ljubljana, was responsible for conduction of the trial, while monitoring was provided by RhoSigma Research Consulting. The trial was registered with ClinicalTrials.gov, number NCT01436747.

Study participants

We recruited a national cohort of adult (>18 years old) kidney transplant recipients with stages 1–4 chronic kidney disease (CKD) and residual proteinuria more than 3 months after transplant. Inclusion criteria were urinary protein-to-creatinine ratio (UPCR) ≥20 mg/mmol (geometric mean of the three consecutive morning voids) despite optimization of the single-agent RAAS blockade during the run-in period and an eGFR ≥15 ml/min/1.73 m^2. Additional inclusion criteria included serum iPTH concentration ≥30 ng/l and serum calcium (adjusted for serum albumin) of <2.60 mmol/l. Exclusion criteria were uncontrolled hypertension (blood pressure [BP] ≥160/100 mmHg), active malignancy, pregnancy or breastfeeding, and treatment with vitamin D analog in the previous 3 months. All patients provided written informed consent.

Study procedures, randomization and masking

The study consisted of the run-in period, treatment period, and follow-up. At the start of a 12-week run-in period, eligibility criteria were verified and informed consent of the potential participants was obtained. During a run-in period, we specifically reviewed existing treatment with RAAS blockade. Patients who were not already receiving RAAS blockade were prescribed with angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB). If the target BP of 140/90 mmHg was not achieved within 4 weeks, doses of RAAS blockers were increased to maximal recommended levels. Additional antihypertensive therapy was prescribed at the physician discretion. In case of graft dysfunction (increase in serum creatinine >30% from baseline), hyperkalemia or symptomatic hypotension after introduction of the RAAS blockade, ACE inhibitor or ARB dose was first decreased and then discontinued if necessary. Doses of ACE inhibitors or ARBs could not be adjusted after randomization. Patients with a BP < 160/100 mmHg could enroll in the treatment period. Any form of vitamin D supplementation was discontinued.

Patients who met the study inclusion criteria at the end of the run-in period were subjected to a treatment period of 24 weeks. The treatment period consisted of

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(i) the vitamin D receptor agonist paricalcitol (19-nor-1,25-dihydroxyvitamin D_2) 2 μg daily (two 1 μg capsules) or (ii) matching placebo (two capsules). The study medication (paricalcitol or placebo) was provided by AbbVie. Placebo capsules had a similar appearance, smell, and taste compared with paricalcitol capsules. Computer-generated random allocation sequence was performed by AbbVie. The investigators (M.A. and G.M.) enrolled participants. Patients received study medication containers labelled with a unique number representing the randomly allocated sequence, whereby all participants and involved investigators remained blinded to the study medication type throughout the entire study. Assignment of the treatment order was not disclosed until the study database was locked.

Patients were examined at the start of randomization (baseline), every 4 weeks during the treatment period, and 8 weeks after treatment completion (follow-up). Vital parameters (BP, pulse rate, and body weight), adherence to drug regimens, blood chemistry, and morning void spot urine specimens were assessed at every visit. Additionally, 24-h urine samples were obtained at baseline and after 24 weeks of treatment. Additional laboratory assessments at baseline and after 24 weeks of treatment included measurements of plasma renin activity (PRA) and aldosterone concentration as biomarkers of RAAS activity, serum concentration of interleukin (IL)-6 as a biomarker of inflammation, and serum concentration of transforming growth factor (TGF)-beta as a biomarker of fibrosis. Certified local laboratories were utilized to process and provide results for all laboratory tests (further details on the study measurements are described in the Appendix S1).

Every 4 weeks during the treatment period, serum albumin, calcium, and intact parathyroid hormone (iPTH) levels were measured for a safety analysis. In case of persistent hypercalcemia (two consecutive corrected serum calcium measurements >2.60 mmol/l) or hypoparathyroidism (iPTH < 15 ng/l), the dose of the study medication (paricalcitol or placebo) was reduced from two capsules to one capsule per day for the remaining treatment period. All patient-reported or observed adverse effects were recorded. Adherence was monitored by counting of returned capsules.

Study endpoints

The primary efficacy measure was the percentage change in geometric mean UPCR from baseline to the last measurement during treatment. Secondary efficacy measures included calculation of the proportion of patients achieving at least 15 mg/mmol reduction in UPCR level and the percentage change in geometric mean urinary albumin-to-creatinine ratio (UACR). Additional secondary efficacy measures recorded between baseline and the end of 24-week treatment was the change in 24-h urine protein excretion, and changes in the levels of serum biomarkers. Changes in systolic and diastolic BP, estimated glomerular filtration rate (eGFR), creatinine clearance, and serum levels of calcium, phosphate, and iPTH, recorded from baseline to the last measurement during treatment were also selected as prespecified secondary endpoints.

Statistical analysis

On the basis of data from our previous study on proteinuria in a national cohort of kidney transplant recipients, a baseline mean UPCR of 50 mg/mmol was suggested for the calculation of sample size [21]. According to Borm *et al.* [22], a sample size of 152 patients was calculated to detect a clinically relevant difference of 15 mg/mmol in UPCR from baseline to last measurement during treatment between paricalcitol and placebo groups, allowing a type 1 error rate of 5%, a type 2 error rate of 20%, two-sided testing, and considering the standard deviation (SD) of 61 mg/mmol (both arms). Assuming a dropout rate of 10%, we aimed to include 168 patients. The intention-to-treat dataset included all randomized patients who had at least one dose of study drug, and was used for all efficacy and safety analyses.

Data are presented as mean (±SD) in case of normally distributed data, geometric mean (95% CI) for non-normally distributed data, and number (percentage) for nominal data. Variable distribution was tested graphically using histograms and probability plots. Data at the end of the run-in period were considered baseline values. Differences in the last-on-treatment values of continuous variables between paricalcitol and placebo groups were assessed using analysis of covariance (ANCOVA), with treatment group as a factor and baseline values as a covariate. Non-normally distributed variables were log-transformed before entering the analysis. When applying ANCOVA for the primary efficacy endpoint, the assumption of homogeneity of regression slopes was not met. Consequently, alternative analytical approach using modified Johnson-Neyman procedure was considered [23]. Point of intersection and simultaneous region of significance (i.e., values of UPCR at baseline, where groups statistically significantly differ in

the last-on-treatment UPCR) were calculated. Two-way mixed analysis of variance (ANOVA) was used to test whether interaction between time and treatment group was statistically significant. Association between two nominal variables was calculated using the chi-square test.

A two-tailed $P < 0.05$ was considered to indicate statistical significance. All analyses were performed using the SPSS statistical software (IBM SPSS statistics, version 23.0, IBM Corporation, Armonk, NY, USA).

Results

Study population

Of 572 patients who were screened for eligibility, 190 (33%) were eligible to participate in the study and were enrolled in the run-in period (Fig. 1). At the beginning of the run-in phase mean UPCR was 64 (95% confidence interval [CI] 54–72) mg/mmol, and 116 patients (61%) were treated with an ACE inhibitor or ARB. During the run-in period, 22 patients discontinued the study. Finally, 168 patients were randomized and assigned to receive placebo ($n = 85$) or 2 μg/day of paricalcitol ($n = 83$). Baseline demographic and clinical characteristics did not differ between the two study groups (Table 1). Approximately 50% of patients had indication kidney biopsy performed prior enrolment, and the most common histological diagnosis was AMR with *de-novo* formation of donor-specific antibodies (DSA). The proportion of patients with previous rejection and *de-novo* DSA was slightly higher in the paricalcitol group and patients assigned to paricalcitol had greater baseline levels of UPCR and 24-h proteinuria (Table 1). Immunosuppression and other concomitant treatments were balanced between the groups. Overall, 156 patients (93%) received background ACE inhibitor or ARB in a fixed dose and there were no significant between-group differences in the mean daily dose (Table 1). Twelve patients (six assigned to paricalcitol and six to placebo) did not tolerate RAAS blockade due to transplant renal artery stenosis (eight patients) or a symptomatic decrease in BP (four patients).

All randomized patients on placebo and 2 μg/day of paricalcitol received at least one dose of study drug, and had measured data at baseline and at least one time-point during treatment, and so were included in the intention-to-treat analysis. Adherence to the assigned treatment was excellent in both groups (≥95% of the prescribed dose). Two patients in the placebo group discontinued from the study, and two patients in the paricalcitol group missed the clinic visit after 24 weeks of treatment (Fig. 1).

Primary efficacy analyses

Compared with baseline, levels of UPCR at last measurement during treatment significantly differ between the paricalcitol and placebo groups ($P < 0.001$). For baseline UPCR >9 (exp 2.17) mg/mmol, paricalcitol provided a significantly higher reduction in end-of-treatment UPCR when compared with placebo, and the difference between groups increased with greater UPCR levels at baseline (Fig. 2). The UPCR-lowering effect of paricalcitol was not influenced by the baseline level of 25-hydroxyvitamin D ($r = 0.16$; $P = 0.164$).

In the 2 μg paricalcitol group, UPCR decreased from baseline to last measurement during treatment (Table 2), with a between-group difference versus placebo of −49% (95% CI −57 to −41; $P < 0.001$; Fig. 3a). UPCR had reduced by week 4 in the paricalcitol group, and this reduction was sustained during the entire treatment phase, peaking at −39% (from 74 to 46 mg/mmol, 95% CI −45 to −31) at week 24 (Fig. 4a). By contrast, UPCR slightly increased in the placebo group (21%, from 55 to 63 mg/mmol; 95% CI −9 to 35; Table 2). Eight weeks after treatment completion, UPCR returned towards baseline, with a between-group difference of −26% (95% CI −37 to −13, $P < 0.001$; Fig. 4a).

Secondary efficacy analyses

The proportion of patients achieving a decrease in UPCR of at least 15 mg/mmol between baseline and the last measurement during treatment was higher in the 2 μg paricalcitol group than in placebo group (57% vs. 9%; $P < 0.001$; Table 2). We also detected significant differences between the paricalcitol and placebo regarding change in UACR, with a between-group difference versus placebo of −52% at last measurement during treatment (95% CI −61 to −40; $P < 0.001$; Fig. 3b). During treatment, paricalcitol provided a sustained reduction in UACR (−47%, from 29 to 16 mg/mmol; 95% CI −54 to −38), while UACR slightly increased in the placebo group (14%, from 20 to 23 mg/mmol; 95% CI −2 to 31; Table 2 and Fig. 4b). Similarly, 24-h proteinuria decreased in the paricalcitol group after 24 weeks of treatment (Table 2), with a between-group difference versus placebo of −27% (95% CI −44 to −3; $P < 0.001$).

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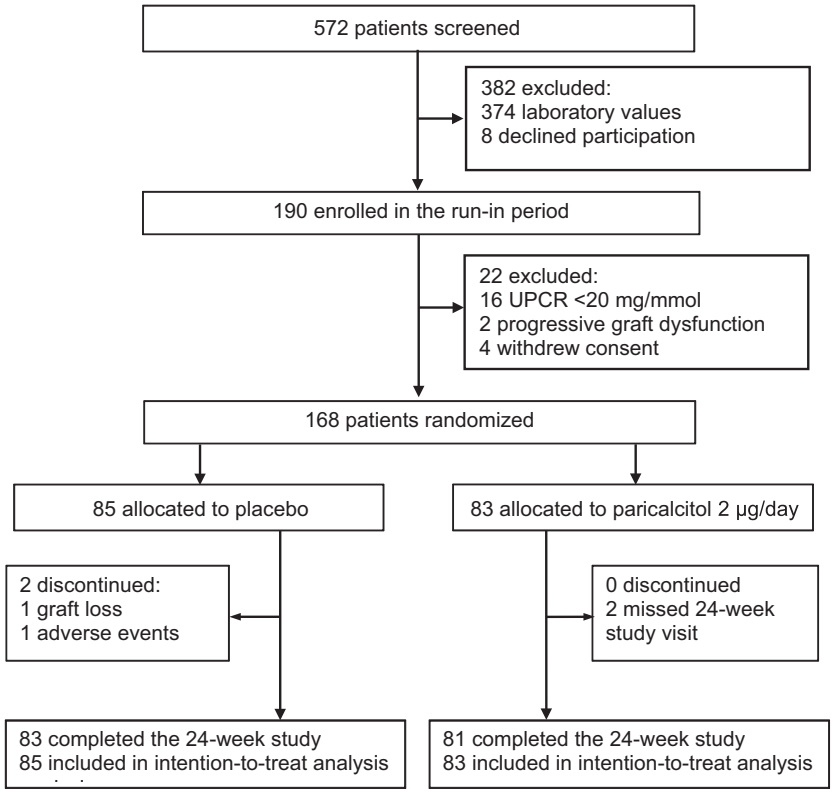

Figure 1 Trial profile indicating the disposition of study participants during screening, enrolment, randomization, and participation in the trial.

Paricalcitol also induced a small but significant reduction in eGFR and systolic BP (Table 2). eGFR had reduced by week 4 in the 2 μg paricalcitol group and remained stable throughout treatment (Fig. 5a), whereas early reduction in systolic BP was followed by a pattern of fluctuations across the treatment phase (Fig. 5b). Creatinine clearance did not change appreciably during the paricalcitol and placebo treatment (Table 2).

Consistent with the known effects of paricalcitol, iPTH levels decreased during treatment in the 2 μg paricalcitol group, and remained stable in the placebo group ($P < 0.001$; Table 2 and Fig. S1A). During the treatment phase, paricalcitol induced a small but significant increase in serum calcium ($P < 0.001$ vs. placebo; Table 2 and Fig. S1B). In contrast, mean serum calcium remained stable in the placebo group. No significant between-group changes were recorded in the measurements of serum phosphate (Table 2 and Fig. S1C).

Paricalcitol treatment resulted in a nonsignificant reduction in PRA ($P = 0.24$ vs. placebo; Table 2). No changes were recorded between groups in serum aldosterone levels. In contrast, IL-6 and TGF-beta serum concentrations decreased from baseline to the end of 24-week treatment in the paricalcitol group and increased in the placebo group ($P < 0.001$; Table 2).

Effect of paricalcitol based on graft function and sodium intake

In a *post hoc* analysis, baseline eGFR (expressed as tertiles) and 24-h sodium excretion (expressed as greater or smaller than geometric mean) had no significant effect on change from baseline in UPCR and UACR in the paricalcitol or placebo group (Fig. S2 and S3), indicating that the anti-proteinuric effect of paricalcitol was present irrespective of the level of graft function and sodium intake.

Effect of paricalcitol based on histologic phenotype

In a subgroup of patients with kidney biopsies performed prior study enrolment (Table 1), there were no significant differences in change of UPCR and UACR by paricalcitol or placebo between non-rejection findings, T cell-mediated rejection, AMR, or AMR and TG (Fig. S4). While UPCR and UACR decreased with paricalcitol in rejection and non-rejection phenotypes, mean UPCR and UACR increased in the placebo group, specifically in patients with prior AMR and TG (Fig. S4).

Adverse events

During treatment, hypercalcemia developed in 16 patients (19%) receiving 2 μg paricalcitol, and in five

Table 1. Baseline patient demographics, clinical and laboratory characteristics*.

Variables	Placebo (n = 85)	Paricalcitol (n = 83)
Demographic characteristics		
Age (years)	54 ± 12	54 ± 11
Male gender (%)	58 (68)	56 (68)
Body mass index (kg/m^2)	25.1 ± 3.7	26.3 ± 4.3
Original kidney disease		
Diabetes (%)	4 (5)	4 (5)
Hypertension (%)	4 (5)	5 (6)
Glomerulonephritis (%)	29 (34)	31 (37)
Polycystic (%)	10 (12)	9 (11)
Pyelonephritis/reflux (%)	6 (7)	9 (11)
Other/undefined (%)	8 (9)/24 (28)	8 (10)/17 (20)
Clinical characteristics		
Time post-transplant (years)	8.5 (2.6–13.2)	8.4 (4.2–13.6)
Graft biopsy prior enrolment† (%)	38 (45)	43 (52)
Histologic findings		
AMR (%)	16 (19)	20 (24)
AMR and TG (%)	10 (12)	11 (13)
T cell-mediated rejection (%)	10 (12)	12 (15)
Recurrent disease (%)	4 (5)	2 (2)
Other findings‡ (%)	8 (9)	9 (11)
De-novo DSA§ (%)	16 (19)	20 (24)
Vital parameters		
Systolic BP (mmHg)	137 ± 17	137 ± 16
Diastolic BP (mmHg)	74 ± 10	77 ± 12
Heart rate (beats/min)	74 ± 13	73 ± 13
Treatments		
ACEi/ARB (%)	79 (93)	77 (93)
Maximum daily ACEi dose¶ (%)	26 (31)	28 (34)
Mean daily ACEi dose (mg)	6.3 ± 3.9	6.2 ± 3.9
Maximum daily ARB dose¶ (%)	21 (25)	20 (24)
Mean daily ARB dose (mg)	71 ± 23	72 ± 21
Diuretic	29 (34)	27 (33)
Other antihypertensives	76 (89)	72 (87)
Lipid-lowering treatments	54 (64)	56 (68)
Glucose-lowering treatments	19 (22)	19 (23)
Calcineurin inhibitor	85 (100)	83 (100)
Mycophenolate	74 (87)	66 (80)
Steroid	48 (57)	46 (55)
Laboratory parameters		
Spot urine		
UPCR	55 (46–65)	74 (62–83)
UACR (mg/mmol)	20 (14–27)	29 (22–40)
24-h urine		
Protein (mg/day)	480 (402–570)	610 (522–720)
Sodium (mmol/day)	180 (165–198)	180 (167–196)
Creatinine clearance (ml/min)	56 ± 22	54 ± 24
Serum		
Creatinine	130 ± 59	132 ± 54
eGFR (ml/min/1.73 m^2)	53 ± 20	52 ± 20
Total cholesterol (mmol/l)	5.0 ± 1.0	4.9 ± 1.1
LDL cholesterol (mmol/l)	2.8 ± 0.8	2.8 ± 0.8
HDL cholesterol (mmol/l)	1.3 ± 0.4	1.3 ± 0.5
Triglycerides (mmol/l)	1.9 ± 1.2	1.8 ± 1.2
Calcium (mmol/l)	2.29 ± 0.15	2.28 ± 0.15
Phosphate (mmol/l)	0.93 ± 0.19	0.97 ± 0.22

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Table 1. Continued.

Variables	Placebo (n = 85)	Paricalcitol (n = 83)
Albumin	43 ± 3	42 ± 4
Intact PTH (ng/l)	111 (97–127)	108 (97–121)
25-OH-vitamin D (nmol/l)	43 ± 18	44 ± 17
Serum biomarkers		
PRA (μg/l/h)	3.05 (2.28–4.07)	2.40 (1.82–3.16)
Aldosterone (nmol/l)	0.32 (0.26–0.40)	0.34 (0.28–0.41)
Interleukin-6 (pg/ml)	2.0 (1.72–2.34)	2.85 (2.40–3.37)
TGF-beta (pg/ml)	7022 (6237–7913)	8578 (7628–9647)

AMR, antibody-mediated rejection; TG, transplant glomerulopathy; ACEi, angiotensin-converting enzyme inhibitor; ARB; angiotensin receptor blocker; BP, blood pressure; UPCR, urinary protein-to-creatinine ratio; UACR, urinary albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; PRA, plasma renin activity; PTH, parathyroid hormone; TGF, transforming growth factor.

*Data are presented as mean ± SD or geometric mean (95% CI) for normally or skewed distributed data, respectively, or as total number (percentage). There were no significant between-group differences at baseline, except with respect to UPCR (P = 0.029), 24-h proteinuria (P = 0.047), and TGF-beta (P = 0.037).

†Kidney graft biopsies performed for cause (increase in serum creatinine >20% from the baseline without other evident causes and/or increase in proteinuria >1 g per day) prior study enrolment. Histologic data represent most recent findings. All patients with rejection findings were treated prior to enrolment.

‡Include calcineurin inhibitor nephrotoxicity, hypertensive glomerulosclerosis, polyomavirus-associated nephropathy, nephrocalcinosis, and reflux nephropathy.

§Determined at the time of most recent graft biopsy for cause prior study enrolment.

¶Maximum recommended daily dose of ACEi (ramipril 10 mg, perindopril 5 mg, trandolapril 4 mg) or ARB (losartan 100 mg, telmisartan 80 mg).

patients (6%) receiving placebo (P = 0.017). Hypercalcemia was mild (serum calcium <2.80 mmol/l), and persisted on repeated measurements in five patients in the paricalcitol and four patients in the placebo group (P = 0.46). A reduction of the study drug dose to one capsule per day lead to acceptable serum calcium levels in all patients. Hypoparathyroidism led to a dose reduction in 14 patients: 13 during 2 μg paricalcitol and one during placebo treatment (P < 0.001). After 24 weeks, iPTH remained suppressed in two patients assigned to paricalcitol.

The overall occurrence of other reported adverse events was similar between the groups (Table 3). Two patients in the placebo group discontinued the study because of adverse events. One patient lost the graft after 12 weeks of treatment due to AMR, and one patient withdrew from the study after 8 weeks due to abdominal bloating and dyspepsia. None of the patients in the paricalcitol group lost the graft or withdrew from the study because of adverse events. However, two patients receiving paricalcitol missed the 24-week clinic visit. During the treatment period, four patients assigned to placebo had kidney biopsy due to graft dysfunction. Two of these patients were

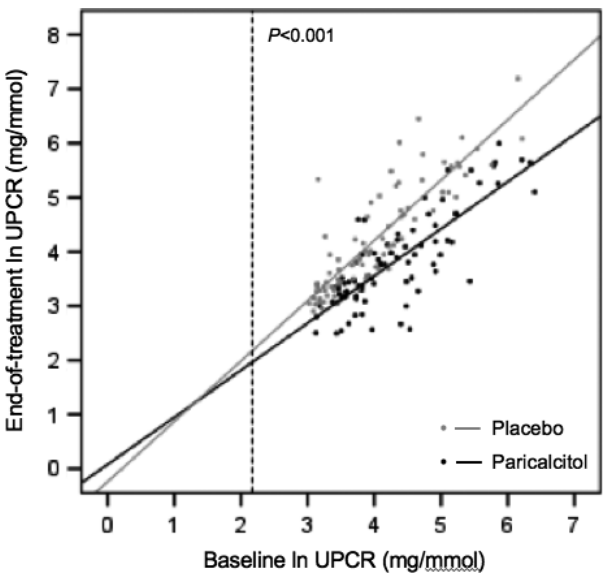

Figure 2 Effects of paricalcitol and placebo on end-of-treatment urinary protein-to-creatinine ratio (UPCR) based on levels at baseline (individual data, logarithmically transformed). For baseline UPCR levels >9 (exp 2.17) mg/mmol (dashed line), patients treated with paricalcitol had significantly lower UPCR at last measurement during treatment (P < 0.001 vs. placebo).

Table 2. Primary and secondary efficacy analyses, by intention-to-treat principles*.

	Placebo (n = 85)			Paricalcitol (n = 83)			
Parameter	Baseline	Last measurement during treatment	% change (95% CI)	Baseline	Last measurement during treatment	% change (95% CI)	P-value
UPCR (mg/mmol)	55 (46 to 65)	63 (50 to 79)	21 (9 to 35)	74 (62 to 83)	46 (38 to 55)	−39 (−45 to −31)	<0.001†
UPCR reduction ≥15 mg/mmol (%)	–	8 (9)	–	–	47 (57)	–	<0.001‡
UACR (mg/mmol)	20 (14 to 27)	23 (16 to 33)	14 (−2 to 31)	29 (22 to 40)	16 (12 to 22)	−47 (−54 to −38)	<0.001§
24-h proteinuria (mg)	480 (402 to 570)	540 (436 to 668)	19 (8 to 30)	610 (522 to 720)	396 (330 to 478)	−35 (−42 to −28)	<0.001§
eGFR (ml/min/1.73 m^2)	53 ± 20	52 ± 21	−1 (−5 to 3)	52 ± 20	48 ± 19	−7 (−11 to −3)	0.035§
Cr-clearance (ml/min)	56 ± 22	55 ± 23	−2 (−6 to 3)	54 ± 24	53 ± 23	−3 (−8 to 2)	0.563§
Systolic BP (mmHg)	137 ± 17	139 ± 17	2 (−1 to 5)	137 ± 17	135 ± 13	−2 (−6 to 1)	0.033§
Diastolic BP (mmHg)	74 ± 10	76 ± 13	2 (−1 to 5)	78 ± 12	76 ± 12	−2 (−4 to 1)	0.263§
iPTH (ng/l)	111 (97 to 127)	101 (85 to 120)	−9 (−20 to 4)	108 (97 to 121)	46 (39 to 54)	−57 (−63 to −51)	<0.001§
25-OH-vitamin D (nmol/l)	43 ± 18	42 ± 20	−3 (−12 to 8)	44 ± 17	45 ± 20	2 (−6 to 12)	0.081§
Calcium (mmol/l)	2.29 ± 0.15	2.29 ± 0.14	0 (−2 to 1)	2.28 ± 0.15	2.38 ± 0.16	4 (3 to 6)	<0.001§
Serum phosphate (mmol/l)	0.93 ± 0.19	1.0 ± 0.23	7 (2 to 11)	0.97 ± 0.22	1.02 ± 0.30	6 (0 to 11)	0.974§
PRA (μg/l/h)	3.05 (2.28 to 4.07)	3.01 (2.23 to 4.06)	4 (−17 to 29)	2.40 (1.82 to 3.16)	2.18 (1.60 to 2.98)	−8 (−28 to 17)	0.240§
Aldosterone (nmol/l)	0.32 (0.26 to 0.40)	0.39 (0.31 to 0.50)	15 (−10 to 46)	0.34 (0.28 to 0.41)	0.39 (0.31 to 0.51)	20 (−1 to 46)	0.840§
Interleukin-6 (pg/ml)	2.0 (1.72 to 2.34)	2.88 (2.42 to 3.42)	44 (19 to 74)	2.85 (2.40 to 3.37)	2.15 (1.78 to 2.58)	−25 (−36 to −11)	<0.001§
TGF-beta (pg/ml)	7022 (6237 to 7913)	8965 (7904 to 9987)	28 (14 to 43)	8578 (7628 to 9647)	7085 (6311 to 7954)	−17 (−25 to −9)	<0.001§

UPCR, urinary protein-to-creatinine ratio; UACR, urinary albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; Cr-clearance, creatinine clearance; BP, blood pressure; PRA, plasma renin activity; TGF, transforming growth factor.

*Data are presented as mean (±SD) or geometric mean (95% CI) for normally or skewed distributed data, respectively, or as total number (percentage).

†Two-way mixed analysis of variance (ANOVA).

‡Chi-square test.

§Analysis of covariance (ANCOVA).

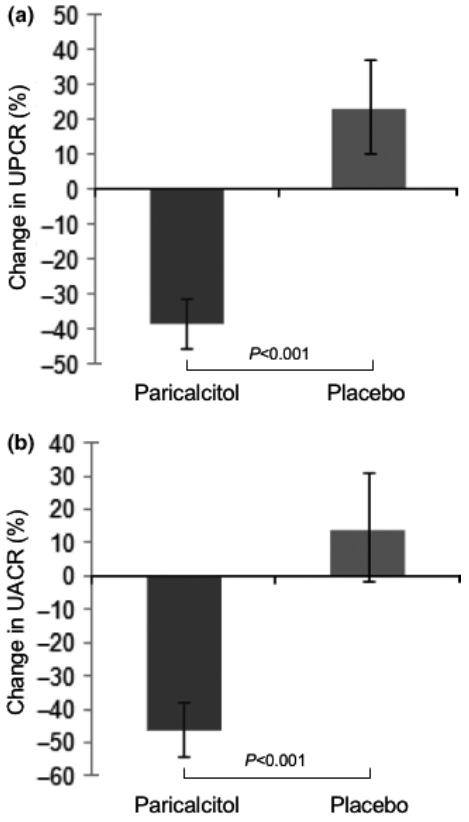

Figure 3 Change in urinary protein-to-creatinine ratio (UPCR) (a) and urinary albumin-to-creatinine ratio (UACR) (b) from baseline to the last measurement during treatment. Error bars represent 95% CI.

diagnosed with AMR, and two patients had non-rejection findings. One patient assigned to 2 μg paricalcitol experienced graft dysfunction and kidney biopsy showed T-cell rejection. None of the patients died during the study.

Discussion

To the best of our knowledge, this is the first randomized, double-blind, placebo-controlled trial evaluating the effect of paricalcitol on urine protein excretion in kidney transplant recipients. We have shown that 24 weeks' treatment with paricalcitol reduced urine protein and albumin excretion despite optimization of the single-agent RAAS blockade. The effects occurred within 4–8 weeks and were sustained during the treatment phase, and measurements returned towards baseline after drug withdrawal, indicating that the effects were real and reversible. Treatment with paricalcitol also suppressed IL-6 and TGF-beta, serum pro-inflammatory and pro-fibrotic markers. Treatment was generally safe and well tolerable.

The capacity of paricalcitol to reduce proteinuria or albuminuria has been suggested in several clinical studies in different CKD populations, predominantly in patients with diabetes [15,24–28]. Two previous studies in kidney transplant recipients have indicated reductions in proteinuria with vitamin D agonist treatment [19,20]. However, these studies were small, open-label and were focused on bone and mineral metabolism, and thus could not show the true size of proteinuria reduction. In our double-blind randomized controlled trial, we have shown that paricalcitol could have unique hemodynamic, antiinflammatory and antifibrotic effects, lowering proteinuria with little hypercalcemia. By contrast, a recent study from Pihlstrøm *et al.* [29] could not demonstrate a significant reduction of albuminuria or modified allograft expression of genes related to fibrosis and inflammation by paricalcitol in *de-novo* transplant recipients. There are several explanations for these seemingly discordant results. Importantly, our study included a larger patient cohort who were on average more than 8 years after transplant, and therefore baseline level of proteinuria was greater than in *de-novo* transplant recipients. As pointed out by Pihlstrøm *et al.* [29], the complexity of what may occur to kidney grafts early after transplant (e.g., delayed graft function, acute rejection) combined with low levels of albuminuria is likely to mask potential antiproteinuric effects of paricalcitol.

Our study aimed to optimize single-agent RAAS blockade during a run-in period, because we were interested in the effect of add-on paricalcitol on residual proteinuria. Only a few patients did not tolerate an ACE inhibitor or ARB where treatment withdrawal was indicated, most commonly due to transplant renal artery stenosis [30]. This is at variance with previous studies where <50% of transplanted patents were treated with RAAS inhibitors [18–20,29]. Furthermore, the majority of our patients had moderately decreased kidney function and were on regular sodium diet. Two previous studies suggested that the extent of antiproteinuric effect of paricalcitol was greater in the presence of higher GFR and higher sodium intake [15,31]. In a *post hoc* analysis of our data, baseline GFR and sodium intake had no significant interaction with UPCR and UACR responses. Several clinical studies in CKD demonstrated that sodium restriction is an effective non-pharmacological intervention to increase RAAS blockade efficacy [32–34]. In line with these observations, a recent prospective study in nondiabetic patients with CKD showed that sodium restriction significantly reduced albuminuria during RAAS blockade, while

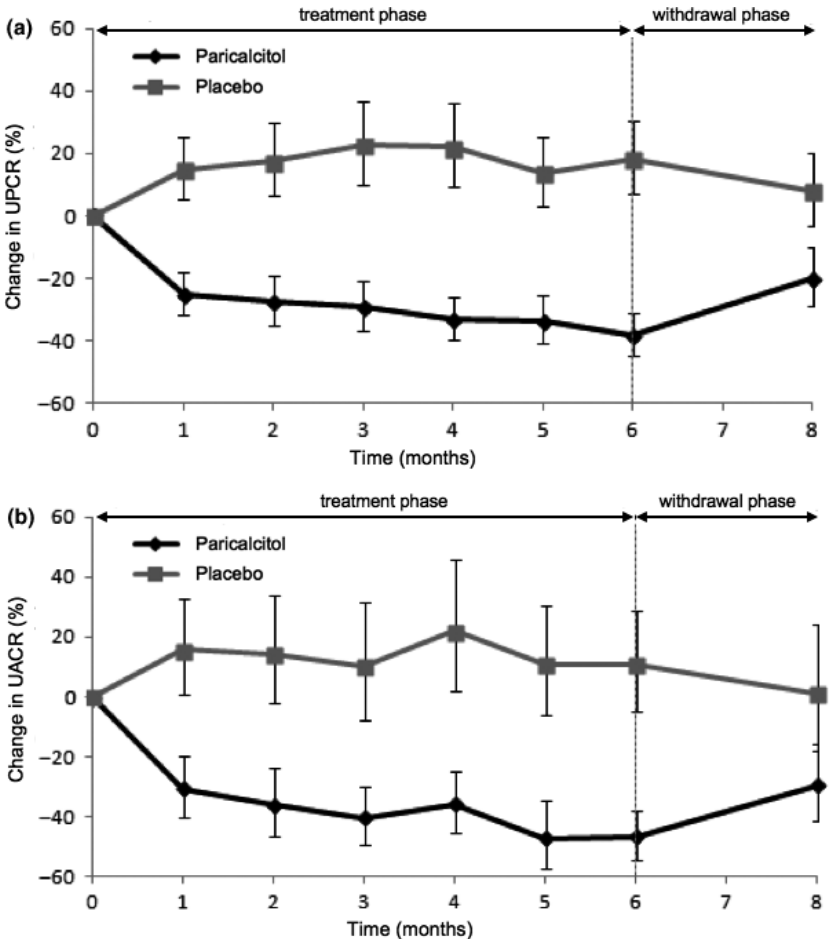

Figure 4 Change in urinary protein-to-creatinine ratio (UPCR) (a) and urinary albumin-to-creatinine ratio (UACR) (b) during treatment and 8 weeks after treatment withdrawal. Error bars represent 95% CI.

paricalcitol provided only a mild further reduction of residual albuminuria [35]. However, the effect of paricalcitol added to sodium restriction remained significant in a per-protocol analysis restricted to patients with more than 95% compliance with study medication [35].

The renoprotective effects of paricalcitol on lowering proteinuria are likely multifactorial and mimic the pattern of responses seen with other renoprotective drug medications like ACE inhibitors and ARBs. Experimental studies have shown that treatment with vitamin D receptor agonists exerts direct protective effects on podocytes [36], negatively regulates the RAAS by suppressing renin production [16,37] and has antiinflammatory and antifibrotic effects [38–40]. These effects most likely explain the antiproteinuric effect of paricalcitol in addition to RAAS blockade, which is also supported by a recent preclinical data showing renal tissue protection during ACE inhibition and paricalcitol in experimental proteinuric nephropathy [41].

A BP lowering effect that we recorded with paricalcitol is supported by previous observational studies, in which systolic BP had an inverse correlation with serum 1,25-dihydroxyvitamin D concentrations [42]. However, the effect of paricalcitol on BP reduction was small and a recent meta-analysis could not demonstrate that vitamin D analogs are effective in lowering BP in patients with CKD [43]. We could also not demonstrate a significant effect of paricalcitol on PRA or circulating aldosterone levels over 24 weeks, although these measurements might not be accurate indicators of renin or angiotensin II activity within the kidney graft in response to paricalcitol. Similar findings were reported in recent studies in patients with nondiabetic CKD where the effect of paricalcitol on endothelial function and RAAS activity was less than expected [27,44,45].

Important for kidney transplanted population, vitamin D receptor is ubiquitously expressed in immune cells and vitamin D has clear effects on immune system functioning, characterized by inhibition of IL-2 and interferon-gamma production by $CD4^+$ T cells and reduced cytotoxicity of $CD8^+$ T cells [46]. Activation of the vitamin D receptor may also induce differentiation

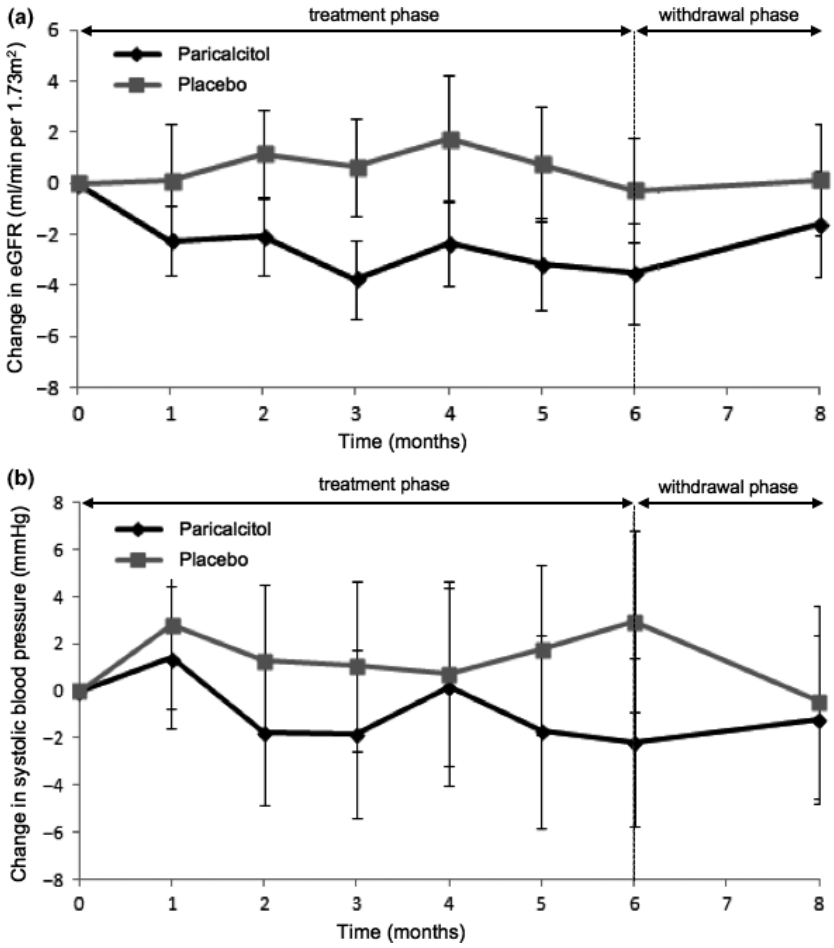

Figure 5 Change in estimated glomerular filtration rate (eGFR) (a) and systolic blood pressure (b) during treatment and 8 weeks after treatment withdrawal. Error bars represent 95% CI.

of suppressive FOXP3$^+$ regulatory T cells [47]. The suppressive effect on IL-6 levels that we observed with paricalcitol may also be important. IL-6 is an essential growth factor of B cells and plasma cells, and blockade of IL-6 receptor signalling induces B cell apoptosis and abrogates plasma cell differentiation [48]. Elevated IL-6 levels in a cohort of kidney transplant recipients were associated with inflammation and graft loss [49], and IL-6-receptor blockade with a monoclonal antibody tocilizumab impaired B cell differentiation and plasma cell development [50], which could prevent or treat AMR [51]. In our subgroup analysis in patients with graft biopsies performed prior study enrolment, proteinuria lowering effect of paricalcitol was similar in rejection and non-rejection histologic findings. However, the total number of individuals with different histologic phenotypes of graft injury was low; hence, results should be interpreted with caution. In contrast to paricalcitol, treatment with placebo resulted in an increase in proteinuria, specifically in patients with preexisting AMR. These results may have clinical implications, because persistent or worsening proteinuria has been associated with increased risk of graft failure, independent of graft function and histology [52].

Paricalcitol treatment was well tolerated. The most common adverse effects were mild hypercalcemia and hypoparathyroidism. In most patients, these events were readily reversible by a decrease in paricalcitol dose. This result may be important, because patients who fail to tolerate the target 2 μg daily dose might be safely maintained on the lower dose to avoid renouncing to the potential benefit of long-term paricalcitol exposure. Unfortunately, biomarkers of bone remodelling were not assessed in this study. To lower the risk of hypoparathyroidism and adynamic bone disease with paricalcitol we ruled out patients with low-normal values of iPTH (<30 ng/l). Nevertheless, a trial with a longer period of therapy and follow-up would be necessary to assess the unfavourable effects of iPTH suppression and of the rise in serum calcium triggered by paricalcitol. Paricalcitol also decreased creatinine based eGFR, while creatinine clearance was not influenced by paricalcitol treatment. An increase in serum creatinine without altering the true GFR has been reported

Table 3. Adverse events registered during treatment*.

Event	Placebo (n = 85)	Paricalcitol (n = 83)
Minor adverse events		
Hypercalcemia	5 (6)	16 (19)
Hypoparathyroidism	1 (1)	13 (16)
Abdominal complaints	3 (4)	2 (2)
Malaise/Myalgia	2 (2)	2 (2)
Blood pressure increase	3 (4)	1 (1)
Paroxysmal arrhythmia	0	1 (1)
Ankle swelling	1 (1)	1 (1)
Thrombophlebitis	1 (1)	1 (1)
Minor bacterial infections	1 (1)	2 (2)
Serious adverse events		
Heart failure/ Myocardial infarction	1 (1)	1 (1)
Infection		
Sepsis	1 (1)	0
Cytomegalovirus	0	2 (2)
Pyelonephritis	2 (2)	2 (2)
Lower respiratory tract	4 (5)	3 (4)
Gastrointestinal	2 (2)	1 (1)
Malignancies	0	0
Rejection		
Antibody-mediated	2 (2)	0
T-cell mediated	0	1 (1)
Graft loss	1 (1)	0
Patient death	0	0

*Data are presented as number (percentage). Some patients had more than one type of adverse event and so have been listed more than once. Patients who had the same type of adverse event more than once are listed only once.

previously for paricalcitol and may be related to an effect on muscle metabolism [53].

This study has some limitations that should be acknowledged. The exposure time to paricalcitol was limited, precluding conclusions on the effect of paricalcitol on long-term transplant outcomes. Moreover, pathophysiologic mechanisms of renoprotection (e.g., intra-glomerular hemodynamic effect, tubular effect or influence on the inflammation and fibrosis) could not be determined based on the results of this study. Second, our findings may not be valid for newly transplanted patients with low baseline levels of proteinuria. Third, several patients did not take maximum doses of RAAS inhibitors, so the results might have varied in patients receiving different types or amounts of these drugs. Also, when iPTH levels were suppressed or calcium levels were increased, the dose of paricalcitol was decreased. While this reflects prudent care, it may have an influence on the levels of proteinuria as well as biomarkers we observed. Finally, surveillance graft biopsies were not performed, so the effect of paricalcitol on histologic scores of inflammation and fibrosis in the kidney graft could not be assessed. Nevertheless, the 24-week period was probably not long enough to expect significant histologic changes in inflammatory or fibrotic processes.

In conclusion, an unmet need exists for drug strategies aimed at preventing long-term graft failure in kidney transplant recipients. Paricalcitol has been extensively used clinically since its introduction more than a decade ago and is well characterized in predialysis and dialysis patients. Observational studies have also suggested a survival benefit among hemodialysis patients receiving long-term treatment with paricalcitol [54]. In this randomized, placebo-controlled clinical trial in kidney transplant recipients, we have shown that administering paricalcitol for 24 weeks suppressed urine protein excretion, as well as IL-6 and TGF-beta, serum proinflammatory and profibrotic markers. Long-term prospective studies are needed to determine if the reduction in proteinuria improves transplant outcomes.

Authorship

All authors participated in research design, performance of the research, and writing of the manuscript. MO, GM, and MA: collected data. MA: participated in data analysis.

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Conflict of Interest

The authors have declared no conflicts of interest.

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All funding sources, including AbbVie, had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data; or preparation, review, or approval of this manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Materials and Methods. Details on the study measurements.

Figure S1. Change in serum intact parathyroid hormone (iPTH) (A), calcium (B) and phosphate (C) concentrations during treatment and 8 weeks after treatment withdrawal. Error bars represent 95% CI.

Figure S2. Change in urinary protein-to-creatinine ratio (UPCR) (A) and urinary albumin-to-creatinine ratio UACR (B) from baseline to the last measurement during treatment, by tertiles of estimated glomerular filtration rate (eGFR) at baseline. Error bars represent 95% CI.

Figure S3. Change in urinary protein-to-creatinine ratio (UPCR) (A) and urinary albumin-to-creatinine ratio UACR (B) from baseline to the last measurement during treatment, by mean 24-h urine sodium excretion at baseline. Error bars represent 95% CI.

Figure S4. Change in urinary protein-to-creatinine ratio (UPCR) (A) and urinary albumin-to-creatinine ratio (UACR) (B) from baseline to the last measurement during treatment in a subgroup of patients with kidney biopsies performed for cause prior study enrolment, by most recent histologic phenotype (non-rejection findings, T cell-mediated rejection [TCR], antibody-mediated rejection [AMR], and AMR and transplant glomerulopathy [TG]). Error bars represent 95% CI.

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