ORIGINAL ARTICLE

Early introduction of oral paricalcitol in renal transplant recipients. An open-label randomized study

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SUMMARY

In stable renal transplant recipients with hyperparathyroidism, previous studies have indicated that vitamin D agonist treatment might have anti-proteinuric effects. Animal studies indicate possible anti-fibrotic and anti-inflammatory effects. Early introduction of paricalcitol in *de novo* renal transplant recipients might reduce proteinuria and prevent progressive allograft fibrosis. We performed a single-center, prospective, randomized, open-label trial investigating effects of paricalcitol 2 μg/day added to standard care. Participants were included 8 weeks after engraftment and followed for 44 weeks. Primary end point was change in spot urine albumin/creatinine ratio. Exploratory microarray analyses of kidney biopsies at study end investigated potential effects on gene expression. Secondary end points included change in glomerular filtration rate (GFR), pulse wave velocity (PWV), and endothelial function measured by peripheral arterial tonometry as reactive hyperemia index (RHI). Seventy-seven de novo transplanted kidney allograft recipients were included, 37 receiving paricalcitol. Paricalcitol treatment lowered PTH levels ($P = 0.01$) but did not significantly reduce albuminuria ($P = 0.76$), change vascular parameters (PWV; $P = 0.98$, RHI; $P = 0.33$), or influence GFR ($P = 0.57$). Allograft gene expression was not influenced. To summarize, in newly transplanted renal allograft recipients, paricalcitol reduced PTH and was well tolerated without negatively affecting kidney function. Paricalcitol did not significantly reduce/prevent albuminuria, improve parameters of vascular health, or influence allograft gene expression.

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Introduction

Proteinuria, present in up till 45% of renal transplant recipients at 1 year after transplantation [1,2], is an independent risk factor for graft loss [3,4]. Persistent derangements in hormone and mineral balance after the immediate post-transplant period may also predict inferior long-term outcome [5–8].

Vitamin D deficiency is a risk factor for persistent hyperparathyroidism [9], and epidemiological studies have shown associations between low 25-hydroxyvitamin D (25(OH)D) and proteinuria [10], as well as increased interstitial fibrosis and tubular atrophy (IFTA) in kidney grafts [11]. There is evidence to suggest that low levels of vitamin D contribute to a lack of suppression of the renin–angiotensin–aldosterone system (RAAS) [12,13]. Consequently, drugs with vitamin D agonist properties have been launched as possible renoprotective agents. Paricalcitol (19-nor-1,25-dihydroxyvitamin D2) is a synthetic, selective third-generation vitamin D receptor agonist (VDRA) associated with low risk of hypercalcemia [14,15]. In CKD, paricalcitol effectively and safely suppresses PTH [16,17] and may reduce proteinuria [18–21]. Similar effects, although less established, have been suggested in renal transplant recipients [22–24].

Calcineurin inhibitors (CNIs: Cyclosporin A (CsA)/tacrolimus (tac)) may be detrimental to long-term graft survival [25,26], although the histological lesions once considered hallmarks of CNI nephrotoxicity may in fact be rather nonspecific signs of injury [27,28]. CNIs activate the intrarenal RAAS [29,30], and the resulting increased levels of angiotensin exert proinflammatory and fibrogenic effects mediated by transforming growth factor beta (TGF-β) signaling [31,32].

Vitamin D agonists downregulate RAAS and lower blood pressure [33]. Experimental data have indicated that paricalcitol may be renoprotective through effects on inflammatory and fibrotic pathways [34–37]. Reductions in renal allograft fibrosis have been suggested in a recent intervention trial in transplant patients [22], and in a rat model, inhibition of TGF-β signaling by paricalcitol seemed to attenuate cyclosporine-induced kidney injury [38].

We hypothesized that treatment with VDRA from an early time point after transplantation might reduce or prevent albuminuria, improve parameters of vascular health, and modify the expression of genes related to pathways of fibrosis or inflammation.

Patients and methods

Inclusion of participants

All study participants were recruited from the National Transplant Centre at Oslo University Hospital, Rikshospitalet, Oslo, Norway. Renal transplant recipients are routinely followed for 8–10 weeks after engraftment and return for a routine one-year surveillance follow-up. Standard maintenance immunosuppressive regimen consists of a CNI (usually tacrolimus), mycophenolate mofetil, and steroids. Patients aged 18 years or more, who had received a kidney transplant or a combined kidney–pancreas transplant, were eligible for randomization 7–8 weeks after transplantation, a time point chosen for practical reasons as it coincided with a scheduled thorough clinical and laboratory evaluation before discharge from Rikshospitalet. CNI treatment, estimated GFR >30 ml/min, and plasma calcium level 2.0–2.6 mmol/l were our inclusion criteria. Exclusion criteria included previous total parathyroidectomy, ongoing (or immediate intent to embark on) treatment with vitamin D, VDRA or calcimimetic drugs, severe osteoporosis in the axial skeleton, a history of allergic reactions or significant sensitivity to paricalcitol or similar drugs, ongoing pregnancy, or donor age >75 years. The study conformed to the principle of the Declaration of Helsinki and the Declaration of Istanbul. The study protocol was approved by the Regional Ethics Committee and the hospital's Research Administration. The Department of Organ Transplantation, Oslo University Hospital, Rikshospitalet, was responsible for the coordination and conduction of the trial, while monitoring was provided by Smerud Medical Consulting A/S. The study has been registered as NCT01694160 (2012/107D) on www.clinicaltrials.gov.

Treatment, randomization, study schedule, and monitoring

Patients who fulfilled the eligibility criteria and gave their written informed consent were randomized 7–8 weeks after transplantation to receive either open-label treatment with oral paricalcitol (Zemplar; Abbvie, North Chicago, IL, USA) 2 μg/day in addition to standard care (paricalcitol group, $n = 37$) or standard care alone (control group, $n = 40$). The random allocation sequence was generated by an independent statistician at the monitoring facility, using computer-generated block-randomization with nonfixed block size. The principal investigator (PI) performed the opening of sealed envelopes and informed the participants of their group assignment. Treatment allocation remained undisclosed

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to the staff performing laboratory measurements and the pathologists scoring transplant biopsies. At discharge from the Transplant Centre around 8 weeks after transplantation, a letter was sent to the local hospitals, informing them of the patients' participation in the trial. Laboratory values, including plasma mineral levels, should be reported to the PI every third month. In the time between the baseline investigation and the final follow-up visit, participants were followed by local nephrologists according to national guidelines recommending a frequency of 2–4 visits a month until 4 months after transplantation, thereafter once a month for the first post-transplant year. If at any time point serum calcium exceeded 2.75 mmol/l, paricalcitol dosage was reduced to 2 μg three times a week, and if patients remained hypercalcemic after dose reduction, the study drug was permanently withdrawn. Study and treatment duration was 44 ± 2 weeks, and the final study visit was coordinated with the one-year standard comprehensive clinical and laboratory evaluation. Adherence was monitored by counting of empty blister packs.

Primary and secondary outcomes

The primary outcome was change in albuminuria (expressed as albumin/creatinine ratio in spot urine) from time of inclusion to end of study. The most important secondary outcomes were gene expression profiles and histopathological changes reflecting inflammation or fibrosis in protocol biopsies at 1 year after transplantation. Other predefined secondary study outcomes included change in proteinuria expressed as fraction of excreted protein (FEPR) [39], and changes in plasma levels of parathyroid hormone (PTH), creatinine, C-reactive protein (CRP), low-density lipoprotein (LDL) cholesterol, alkaline phosphatases, calcium, and phosphate. Endothelial function measured as RHI by peripheral arterial tonometry (PAT), arterial stiffness reflected by pulse wave velocity (PWV) measured by applanation tonometry, as well as glomerular filtration rate measured by iohexol clearance (mGFR) were also selected as prespecified secondary end points. As a supplementary analysis, we also measured change in serum levels of 25 (OH)D, as knowledge about calcidiol levels in both study groups was considered relevant for the interpretation of a possible treatment effect of paricalcitol.

Baseline and final visit evaluations

Blood samples were drawn in the morning after an overnight fast. Spot albumin/creatinine ratio and FEPR were measured in the first morning urine sample. Intact PTH (1–84) was measured by the Roche Modular E170 PTH immunoassay (Roche Diagnostics, Indianapolis, IN, USA). PWV was calculated by applanation tonometry using "Sphygmocor™" (Actor Medical, Sidney, Australia), detailed technique described elsewhere [40]. Endothelial function was assessed by peripheral arterial tone signal technology ("EndoPAT"; ITAMAR Medical Ltd, Franklin, MA, USA), a noninvasive plethysmographic method based on measuring pulsatile volume changes, in the digital bed prior to and during reactive hyperemia [41]. RHI was measured in agreement with current recommendations [42], avoiding the exposure to caffeine and smoking prior to testing. Routine baseline (8 week) and one-year kidney allograft protocol biopsies were scored according to the updated Banff criteria [43] by experienced transplant pathologists. Interstitial fibrosis was evaluated in Masson Trichrome stained sections by assessing percentage area affected by fibrosis in consecutive high power fields/HPFs (40× objective). The values from all HPFs were added and subsequently divided by the number of HPFs. Chronic allograft damage index (CADI score) consisting of vascular intimal sclerosis, tubular atrophy, interstitial fibrosis, interstitial inflammation, mesangial matrix, and glomerulosclerosis was calculated for each biopsy [44,45]. Each parameter was evaluated with a score from 0 to 3, rendering a possible range of total scores between 0 and 18.

Patients' mGFR was determined by iohexol clearance (Omnipaque™, 300 mg iodine/ml; GE Healthcare, Chicago, IL, USA) with blood sampling 2 and 5–8 h after the iohexol injection (depending on eGFR) Serum samples were analyzed by a high-performance liquid chromatography (HPLC) system and calculated according to the Bröchner Mortensen method as previously described [46].

Gene expression analysis

One part of the retrieved biopsy tissue was stored in RNA*later*® solution (AMBION Inc., Austin, TX, USA) and kept frozen at −80 °C until the end of study. Seventy available one-year biopsies were thawed, and its ribonucleic acid (RNA) extracted with the RNeasy kit (QIAGEN, Germantown, MD, USA), according to manufacturer's instructions. Samples were delivered to the Norwegian Genomics Consortium in Oslo and kept at 80 °C until use. Concentration and purity of the RNA samples were measured using the Nano Drop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The quality (RNA integrity) of the samples was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies,

Waldbronn, Germany). Sixty samples, equally divided between study groups, were found to have sufficient quality to continue with amplification and labeling. For each sample, 500 ng of total RNA was amplified and labeled. The quantity of the labeled complementary RNA (cRNA) was measured using the NanoDrop Spectrophotometer, and the quality and size distribution of the labeled cRNA assessed using the 2100 Bioanalyzer. This procedure enabled hybridization of equal amounts of successfully labeled cRNA to the arrays. For each sample, 750 ng of biotin labeled cRNA was hybridized to the Illumina's HumanHT-12 v4 Expression BeadChip (Illumina Inc., San Diego, CA, USA). Quant normalization and missing value imputation were applied on the raw microarray data (~47 323 genes). Genes with less expression variation across the samples were removed (i.e., maximum intensity of a gene is less than 1.5-fold the minimum intensity in 60 samples) before log transforming and converting the normalized intensities to *Z*-scores. In this way, ~12 754 genes remained for further data analysis, where *Z*-scores were used to identify differentially expressed genes between paricalcitol group and controls. For each gene, a relative ratio of the mean *Z*-scores between the two groups was computed, and the statistical significance of relative ratios (*P*-value) was estimated by Gaussian distribution. A detailed description of the method has previously been published [47]. In a supplementary analysis, pairwise Fishers' linear discriminant [48] was used to identify differentially expressed genes between samples showing evidence of immunological activity and the remainder.

Sample size estimation

From clinical experience with our transplant patients, a low baseline mean albumin/creatinine ratio of 15.0 mg/mmol was suggested for the calculations of sample size. Allowing a type 1 error rate of 5% and a type 2 error rate of 20%, and regarding as clinically relevant a 5.2 (SD 10.0) mg/mmol difference between treatment arms in Δ albumin/creatinine ratio from baseline to end of study, the estimated number of patients in each arm should be 30. Accounting for possible early dropouts, the study group decided that additional participants should be included to ensure a total number of at least 70 randomized patients.

Statistical methods

The intention-to-treat population consisted of all randomized participants who received at least one dose of study drug (applicable to the treatment group), irrespective of any violations to the study protocol. The per-protocol population consisted of all participants fulfilling the protocol in terms of eligibility, intervention, and outcome assessment. When expressed as *individual absolute change from baseline to the final study visit*, the selected primary and secondary study outcomes showed only negligible deviations from the normal distribution and were therefore analyzed by *t*-test for independent observations. Sensitivity analyses were performed using ANCOVA investigating potential group differences in outcomes at study end with adjustments for baseline values of each outcome. Non-normally distributed variables were log-transformed. Histological scores were highly non-normally distributed, even after attempt of logarithmic transformation; hence, ANCOVA was not performed for these parameters. For categorical data, Fisher's exact test was applied. A two-tailed *P*-value ≤ 0.05 was considered to represent statistical significance. General statistical analyses were performed by SPSS version 21 and GRAPHPADPRISM 7. Gene expression analyses were based on the results from the Illumina microarray, and statistical tests for identifying differentially expressed genes were performed using MATLAB toolbox. The array dataset has been made public in the NCBIs Gene Expression Omnibus (GSE83486).

Results

Study population

From January 2013 to February 2014, a total of 208 patients were screened for participation in the study, as shown in the Consort Diagram (Fig. 1). Forty-three patients did not meet selection criteria, and 38 were not included due to practical reasons. Thirty-three did not want to participate, and 17 patients were excluded due to complex comorbidities. The remaining 77 patients were included in the study and randomized. No patient withdrew consent or was lost to follow-up. Adherence in the treatment group was excellent (92–108% of the prescribed dose). Two patients in the treatment group were withdrawn from the study due to persistent hypercalcemia refractory to reductions in paricalcitol doses, while six patients completed the study using 2 μg paricalcitol three times a week. No patient in the treatment group was given native vitamin D to correct 25(OH)D insufficiency, but six patients in the control group were treated with cholecalciferol prescribed by local physicians and two controls started treatment with calcitriol during follow-up. Hence, the per-protocol population

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consisted of 70 patients. All participants completed end of study investigations 1 year after transplant, including those who had to withdraw, rendering 77 patients available for subsequent intention-to-treat analyses.

Baseline demographics

Demographic, clinical, and biochemical characteristics did not differ significantly between the two study groups (Table 1). In particular, the 32.5% lower median albumin/creatinine ratio in the treatment group at baseline did not reach statistical significance ($P = 0.635$ by Mann–Whitney *U*-test). The control group had slightly more hypertension and was more often treated with drugs blocking the RAAS. Mean Banff score for interstitial fibrosis in baseline protocol biopsies was somewhat higher in the treatment group than in controls (0.57 vs. 0.38). There were more men among controls, and they had slightly lower GFR and lower baseline vitamin D levels. Baseline 25(OH)D levels ranged between 17 and 107 nmol/l. Ninety-two percent of patients had levels <75 nmol/l, while 64% had levels <50 nmol/l.

Primary end point analyses

Table 2 summarizes the results of comparisons between patients treated with paricalcitol and the control group with respect to the primary and secondary study outcomes. We did not detect significant differences between the groups regarding *change in* urinary albumin/creatinine ratio from baseline to study end ($P = 0.76$), and the result was identical if the variable was logarithmically transformed ($P = 0.43$, not shown in table) or if FEPR was used in the calculations ($P = 0.75$). *Absolute levels* of albuminuria and FEPR at study end were not different between groups either. ANCOVA sensitivity analyses with adjustment for baseline values of each variable rendered essentially the same results as the *t*-test. Restricting analyses to the per-protocol population did not change the results ($P = 0.70$ for primary end point, data not shown). Among patients with baseline 25(OH)D values <50 nmol/l, there was slightly higher reductions in albumin/creatinine ratio with paricalcitol (−3.9 nmol/l) than no treatment (−1.5 nmol/l), but results were still non-significant ($P = 0.62$). Exclusion of patients who experienced relapse of glomerulonephritis or clinical or borderline biopsy proven rejection from the analyses did not affect results (data not shown).

Histopathological changes and gene expression analysis

There were no significant group differences in development of fibrosis or chronic allograft damage index (CADI) from baseline to end of follow-up (Table 2). Neither were there any differences in C4d positivity in protocol biopsies, or in development of circulating donor-specific antibodies at 1 year (Table 3). Per-protocol analyses yielded equivalent results. Not shown in table, the proportion of patients with *moderate-to-severe* interstitial fibrosis at 1 year (ci ≥ 2), was also similar between study arms (two patients in the treatment group versus three patients in the control group, $P = 1.00$).

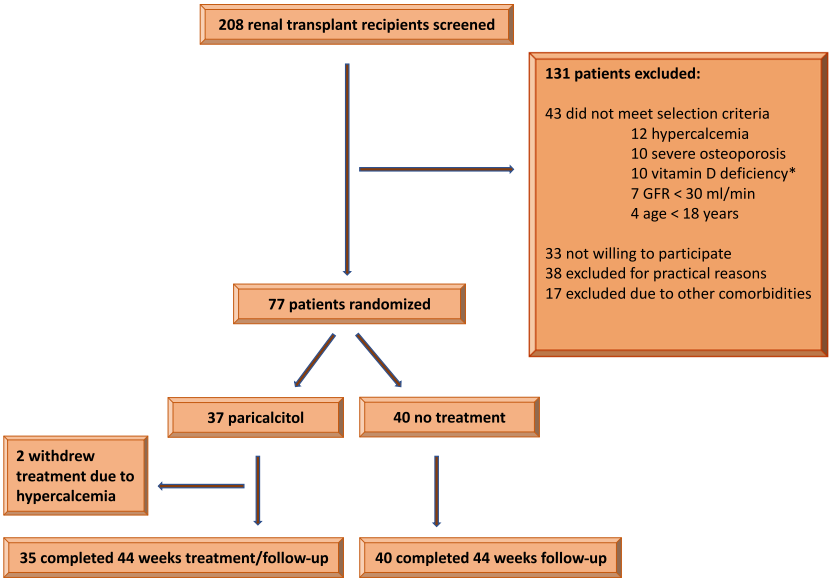

Figure 1 Consort diagram of screening process and patient flow through the trial. * Vitamin D deficiency here defined as s-25-OH-vitamin D levels <25 nmol/L

Table 1. Baseline characteristics of the study population.

Variables	Paricalcitol (n = 37)	Control (n = 40)
Age, years	55.6 (13.3)	55.1 (12.6)
Male gender	27 (73.0%)	34 (85.0%)
Caucasian ethnicity	35 (94.6%)	38 (95.0%)
Phys.exercise. >1/week	22 (59.4%)	19 (47.5%)
BMI, kg/m^2	26.1 (3.2)	25.4 (3.9)
Current smoking	5 (13.5%)	5 (12.5%)
Living donor	10 (27.0%)	13 (32.5%)
Cold ischemia time, h	10.5 (6.3)	9.9 (5.8)
Predialytic	13 (35.1%)	13 (32.5%)
HLA mismatches, number	3.1 (1.3)	3.0 (1.4)
CMV neg recipient	12 (32.4%)	12 (30.0%)
Acute rejection <8 weeks post tx, %	1 (2.7%)	2 (5.0%)
Interst.fibr.(ci) at baseline biopsy	0.57 (0.56)	0.38 (0.54)
% Sclerotic glomeruli, baseline biopsy*	0 (10)	2 (10)
Hypertension	31 (83.8%)	37 (92.5%)
Chronic heart disease	11 (29.7%)	13 (32.5%)
Pre-tx diabetes mellitus	7 (18.9%)	6 (15.0%)
PTDM by OGTT at 8 weeks, %	2 (5.4%)	3 (7.5%)
Systolic blood pressure, mmHg	145 (21)	142 (22)
Diastolic blood pressure, mmHg	83 (10)	84 (11)
Resting heart rate, bpm	69 (9)	67 (9)
RHI at baseline (EndoPAT)	2.49 (0.79)	2.34 (0.72)
P-Cholesterol, mmol/l	5.8 (1.1)	5.9 (0.9)
P-HDL cholesterol, mmol/l	1.6 (0.5)	1.6 (0.4)
P-LDL cholesterol, mmol/l	3.8 (1.0)	3.9 (0.9)
P-Triglycerides, mmol/l*	1.3 (1.0)	1.4 (0.5)
P-Creatinine, µmol/l	114 (25)	121 (30)
Measured GFR, ml/min	52.9 (14.0)	48.7 (15.2)
B-Hemoglobin, g/l	12.5 (1.2)	12.3 (1.2)
P-C-reactive protein, mg/l*,†	0.83 (2.40)	1.00 (1.20)
P-Calcium total, mmol/l	2.39 (0.09)	2.37 (0.11)
P-Phosphate, mmol/l*	0.9 (0.3)	0.8 (0.4)
P-Albumin, g/l	42.4 (2.4)	41.7 (2.5)
P-PTH, pmol/l*	10.1 (7.8)	10.1 (5.5)
S-25-OH-vitamin D, nmol/l	50.6 (18.0)	44.9 (17.0)
S-1,25-OH-vitamin D, nmol/l	101.5 (35.3)	96.3 (37.2)
P-Alkaline phosphatase, U/l	61.4 (21.7)	69.2 (28.3)
U-Albumin/creat ratio, mg/mmol*	2.7 (6.9)	4.0 (7.8)
Treatment with ACEi/ARB, %	9 (24.3%)	15 (37.5%)

BMI, body mass index; HLA, human leukocyte antigen; PTDM, post-transplant diabetes mellitus; OGTT, oral glucose tolerance test; RHI, reactive hyperemia index; PAT, peripheral arterial tonometry; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GFR, glomerular filtration rate; PTH, parathyroid hormone; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Categorical data expressed as number (percentage frequency).

Continuous data expressed as mean (standard deviation) or *median (interquartile range).

†Values <0.60 mg/l (laboratory detection cutoff) are all given the value 0.30. Values >15 mg/l are rounded down to this value.

Thirty patients from each treatment group were compared for differential gene expression in renal allograft biopsy tissue by genome-wide gene expression microarray analysis. Due to financial restrictions, only one-year biopsy samples were analyzed. The top 82 significantly differentially expressed genes ($P < 0.05$ after Bonferroni's correction) between treated and untreated patients are illustrated in a heat map (Fig. 2), where the red and blue color scales represent the positive and negative Z-scores, respectively. By visual inspection, the figure

Table 2. Primary and secondary outcomes, by intention-to-treat principles.

	Paricalcitol (n = 37)			Control (n = 40)			Group differences in change		
Variable	Baseline Mean (SD)	Study End Mean (SD)	Mean Change (%)	Baseline Mean (SD)	Study End Mean (SD)	Mean Change (%)	Point est. (95% CI)	P† t-test	P‡ ANCOVA
U-Albumin/creat ratio, mg/mmol	9.3 (22.6)	7.2 (12.3)	−2.1 (−23)	8.2 (13.8)	7.1 (20.4)	−1.1 (−13)	−1.0 (−7.4, 5.5)	0.76	0.58*
FEPR, %	3.7 (5.1) × 10^{-3}	2.7 (3.0) × 10^{-3}	−1.0 (−27)	3.7 (3.2) × 10^{-3}	3.0 (4.2) × 10^{-3}	−0.8 (−20)	−0.2 (−1.7, 1.2)	0.75	0.25*
Measured GFR, ml/min, n = 68	53 (14)	60 (13)	+7 (+13)	49 (15)	58 (15)	+9 (+18)	−2 (−4.2, 2.5)	0.57	0.91
P-creatinine, mmol/l	114.4 (24.5)	110.3 (26.7)	−4.1 (−4)	121.8 (29.9)	114.8 (27.1)	−7.0 (−6)	+2.9 (−6.7, 12.6)	0.54	0.89
P-PTH, pmol/l	11.5 (5.7)	7.9 (4.8)	−3.6 (−31)	11.2 (5.3)	9.9 (3.8)	−1.3 (−12)	−2.3 (−4.1, −0.6)	0.01	<0.001*
P-Alkaline phosphatase, U/l	61.4 (21.7)	58.4 (20.5)	−3.0 (−5)	69.2 (28.3)	70.4 (33.4)	+1.2 (+2)	−4.2 (−15.2, 6.7)	0.44	0.19
P-calcium, mmol/l	2.39 (0.09)	2.43 (0.11)	+0.04 (+2)	2.37 (0.11)	2.39 (0.10)	+0.02 (+1)	+0.02 (−0.03, 0.06)	0.49	0.26
P-phosphate, mmol/l	0.89 (0.23)	0.92 (0.15)	+0.03 (+3)	0.93 (0.34)	0.95 (0.15)	+0.03 (+3)	0.0 (−0.11, 0.12)	0.90	0.32*
S-25-OH-vitamin D, nmol/l, n = 74	51 (18)	57 (21)	+6 (+12)	45 (17)	59 (22)	+14 (+31)	−8 (−18.0, 1.6)	0.10	0.23
P-C-reactive protein, mg/l	2.26 (3.27)	3.69 (4.57)	+1.43 (+63)	2.11 (3.06)	2.57 (2.75)	+0.46 (+22)	+0.97 (−1.00, 2.89)	0.33	0.35*
P-LDL, mmol/l	3.8 (1.0)	2.7 (0.9)	−1.1 (−29)	3.9 (1.0)	3.2 (0.8)	−0.7 (−18)	−0.4 (−0.8, 0.0)	0.06	0.01
Reactive hyperemia index, n = 64	2.44 (0.70)	2.11 (0.67)	−0.33 (−14)	2.32 (0.72)	2.16 (0.68)	−0.16 (−7)	−0.17 (−0.42, 0.14)	0.33	0.47
Pulse wave velocity, m/s, n = 71	10.02 (2.67)	10.39 (2.81)	+0.37 (+4)	10.06 (3.36)	10.41 (3.74)	+0.36 (+4)	+0.01 (−0.67, 0.69)	0.98	0.98
Biopsy Interstit. fibrosis (%), n = 68	7.9 (14.2)	11.6 (12.7)	+3.7 (+47)	8.2 (11.7)	9.3 (13.9)	+1.2 (+15)	+2.5 (−5.2, 9.9)	0.53	NA
Biopsy CADI score§, n = 67	3.2 (2.3)	3.7 (2.0)	+0.4 (+13)	3.5 (2.3)	3.7 (2.5)	+0.2 (+6)	+0.2 (−1.3, 1.3)	0.98	NA

FEPR, fraction of excreted protein; GFR, glomerular filtration rate; PTH, parathyroid hormone; LDL, low-density lipoprotein; CADI, Chronic allograft damage index: §a combined score consisting of vascular intimal sclerosis, tubular atrophy, interstitial fibrosis, interstitial inflammation, mesangial matrix, glomerular sclerosis. Measurements given as mean with standard deviations; mean differences are given in absolute values and percentages of baseline values.

**P*-values are calculated based on logarithmically transformed baseline and study end variables.

†Comparison of mean change from baseline values between study groups for all selected outcomes. *P*-values are based on *t*-test for independent samples, as all measured change variables were found be normally, or close to normally distributed.

‡Sensitivity analyses for group differences performed with ANCOVA with study end values as outcomes and adjustments made for baseline values, except for those not normally distributed despite logarithmic transformation.

indicated no significant difference in gene expression levels between study groups, and the relative ratio of the mean *Z*-scores in the two groups did not reach significance level for any gene. The upregulation of ACTA1 (actin, alpha 1, skeletal muscle) and ENO3 (enolase 3) in a few samples in both groups probably relates to contamination of extrarenal skeletal muscle tissue in the biopsy. Subsequently, a principal component analysis (PCA) was applied on the 12 754 genes, and the 60 patients were plotted in three two-dimentional figures based on the first three principal components of gene expression variations (Fig. 3). There was lack of separation between groups, and we failed to detect clear differences between treated and untreated patients.

However, the PCA plots revealed "outlier" samples which were found to share a common feature of immunological activity in the biopsy: borderline changes suspicious of T-cell-mediated rejection, chronic antibody-mediated rejection or polyoma virus-associated nephropathy (Fig. S1). These "outliers" were grouped together with two additional samples (located in the main cluster) showing borderline histological changes and samples with upregulation of immunoglobulin kappa variable 3D-20 and 1D-33, as immunoglobulin light chains will be upregulated upon inflammation [49]. We then compared gene expression profiles between this new group and the remaining samples. Fig. S2 presents the 21 top differentially expressed genes, where an upregulation of genes related to inflammation such as IFN-γ inducible genes CXCL9 and CXCL10, IRF-1 (interferon regulating factor), CCL5 (RANTES), granzyme A and K, ITGB2 was confirmed.

Other secondary end points

Consistent with available knowledge of paricalcitol, we found a significant reduction in serum PTH ($P = 0.01$) in the treatment group, compared with controls (Table 2). Treatment did not influence change in serum calcium ($P = 0.49$) or phosphate ($P = 0.90$) on a group basis. Measured glomerular filtration rate was not significantly affected by paricalcitol treatment ($P = 0.57$), nor was serum creatinine ($P = 0.54$). There were no significant differences in C-reactive protein (CRP) or measures of arterial stiffness or endothelial function between the groups. Restricting analyses to the per-protocol population did not change results (data not shown).

Safety

The study drug was generally well tolerated. Eight patients in the paricalcitol group developed hypercalcemia (serum Ca ≥2.75 mmol/l on repeated measurements). Dose reduction lead to acceptable s-Ca values in six of these patients, while two patients had to permanently stop the treatment. Four patients in the treatment group, of whom three experienced mild hypercalcemia during follow-up, developed focal calcifications in their one-year graft biopsy. Focal calcifications were not seen among controls. Five patients in the paricalcitol group and one patient in the control group were hospitalized for serious infectious disease (septicemia/upper UTI/pneumonia). Other registered adverse events (AE) such as viral reactivation, rejection episodes, post-transplant diabetes mellitus (PTDM), and malignancy were evenly distributed between treatment groups (Table 4). No patients died during follow-up.

Discussion

To the best of our knowledge, this is the first study evaluating the effect of vitamin D agonist treatment on allograft gene expression in transplantation. In this randomized clinical trial, paricalcitol had no effect on albuminuria or transcriptome profiles at 1 year after transplantation. As might be anticipated, PTH was significantly reduced. Treatment was safe and not associated with negative impacts on renal function.

A few previous studies in transplant patients have indicated reductions in proteinuria with vitamin D agonist treatment [23,24]. Trillini *et al.* [24] demonstrated that paricalcitol treatment in selected renal allograft recipients with known HPT, transplanted >5 years prior

Table 3. Markers of antibody-mediated allograft damage, by intention-to-treat principles.

Markers of ABMR at 1 year	Paricalcitol (%)	Controls (%)	*P*
Donor-specific antibodies	3 (9)	6 (15)	0.69
C4d positivity	0 (0)	1 (3)	0.17

ABMR, antibody mediated rejection; *P*-values calculated by Fisher's exact test.

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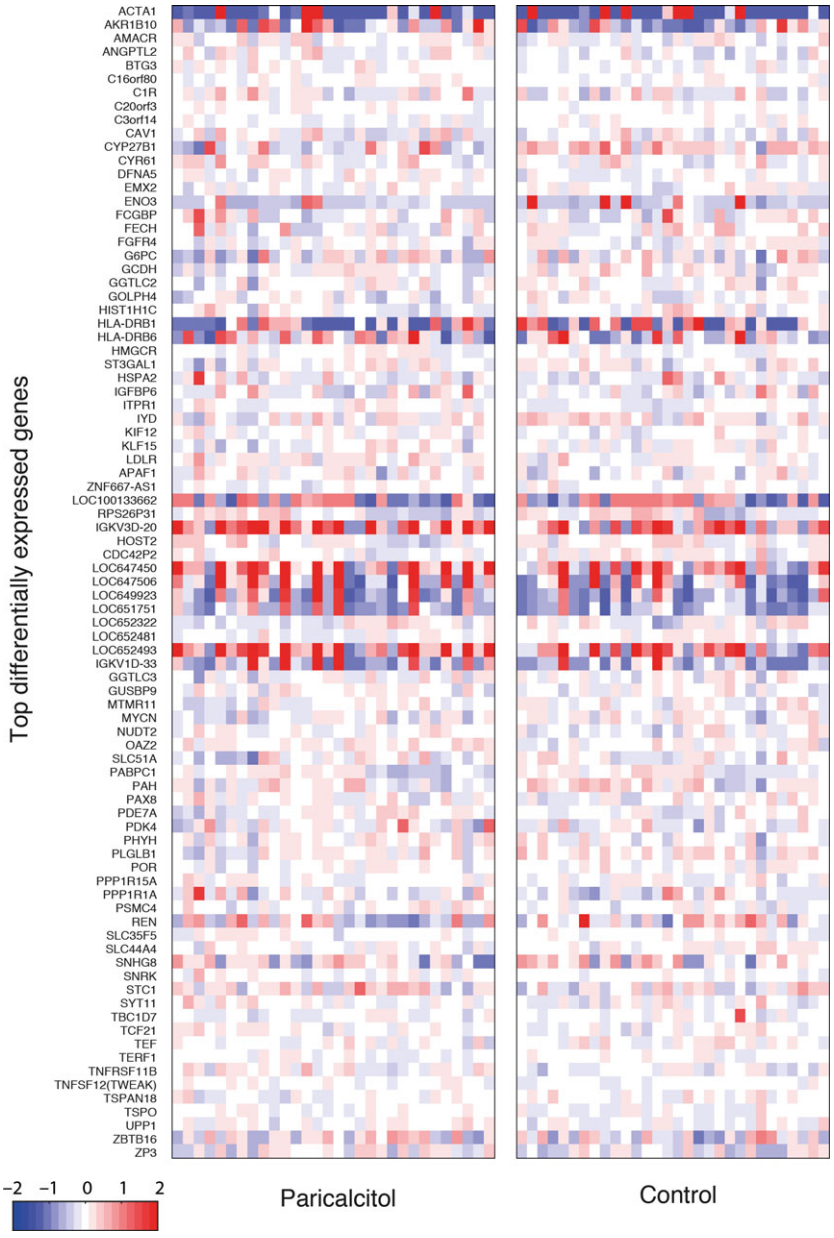

Figure 2 Heat map comparison between study groups across the 86 most differentially expressed genes. Gene names on the *Y*-axis and individual RNA samples on the *X*-axis, grouped by treatment received. Red and blue color scales represent positive and negative *Z*-scores, respectively.

to study participation, lowered proteinuria. A similar observation was recorded in a recent study performed in recipients with secondary HPT transplanted on average 6 years prior to study participation [23]. Despite having conducted a trial of comparable size, we could not corroborate these findings. Importantly, these studies included patients at a later time point after transplant, all participants had HPT, and baseline level of proteinuria was generally higher than in de novo transplant recipients. Our results are more in line with those of Amer *et al.* [22], who also studied de novo transplant recipients irrespective of PTH-levels, not finding a persistent anti-proteinuric effect. In another study of 110 renal transplant recipients with vitamin D levels in the lower range, oral calcidiol did not reduce proteinuria [50]. The complexity of what may occur to renal allografts during the early phase after transplant, combined with very modest baseline levels of albuminuria is likely to mask potential anti-proteinuric effects of VDRA treatment in de novo allograft recipients. Events like acute rejection or urinary tract infection may transiently increase proteinuria. Moreover, as early as 8 weeks after transplantation, some protein leakage from native kidneys may also be present. Furthermore, our sample size was most likely too limited to demonstrate an effect on the primary end point, given that 22% of patients in the paricalcitol group had to reduce or stop treatment, and 13% of patients in the control group were

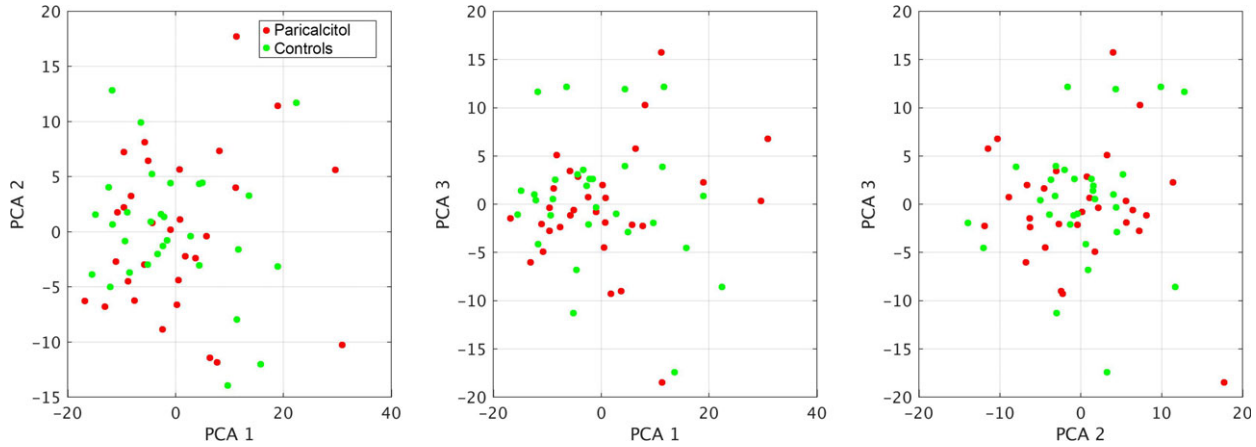

Figure 3 Principal component analysis (PCA, two-dimensional) of gene expression profiles of all 60 samples. Red dots represent patients treated with paricalcitol, and green dots represent the control group.

Table 4. Adverse events registered during follow-up.

Adverse events (AE)	Total (*n*)	Paricalcitol (*n*)	Control (*n*)
Total	45	27	18
Serious AE	14	9	5
Sepsis/febrile neutropenia	3	2	1
Upper urinary tract infection	3	3	0
Lower respiratory tract inf.	1	1	0
Invasive CMV disease	1	0	1
Hospitalization for rejection	4	2	2
Hospitalization for syncope	1	0	1
Malignancy (nonskin)	1	1	0
Minor AE	31	18	13
Hypercalcemia	9	8	1
Viral reactivation	9	4	5
PTDM	3	2	1
Recurring GN	2	0	2
Malignancy skin	1	0	1
Minor bacterial infections	3	2	1
Surgical complication	2	1	1
Arrytmia (cardioversion)	1	0	1
Venous thromboembolism	1	1	0

CMV, cytomegalovirus; GN, glomerulonephritis; PTDM, post-transplant diabetes mellitus.

prescribed drugs with vitamin D agonist effects by clinicians outside the study, possibly leading to a dilution effect.

The proteinuria lowering effect of ergocalciferol in CKD patients with vitamin D deficiency was recently demonstrated [51]. Based on our subgroup analyses, one might speculate that in a more Vitamin D-sufficient population, there be little room for additional anti-proteinuric effects exerted by VRDA. The fact that the control group experienced an increase in mean 25-OH-vitamin D serum levels at study end, notably larger than patients in the treatment group, suggests these protocol violations be relevant for the interpretation of negative results. Patients in the control group admitting to having used drugs with VDRA agonist effects during the study were, however, excluded from the per-protocol analyses, without results being significantly different. About one-third of our patients were treated with drugs inhibiting the RAAS, and such treatment was more frequent among controls than in the paricalcitol group. However, no patients stopped or started these drugs over the course of the trial; hence, large influences on

change in albuminuria or GFR would be unlikely. All of the above-mentioned aspects should be acknowledged when interpreting the nonconfirmatory results regarding anti-proteinuric effects of paricalcitol.

Early histological signs of chronic injury to the allograft are independently associated with late graft loss [52,53]. Gene expression analysis of allograft tissue may suggest injury before damage can be detected in histological samples [54]. In a recent microarray analysis of kidney biopsies from 53 patients with CKD, Nakagawa *et al.* [55] identified a set of upregulated genes showing a significant correlation with histological tubular cell damage and tubulointerstitial fibrosis. Slattery *et al.* [56] identified 128 genes which were differentially expressed in renal tubular cells after treatment with CsA, including established profibrotic factors, oncogenes, and transcriptional regulators. In mice, significantly increased mRNA levels of the fibroblast markers FSP-1 and α-SMA were detected after 4 weeks of treatment with CsA [57]. From a more general viewpoint, gene expression data have also proven valuable for the prediction of renal function [58] and graft survival [59] in transplant patients, and genes reflecting tissue injury, dedifferentiation of the epithelium, and tissue remodeling showed the highest predictive ability.

Our study aimed to investigate whether paricalcitol might modify allograft expression of genes related to fibrotic or inflammatory processes. However, in this genome-wide survey of mRNA transcripts in 60 kidney biopsies, we were not able to demonstrate differentially expressed genes related to pathways of fibrosis or inflammation between study groups. Neither did pathologist scoring of the biopsies according to the Banff criteria reveal significant group differences. This is in contrast with the study of Amer *et al.* [22], who found significantly more moderate-to-severe interstitial fibrosis (ci $\geq$ 2) at 1 year in the control group. However, the total number of individuals with this degree of fibrosis was low (n = 4), as was the case in our study (n = 5); hence, results should be interpreted with caution. There may be several possible reasons for lack of treatment effects: First, several study patients experienced adverse events during follow-up; clinical (n = 4) or subclinical (n = 7) rejections, viral reactivation (n = 9), relapse of primary glomerulonephritis (n = 2), which might influence histological patterns and gene expression profiles more profoundly than what would be expected from treatment with paricalcitol (Figs S1 and S2), thus reducing the power to detect a significant treatment effect. Second, while Amer *et al.* reported 19.5% of participants with some degree of IFTA (ci > 0 and/or ct > 0) at baseline, corresponding IFTA was present in 72% of our baseline biopsies. This difference likely reflects that their cohort consisted mainly of recipients receiving a kidney from a living donor, while there were 30% LD recipients in our study. Variable distribution of pre-existing fibrotic changes throughout deceased donor kidneys may to some extent obscure the evaluation of development of new fibrotic lesions. Lastly, there were group differences with regard to IFTA at baseline, and microarray results would be less affected by interindividual differences if transcriptome profiles at study end were analyzed in conjunction with gene expression profiles prior to paricalcitol treatment.

General anti-inflammatory effects of paricalcitol have been suggested in pilot studies on patients with varying degrees of renal dysfunction [60–63]. However, like in several of the smaller clinical studies in patients with CKD [21,64–66], we were not able to demonstrate ameliorating effects of paricalcitol on systemic inflammation as measured by serum levels of C-reactive protein (CRP).

Persistent derangements in bone and mineral health after renal transplantation are frequent [67], and hyperparathyroidism may be a contributing factor [68]. In addition to increasing the risk of fractures in renal transplant recipients [68], persistent hyperparathyroidism may negatively affect graft function [5,6]. In concert with two recent clinical trials in transplant patients [22,24], we found significantly lowered PTH levels in the treatment group.

Preclinical and clinical studies have suggested vitamin D activation to be essential in the prevention of arterial aging [65,69,70]. We were not able to demonstrate differences in endothelial function between allocation groups. Given that endothelial function is shown to improve after renal transplantation [71], a potential additional effect of treatment with a vitamin D agonist might prove more difficult to establish in this setting. The finding that paricalcitol did not affect arterial stiffness as measured by PWV is in line with an RCT in type 1 diabetic patients with nephropathy [72].

There has been some concern about VDRA treatment causing modest reductions in renal function [24,73]. Using measured GFR, we found no significant differences between treatment groups with respect to change in renal function from baseline to end of study. Paricalcitol does not seem to have any detrimental effect on renal function in kidney allograft recipients, a finding which should be reassuring in the planning of further

studies on vitamin D agonists in renal transplantation. Future studies should, however, address the possible association between paricalcitol treatment and focal calcium deposits in the allograft, which might be related to even mild hypercalcemia.

The study has some limitations which should be acknowledged. There is a risk of introducing investigational bias in open-label trials, but for administrative reasons, placebo drugs were unfortunately not available. Also, albumin/creatinine ratio was measured only once each visit. Urinary calcium measurements are lacking, which would have been valuable in the evaluation of safety regarding paricalcitol treatment. Although serum calcium and phosphate were included as secondary study outcomes, changes in serum mineral levels during follow-up were not reported systematically from local nephrologists. Our findings may be valid for those with a fair to excellent allograft function, but little can be concluded for transplant patients with more advanced CKD. Ninety-five percent of participants were Caucasian; hence, results should be interpreted as valid for a white European population. Major strengths of this trial include a high level of adherence to treatment in the paricalcitol group and no patient-initiated withdrawals. Another advantage is evaluation of graft function by measured GFR. Also, we present a thorough evaluation of surveillance biopsies including transcriptome profiling as well as histological scoring.

Conclusions

In this randomized controlled clinical trial in de novo transplanted kidney allograft recipients, we found paricalcitol therapy to significantly reduce PTH. No significant effects on albuminuria, systemic inflammation, vascular health, or allograft gene expression profiles were detected. There was no evidence of paricalcitol negatively affecting GFR.

Authorship

All authors participated in the research design, writing of the manuscript, and performance of the research. HP, CH, FG, JW, MK, GH, and HH performed data analysis.

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Conflict of Interest

The authors declare no conflicts of interest.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Principal component analysis (PCA, 2 dimensional) of gene expression profiles where red dots represent samples with signs of immunological activation*, green dots represent samples without such signs.

Figure S2. Top differentially expressed genes between 15 samples showing signs of immunological activation and the remaining 45 samples.

REFERENCES

1. Tsampalieros A, Knoll GA. Evaluation and management of proteinuria after kidney transplantation. *Transplantation* 2015; **99**: 2049.
2. Barnas U, Schmidt A, Haas M, *et al.* Parameters associated with chronic renal transplant failure. *Nephrol Dial Transplant* 1997; **12**(Suppl. 2): 82.
3. Amer H, Fidler ME, Myslak M, *et al.* Proteinuria after kidney transplantation, relationship to allograft histology and survival. *Am J Transplant* 2007; **7**: 2748.
4. Cherukuri A, Welberry-Smith MP, Tattersall JE, *et al.* The clinical significance of early proteinuria after renal transplantation. *Transplantation* 2010; **89**: 200.
5. Gwinner W, Suppa S, Mengel M, *et al.* Early calcification of renal allografts detected by protocol biopsies: causes and clinical implications. *Am J Transplant* 2005; **5**: 1934.
6. Pihlstrom H, Dahle DO, Mjoen G, *et al.* Increased risk of all-cause mortality and renal graft loss in stable renal transplant recipients with hyperparathyroidism. *Transplantation* 2015; **99**: 351.
7. Egbuna OI, Taylor JG, Bushinsky DA, Zand MS. Elevated calcium phosphate product after renal transplantation is a risk factor for graft failure. *Clin Transplant* 2007; **21**: 558.
8. Ozdemir FN, Afsar B, Akgul A, Usluogullari C, Akcay A, Haberal M. Persistent hypercalcemia is a significant risk factor for graft dysfunction in renal

transplantation recipients. *Transplant Proc* 2006; **38**: 480.
9. Giannini S, Sella S, Silva Netto F, *et al.* Persistent secondary hyperparathyroidism and vertebral fractures in kidney transplantation: role of calcium-sensing receptor polymorphisms and vitamin D deficiency. *J Bone Miner Res* 2010; **25**: 841.
10. Lee DR, Kong JM, Cho KI, Chan L. Impact of vitamin D on proteinuria, insulin resistance, and cardiovascular parameters in kidney transplant recipients. *Transplant Proc* 2011; **43**: 3723.
11. Bienaime F, Girard D, Anglicheau D, *et al.* Vitamin D status and outcomes after renal transplantation. *J Am Soc Nephrol* 2013; **24**: 831.
12. de Borst MH, Vervloet MG, ter Wee PM, Navis G. Cross talk between the renin-angiotensin-aldosterone system and vitamin D-FGF-23-klotho in chronic kidney disease. *J Am Soc Nephrol* 2011; **22**: 1603.
13. Santoro D, Caccamo D, Lucisano S, *et al.* Interplay of vitamin D, erythropoiesis, and the renin-angiotensin system. *Biomed Res Int* 2015; **2015**: 145828.
14. Hadjiyannakos D, Filiopoulos V, Trompouki S, *et al.* Treatment with oral paricalcitol in daily clinical practice for patients with chronic kidney disease stage 3–4: a preliminary study. *Clin Kidney J* 2013; **6**: 164.
15. Cozzolino M, Brancaccio D. Emerging role for the vitamin D receptor activator (VDRA), paricalcitol, in the treatment of secondary hyperparathyroidism. *Expert Opin Pharmacother* 2008; **9**: 947.
16. Ross EA, Tian J, Abboud H, *et al.* Oral paricalcitol for the treatment of secondary hyperparathyroidism in patients on hemodialysis or peritoneal dialysis. *Am J Nephrol* 2008; **28**: 97.
17. Martin KJ, Gonzalez EA, Gellens M, Hamm LL, Abboud H, Lindberg J. 19-Nor-1-alpha-25-dihydroxyvitamin D2 (Paricalcitol) safely and effectively reduces the levels of intact parathyroid hormone in patients on hemodialysis. *J Am Soc Nephrol* 1998; **9**: 1427.
18. Agarwal R, Acharya M, Tian J, *et al.* Antiproteinuric effect of oral paricalcitol in chronic kidney disease. *Kidney Int* 2005; **68**: 2823.
19. Fishbane S, Chittineni H, Packman M, Dutka P, Ali N, Durie N. Oral paricalcitol in the treatment of patients with CKD and proteinuria: a randomized trial. *Am J Kidney Dis* 2009; **54**: 647.
20. Cheng J, Zhang W, Zhang X, Li X, Chen J. Efficacy and safety of paricalcitol therapy for chronic kidney disease: a meta-analysis. *Clin J Am Soc Nephrol* 2012; **7**: 391.
21. de Lorenzo A, Salanova L, Bomback AS, *et al.* Oral paricalcitol as antiproteinuric agent in chronic kidney disease. *Nefrologia* 2013; **33**: 709.
22. Amer H, Griffin MD, Stegall MD, *et al.* Oral paricalcitol reduces the prevalence of posttransplant hyperparathyroidism: results of an open label randomized trial. *Am J Transplant* 2013; **13**: 1576.
23. Gonzalez E, Rojas-Rivera J, Polanco N, *et al.* Effects of oral paricalcitol on secondary hyperparathyroidism and proteinuria of kidney transplant patients. *Transplantation* 2013; **95**: e49.
24. Trillini M, Cortinovis M, Ruggenenti P, *et al.* Paricalcitol for secondary hyperparathyroidism in renal transplantation. *J Am Soc Nephrol* 2015; **26**: 1205.
25. Chapman JR. Chronic calcineurin inhibitor nephrotoxicity-lest we forget. *Am J Transplant* 2011; **11**: 693.
26. Casey MJ, Meier-Kriesche HU. Calcineurin inhibitors in kidney transplantation: friend or foe? *Curr Opin Nephrol Hypertens* 2011; **20**: 610.
27. Snanoudj R, Royal V, Elie C, *et al.* Specificity of histological markers of long-term CNI nephrotoxicity in kidney-transplant recipients under low-dose cyclosporine therapy. *Am J Transplant* 2011; **11**: 2635.
28. Brocker V, Schubert V, Scheffner I, *et al.* Arteriolar lesions in renal transplant biopsies: prevalence, progression, and clinical significance. *Am J Pathol* 2012; **180**: 1852.
29. Bobadilla NA, Gamba G. New insights into the pathophysiology of cyclosporine nephrotoxicity: a role of aldosterone. *Am J Physiol Renal Physiol* 2007; **293**: F2.
30. Prokai A, Csohany R, Sziksz E, *et al.* Calcineurin-inhibition results in upregulation of local renin and subsequent vascular endothelial growth factor production in renal collecting ducts. *Transplantation* 2016; **100**: 325.
31. Wolf G. Renal injury due to renin-angiotensin-aldosterone system activation of the transforming growth factor-beta pathway. *Kidney Int* 2006; **70**: 1914.
32. Sutariya B, Jhonsa D, Saraf MN. TGF-beta: the connecting link between nephropathy and fibrosis. *Immunopharmacol Immunotoxicol* 2016; **38**: 39.
33. Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J. Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. *J Steroid Biochem Mol Biol* 2004; **89–90**: 387.
34. Tan X, Wen X, Liu Y. Paricalcitol inhibits renal inflammation by promoting vitamin D receptor-mediated sequestration of NF-kappaB signaling. *J Am Soc Nephrol* 2008; **19**: 1741.
35. Tan X, He W, Liu Y. Combination therapy with paricalcitol and trandolapril reduces renal fibrosis in obstructive nephropathy. *Kidney Int* 2009; **76**: 1248.
36. Mizobuchi M, Morrissey J, Finch JL, *et al.* Combination therapy with an angiotensin-converting enzyme inhibitor and a vitamin D analog suppresses the progression of renal insufficiency in uremic rats. *J Am Soc Nephrol* 2007; **18**: 1796.
37. Nolan KA, Brennan EP, Scholz CC, *et al.* Paricalcitol protects against TGF-beta1-induced fibrotic responses in hypoxia and stabilises HIF-alpha in renal epithelia. *Exp Cell Res* 2015; **330**: 371.
38. Park JW, Bae EH, Kim IJ, *et al.* Paricalcitol attenuates cyclosporine-induced kidney injury in rats. *Kidney Int* 2010; **77**: 1076.
39. Stevens KK, Patel RK, Methven S, *et al.* Proteinuria and outcome after renal transplantation: ratios or fractions? *Transplantation* 2013; **96**: 65.
40. Wilkinson IB, Fuchs SA, Jansen IM, *et al.* Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. *J Hypertens* 1998; **16**: 2079.
41. Kuvin JT, Patel AR, Sliney KA, *et al.* Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J* 2003; **146**: 168.
42. Bruyndonckx L, Radtke T, Eser P, *et al.* Methodological considerations and practical recommendations for the application of peripheral arterial tonometry in children and adolescents. *Int J Cardiol* 2013; **168**: 3183.
43. Haas M. The revised (2013) Banff classification for antibody-mediated rejection of renal allografts: update, difficulties, and future considerations. *Am J Transplant* 2016; **16**: 1352.
44. Isoniemi H, Taskinen E, Hayry P. Histological chronic allograft damage index accurately predicts chronic renal allograft rejection. *Transplantation* 1994; **58**: 1195.
45. Helanterä I, Ortiz F, Koskinen P. *Chronic Allograft Damage Index (CADI) as a Biomarker in Kidney Transplantation. Biomarkers in Kidney Disease*. Dordrecht: Springer Science + Business Media, 2015.
46. Salvador CL, Tondel C, Morkrid L, *et al.* Glomerular filtration rate measured by iohexol clearance: a comparison of venous samples and capillary blood spots. *Scand J Clin Lab Invest* 2015; **75**: 710.

47. Wang J, Lan X, Hsu PY, *et al.* Genome-wide analysis uncovers high frequency, strong differential chromosomal interactions and their associated epigenetic patterns in E2-mediated gene regulation. *BMC Genom* 2013; **14**: 70.
48. Wang J, Bo TH, Jonassen I, Myklebost O, Hovig E. Tumor classification and marker gene prediction by feature selection and fuzzy c-means clustering using microarray data. *BMC Bioinform* 2003; **4**: 60.
49. Redegeld FA, Thio M, Groot Kormelink T. Polyclonal immunoglobulin free light chain and chronic inflammation. *Mayo Clin Proc* 2012; **87**: 1032.
50. Kanter Berga J, Crespo Albiach J, Beltran Catalan S, *et al.* Vitamin D deficiency in a renal transplant population: safe repletion with moderate doses of calcidiol. *Transplant Proc* 2010; **42**: 2917.
51. Susantitaphong P, Nakwan S, Peerapornratana S, *et al.* A double-blind, randomized, placebo-controlled trial of combined calcitriol and ergocalciferol versus ergocalciferol alone in chronic kidney disease with proteinuria. *BMC Nephrol* 2017; **18**: 19.
52. Naesens M, Kuypers DR, De Vusser K, *et al.* Chronic histological damage in early indication biopsies is an independent risk factor for late renal allograft failure. *Am J Transplant* 2013; **13**: 86.
53. Mannon RB, Matas AJ, Grande J, *et al.* Inflammation in areas of tubular atrophy in kidney allograft biopsies: a potent predictor of allograft failure. *Am J Transplant* 2010; **10**: 2066.
54. Mueller TF, Mas VR. Microarray applications in nephrology with special focus on transplantation. *J Nephrol* 2012; **25**: 589.
55. Nakagawa S, Nishihara K, Miyata H, *et al.* Molecular markers of tubulointerstitial fibrosis and tubular cell damage in patients with chronic kidney disease. *PLoS One* 2015; **10**: e0136994.
56. Slattery C, Campbell E, McMorrow T, Ryan MP. Cyclosporine A-induced renal fibrosis: a role for epithelial-mesenchymal transition. *Am J Pathol* 2005; **167**: 395.
57. O'Connell S, Slattery C, Ryan MP, McMorrow T. Identification of novel indicators of cyclosporine A nephrotoxicity in a CD-1 mouse model. *Toxicol Appl Pharmacol* 2011; **252**: 201.
58. Bunnag S, Einecke G, Reeve J, *et al.* Molecular correlates of renal function in kidney transplant biopsies. *J Am Soc Nephrol* 2009; **20**: 1149.
59. Einecke G, Reeve J, Sis B, *et al.* A molecular classifier for predicting future graft loss in late kidney transplant biopsies. *J Clin Invest* 2010; **120**: 1862.
60. Alborzi P, Patel NA, Peterson C, *et al.* Paricalcitol reduces albuminuria and inflammation in chronic kidney disease: a randomized double-blind pilot trial. *Hypertension* 2008; **52**: 249.
61. Izquierdo MJ, Cavia M, Muniz P, *et al.* Paricalcitol reduces oxidative stress and inflammation in hemodialysis patients. *BMC Nephrol* 2012; **13**: 159.
62. Navarro-Gonzalez JF, Donate-Correa J, Mendez ML, de Fuentes MM, Garcia-Perez J, Mora-Fernandez C. Anti-inflammatory profile of paricalcitol in hemodialysis patients: a prospective, open-label, pilot study. *J Clin Pharmacol* 2013; **53**: 421.
63. Donate-Correa J, Dominguez-Pimentel V, Mendez-Perez ML, *et al.* Selective vitamin D receptor activation as anti-inflammatory target in chronic kidney disease. *Med Inflamm* 2014; **2014**: 670475.
64. Thethi TK, Bajwa MA, Ghanim H, *et al.* Effect of paricalcitol on endothelial function and inflammation in type 2 diabetes and chronic kidney disease. *J Diabetes Complications* 2015; **29**: 433.
65. Lundwall K, Jorneskog G, Jacobson SH, Spaak J. Paricalcitol, microvascular and endothelial function in non-diabetic chronic kidney disease: a randomized trial. *Am J Nephrol* 2015; **42**: 265.
66. Hansen D, Rasmussen K, Rasmussen LM, Bruunsgaard H, Brandi L. The influence of vitamin D analogs on calcification modulators, N-terminal pro-B-type natriuretic peptide and inflammatory markers in hemodialysis patients: a randomized crossover study. *BMC Nephrol* 2014; **15**: 130.
67. Evenepoel P. Recovery versus persistence of disordered mineral metabolism in kidney transplant recipients. *Semin Nephrol* 2013; **33**: 191.
68. Perrin P, Caillard S, Javier RM, *et al.* Persistent hyperparathyroidism is a major risk factor for fractures in the five years after kidney transplantation. *Am J Transplant* 2013; **13**: 2653.
69. Cozzolino M, Stucchi A, Rizzo MA, *et al.* Vitamin D receptor activation and prevention of arterial ageing. *Nutr Metab Cardiovasc Dis* 2012; **22**: 547.
70. Zoccali C, Curatola G, Panuccio V, *et al.* Paricalcitol and endothelial function in chronic kidney disease trial. *Hypertension* 2014; **64**: 1005.
71. Yilmaz MI, Sonmez A, Saglam M, *et al.* Soluble TWEAK plasma levels increase after renal transplantation and associate with the improvement of endothelial function. *Eur J Clin Invest* 2013; **43**: 1250.
72. Joergensen C, Tarnow L, Goetze JP, Rossing P. Vitamin D analogue therapy, cardiovascular risk and kidney function in people with Type 1 diabetes mellitus and diabetic nephropathy: a randomized trial. *Diabet Med* 2014; **32**: 374.
73. Zhang Q, Li M, Zhang T, Chen J. Effect of vitamin D receptor activators on glomerular filtration rate: a meta-analysis and systematic review. *PLoS One* 2016; **11**: e0147347.