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ORIGINAL ARTICLE

The effect of cholecalciferol supplementation on allograft function in incident kidney transplant recipients: A randomized controlled study

Yohei Doi[1] | Makoto Tsujita[2,3] | Takayuki Hamano[1,4] | Yoshitsugu Obi[5] | Tomoko Namba-Hamano[1] | Toshihide Tomosugi[2] | Kenta Futamura[2] | Manabu Okada[2] | Takahisa Hiramitsu[2] | Norihiko Goto[2] | Akira Nishiyama[6] | Asami Takeda[7] | Shunji Narumi[2] | Yoshihiko Watarai[2] | Yoshitaka Isaka[1]

[1]Department of Nephrology, Osaka University Graduate School of Medicine, Suita, Japan

[2]Department of Transplant Nephrology and Surgery, Nagoya Daini Red Cross Hospital, Nagoya, Japan

[3]Department of Kidney Transplantation, Masuko Memorial Hospital, Nagoya, Japan

[4]Department of Nephrology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

[5]Division of Nephrology, University of Tennessee Health Science Center, Memphis, Tennessee

[6]Department of Pharmacology, Faculty of Medicine, Kagawa University, Kagawa, Japan

[7]Department of Nephrology, Nagoya Daini Red Cross Hospital, Nagoya, Japan

Correspondence
Takayuki Hamano, 1 Kawasumi Mizuho-cho Mizuho-ku Nagoya, Aichi, Japan.
Email: hamatea@kid.med.osaka-u.ac.jp

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It is unknown whether cholecalciferol supplementation improves allograft outcomes in kidney transplant recipients (KTRs). We conducted a single-center randomized, double-blind, placebo-controlled trial of daily 4000 IU cholecalciferol supplementation in KTRs at 1-month posttransplant. The primary endpoint was the change in eGFR from baseline to 12-month posttransplant. Secondary endpoints included severity of interstitial fibrosis and tubular atrophy (IFTA) at 12-month posttransplant and changes in urinary biomarkers. Of 193 randomized patients, 180 participants completed the study. Changes in eGFR were 1.2 mL/min/1.73 m^2 (95% CI; −0.7 to 3.1) in the cholecalciferol group and 1.8 mL/min/1.73 m^2 (95% CI, −0.02 to 3.7) in the placebo group, with no significant between-group difference (−0.7 mL/min/1.73 m^2 [95% CI; −3.3 to 2.0], p = 0.63). Subgroup analyses showed detrimental effects of cholecalciferol in patients with eGFR <45 mL/min/1.73 m^2 ($P_{interaction}$ <0.05, between-group difference; −4.3 mL/min/1.73 m^2 [95% CI; −7.3 to −1.3]). The degree of IFTA, changes in urine albumin-to-creatinine ratio, or adverse events including hypercalcemia and infections requiring hospitalization did not differ between groups. In conclusion, cholecalciferol supplementation did not affect eGFR change compared to placebo among incident KTRs. These findings do not support cholecalciferol supplementation for improving allograft function in incident KTRs.

Clinical trial registry: This study was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) as UMIN000020597 (please refer to the links below). UMIN-CTR: https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000023776

Abbreviations: 1,25(OH)2D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; ANCOVA, analysis of covariance; CKD-EPI, Chronic Kidney Disease Epidemiology; eGFR, estimated GFR; GFR, glomerular filtration rate; IFTA, interstitial fibrosis and tubular atrophy; KTRs, kidney transplant recipients; PRA, plasma renin activity; PTH, parathyroid hormone; RAS, renin-angiotensin system; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; UACR, urine albumin-creatinine ratio; u-AGT, urinary angiotensinogen; u-LFABP, Urinary liver-type fatty acid-binding protein; UPCR, urine protein-creatinine ratio; u-TGF-β1, Urinary TGF-β1; VITAL-DKD, Vitamin D and Omega-3 Trial to Prevent and Treat Diabetic Kidney Disease.

Yohei Doi and Makoto Tsujita contributed equally.

KEYWORDS
clinical research/practice, clinical trial, kidney (allograft) function/dysfunction, kidney transplantation/nephrology

1 | INTRODUCTION

Vitamin D deficiency (i.e., low 25-hydroxyvitamin D [25(OH)D] level) is commonly observed in kidney transplant recipients (KTRs), particularly in the early phase postransplant, due to catabolic effect of glucocorticoid and/or sun-avoidance behavior.[1,2] We previously reported that low 25(OH)D levels predicted a rapid decline in estimated GFR (eGFR) in patients with less than 10 years after transplantation.[3] Bienaimé et al presented that low 25(OH)D levels at 3-month posttransplant were associated with lower glomerular filtration rate (GFR) and progression of allograft interstitial fibrosis at 12-month posttransplant.[4] From the results of basic researches, vitamin D is expected to exert protective effects on allograft function via reducing kidney inflammation and fibrosis, suppressing the renin-angiotensin system (RAS), and exerting prosurvival effects on podocytes resulting in reduced albuminuria and glomerulosclerosis.[5,6]

In Japan, it is quite distinctive that living kidney donation accounts for about 90% of the total kidney transplantation, and the most common age group of living donors is 60–70 years.[7] The number of living donors aged 70 and older has increased.[7] An aging allograft is a limiting factor of long-term graft survival and is associated with interstitial fibrosis and tubular atrophy (IFTA).[8] The degree of IFTA is a proxy of allograft survival and progresses especially during the first year after transplantation.[9-11]

Among non-KTRs, nutritional vitamin D supplementation was reported to reduce urinary TGF-β1 (u-TGF-β1), albuminuria, and suppress RAS activity.[12-14] Hence, we hypothesized that nutritional vitamin D supplementation provides a beneficial effect on allograft function, if any, via inhibiting the progression of IFTA. No published randomized control trial exists to date focusing on the renoprotective effects of nutritional vitamin D in incident KTRs. Hence, we conducted a randomized trial to demonstrate the effects of cholecalciferol supplementation on changes in eGFR among incident KTRs who are at high risk of vitamin D deficiency. We also evaluated its effect on kidney fibrosis and several biomarkers related to kidney damage.

2 | METHODS

2.1 | Study design and participants

This study was a randomized, double-blind, placebo-controlled trial conducted at Nagoya Daini Red Cross Hospital, Japan. Eligible criteria included age between 20 and 80 years, eGFR ≥30 mL/min/1.73 m^2, and 1 month after living kidney transplantation. Key exclusion criteria included patients receiving nutritional vitamin D (ergo- or cholecalciferol) or patients with hypercalcemia, defined as serum-corrected calcium level ≥11 mg/dL. Patients were also excluded if they were deemed ineligible based on the judgment of the attending physicians and no criteria were provided for 25(OH)D levels. All patients provided written informed consent, and the study adhered to the Declaration of Helsinki and was registered in the UMIN Clinical Trials Registry (ID: UMIN000020597). This study was approved by the Nagoya Daini Red Cross Hospital Ethics Committee (IRB approval number: 1038).

2.2 | Randomization and intervention

With the use of a computer-generated random sequence, stratified block randomization (block size: 8) according to age (<50 or ≥50 year) and gender was applied within 1 month posttransplant at an allocation ratio of 1:1. Participants received one capsule of either cholecalciferol 4000 IU or a matching placebo daily from 1 month to 12 months posttransplant (an 11-month intervention). Participants and investigators were masked to treatment allocation throughout the entire study. Adherence to the study drugs was monitored by interviewing the patients at every visit. Poor adherence was defined as taking less than half the prescribed drugs throughout the study period.

2.3 | Endpoints

The primary endpoint was a change in eGFR from baseline to 12 months posttransplant. GFR was estimated using the Chronic Kidney Disease Epidemiology (CKD-EPI) creatinine–cystatin C equation.[15] Prespecified secondary endpoints included (a) IFTA on transplanted kidney at 12 months posttransplant; (b) changes in urinary biomarkers including urine albumin-to-creatinine ratio (UACR), urine protein-creatinine ratio (UPCR), urinary liver-type fatty acid-binding protein (u-LFABP), and u-TGF-β1; (c) incidence of hypercalcemia, defined as corrected serum calcium ≥11 mg/dL; and (d) incidence of infections requiring hospitalization. Post hoc exploratory endpoints were changes in blood pressure, urinary angiotensinogen (u-AGT), and plasma renin activity (PRA) at the end of the study.

2.4 | Kidney histology

We performed protocol kidney allograft biopsies at 12 months posttransplant. Two trained nephrologists (M.T. and A.T.), who were unaware of the patients' treatment, scored the biopsy samples based on the Banff scheme. Banff's chronic interstitial score (ci) and chronic tubular score (ct) were combined as IFTA score in order to assess the effects of cholecalciferol on IFTA.[4]

2.5 | Measurements

Blood and urine samples were obtained at baseline, 6, and 12 months posttransplant and samples were stored at −80°C until they were analyzed. Chemical parameters were measured using standard automated techniques. Serum 25(OH)D levels were determined through chemiluminescent immunoassay (DiaSorin Inc). Serum 1,25-dihydroxyvitamin D (1,25[OH]$_2$D) concentrations were determined with radioimmunoassay (Immunodiagnostic Systems Ltd). PRA was measured with an enzyme immunoassay (Yamasa Co. Ltd). Serum intact PTH (iPTH) was measured with electrochemiluminescence immunoassay (Roche Diagnostics K. K.). u-LFABP was evaluated using a two-step sandwich enzyme-linked immunosorbent assay (ELISA) (CMIC Holdings). u-TGF-β1 and u-AGT were measured with ELISA using the Quantikine Human TGF-beta 1 Immunoassay (R&D Systems, Inc, USA) and Human Total Angiotensinogen Assay (IBL Co. Ltd), respectively. Stored samples were used to measure 25(OH)D, PRA, urinary albumin, u-TGF-β1, and u-AGT levels after the end of the study.

2.6 | Immunosuppressive therapy

All participants received standard induction therapies based on their risk of developing acute rejection. Maintenance regimens consisted of triple immunosuppression therapies with the following agents: (a) prednisolone, (b) calcineurin inhibitor (cyclosporine or tacrolimus), and (c) mycophenolate mofetil or everolimus. We administered 500 mg methylprednisolone intravenously on the day of transplantation, then initiated 60 mg oral prednisolone, which was lowered to 10 mg/day by 3 weeks posttransplant. Thereafter, the daily dose of prednisolone was tapered to 5 mg (maintenance dose) by 3 months following transplantation.

2.7 | Statistical analyses

Sample size was calculated as follows: The expected group difference in 25(OH)D levels was 23.4 ng/mL based on a previous interventional study.[16] In the observational study, 25(OH)D levels at 3 months posttransplant were positively associated with GFR at 12 months posttransplant (coefficient = 0.17 per 1 ng/dL increase in 25[OH]D).[4] Assuming that the intervention brings 80% of the estimated effects, the expected group difference in eGFR at 12 months posttransplant was 3.2 mL/min/1.73 m^2. We used data from 40 KTRs seen at our facility to estimate the standard deviation of change in eGFR, which was 7.32 mL/min/1.73 m^2. The sample size of 95 for each arm was calculated based on a power of 85% to show a between-group difference in eGFR of 3.2 mL/min/1.73 m^2 and a standard deviation of 7.32 mL/min/1.73 m^2 at a two-sided significance level of less than 0.05. We planned to enroll a total of 200 patients assuming a dropout rate of 5%.

An analysis of covariance (ANCOVA) with adjustment for baseline values was used in the primary endpoint analysis based on the modified intention-to-treat set which included all randomized patients who

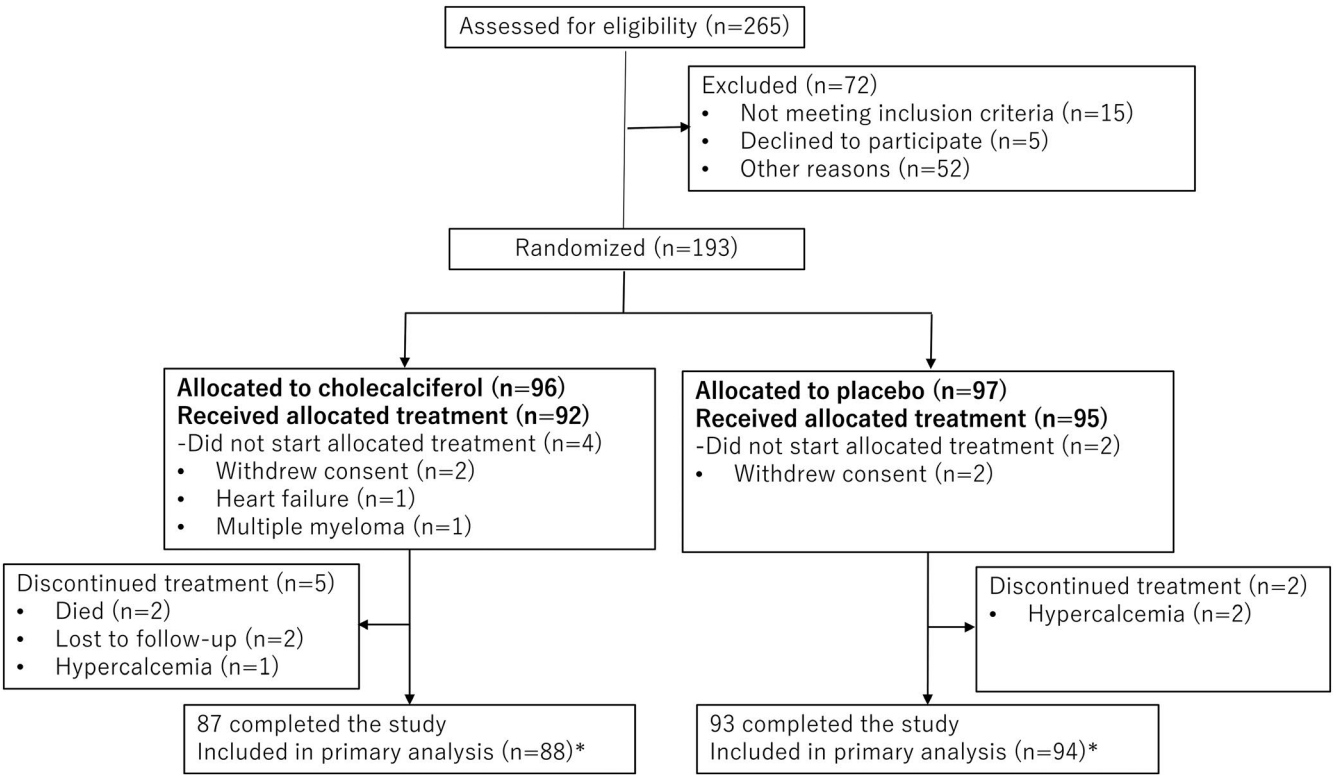

FIGURE 1 Flowchart of the study population. *The primary analysis included all randomized patients who had a final eGFR value

TABLE 1 Baseline characteristics

	Cholecalciferol (N = 92)	Placebo (N = 95)
Basic information		
Age, years	52 (42, 63)	52 (43, 62)
Male gender, n (%)	64 (70)	65 (68)
BMI, kg/m^2	22.5 (20.4, 24.5)	22.1 (20.3, 24.7)
SBP, mmHg	134 (124, 141)	130 (121, 140)
PEKT, n (%)	45 (49)	52 (55)
Dialysis vintage, months	2 (0, 17)	0 (0, 13)
ABO compatible transplantation, n (%)	59 (64)	61 (64)
Preformed DSA, n (%)[a]	2 (2)	5 (5)
Second transplantation, n (%)	3 (3)	1 (1)
Donor age, years	65 (57, 71)	63 (55, 71)
Immunosuppressive drugs, n (%)		
Cyclosporine	32 (35)	29 (31)
Tacrolimus	60 (65)	66 (69)
Everolimus	24 (26)	22 (23)
Mycophenolate mofetil	68 (74)	73 (77)
Antihypertensive drugs, n (%)[b]		
Angiotensin II receptor blocker	52 (57)	47 (49)
β-Blocker	21 (23)	22 (23)
Calcium channel blocker	51 (55)	62 (65)
Other antihypertensive drugs	14 (15)	22 (23)
Primary renal disease, n (%)		
Chronic glomerulonephritis	29 (32)	27 (28)
Diabetic nephropathy	24 (26)	27 (28)
Polycystic kidney disease	7 (8)	11 (12)
Hypertensive nephropathy	7 (8)	6 (6)
Others	25 (27)	24 (25)
Laboratory data		
Hemoglobin, g/dL	11.4 (10.6, 12.2)	11.3 (10.4, 12.0)
Albumin, g/dL	4.0 (3.8, 4.3)	4.0 (3.8, 4.2)
eGFR, mL/min per 1.73 m^2	46 (37, 55)	46 (36, 57)
Corrected calcium, mg/dL	9.5 (9.2, 9.7)	9.4 (9.2, 9.8)
Phosphate, mg/dL	2.7 (2.1, 3.3)	2.8 (2.1, 3.2)
25(OH)D, ng/mL	10 (9, 14)	10 (8, 13)
1,25(OH)$_2$D, pg/mL	46 (30, 59)	42 (34, 51)
Intact PTH, pg/mL	127 (91, 87)	113 (74, 147)
UACR, mg/g Cre	27 (9, 56)	26 (10, 56)
UPCR, g/g Cre	0.09 (0.06, 0.17)	0.08 (0.04, 0.15)
Urinary LFABP, μg/g Cre	10.5 (4.5, 23.9)	7.2 (3.3, 15.1)
Urinary TGF-β1, ng/g Cre	36 (20, 60)	41 (22, 79)
Urinary AGT, μg/g Cre	40 (16, 87)	29 (16, 53)
Urinary calcium-creatinine ratio, g/g Cre	0.07 (0.04, 0.11)	0.06 (0.03, 0.11)

Note: Data are based on the intention to treat population. Continuous variables are presented as median (25th, 75th percentile).

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; AGT, angiotensinogen; BMI, body mass index; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; LFABP, liver-type fatty-acid binding protein; PEKT, preemptive kidney transplantation; PRA, plasma renin activity; PTH, parathyroid hormone; SBP, systolic blood pressure; TGF, transforming growth factor; UPCR, UACR, urine albumin-to-creatinine ratio; urine protein-to-creatinine ratio.

[a]All positive patients had class 1 DSA. Three patients in the placebo group had both classes I and II DSA.

[b]Before transplantation.

received the study drug at least once. Changes in other continuous parameters were also analyzed in a manner similar to that of the eGFR changes. We employed Fisher's exact test to detect group differences in histological findings, the incidence of hypercalcemia, and infections requiring hospitalization. PRA at 12 months posttransplant was compared between the study groups using Wilcoxon rank-sum test. Prespecified subgroup analyses were conducted for the primary endpoint based on baseline eGFR (<45, ≥45 mL/min/1.73 m^2), hemoglobin (≤ the median, > the median), gender, presence or absence of diabetes mellitus, and the use of mycophenolate mofetil or everolimus. We also carried out post hoc subgroup analyses according to the UACR (<30, ≥30 mg/g Cre), 25(OH)D (<12, ≥12 ng/mL), and iPTH levels (≤the median, >the median). For continuous variables in the secondary endpoints, effect modifications by their baseline values, 25(OH)D, eGFR, and UACR levels were tested incorporating interaction terms with an intervention into the models. The stratified analyses were performed only when the interaction term was significant.

We used mixed-effects models with repeated measures, including treatment, time, and treatment by time interaction, as fixed effects with unstructured variance–covariance using data collected at baseline, 6, and 12 months posttransplant for the prespecified sensitivity analysis of the primary endpoint. Furthermore, we imputed missing values according to the last observation carried forward method. Post hoc sensitivity analysis was also conducted using eGFR based on the CKD-EPI cystatin C equation because vitamin D supplementation may alter creatinine metabolism.[17] Additionally, we performed a post hoc sensitivity analysis, with missing data handled with multivariate imputation by chained equations. We included only patients with ≥50% compliance, as a per-protocol analysis. Imputation for missing data was not used unless otherwise stated and we did not control for multiple comparisons. Therefore, the results of subgroup and sensitivity analyses should be interpreted with caution. The statistical test was two-tailed, and $p < 0.05$ was considered statistically significant. All statistical analyses were conducted using the Stata/SE 15 statistical software package (Stata Corp.).

TABLE 2 Clinical and laboratory parameters at baseline, 6 months, and 12 months posttransplant

Parameters	Treatment	Baseline	6 months	12 months	Between-group difference at 12 months posttransplant (95% CI)[a]	*p*-value[b]
25(OH)D, ng/mL	Cholecalciferol	10 (9, 14)	38 (31, 45)	40 (30–49)	25 ng/mL (22 to 28)	<0.01
	Placebo	10 (8, 13)	13 (10, 17)	14 (10, 18)		
$1,25(OH)_2D$, pg/mL	Cholecalciferol	46 (30, 59)	68 (47, 89)	69 (50, 62)	14 pg/mL (9 to 20)	<0.01
	Placebo	42 (34, 51)	47 (39, 64)	51 (43, 62)		
eGFR, mL/min per 1.73 m^2	Cholecalciferol	46 (37, 55)	45 (36, 53)	46 (37, 57)	−0.7 mL/min/1.73 m^2 (−3.3 to2.0)	0.63
	Placebo	46 (36, 57)	44 (38, 54)	48 (40, 55)		
UACR, mg/g Cre	Cholecalciferol	27 (9, 56)		30 (11, 83)	12% (−30 to 79)	0.64
	Placebo	26 (10, 56)		23 (9, 61)		
UPCR, g/g Cre	Cholecalciferol	0.09 (0.06, 0.17)	0.06 (0.04, 0.13)	0.07 (0.04, 0.14)	6% (−19 to 39)	0.65
	Placebo	0.08 (0.04, 0.15)	0.05 (0.03, 0.11)	0.06 (0.04, 0.12)		
Urinary LFABP, μg/g Cre	Cholecalciferol	10.5 (4.5, 23.9)	8.4 (3.5, 19.3)	9.9 (3.9, 24.9)	14% (−22 to 64)	0.50
	Placebo	7.2 (3.3, 15.1)	7.3 (3.3, 15.1)	7.3 (3.5, 17.7)		
Urinary TGF-β1, ng/g Cre	Cholecalciferol	36 (20, 60)		35 (20, 56)	14% (−9 to 43)	0.25
	Placebo	41 (22, 79)		29 (20, 51)		
Urinary calcium-creatinine ratio, g/g Cre	Cholecalciferol	0.07 (0.04, 0.11)	0.05 (0.03, 0.10)	0.06 (0.03, 0.11)	0% (−24 to 32)	0.98
	Placebo	0.06 (0.03, 0.11)	0.05 (0.03, 0.08)	0.06 (0.03, 0.09)		
Corrected calcium, mg/dL	Cholecalciferol	9.5 (9.2, 9.7)	9.6 (9.3, 9.9)	9.6 (9.3, 9.9)	0.1 mg/dL (−0.1 to 0.2)	0.33
	Placebo	9.4 (9.2, 9.8)	9.5 (9.2, 9.7)	9.5 (9.2, 9.8)		
Phosphate, mg/dL	Cholecalciferol	2.7 (2.1, 3.3)	3.2 (2.9, 3.5)	3.1 (2.8, 3.4)	0.03 mg/dL (−0.1 to 0.2)	0.63
	Placebo	2.8 (2.1, 3.2)	3.2 (2.9, 3.5)	3.0 (2.8, 3.5)		
SBP, mmHg	Cholecalciferol	134 (124, 141)	126 (120, 133)	128 (119, 135)	−0.7 mmHg (−4.3 to 2.9)	0.70
	Placebo	130 (121, 140)	127 (123, 133)	127 (118, 134)		
PRA, ng/mL/h[c]	Cholecalciferol			2.2 (1.3, 5.2)		0.20
	Placebo			2.1 (1.0, 4.1)		
Urinary AGT, μg/g Cre	Cholecalciferol	40 (16, 87)		28 (11, 77)	21% (−23 to 91)	0.40
	Placebo	29 (16, 53)		25 (8, 71)		
Intact PTH, pg/mL	Cholecalciferol	127 (91, 87)	68 (53, 100)	65 (50, 90)	−12% (−23 to −0.5)	0.04
	Placebo	113 (74, 147)	80 (55, 107)	70 (53, 95)		

Note: Data are presented as median (25th, 75th percentile).

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; AGT, angiotensinogen; eGFR, estimated glomerular filtration rate; LFABP, liver-type fatty-acid binding protein; PRA, plasma renin activity; PTH, parathyroid hormone; SBP, systolic blood pressure; TGF, transforming growth factor; UACR, urine albumin-to-creatinine ratio; UPCR, urine protein-to-creatinine ratio.

[a]Adjustment for baseline values.

[b]*p*-values were tested with analysis of covariance except for PRA.

[c]Between-group difference was analyzed using the Wilcoxon rank-sum test.

3 | RESULTS

3.1 | Study patients and study flow

A total of 193 patients underwent randomization from January 2016 to July 2018 (Figure 1). Four patients withdrew consent and two patients withdrew from the study due to heart failure or multiple myeloma before receiving the allocated drug. During the study, two patients in the cholecalciferol group died and five others discontinued the allocated drug and, therefore, 180 (96%) patients completed the study. In the trial population as a whole, median age, eGFR, and 25(OH)D levels, were 52 years, 47 mL/min/1.73 m^2, and 10 ng/mL, respectively. All except for one participant in the placebo group were vitamin D insufficient (<30 ng/mL) according to an Endocrine Society clinical practice guideline.[18] Baseline characteristics were well balanced between groups, except for the u-LFABP creatinine ratio in the cholecalciferol group (Table 1). Median 25(OH)D level was increased from 10

to 40 ng/mL in the cholecalciferol group and changed minimally in the placebo group at the end of the study (Table 2, Figure 2A). No patients in the placebo group but 67 (77%) participants in the cholecalciferol group had a 25(OH)D above 30 ng/mL. Overall, the between-group difference in the 25(OH)D levels, with adjustment for baseline values, was 25 ng/mL (95% CI; 22–28) (Table 2). Serum $1,25(OH)_2D$ levels were also significantly increased in the cholecalciferol group (between-group difference 14 pg/mL [95% CI; 9–20], Table 2, Figure 2B). Poor adherence was observed in only two participants (one patient in each group) throughout the study.

3.2 | Primary endpoint: Change in eGFR

The mean change in eGFR from baseline to 12 months posttransplant was 1.2 mL/min/1.73 m^2 (95% CI; −0.7 to 3.1) in the cholecalciferol group and 1.8 mL/min/1.73 m^2 (95% CI; −0.02 to 3.1) in the placebo group, with no significant between-group differences (−0.7 mL/min/1.73 m^2 [95% CI; −3.3 to 2.0, p = 0.63], Table 2, Figure 3A). Similar results were obtained from several sensitivity analyses (Figure S1). The result was not substantially unchanged in the per-protocol analysis, excluding subjects who discontinued the study drug, with an estimated treatment difference of −0.7 mL/min/1.73 m^2 (95% CI; −3.4 to 1.9). Stratified analyses by baseline eGFR and UACR revealed significant heterogeneities in the treatment effect (Figure 4). Patients on cholecalciferol had significantly less improvement in eGFR than those on placebo among those with an eGFR of less than 45 mL/min/1.73 m^2 (between-group difference of −4.3 mL/min/1.73 m^2 [95% CI; −7.3 to −1.3], Figure 3B, Figure 4). Also, cholecalciferol reduced eGFR significantly in patients with UACR greater than or equal to 30 mg/g Cre, as compared with the placebo group (between-group difference of −4.7 mL/min/1.73 m^2 [95% CI; −8.4 to −0.9], Figure 3C, Figure 4).

3.3 | Kidney pathology

An evaluation of the kidney biopsies at 12 months posttransplant is summarized in Table 3 and Table S1. The tubulointerstitial area was preserved, as a whole, and approximately 70% of patients showed no interstitial fibrosis. The severity of IFTA at 12 months did not differ between groups (Table 3). Furthermore, no significant differences were observed when comparing the scores of IFTA individually (Table 3). Besides, inflammation in each kidney compartments was similar between the groups (Table S1). No one showed moderate-to-severe calcification, defined as the number of calcification foci of ≥3 in serial histological slides, in the tubulointerstitial region.

3.4 | Urinary biomarkers

Table 2 depicts a series of urinary biomarkers used during the study period. Overall, cholecalciferol supplementation did not reduce UACR, UPCR, u-LFABP, or u-TGF-β1 compared to placebo (Table 2), with no effect modification by their baseline values, 25(OH)D levels, or UACR (all $p_{interaction}$ > 0.15). Besides, changes in the urinary calcium-creatinine ratio were similar between the groups (Table 2).

3.5 | Renin-angiotensin system, blood pressure, and parathyroid hormone

We conducted post hoc exploratory analyses regarding RAS, blood pressure, and iPTH to search for mechanisms of decline in eGFR with cholecalciferol among patients with low eGFR (i.e., eGFR <45 mL/min/1.73 m^2) or elevated UACR (i.e., UACR ≥30 mg/g Cre). To increase the statistical power of stratified analyses, we employed mixed-effects models using all available data to test between-group

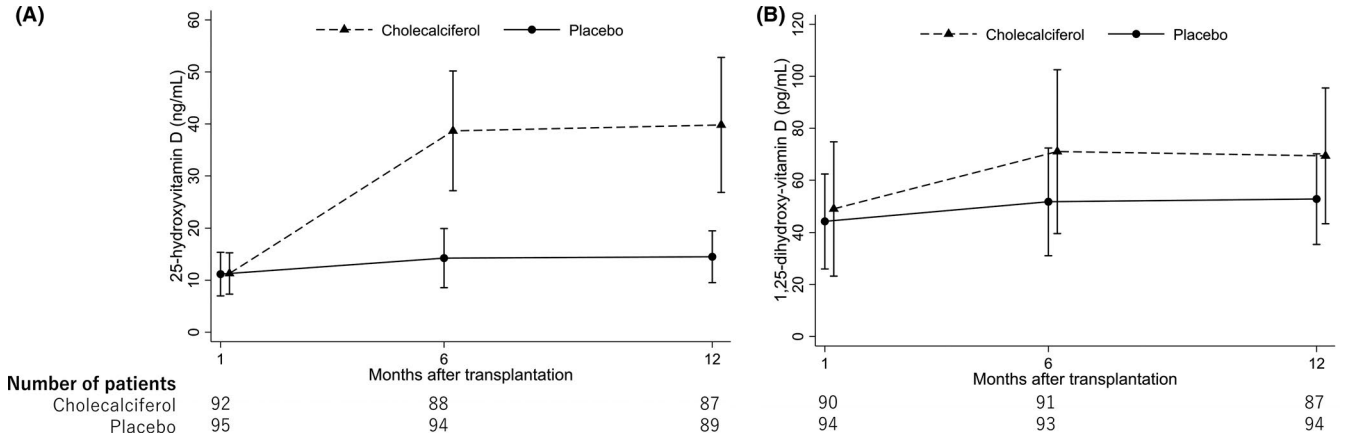

FIGURE 2 The changes in (A) 25-hydroxyvitamin D and (B) 1,25-dihydroxyvitamin D. The mean levels of (A) 25-hydroxyvitamin D and (B) 1,25-dihydroxyvitamin D are shown at different time points during the study. The I bars indicate standard deviations

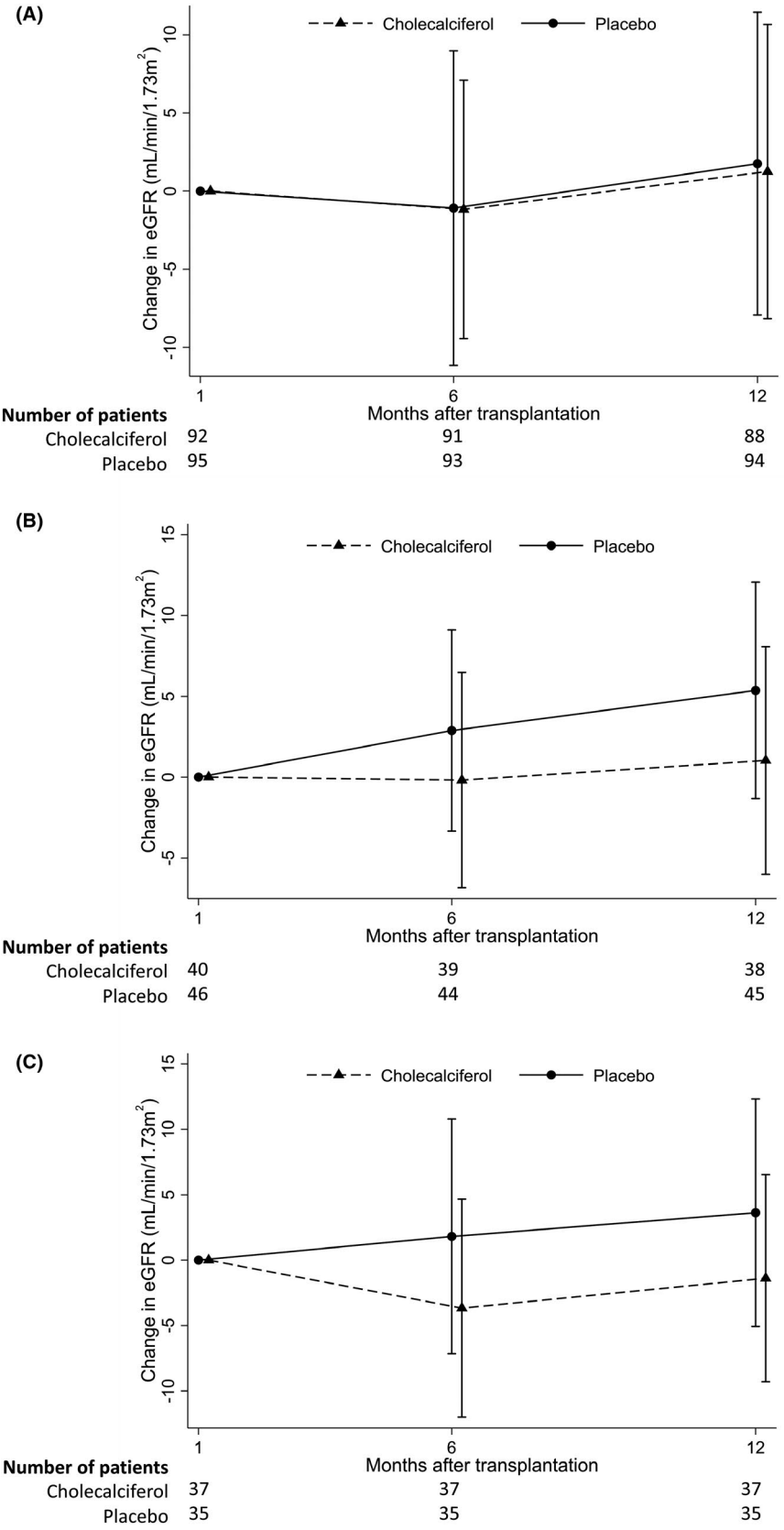

FIGURE 3 Relative mean changes in eGFR according to time and trial group among (A) the whole population, (B) patients with eGFR less than 45 mL/min/1.73 m^2, and (C) patients with UACR greater than or equal to 30 mg/g Cre. The change in eGFR denotes eGFR at a given time minus its baseline value. The I bars indicate standard deviation. eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio

differences in systolic blood pressure (SBP) and iPTH. As a whole group, the changes in systolic blood pressure (SBP), u-AGT creatinine ratio, and PRA levels at 12 months posttransplant did not differ between groups (Table 2, Figure 5A), whereas iPTH levels were mildly suppressed in the cholecalciferol group (between-group difference of −12% [95%CI; −23 to −0.5], Table 2). The change in u-AGT

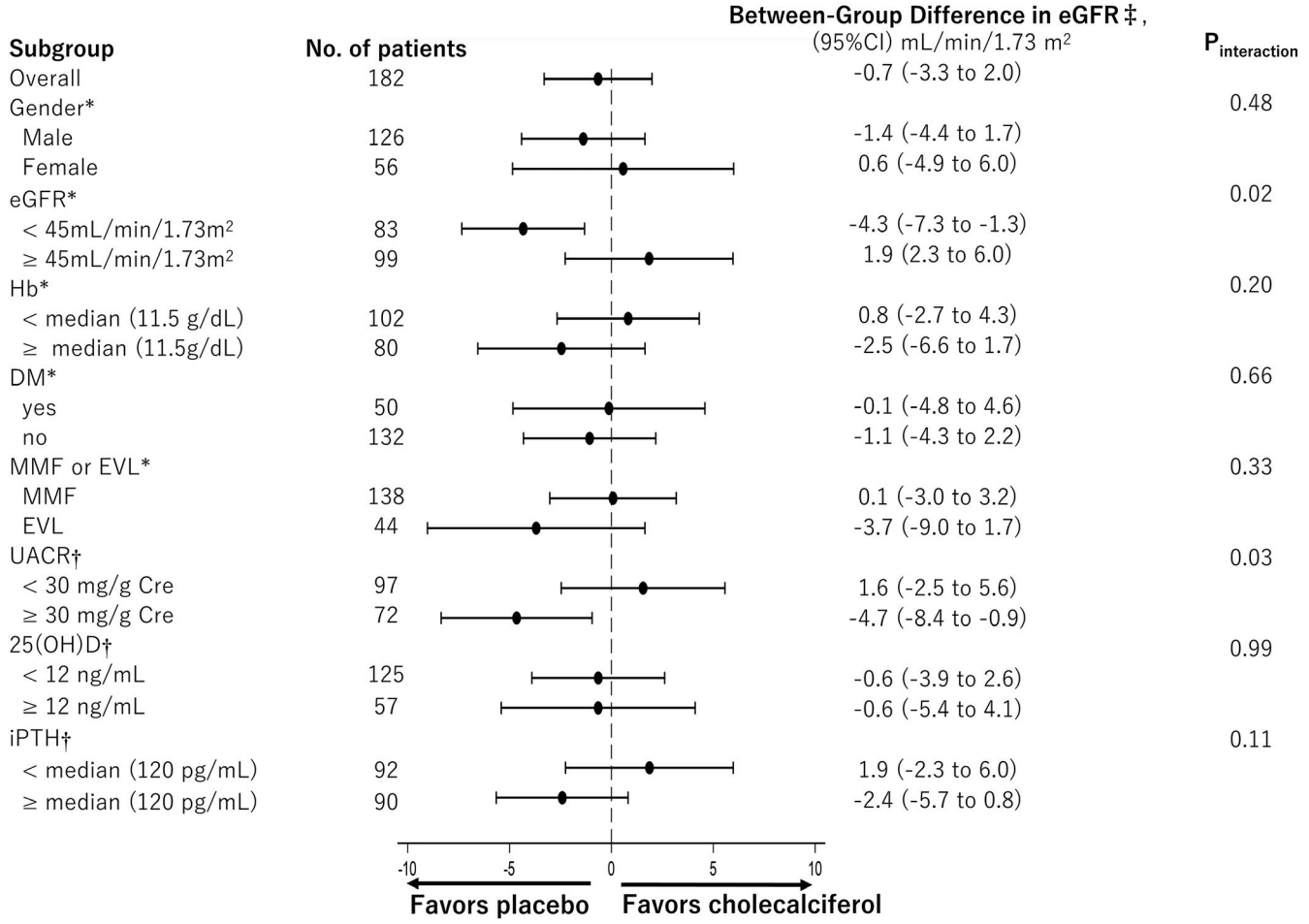

FIGURE 4 Effects of cholecalciferol on changes in eGFR among participant subgroups. *Prespecified subgroup analyses. †Post hoc subgroup analyses. ‡Adjustment for baseline eGFR. eGFR, estimated glomerular filtration rate; Hb, hemoglobin; DM, diabetes mellitus; MMF, mycophenolate mofetil; EVL, everolimus; UACR, urine albumin-to-creatinine ratio; 25(OH)D, 25-hydroxyvitamin D; iPTH, intact parathyroid hormone

creatinine ratio and PRA levels at 12 months posttransplant was comparable in subgroups with low eGFR or elevated UACR (data not shown). Suppressions of iPTH by cholecalciferol in two subgroups were similar to that of the whole population (between-group difference of −11% [95%CI; −31 to 14] in those with low eGFR and −17% [95%CI; −31 to 0.5] in those with elevated UACR, respectively, Table S2). Meanwhile, cholecalciferol lowered SBP significantly compared to placebo in subjects with low eGFR (between-group difference of −5.8 mmHg [95%CI; −11.4 to −0.2], Figure 5B, Table S2) and patients with elevated UACR (between-group difference of −6.7 mmHg [95%CI; −12.3 to −1.2], Figure 5C, Table S2).

3.6 | Adverse events

The incidence of hypercalcemia, defined as a corrected calcium level above 11 mg/dL, was uncommon and did not differ between treatment groups ($p > 0.99$, Table 4). Seven infections requiring hospitalization, of which more than half were due to cytomegalovirus, occurred in the cholecalciferol group and 10 occurred in the placebo group ($p = 0.61$, Table S3). No significant differences were observed between groups for serious adverse events including death, biopsy-proven rejection, and fracture. The most frequent adverse events were nasopharyngitis, followed by cytomegalovirus and varicella zoster virus infection.

4 | DISCUSSION

This randomized clinical trial among incident KTRs showed no evidence of the beneficial effects of an 11-month intervention with cholecalciferol on changes in eGFR. Furthermore, cholecalciferol supplementation did not provide favorable results in histology, urinary biomarkers related to kidney damage. Contrary to our hypothesis, subgroup analyses indicated that subjects with low eGFR or elevated UACR at baseline had a significant decline in eGFR with cholecalciferol compared to placebo. In contrast, oral cholecalciferol supplementation at a daily dose of 4000 IU elevated 25(OH)D levels into the physiological range and the incidence of adverse events did not differ between the two groups.

TABLE 3 Results of interstitial fibrosis and tubular atrophy score at 12 months posttransplant

Histology	Cholecalciferol (N = 79)	Placebo (N = 88)	*p*-value
	No. of patients (%)		
Interstitial Fibrosis (ci)			0.44
0	56 (71)	57 (65)	
1	20 (25)	30 (34)	
2	2 (3)	1 (1)	
3	1 (1)	0 (0)	
Tubular Atrophy (ct)			0.29
0	20 (25)	32 (36)	
1	56 (71)	55 (63)	
2	2 (3)	1 (1)	
3	1 (1)	0 (0)	
IFTA (ci+ct)			0.40
0 or 1	56 (71)	57 (65)	
2 to 6	23 (29)	31 (35)	

Note: IFTA scores were calculated using the total of "ct" and "ci" scores based on the Banff scheme. Between-group differences were compared using Fisher's exact test. IFTA, interstitial fibrosis and tubular atrophy.

Our findings are consistent with those of the VITAL-DKD (Vitamin D and Omega-3 Trial to Prevent and Treat Diabetic Kidney Disease) trial conducted to assess the efficacy of vitamin D among patients with type 2 diabetes mellitus (T2DM).[19] A total of 1312 adults with T2DM were randomized to receive 2000 IU cholecalciferol or placebo daily during the VITAL-DKD trial, resulting in no significant between-group differences in eGFR change from baseline to year 5 (0.9 mL/min/1.73 m^2 [95%CI; −0.7 to 2.5]). Nonetheless, the results of the VITAL-DKD did not preclude the possibility that cholecalciferol supplementation might be effective in vitamin D-deficient patients since baseline mean 25(OH)D level in the VITAL-DKD was 30 ng/dL. Our trial (median 25[OH]D level; 10 ng/dL) suggested that cholecalciferol did not have renoprotective effects in terms of eGFR or UACR even in patients with vitamin D deficiency. Unexpectedly, a significant decline in eGFR with cholecalciferol compared with the placebo was observed in patients with low eGFR (<45 mL/min/1.73 m^2) or elevated UACR (≥30 mg/g Cre). The mechanism for the detrimental effect of cholecalciferol remains uncertain. Based on our exploratory analyses, at least in part, lower SBP in those subgroups with cholecalciferol might have led to a decline in GFR. The results should be confirmed by future studies, considering the exploratory nature of subgroup analysis and the lack of multiplicity adjustment.

IFTA on kidney allograft biopsy is a strong predictor of graft loss.[20] An observational study in T2DM showed that cholecalciferol supplementation reduced u-TGF-β1, which is an indicator of kidney fibrosis.[14] The degree of IFTA and change in u-TGF-β1 did not differ between groups in this study. Our findings may be considered as inconsistent with the observational study reporting the link between low 25(OH)D levels and the progression of IFTA.[4] Proteinuria could be a candidate for residual confounding in that observational study,[4] given the negative relationship between circulating 25(OH)D levels and proteinuria.[21] In line with our results, two randomized trials with incident KTRs demonstrated that vitamin D analog provided little or no anti-fibrotic effect on kidney allograft.[22,23]

Cholecalciferol supplementation did not affect urinary biomarkers, including UACR, u-LFABP, or UPCR in our investigation, despite the increase in $1,25(OH)_2D$ concentrations. Again, no anti-proteinuric effects of cholecalciferol in our study were consistent with the VITAL-DKD trial.[19] However, a meta-analysis among non-KTRs concluded that active vitamin D and its analog reduced proteinuria.[24] Two possible reasons might explain this discrepancy. First, much higher levels of 25(OH)D may be needed to obtain the efficacy of treatment. Considering that there was no calcemic action by cholecalciferol in our trial, vitamin D receptor activation by cholecalciferol seems to be weak compared to active vitamin D or its analog, which were reported to increase serum calcium levels.[22] Second, low baseline proteinuria may mask the anti-proteinuric effects of cholecalciferol. The median UACR values were less than 30 mg/g Cre in our trial and the VITAL-DKD.[19]

The negative regulatory role of vitamin D in renin gene transcription [25,26] was postulated as one potential explanation of why vitamin D deficiency is associated with the development of hypertension, and cardiovascular and kidney disease. Some observational studies demonstrated an association between low 25(OH)D levels and higher RAS activity.[27,28] However, most interventional trials reported negligible effects of nutritional vitamin D on blood pressure and RAS activity, which is consistent with our results.[29-31] Notably, we observed lower SBP at the end of the study with cholecalciferol than with placebo in the subgroup with low eGFR or elevated UACR. Further studies are warranted to confirm this finding.

The null finding for the primary endpoint is noteworthy because our participants had very low levels of 25(OH)D at baseline, which can maximize the potential to demonstrate the beneficial effect of cholecalciferol supplementation. Other strengths of our trial included an adequate protocol and high participant adherence, which resulted in the expected group difference in 25(OH)D levels. Also, various evaluations of the allograft were performed including eGFR, histology, and urinary biomarkers. In contrast, there were several limitations. First, GFR was not measured but estimated with equations. Because vitamin D supplementation may change creatine metabolism,[17] it is uncertain whether changes in eGFR reflect true changes in GFR. However, using the GFR equation with cystatin C alone yielded robust results in our investigation. Second, the study follow-up duration may be too short to fully evaluate the renoprotection effects of cholecalciferol. Third, incident KTRs might not be a suitable population to observe the renoprotective effects of cholecalciferol because of the intensive immunosuppressive therapy and low proteinuria. Fourth, drug adherence monitoring was solely dependent on self-report. However, the achievement of expected group difference in 25(OH)D levels suggested good participant adherence as reported by themselves. Fifth, given the limited size of participants and nonadjustment for multiple comparisons,

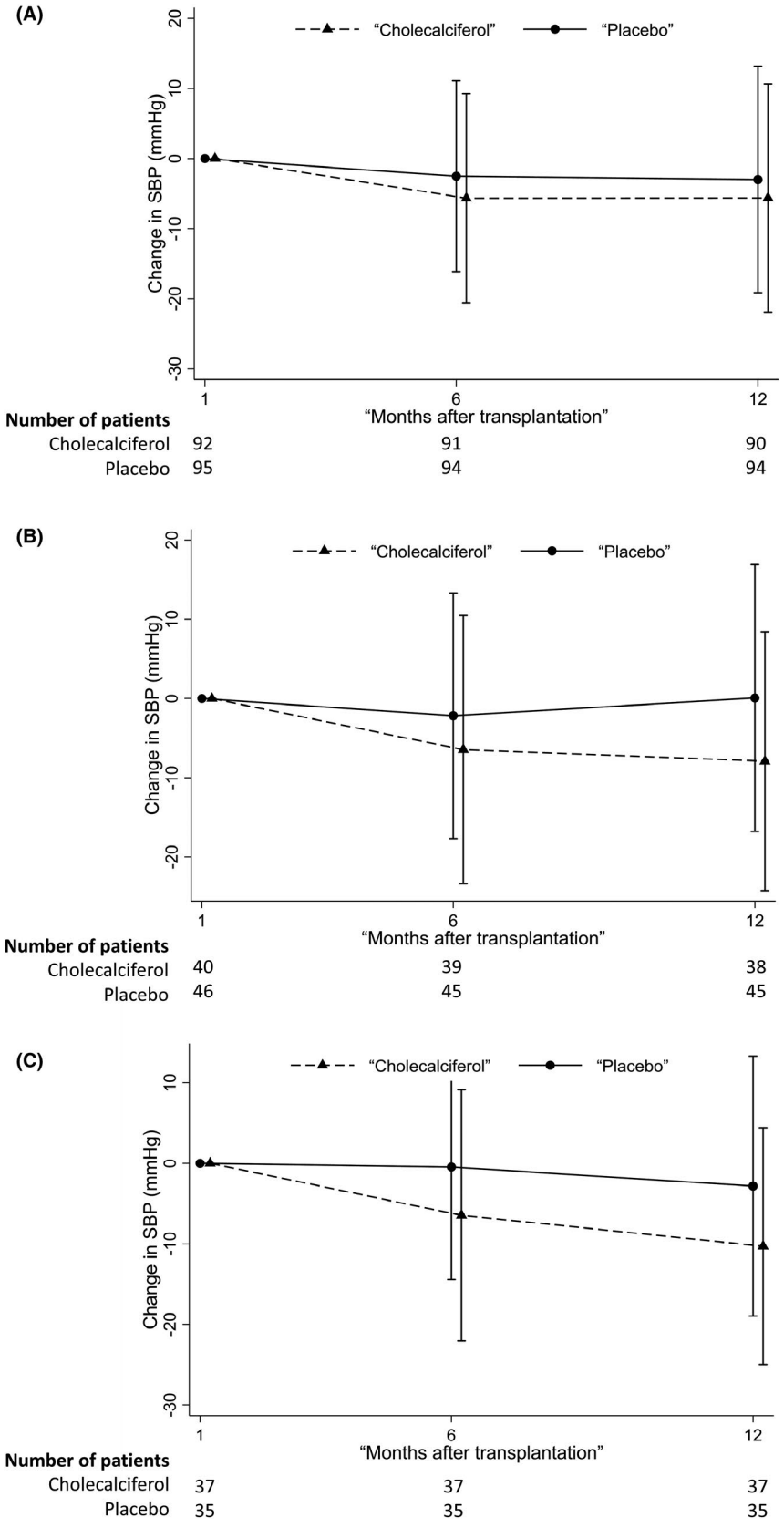

FIGURE 5 The relative mean changes in SBP based on time and trial group among (A) the entire population, (B) patients with eGFR less than 45 mL/min/1.73 m^2, and (C) patients with UACR greater than or equal to 30 mg/g Cre. The change in SBP denotes SBP at a given time point minus its baseline value. The I bars indicate standard deviations. SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio

TABLE 4 Adverse events

	Cholecalciferol (N = 92)	Placebo (N = 95)
	No. of patients	
Any AEs	50	53
De novo DSA	1	0
Hypercalcemia	3	4
Serious AE		
Death	2	0
Ischemic stroke	1	0
Heart failure	0	1
Infections requiring hospitalization	7	10
Biopsy-proven rejection[a]	3	0
Fracture	2	1
Osteonecrosis	2	0
Intestinal bleeding	1	0
	1	0
Kidney aneurysm rupture	1	0
Vesicovaginal fistula closure	1	0
Orthopedic surgery	1	0
AEs occurring in more than 5%		
Nasopharyngitis	13	14
Cytomegalovirus infection	11	12
Varicella zoster virus Infection	5	8
Leg edema	4	6

Abbreviations: AEs, adverse events; DSA, donor-specific antibody.
[a]Two acute T cell–mediated rejections and one chronic active antibody-mediated rejection.

the results of subgroup analysis should be considered hypothesis generating.

In summary, this trial provided the first evidence that a daily dose of 4000 IU cholecalciferol was effective in achieving sufficient 25(OH)D levels with good tolerability and safety profile among incident KTRs. However, we did not find evidence of the clinically meaningful benefit of cholecalciferol on allograft outcomes. Caution is required when prescribing cholecalciferol in KTRs with low eGFR or elevated UACR.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

AUTHOR CONTRIBUTIONS

M.T., T.H., Y.O., A.T., S.N., and Y.W. conceptualized the study. M.T., T.H., and Y.O. designed the study and were involved in the drafting and editing of the study protocol. Y.D. and T.H. analyzed the data. Y.D., M.T., T.H., and Y.I. drafted the manuscript. T.N., T.T., K.F., M.O., T.H., N.G., and A.N. reviewed and edited the manuscript. Y.W. was the overall study principal investigator.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, T.H, upon reasonable request.

ORCID

Yohei Doi https://orcid.org/0000-0002-5165-291X
Makoto Tsujita https://orcid.org/0000-0001-6927-6353
Takayuki Hamano https://orcid.org/0000-0002-2100-9106
Yoshitsugu Obi https://orcid.org/0000-0001-7032-4383
Toshihide Tomosugi https://orcid.org/0000-0001-8715-9134
Manabu Okada https://orcid.org/0000-0002-8446-7841
Norihiko Goto https://orcid.org/0000-0003-4931-3203
Yoshitaka Isaka https://orcid.org/0000-0002-0820-7167

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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