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ORIGINAL ARTICLE

# Correcting anemia and native vitamin D supplementation in kidney transplant recipients: a multicenter, 2 × 2 factorial, open-label, randomized clinical trial

Yoshitsugu Obi[1,2,3], Naotsugu Ichimaru[4], Yusuke Sakaguchi[5], Kazuhiro Iwadoh[6,7], Daisuke Ishii[8], Ken Sakai[9], Daiki Iwami[10,11], Hiroshi Harada[12], Keiichi Sumida[3,13], Akinari Sekine[13], Kosuke Masutani[14], Naotake Akutsu[15], Takamitsu Inoue[16], Morikuni Nishihira[17], Tatsuo Yoneda[18], Shinichi Ito[19], Motoo Araki[20], Jun-Ya Kaimori[4,5], Katsunori Yoshida[18], Shigeru Satoh[21], Yoshifumi Ubara[13], Yoshitaka Isaka[1], Kazunari Yoshida[8,22], Yoshiharu Tsubakihara[23], Shiro Takahara[4,24], & Takayuki Hamano[1,5,25] on behalf of the CANDLE-KIT Trial Investigators*

1 Department of Nephrology, Osaka University Graduate School of Medicine, Suita, Japan
2 Obi Clinic, Osaka, Japan
3 Division of Nephrology, University of Tennessee Health Science Center, Memphis, TN, USA
4 Department of Advanced Technology for Transplantation, Osaka University Graduate School of Medicine, Suita, Japan
5 Department of Inter-Organ Communication Research in Kidney Disease, Osaka University Graduate School of Medicine, Suita, Japan
6 Department of Surgery III, Tokyo Women's Medical University, Tokyo, Japan
7 Department of Blood Purification, Tokyo Women's Medical University, Tokyo, Japan
8 Department of Urology, Kitasato University School of Medicine, Sagamihara, Japan
9 Department of Nephrology, Omori Medical Center, Toho University School of Medicine, Tokyo, Japan
10 Department of Urology, Hokkaido University, Sapporo, Japan
11 Division of Renal Surgery and Transplantation, Department of Urology, Jichi Medical University, Shimotsuke, Japan
12 Department of Kidney Transplant Surgery, Sapporo City General Hospital, Sapporo, Japan

## SUMMARY

Anemia and vitamin D deficiency are associated with allograft failure, and hence, are potential therapeutic targets among kidney transplant recipients (KTRs). We conducted a multicenter, two-by-two factorial, open-label, randomized clinical trial to examine the effects of anemia correction and vitamin D supplementation on 2-year change in eGFR among KTRs (CANDLE-KIT). We enrolled 153 patients with anemia and >1-year history of transplantation across 23 facilities in Japan, and randomly assigned them to either a high or low hemoglobin target (>12.5 vs. <10.5 g/dl) and to either cholecalciferol 1000 IU/day or control. This trial was terminated early based on the planned interim intention-to-treat analyses ($\alpha = 0.034$). Among 125 patients who completed the study, 2-year decline in eGFR was smaller in the high vs. low hemoglobin group (i.e., $-1.6 \pm 4.5$ vs. $-4.0 \pm 6.9$ ml/min/1.73 m$^2$; $P = 0.021$), but did not differ between the cholecalciferol and control groups. These findings were supported by the fully adjusted mixed effects model evaluating the rate of eGFR decline among all 153 participants. There were no significant between-group differences in all-cause death or the renal composite outcome in either arm. In conclusion, aggressive anemia correction showed a potential to preserve allograft kidney function.

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**Key words**
allograft function, anemia, cancer, erythropoiesis-stimulating agents, kidney transplant, malignancy, vitamin D

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**Correspondence**
Takayuki Hamano MD, PhD, Department of Nephrology, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi Mizuho-cho Mizuho-ku Nagoya, Aichi 467-8601, Japan.
Tel./fax: +81-52-858-7366;
e-mail: hamatea@med.nagoya-cu.ac.jp




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13 Nephrology Center, Toranomon Hospital Kajigaya, Kawasaki, Japan
14 Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
15 Department of Surgery and Clinical Research Center, Chibahigashi National Hospital, National Hospital Organization, Chiba, Japan
16 Department of Urology, Akita University Graduate School of Medicine, Akita, Japan
17 Department of Nephrology, Tomishiro Central Hospital, Tomigusuku, Japan
18 Departments of Urology, Nara Medical University, Kashihara, Nara, Japan
19 Sunshine M&D Clinic, Mizuho, Gifu, Japan
20 Department of Urology, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, Okayama, Japan
21 Center for Kidney Disease and Transplantation, Akita University Hospital, Akita, Japan
22 Research and Development Center for New Medical Frontiers, Organ Transplant Medicine, Kitasato University School of Medicine, Sagamihara, Japan
23 Department of Safety Management in Health Care Sciences, Graduate School of Health Care Sciences, Jikei Institute, Osaka, Japan
24 Department of Renal Transplantation, Kansai Medical Hospital, Toyonaka, Japan
25 Department of Nephrology, Graduate School of Medical Science, Nagoya City University, Nagoya, Japan

*See Appendix 1 for CANDLE-KIT Trial Investigators.Clinical Trial Registry: This study was registered at ClinicalTrials.gov and University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) as NCT01817699 and UMIN000009970, respectively.

## Introduction

Chronic renal allograft dysfunction represents cumulative and incremental damage to nephrons from immunologic and nonimmunologic causes [1] and is the most common cause of graft failure after the first year [2,3]. It results in a gradual decline in kidney allograft function typically with proteinuria and its pathological features include interstitial fibrosis and tubular atrophy [4]. Anemia [5–7] and vitamin D deficiency [8–12] are potential therapeutic targets as they are adversely associated with clinical outcomes among patients with CKD and kidney transplant recipients (KTRs).

Erythropoiesis-stimulating agents (ESAs) have been proposed to enhance survival of renal tissues through direct effects on renal cells leading to reduced cell apoptosis, or indirect effects via greater oxygen delivery with increased hemoglobin [13]. However, large randomized clinical trials (RCTs) in the CKD population have shown that correcting anemia with ESAs does not provide renoprotective benefit but did increase cardiovascular and thrombotic events [14–16]. Furthermore, a cancer signal was observed in the largest of these trials [16], and trials of ESA in some cancers suggest they increase the risk of death [17]. Accordingly, the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines suggest not initiating ESAs with hemoglobin (Hb) ≥10 g/dl and not using ESAs to maintain Hb ≥11.5 g/dl [18]. In the kidney transplant population, a previous RCT reported in 2013 from France (i.e., the CAPRIT study) [19] demonstrated that targeting hemoglobin levels at 13.0–15.0 g/dl (vs. 10.5–11.5 g/dl) using epoetin-β slowed the rate of decline in kidney allograft function. However, the external validity of this finding remains unclear.

Vitamin D does not only play a pivotal role in maintaining bone and mineral homeostasis but may exert pleiotropic effects via RAAS inhibition and immunomodulation [8]. Among patients with CKD or ESRD, vitamin D deficiency is associated with adverse clinical outcomes such as anemia, kidney function decline, cardiovascular events, and mortality [8–12]. Vitamin D deficiency is highly prevalent among KTRs who are advised to avoid UV exposure for skin cancer prevention [20,21]. Transplant patients with vitamin D deficiency also have an increased risk of allograft function decline and graft failure as well as cancer, infection, cardiovascular disease, and mortality [20–26]. Nevertheless, there is scarce evidence showing the effects of vitamin D supplementation on clinical outcomes. Clinical trials for vitamin D supplementation were feasible particularly in Japan where most patients were not evaluated for vitamin D status and hence not supplemented with nutritional vitamin D because they are reimbursed only for vitamin D deficiency-induced osteomalacia/rickets and the prevention of denosumab-induced hypocalcaemia, respectively.

Therefore, we aimed to evaluate the effectiveness of achieving a higher hemoglobin target through ESA therapy and the effectiveness of cholecalciferol supplementation on preserving kidney allograft function among KTRs.

## Methods

### Study design

In this multicenter open-label RCT with a 2-by-2 factorial design, we evaluated the efficacy of maintaining high hemoglobin levels and cholecalciferol supplementation, separately, on the change in estimated glomerular filtration rate (eGFR) over a 2-year intervention period (Trial registration: NCT01817699 at ClinicalTrials.gov and UMIN000009970 at University Hospital Medical Information Network Clinical Trials Registry). This study was conducted at 23 facilities across Japan from April of 2013 to December of 2018. The rationale for the factorial design was based on several arguments. Most importantly, both interventions are readily available in clinical practice but have limited evidence in its efficacy, and hence, are worthy of study in their own right. All participating centers had no issues in providing either intervention. Additionally, we did not anticipate substantial interactive effect between these interventions which would compromise the sample size calculation. Therefore, the factorial design was expected to provide substantial efficiencies in costs and time needed to study the two interventions separately. All patients provided written informed consent before study enrollment. This study was conducted in accordance with the principles of the Declaration of Helsinki, and the study protocol was approved by local ethics committees and the Ethics Committee of Osaka University Hospital (Approval No. 12207).

### Study participants

Patients were eligible if they were aged 20–79 years, had eGFR of ≥15 and <60 ml/min per 1.73 $m^2$), received kidney transplant >1-year prior to the enrollment, and had anemia defined as <10.5 g/dl of hemoglobin without iron deficiency (serum ferritin level ≥50 ng/ml) or on any erythropoiesis-stimulating agent regardless of iron status. eGFR was calculated using an equation for the Japanese population [27]. Exclusion criteria included (i) on anticancer treatment, (ii) history of ischemic stroke or transient ischemic attack, (iii) corrected serum calcium ≥10.5 mg/dl, (iv) HIV virus infection, (v) anticipated refractory hypertension with the high hemoglobin target, (vi) were pregnant or lactating, and (vii) current use of native vitamin D supplement. Patients were also excluded if they were considered ineligible according to physician' judgment.

### Randomization and intervention

Participants were randomly assigned in a 1:1 ratio to either a high or low hemoglobin target (i.e., ≥12.5 vs. <10.5 g/dl) and to either a cholecalciferol group or a control group by using an online-based block randomization program (block size: 8) with stratification by transplant date (before or after November 1999 when mycophenolate mofetil became available in Japan) and urine protein-to-creatinine ratio. This was an open-label trial, and treatment allocation was not concealed from the patients and investigators. All patients were given subcutaneous methoxy polyethylene glycol epoetin beta (MPG-EPO or Mircera®; Chugai Pharmaceutical Co. Ltd., Chuo, Tokyo, Japan) 0–250 μg at each visit, and the doses and interval were adjusted according to hemoglobin level and its target. Epoetin-α, epoetin-β, and darbepoetin-α were not used during the study period. Iron status was evaluated every 6 months, and iron was supplemented if deficient. Patients in the cholecalciferol group received 1000 IU/day of cholecalciferol supplementation. Patients in the control group received standard care alone. Adherence to the hemoglobin targets was periodically evaluated by the central study team who made intervention recommendations to the local study investigators if necessary. Adherence to cholecalciferol was assessed by interviewing patients at every follow-up visit, and good compliance was defined as taking a prescribed dose for >50% of days since the prior visit.

### Outcomes

Serum creatinine levels were measured at the study entry, month 1, 6, 12, and 24. The primary outcome was a change in creatinine-based eGFR (eGFRcr) over the 2-year study period. Changes in hemoglobin and serum 25(OH)D over the study period were also measured to assess compliance to the study protocol. Cystatin C-based eGFR (eGFRcys) was used as a post hoc exploratory outcome [28]. The prespecified secondary outcomes included MPG-EPO dose, blood pressure, all-cause death, cancer development or recurrence, and biopsy-proven acute cellular rejection as well as the renal composite endpoint consisting of 50% increase in serum creatinine, subsequent transplantation, and re-initiation of dialysis. Changes in whole parathyroid hormone (PTH), infection requiring hospitalization, and hypercalcemia defined as albumin-corrected calcium ≥11 mg/dl were also compared between the cholecalciferol and control groups.



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Blood samples were stored at −80 °C at each facility, collected after the end of the trial, and then assayed for serum 25(OH)D using Elecsys Vitamin D total II immunoassay.

### Statistical analyses

Details of the sample size calculation are described elsewhere [29]. In short, based on previous studies [6,19], we hypothesized that the 2-year eGFR decline in the high and low hemoglobin groups would be 1.03 (SD, 4.94) and 3.32 (SD, 4.94) ml/min per 1.73 $m^2$, respectively, and estimated that a sample size of 218 patients (109 in each group) would provide a power of 90% for the group comparison between the high vs. low hemoglobin target by using *t*-test with a type I error of 5%. We assumed 20% of dropout or lost-to-follow and planned to enroll 272 patients. Regarding the study power for cholecalciferol supplementation, we hypothesized that 1.77 ml/min per 1.73 $m^2$ in eGFR would be preserved by 11.8 ng/ml increase in serum 25(OH)D levels with 1000 IU/day of cholecalciferol supplementation [21,30], and the study size above was considered to provide a power of 70%.

Given slow recruitment and regulatory changes with the new Clinical Trials Act in Japan, an interim analysis plan with the Lan-DeMets α-spending function approach (Pocock type) was added to the protocol, which was authorized by the Ethics Committee on August 27, 2018 [31]. The interim analysis was performed by a member of the data and safety monitoring board who was independent of the execution of this study.

In the primary analysis, we performed t-test for group comparison of the primary outcome (i.e., changes in eGFR from baseline) on the full analysis set which included all randomized patients who underwent the final eGFR measurement at Month 24. Effect modifications were evaluated by including an interaction term between anemia management and cholecalciferol supplementation into linear regression model. We also evaluated effect modifications for each intervention by baseline levels of urinary protein, eGFR, and the length of time since transplantation as well as baseline hemoglobin or 25(OH)D. As nonprespecified sensitivity analyses in order to account for patients who dropped during the trial for any reason, we estimated eGFR decline using linear mixed effects model for repeated measures with both fixed and random intercept and slope, and then compared its difference by an interaction term between time and treatment group. Creatinine-based eGFR levels at Month 18 were added to this model during the review process, which did not change the results significantly. Missing eGFR levels at the following visit after dialysis initiation were imputed with those levels at dialysis initiation if <10 ml/min per 1.73 $m^2$ or 10 ml/min per 1.73 $m^2$ if 10 ml/min per 1.73 $m^2$ or greater.

Prespecified sensitivity analyses included multivariable adjustment for age, gender, transplant vintage, mean arterial blood pressure, baseline eGFR, urine protein creatinine ratio, and types of diabetes (i.e., type 1, type 2, or new onset diabetes after transplant). Among those covariates, baseline blood pressure was missing in seven patients (5%) and was imputed with multiple imputation method with five data sets.

For secondary outcomes, we used *t*-test, Wilcoxon rank-sum test, or log-rank test for group comparison, and generalized linear models or Cox proportional hazards model with adjustment for baseline levels or history of respective outcome event, appropriately.

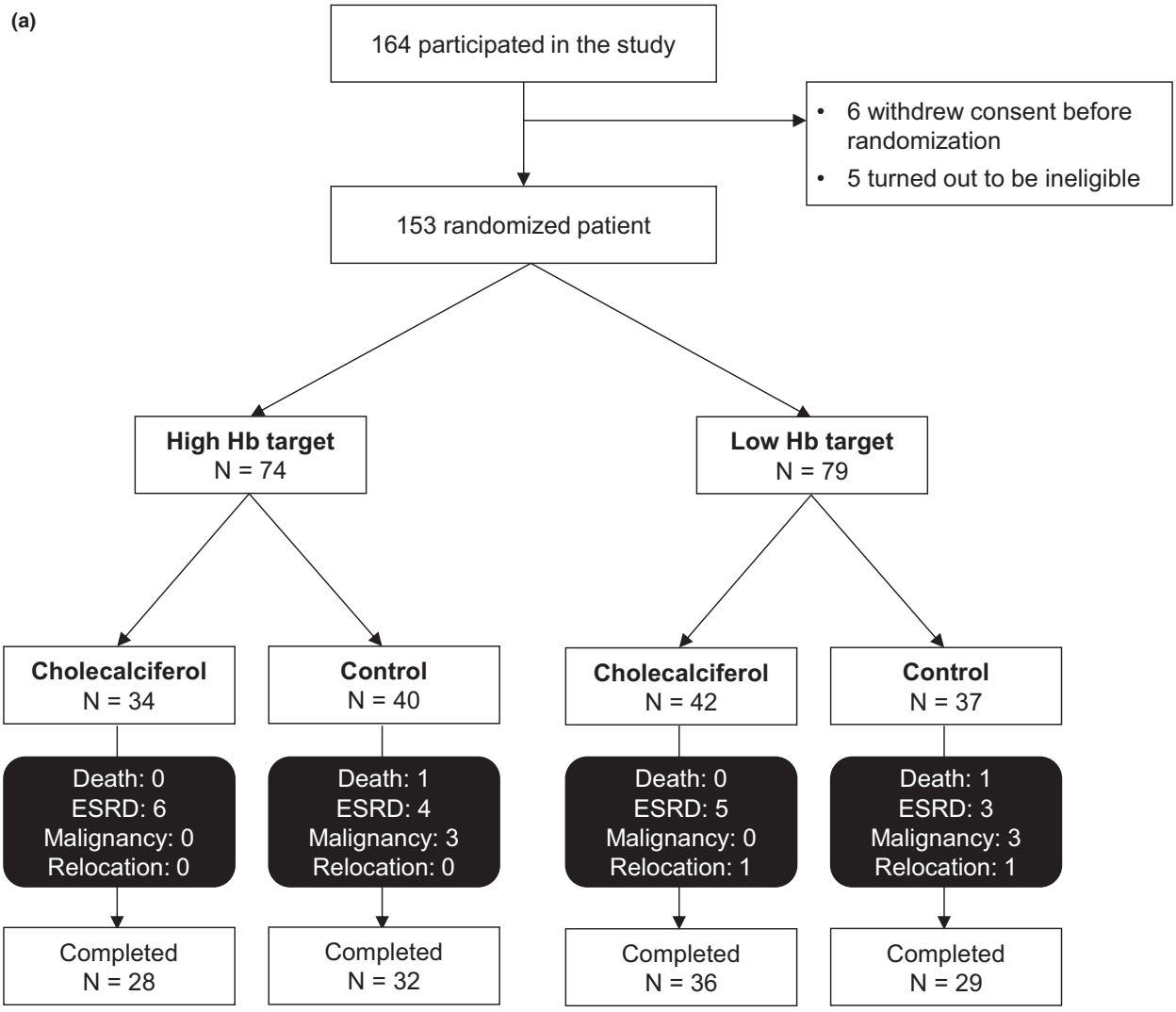
All analyses were done by intention to treat. Missing longitudinal data were in 2–3% for serum calcium and phosphorus, 5–10% for blood pressure, serum cystatin C, and whole PTH, 10–12% for $1,25(OH)_2D$, and 10–18% for 25(OH)D. Those results were presented without imputation. All statistical analyses were conducted using STATA IC 14 (Stata Corp., College Station, TX, USA).

## Results

The interim analysis was performed when a total of 153 were enrolled in the study. Approximately 4990 outpatients were screened and about 28% of eligible patients gave written informed consent. A flow chart of participants is shown in Fig. 1. Overall, the mean age was 51 ± 12 years, among whom 54% were male. The median (interquartile range, IQR) of transplant vintage was 8 (5, 12) years. The mean eGFR was 30.6 ± 11.0 ml/min/1.73 $m^2$, and the median (IQR) of urinary protein was 0.33 (0.12–1.04) g/g·Cr. The mean levels of hemoglobin and serum 25(OH)D were 10.7 ± 1.2 g/dl and 14.5 ± 5.2 ng/ml, respectively. Chronic glomerulonephritis accounted for approximately one half of the ESRD origins, followed by congenital anomalies of the kidney and urinary tract (12%). Most participants were on mycophenolate mofetil. The baseline characteristics including the levels of eGFR, hemoglobin, and serum 25(OH)D were similar across all groups (Table 1).

This study was terminated early based on the interim analyses done when 125 patients (82%) completed the study; the stopping boundary *P* value was <0.0343. The reasons for dropout from the study were death, ESRD, newly diagnosed malignancy, or relocation (Fig. 1). The

**(a)**




**(b)**

| | | *Hb target arm* | | |
|---|---|---|---|---|
| | | **≥12.5 g/dL** | **<10.5 g/dL** | **Total** |
| ***Cholecalciferol arm*** | **1000 IU/day** | 34 (22%) | 42 (27%) | **76 (50%)** |
| | **Control** | 40 (26%) | 37 (24%) | **77 (50%)** |
| | **Total** | **74 (48%)** | **79 (52%)** | 153 |

**Figure 1** (a) Flow chart of the 153 enrolled patients. (b) The numbers of participants randomized to each group (2 × 2 factorial design).

dropout rate was similar between respective arms (i.e., 18% [14 of 79] and 19% [14 of 74] in the low and high hemoglobin target groups, respectively, and 21% [16 of 77] and 16% [12 of 76] in the control and cholecalciferol groups, respectively).

### High vs. low hemoglobin target arm

Trajectories of hemoglobin and eGFRcr over the study period are shown in Fig. 2. Figure 2a,b contains all available data at each study period whereas data in

**Table 1.** Baseline characteristics of study participants.

| Characteristics | Hb target arm | | Cholecalciferol arm | |
|---|---|---|---|---|
| | <10.5 g/dl *N* = 79 | ≥12.5 g/dl *N* = 74 | Control *N* = 77 | 1000 IU/day *N* = 76 |
| Age, year | 52 ± 12 | 50 ± 12 | 52 ± 12 | 50 ± 12 |
| Male, *n* | 38 (48%) | 42 (57%) | 39 (51%) | 41 (54%) |
| Body weight, kg | 55 ± 12 | 57 ± 12 | 55 ± 12 | 57 ± 11 |
| ESRD origin, *n* | | | | |
| Chronic glomerulonephritis | 35 (44%) | 42 (57%) | 44 (57%) | 33 (43%) |
| CAKUT | 11 (14%) | 67 (10%) | 12 (15%) | 8 (10%) |
| Diabetes | 7 (9%) | 5 (7%) | 4 (5%) | 8 (11%) |
| Polycystic kidney disease | 7 (9%) | 3 (4%) | 4 (5%) | 6 (8%) |
| Other | 19 (24%) | 16 (22%) | 13 (17%) | 22 (29%) |
| Diabetes, *n* | | | | |
| NODAT | 1 (1%) | 4 (5%) | 3 (4%) | 2 (3%) |
| Type 1 | 2 (3%) | 3 (4%) | 2 (3%) | 3 (4%) |
| Type 2 | 6 (8%) | 7 (9%) | 7 (9%) | 6 (8%) |
| Transplant vintage, year | 8 [4, 13] | 7 [3, 13] | 7 [3, 14] | 8 [5, 12] |
| History of CVD, *n* | 4 (5%) | 8 (11%) | 6 (8%) | 6 (8%) |
| History of cancer, *n* | 5 (6%) | 4 (5%) | 5 (6%) | 4 (5%) |
| Calcineurin inhibitor, *n* | | | | |
| Tacrolimus | 49 (62%) | 40 (54%) | 49 (64%) | 40 (52%) |
| Cyclosporine | 27 (34%) | 31 (42%) | 26 (34%) | 32 (42%) |
| None | 3 (4%) | 3 (4%) | 2 (2%) | 4 (6%) |
| Antimetabolites, *n* | | | | |
| Mycophenolate mofetil | 63 (80%) | 60 (81%) | 60 (78%) | 63 (83%) |
| Azathioprine | 2 (3%) | 4 (5%) | 4 (5%) | 2 (3%) |
| Mizoribine | 9 (11%) | 6 (8%) | 6 (8%) | 9 (12%) |
| None | 5 (6%) | 4 (5%) | 7 (9%) | 2 (3%) |
| Everolimus, *n* | 18 (23%) | 11 (15%) | 20 (26%) | 9 (12%) |
| Steroids, *n* | 67 (85%) | 59 (80%) | 63 (82%) | 63 (83%) |
| RAAS inhibitor, *n* | 54 (68%) | 46 (62%) | 48 (62%) | 52 (68%) |
| Active vitamin D analogues, *n* | 13 (16%) | 9 (12%) | 14 (18%) | 8 (11%) |
| Systolic BP, mmHg | 126 ± 14 | 129 ± 15 | 125 ± 15 | 130 ± 13 |
| Diastolic BP, mmHg | 75 ± 9 | 78 ± 11 | 75 ± 11 | 77 ± 10 |
| Laboratory tests | | | | |
| Serum creatinine, mg/dl | 1.9 ± 0.7 | 2.0 ± 0.7 | 1.9 ± 0.7 | 2.0 ± 0.7 |
| eGFR, ml/min per 1.73 $m^2$ | 30.6 ± 10.8 | 30.5 ± 11.3 | 30.4 ± 11.0 | 30.7 ± 11.0 |
| Hemoglobin, g/dl | 10.6 ± 1.3 | 10.8 ± 1.2 | 10.4 ± 1.2 | 10.9 ± 1.2 |
| Iron saturation, % | 34 ± 14 | 34 ± 16 | 35 ± 16 | 33 ± 14 |
| Ferritin, ng/ml | 92 [56, 194] | 68 [31, 148] | 88 [46, 195] | 75 [44, 141] |
| Serum 25(OH)D, ng/ml | 14.5 ± 5.1 | 14.5 ± 5.3 | 14.3 ± 5.3 | 14.6 ± 5.2 |
| Urine protein, g/g·Cr | 0.3 [0.1, 1.1] | 0.4 [0.1, 1.0] | 0.3 [0.1, 0.7] | 0.3 [0.1, 1.6] |

25(OH)D, 25-hydroxy vitamin D; CAKUT, congenital anomalies of kidney and urinary tract; CVD, cardiovascular disease; ESA, erythropoiesis-stimulating agent.

Data are on the basis of all randomized patients. Continuous variables are presented as mean ± SD or median [25th, 75th percentile].

Fig. 2c,d were limited to those obtained from 125 patients who completed the trial.

Despite periodical monitoring and recommendations made by the central study team, the high hemoglobin target group showed lower mean levels (i.e., 11.4 g/dl; Fig. 2a) than originally planned with suboptimal dose of MPG-EPO (i.e., median 125–150 μg per month; Table S1). The low hemoglobin target group showed slightly higher mean levels (i.e., 10.6 g/dl) than planned with median 0–50 μg per month of MPG-EPO. Consequently, the mean hemoglobin levels of both groups were within the range recommended by

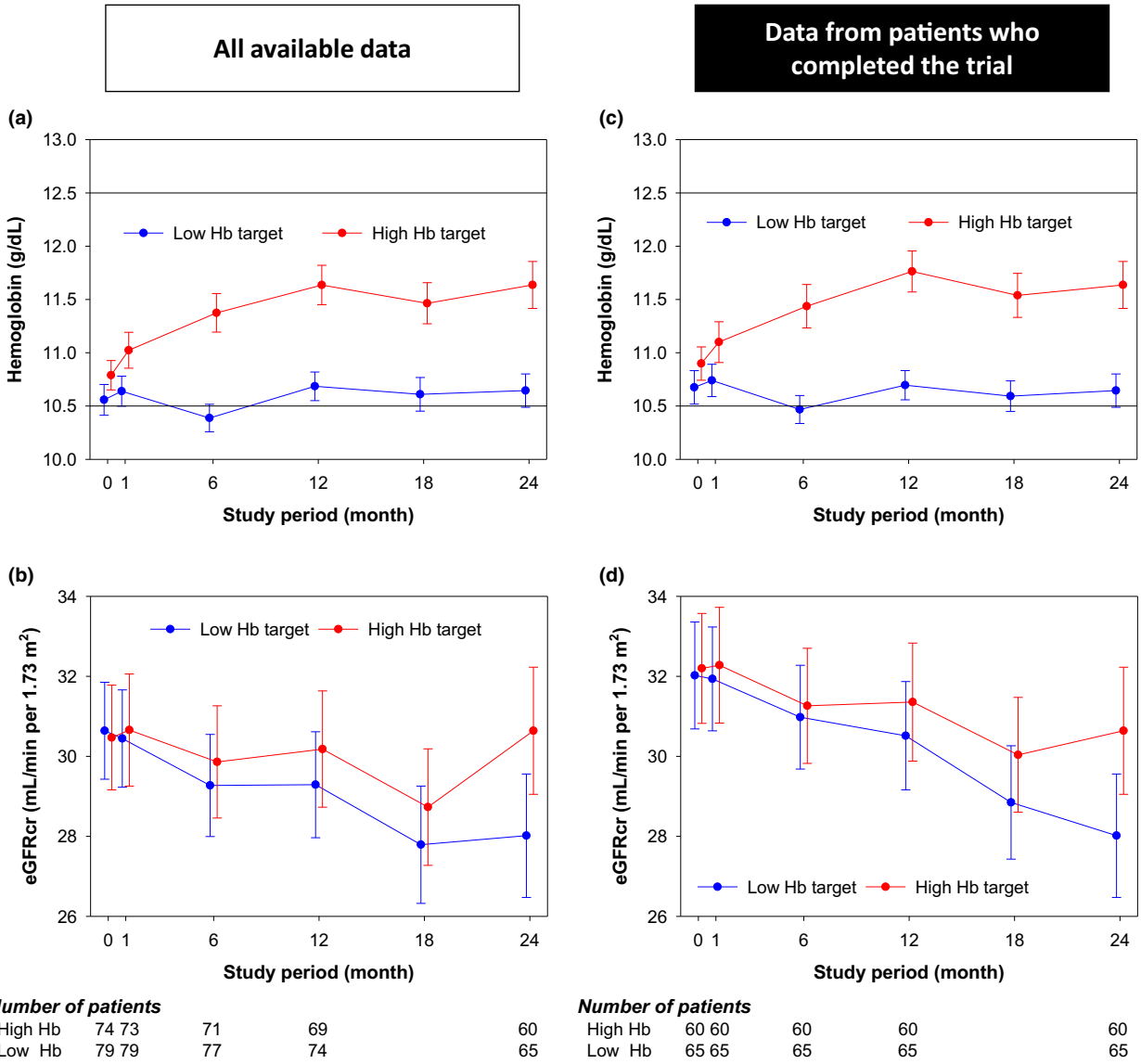



**Figure 2** Changes in mean hemoglobin and creatinine-based eGFR (eGFRcr) based on all available data (panels a and b, respectively) and data from 125 patients who completed the trial (panels c and d, respectively) in the high vs. low hemoglobin target arm. There were no missing data in hemoglobin and creatinine-based eGFR. Bars represent SE.

the KDIGO guidelines [18], and the between-group difference in hemoglobin was limited to 0.8 (95% CI, 0.5–1.2) g/dl, smaller than the original assumption of 2.0 g/dl. During the study period, the iron saturation and ferritin levels were stable in the low Hb group but the high hemoglobin group showed a small increase in the mean iron saturation levels from 34% to 38% at and after the 6-month visit and a gradual increase in the median ferritin levels from 68 to 140 ng/ml. However, these iron indices were not significantly different between groups throughout the study period except for iron saturation at 12 months. There were no significant between-group differences in MAP or MMF dose throughout the study period (Table S1).

Among 125 participants who completed the trial, the decline in eGFRcr over 2 years was significantly smaller in the high hemoglobin target group ($N = 60$; −1.6 [95% CI, −2.7 to −0.4] ml/min/1.73 m$^2$) than the low hemoglobin target group ($N = 65$; −4.0 [95% CI, −5.7 to −2.3] ml/min/1.73 m$^2$) with the between-group difference of 2.4 (0.4–4.5) ml/min/1.73 m$^2$ ($P = 0.021$). Consistent results were obtained for eGFRcys (Fig. S1), and these findings were robust against baseline and multivariate adjustments (Table S2). The interaction term between the hemoglobin group and time in the



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mixed effects model showed a $P$ value of 0.044. Estimated annual change in eGFRcr was −1.6 (95% CI, −2.1 to −1.2) ml/min/1.73 $m^2$ per year and −2.3 (95% CI, −2.8 to −1.8) ml/min/1.73 $m^2$ per year for the high and low hemoglobin target groups, respectively. The between-group difference was 0.7 (0.0–1.4) ml/min/1.73 $m^2$ per year in unadjusted and baseline-adjusted model ($P$ = 0.059 and 0.046, respectively) and was 0.8 (0.2–1.4) ml/min/1.73 $m^2$ per year in the fully adjusted model ($P$ = 0.013; Table S2). There were no differences in secondary efficacy and safety endpoints between low vs. high hemoglobin target groups (Table 2).

Prespecified stratified analyses revealed that the effect of the high hemoglobin target regimen was not modified by transplant vintage, baseline eGFRcr, or baseline hemoglobin but was significantly pronounced among patients with urine protein creatinine ratio of ≥0.5 g/g·Cr vs. <0.5 g/g·Cr ($P_{interaction}$ = 0.029; Fig. 3 and Table S3). Additionally, cholecalciferol supplementation did not modify the effect of the high Hb target strategy ($P_{interaction}$ = 0.74).

### Cholecalciferol vs. control arm

Among patients in the cholecalciferol group, all but one reported good adherence to vitamin D. Trajectories of serum 25(OH)D and eGFRcr over the study period are shown in Fig. 4. Figure 4a,b contains all available data at each study period whereas data in Fig. 4c,d is limited to those obtained from 125 patients who completed the trial.

The cholecalciferol 1000 IU/day group showed higher mean 25(OH)D levels than the control group and its mean level achieved >30 ng/ml after Month 6 (Fig. 4a) whereas the control group showed a mean level of 14.8 ng/ml. Consequently, the between-group difference in serum 25(OH)D was 13.8 ng/ml, slightly greater than the original assumption of 11.8 ng/ml. There were no significant differences in MPG-EPO dose, MMF dose, or MAP throughout the study period except for MAP at Month 24 being higher in the cholecalciferol group (i.e., 97 ± 10 mmHg vs. 92 ± 10 mmHg in the control group; $P$ = 0.011; Table S1).

Among 125 participants who completed the trial, the decline in eGFRcr over 2 years was not significantly different between the cholecalciferol group ($N$ = 64; −3.7 [95% CI, −5.2 to −2.2] ml/min/1.73 $m^2$) and the control group ($N$ = 61; −1.9 [95% CI, −3.4 to −0.4] ml/min/1.73 $m^2$; $P$ = 0.097). A sensitivity analysis based on the mixed effects model showed a consistent result ($P$ = 0.11). Estimated annual change in eGFRcr was −2.2 (95% CI, −2.8 to −1.7) ml/min/1.73 $m^2$ per year and −1.7 (95% CI, −2.2 to −1.2) ml/min/1.73 $m^2$ per year for the cholecalciferol and control groups, respectively ($P$ = 0.14). Consistent results were obtained for eGFRcys (Fig. S1), and these findings were robust against baseline and multivariate adjustments (Table S2).

**Table 2.** Secondary efficacy and safety endpoints.

| | Hemoglobin target arm | | | Cholecalciferol arm | | |
|---|---|---|---|---|---|---|
| Secondary outcomes | <10.5 g/dl $N$ = 79 (%) | ≥12.5 g/dl $N$ = 74 (%) | $P$ | Control $N$ = 77 (%) | 1000 IU/day $N$ = 76 (%) | $P$ |
| 50% increase in serum creatinine | 21 (27) | 12 (16) | 0.12 | 14 (18) | 19 (25) | 0.31 |
| Initiation of dialysis or kidney transplant* | 8 (10) | 10 (14) | 0.52 | 7 (9) | 11 (14) | 0.30 |
| Renal composite† | 22 (28) | 14 (19) | 0.19 | 14 (18) | 22 (29) | 0.12 |
| All-cause death‡ | 2 (3) | 1 (1) | 0.60 | 3 (4) | 0 (0) | 0.08 |
| Malignancy*,‡ | 4 (5) | 3 (4) | 0.77 | 7 (9) | 0 (0) | 0.007 |
| Stroke | 1 (1) | 0 (0) | 0.33 | 1 (1) | 0 (0) | 0.32 |
| Blood transfusion | 1 (1) | 0 (0) | 0.33 | 1 (1) | 0 (0) | 0.32 |
| Biopsy-proven acute cellular rejection | 1 (1) | 1 (1) | 0.96 | 0 (0) | 2 (3) | 0.15 |
| Infection requiring hospitalization | 7 (9) | 4 (5) | 0.41 | 7 (9) | 4 (5) | 0.36 |
| Hypercalcemia¶ | 2 (3) | 1 (1) | 0.60 | 2 (3) | 1 (1) | 0.57 |

*One patient in the low Hb target group and the no cholecalciferol group was diagnosed with malignancy after developing ESRD.

†50% increase in serum creatinine, initiation of dialysis or kidney transplant

‡One patient in the low Hb target group and the no cholecalciferol group died after being diagnosed with malignancy.

¶Defined as albumin-corrected calcium ≥11 mg/dl.

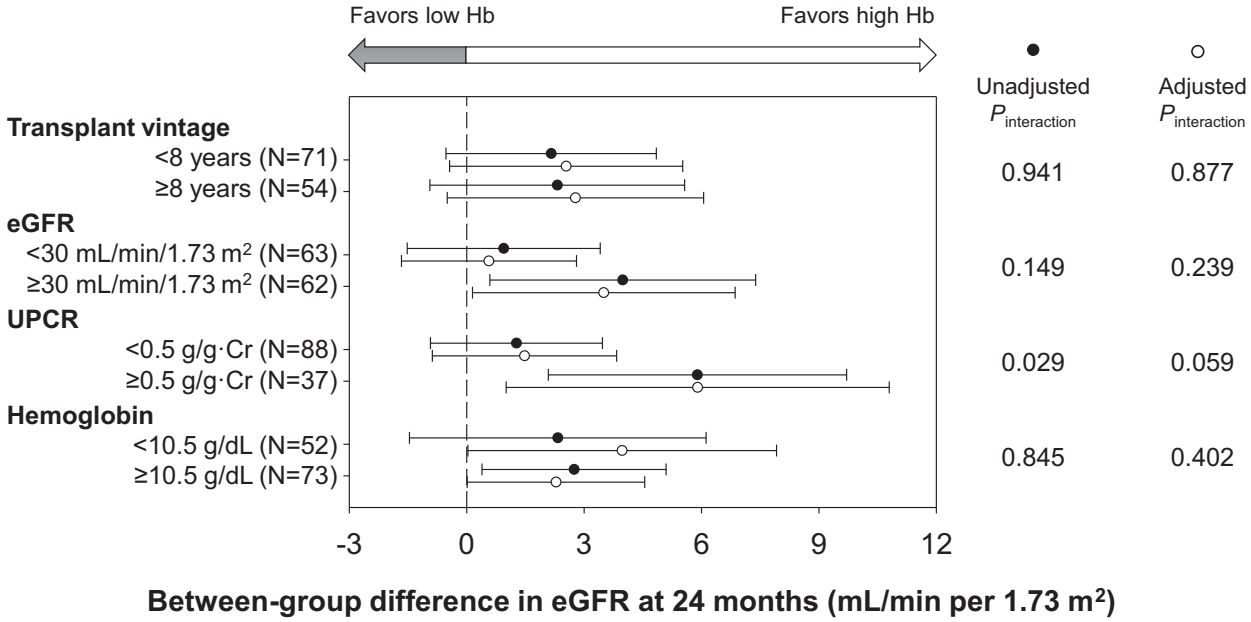


**Figure 3** Stratified analyses for the between-group difference in 2-year changes in creatinine-based eGFR (eGFRcr) among 125 participants who completed the study. eGFR, estimated glomerular filtration rate; Hb, hemoglobin; UPCR, urine protein creatinine rate.

Prespecified stratified analyses indicated that cholecalciferol supplementation may accelerate the decline in allograft function among patients with eGFR ≥30 ml/min/1.73 $m^2$ but not among those with eGFR <30 ml/min/1.73 $m^2$ ($P_{interaction}$ = 0.024; Fig. 5 and Table S4). Otherwise, the effect of cholecalciferol was not modified by transplant vintage, urinary creatinine-protein ratio, or baseline 25(OH)D.

No one in the cholecalciferol group but seven patients (9%) in the control group developed malignancy ($P$ = 0.007); two lung cancers, one breast cancer, one gastric cancer, one testicular cancer, 1 renal cell carcinoma, and one myelodysplastic syndrome. There were no differences in the other secondary efficacy and safety endpoints between cholecalciferol and control groups (Table 2).

Table S5 shows the trajectories of the parameters of CKD-MBD. In the cholecalciferol group, 1,25(OH)2D increased with 25(OH)D, and the levels of 25(OH)D and 1,25(OH)2D were greater than the control group after Month 1 ($P$ < 0.001 and <0.025, respectively). However, there were no significant differences in the levels of calcium, phosphorus, or whole PTH at any point.

## Discussion

This nation-wide, open-label RCT demonstrated that maintaining high hemoglobin level using MPG-EPO slowed the decline in eGFR over 2 years among KTRs. Cholecalciferol supplementation did not show such renoprotective effect, but the 2-year incidence of malignancy was significantly less in the cholecalciferol group vs. the control group (i.e., 0/76 vs. 7/77, respectively). Cholecalciferol supplementation also effectively increased $1,25(OH)_2D$, but did not affect serum calcium or whole PTH levels.

The slower decline in eGFR in the high hemoglobin target group is consistent with two previous RCTs; one is the CAPRIT study [19] and the other is a single center trial by Tsujita, *et al.* from Japan [32]. In addition to our study being the largest RCT, the major differences among those studies include the type of ESA and the achieved hemoglobin levels. Our study and Tsujita *et al.* used long-acting ESAs (i.e., MPG-EPO and darbepoetin-α, respectively), not epoetin-β as in the CAPRIT study, as longer administration intervals, reduce the need for office visit and hence is preferred for outpatients. The dose of ESAs used was equivalent among those three studies, but compared to the other studies, achieved hemoglobin levels in our study were lower in both high and low hemoglobin target groups, and the between-group difference was also smaller. The higher resistance to ESA treatment in our study likely reflects the difference in the inclusion criteria for baseline hemoglobin levels; we included only KTRs who had hemoglobin <10.5 g/dl or who were already on any ESA treatment while the other studies included those with hemoglobin <11.5 g/dl.



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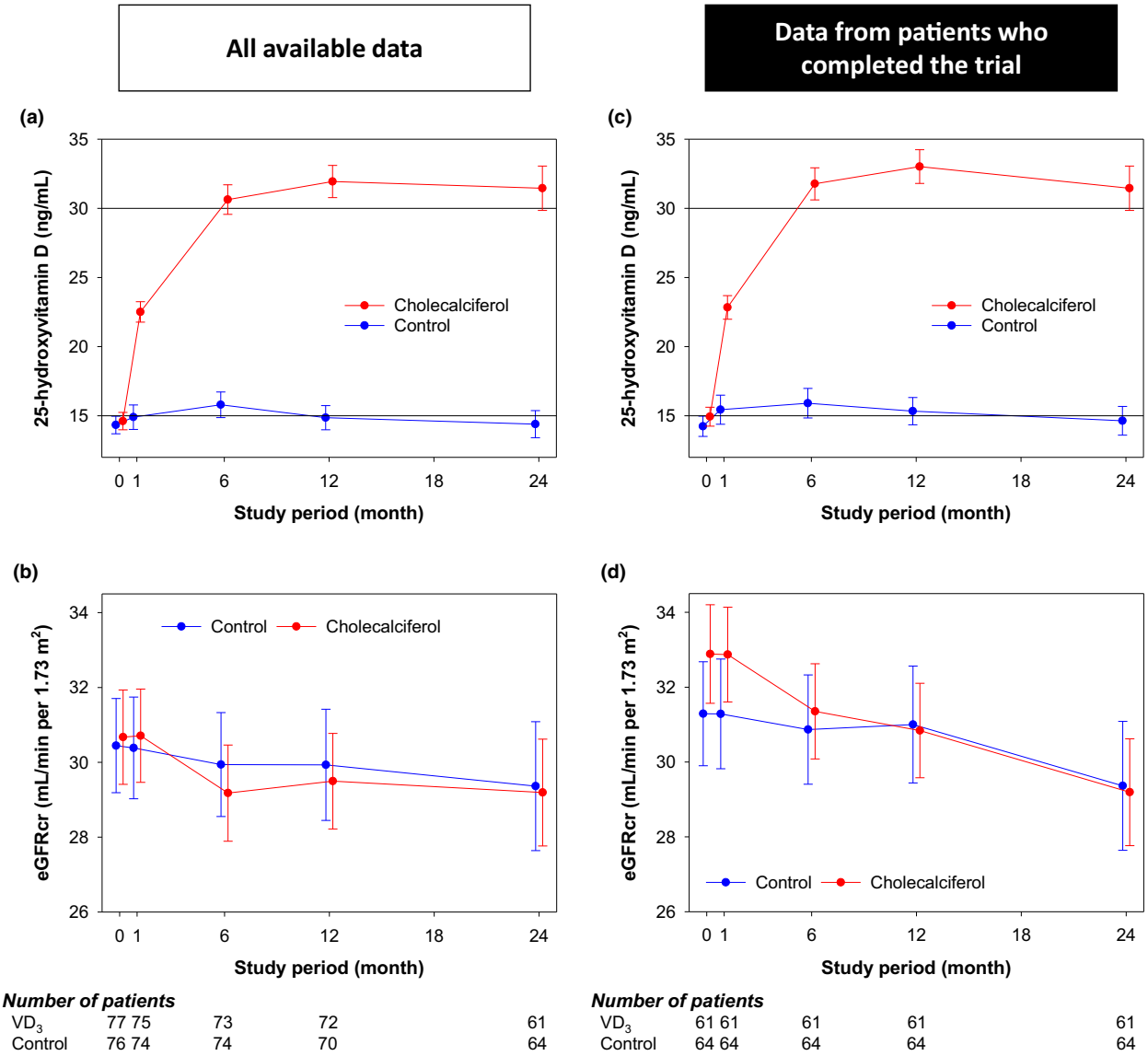


**Figure 4** Changes in mean 25-hydroxyvitamin D and creatinine-based eGFR (eGFRcr) based on all available data (panels a and b, respectively) and data from 125 patients who completed the trial (panels c and d, respectively) in the cholecalciferol vs. control arm. Missing data on 25-hydroxyvitamin D were 10%, 15%, 16%, 17%, and 10% at Month 0, 1, 6, 12, and 24, respectively. There were no missing data on creatinine-based eGFR. Bars represent SE.

Since high-dose ESA treatment entails unclear long-term safety profile along with greater cost, it is imperative to identify populations that would benefit most from this intervention. The effect of the high hemoglobin target was subdued among participants with less proteinuria, which can be explained by the fact that they are less likely to lose their kidney function without intervention anyway. Additionally, the high hemoglobin target is neither likely to be effective for those with advanced chronic allograft dysfunction. The number of patients who reached ESRD between the high vs. low hemoglobin target group were not different, and their median eGFRcr and urine protein creatinine ratio were 18.0 (15.9, 22.2) ml/min/1.73 $m^2$ and 2.4 (1.0, 4.9) g/g·Cr at baseline. Our study results suggest proteinuric KTRs may benefit from early intervention in the anemia management.

The dose of MPG-EPO in the high hemoglobin group was often not increased by participating physicians to reach its target level, and the achieved hemoglobin levels in both groups resulted in the range suggested by the KDIGO Clinical Practice Guidelines [18]. This is likely one of the limitations resulting from the open-label study design. Although the CAPRIT

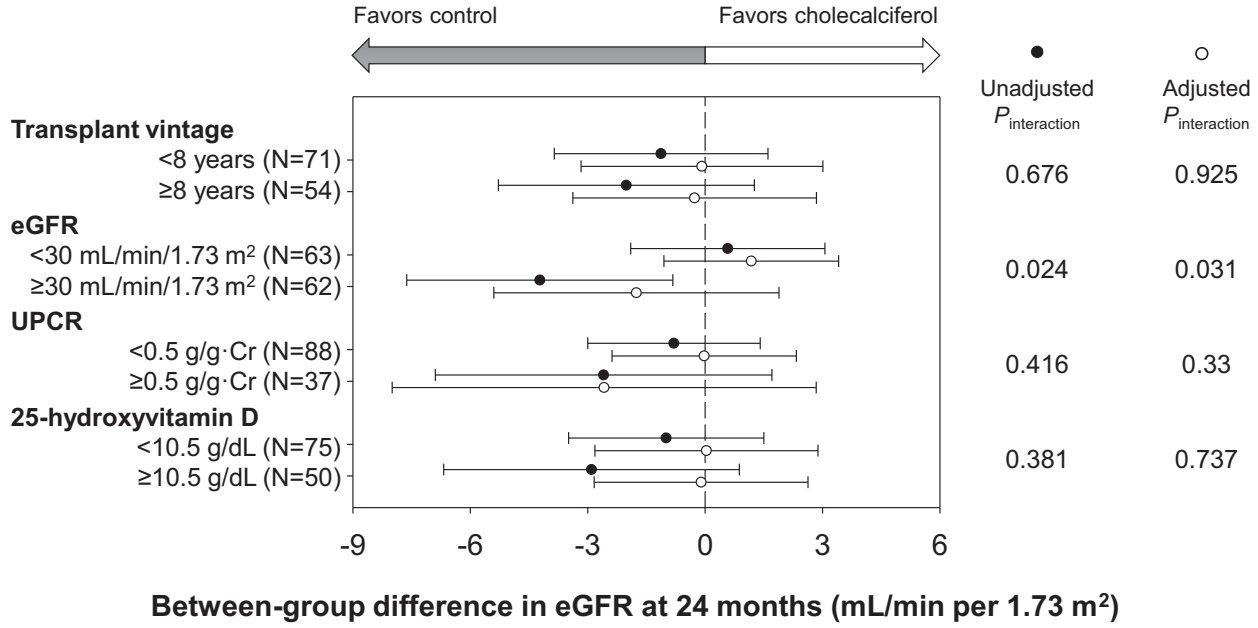


**Figure 5** Stratified analyses for the between-group difference in 2-year changes in creatinine-based eGFR (eGFRcr) among 125 participants who completed the study. eGFR, estimated glomerular filtration rate; Hb, hemoglobin; UPCR, urine protein creatinine rate. Bars represent 95% confidence intervals.

study and Tsujita *et al*. reported larger between-group differences in achieved hemoglobin levels and greater efficacy of the high hemoglobin target strategy on eGFR than our study [19,32], which suggests a dose-dependent effect of ESA, it remains unclear at which level of hemoglobin and with what dose KTRs can most benefit from ESA treatment. Nevertheless, our study confirmed that post-transplant anemia needs to be managed differently from anemia in CKD and that hemoglobin levels of KTRs should be maintained higher than what is currently recommended in the general CKD population.

Cholecalciferol supplementation 1000 IU/day effectively increased the mean serum 25(OH)D levels to the target >30 ng/ml. However, among patients with baseline eGFRcr ≥30 ml/min/1.73 $m^2$, the cholecalciferol group experienced more rapid decline in eGFRcr than the control group, which goes against our original hypothesis. A previous study showed that vitamin D receptor activation increases serum creatinine due to increased production, leading to false decrease in eGFRcr [33]. Our patients with baseline eGFRcr ≥30 ml/min/1.73 $m^2$ did increase their $1,25(OH)_2D$ levels to a greater extent than those with eGFRcr <30 ml/min/1.73 $m^2$, but we confirmed consistent negative effects of cholecalciferol on cystatin C-based eGFR (data not shown). Excessive vitamin D may actually promote kidney fibrosis by modulating macrophage phenotype [34]. Initial preliminary data from the VITA-D study, a placebo-controlled RCT evaluating the effect of cholecalciferol among KTRs, also showed negative effects on allograft function [35].

No patients in the cholecalciferol group but 9% of patients in the control group developed malignancy. Malignancy is one of the prespecified secondary outcomes based on previous publications [22,23]. However, this effect size appears too good to be true. Large RCTs with long follow-up periods are necessary to confirm this cancer preventive effect and to assess the risk-benefit balance against potential negative effects on kidney allograft function.

Several limitations should be noted in our study. First, our study was not designed to evaluate hard clinical outcomes such as graft failure or all-cause mortality. Second, patients were screened at the discretion of the local investigators, and the number of screened patients is not clear. Third, our open-label study design was susceptible to study protocol violation as discussed above. Fourth, it is unclear whether hypoxia-inducible factor prolyl hydroxylase inhibitors have a similar renoprotective effect to ESAs [36]. This new class of agents can be given orally, which may be a more favorable route for patients not undergoing hemodialysis. However, their possible effect on tumor growth remains a concern especially among KTRs. Lastly, data on 25(OH)D, 1,25 $(OH)_2D$, and whole PTH were missing up to 20% during the follow-up.

## Conclusion

Maintaining high hemoglobin levels with MPG-EPO showed a potential to preserve allograft kidney function. Further large long-term RCTs are needed to examine the effects of anemia correction and vitamin D supplementation on hard outcomes and to evaluate external validity in other races/ethnicities.

## Authorship

The authors' responsibilities were as follows—YO and TH conceived and designed the study. YO, NI, KI, DI, KS, DI, HH, KS, SS, KM, NA, TI, MN, TY, SI, MA, JK, KY, SS, YU, YI, KY, YT and ST: involved in the provision of study materials or patients and data acquisition. YO, YS and TH: analyzed and interpreted the results. YO: drafted the report, and the other authors gave critical revision of the manuscript for scientific and factual content. TH: principal investigator, involved in full access to all the data in the study, and the final responsibility for the decision to submit for publication. All authors read and approved the final manuscript.

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## Conflict of interest

Dr. Hamano has received honoraria and/or support from Chugai, Otsuka, Torii, Kissei, Kyowa Hakko Kirin, Terumo, Fuso, Eisai, and Takeda, outside the submitted work. TH have received honoraria and research grant support from Chugai Pharmaceutical Co., Ltd. and Kissei Pharmaceutical Co., Ltd. All other authors declare no conflicts of interest.

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## Previous presentation

Preliminary results of this study have been partly presented as posters at the Kidney Week 2019 of American Society of Nephrology, Washington, DC, United States and at the Kidney Week 2020 of American Society of Nephrology, Denver, CO, United States.

## Data availability statement

The deidentified participant-level data collected during the trial that underlie the results reported in this article will be made available 6 months after publication and ending 3 years after publication of this article. The data

will be provided mainly for individual participant data meta-analysis and will be shared with researchers whose proposed use of the data has been approved by an independent review committee identified for this purpose to achieve aims in the approved proposal. Requestors will need to sign a data access agreement and data will be sent through a third-party website. Other supporting documents (study protocol, statistical analysis plan, informed consent form, clinical study report, and analytic code) will not be available. Study design is available on ClinicalTrials.gov (NCT01817699).

## SUPPORTING INFORMATION

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

**Table S1**. Changes in the dose of Methoxy polyethylene glycol epoetin beta, mycophenolate mofetil, and mean arterial pressure in the hemoglobin target arm and the cholecalciferol arm.

**Table S2**. Between-group differences in 2-year change in eGFR and estimated annual decline in eGFR in the hemoglobin target arm and the cholecalciferol arm.

**Table S3**. Changes in creatinine-based eGFR over 2 years according to pre-specified stratification in the hemoglobin target arm.

**Table S4**. Changes in creatinine-based eGFR over 2 years according to pre-specified stratification in the cholecalciferol arm.

**Table S5**. Pre-specified stratified analyses in the cholecalciferol arm.

**Figure S1**. Changes in cystatin C-based eGFR (eGFRcys) based on all available data (panel a) and data from 125 patients who completed the trial (panel b) in the high vs. low hemoglobin target arm.

**Figure S2**. Changes in cystatin C-based eGFR (eGFRcys) based on all available data (panel a) and data from 125 patients who completed the trial (panel b) in the high vs. low hemoglobin target arm.

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## APPENDIX 1

## CANDLE-KIT trial investigators

*Steering Committee Members*: Yoshitsugu Obi, Naotsugu Ichimaru, Yusuke Sakaguchi, Yoshiharu Tsubakihara, Shiro Takahara, and Takayuki Hamano (Osaka University); *Independent Data and Safety Monitoring Committee Members*: Shinsuke Nanto (Hyogo Prefectural Nishinomiya Hospital), Masamitsu Fujii and Satoshi Mikami (Higashikouri Hospital), and Takahito Ito (Kataguilli Medical Center); *Principal Investigator*: Takayuki Hamano (Osaka University); *Participating Centers*: Hokkaido University Hospital, Sapporo, Hokkaido, Japan; Sapporo City General Hospital, Sapporo, Hokkaido, Japan; Akita University Hospital, Akita, Akita, Japan; National Hospital Organization Mito Medical Center, Higashiibarakigun, Ibaraki, Japan; Tokyo Women's Medical University Hospital, Tokyo, Japan; Omori Medical Center, Toho University School of Medicine, Tokyo, Japan; Toranomon Hospital Kajigaya, Kawasaki, Kanagawa, Japan; Kitasato University Hospital, Sagamihara, Kanagawa, Japan; Chiba-East National Hospital, Chiba, Chiba, Japan; Gifu University Hospital, Gifu, Gifu, Japan; Sunshine M&D Clinic, Mizuho, Gifu, Japan; Nagoya Daini Red Cross Hospital, Nagoya, Aichi, Japan; Inoue Hospital, Suita, Osaka; Takatsuki General Hospital, Takatsuki, Osaka, Japan; Nara Medical University, Kashihara, Nara, Japan; Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Hyogo, Japan; Okayama Medical Center, Okayama, Okayama, Japan; Okayama University Hospital, Okayama, Okayama, Japan; Kagawa University, Kita, Kagawa, Japan; Kyushu University Hospital, Fukuoka, Fukuoka, Japan; Harasanshin Hospital, Fukuoka, Fukuoka, Japan; Japanese Red Cross Kumamoto Hospital, Kumamoto, Kumamoto, Japan; Tomishiro Central Hospital, Tomigusuku, Okinawa, Japan.