

# ELEVATE: an innovative study design to assess the efficacy, safety, and evolution of cardiovascular parameters in de novo kidney transplant recipients after early conversion from a calcineurin inhibitor to everolimus

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**Abstract:** Progressive decline in allograft function and cardiovascular mortality after kidney transplantation remain major clinical challenges that can potentially be addressed by the mammalian target of rapamycin (mTOR) inhibitors, everolimus and sirolimus. mTOR inhibitors maintain immunosuppressive efficacy after minimization of calcineurin inhibitor (CNI) therapy and can achieve significant long-term improvements in renal function. Recently, data have accumulated that suggest mTOR inhibitors may offer cardioprotective effects. In animal models, inhibition of mTOR leads to regression of cardiac hypertrophy, and the limited data consistently point to a remodeling benefit following heart transplantation. Experimentally, mTOR inhibitors restrict atherogenesis, confirmed clinically by intravascular ultrasound data demonstrating lower rates of transplant vasculopathy in heart transplant recipients on everolimus. Lastly, mTOR inhibitors appear to ameliorate arterial stiffness, a known risk factor for post-transplant cardiovascular events, but data remain sparse. The ELEVATE study will examine the renal effect of early conversion from CNI therapy to everolimus after kidney transplantation. Key secondary endpoints include the change in left ventricular mass index, the first time this endpoint has been included in a prospective study of an mTOR inhibitor. The occurrence of cardiovascular events will be rigorously documented and pulse wave velocity is being measured in a subpopulation of patients. Results from this innovative trial are awaited with interest.

**Keywords:** cardiovascular, calcineurin inhibitors, ELEVATE, everolimus, kidney transplantation, mammalian target of rapamycin

## Introduction

One of the most promising developments regarding immunosuppression after solid organ transplantation in recent years has been the introduction of the mammalian target of rapamycin (mTOR) inhibitors, everolimus and sirolimus. A series of randomized clinical trials employing mTOR inhibitors within various regimens in different patient populations has examined the efficacy and safety of this class of immunosuppressants.<sup>1,2</sup> The results have shown that mTOR inhibitors not only permit minimization of exposure to calcineurin inhibitor (CNI) therapy, with an associated reduction in CNI-related nephrotoxicity, but that their pleiotropic effects offer the potential to reduce post-transplant malignancy and transplant vasculopathy.<sup>3-5</sup> Use of mTOR inhibitors may also reduce post-transplant viral infections.<sup>6,7</sup> Research efforts are now focused

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on examining the long-term effect of immunosuppression based on mTOR inhibitors. Is the renal benefit conferred by mTOR inhibitors versus conventional CNI-based therapy sustained over time? Does the evidence for a cardioprotective influence of mTOR inhibitors translate to a clinically relevant effect?

ELEVATE is a prospective, randomized trial that addresses key questions relating to use of everolimus in de novo kidney transplant recipients. It assesses the renal effect of early conversion from CNI therapy to everolimus after kidney transplantation as the primary endpoint. In addition, a key secondary endpoint is the change in left ventricular mass index under everolimus or CNI therapy, the first time a cardiac variable of this type has been included in a trial of mTOR inhibition after kidney transplantation. Furthermore, in a preplanned substudy, pulse wave velocity is being measured prospectively at centers of excellence to substantiate or refute preliminary evidence that conversion to mTOR inhibitors can improve conduit artery stiffness.<sup>8,9</sup> Lastly, the occurrence of cardiovascular events is being carefully documented in a more systematic manner than is standard in clinical trials of kidney transplant patients. This paper considers the issues underpinning the rationale for the ELEVATE study, and describes the key methodological features of the trial.

## Barriers to improving long-term outcomes after kidney transplantation

The improvement in short-term outcomes after kidney transplantation over the last decade is well documented, with a marked improvement in both graft function and survival during the first year post-transplant.<sup>10</sup> One-year graft survival now exceeds 90% after deceased donation and 95% after living donation.<sup>10,11</sup> However, although long-term graft survival rates have also increased somewhat over time, there continues to be substantial attrition, with approximately one in three deceased donor recipients and one in four living donor recipients returning to dialysis within 10 years.<sup>10</sup> Even more striking is the proportion of kidney transplant patients who die with a functioning graft, which has not improved at all over the last decade.<sup>10</sup> Approximately 20% of kidney transplant patients die within 10 years of engraftment.<sup>10</sup> Data from the international Collaborative Transplant Study indicate that during the first year, almost 5% of kidney transplant recipients die with a functioning graft, with a further 2% per year subsequently.<sup>12</sup> After the first year post-transplant, cardiovascular disease is the most common

cause of death,<sup>12,13</sup> followed by infectious death and, latterly, malignancy.<sup>14,15</sup> It has been estimated that the annual rate of fatal or nonfatal cardiovascular events is 3.5%–5.0% following kidney transplantation, which is 50-fold higher than in the nontransplanted general population.<sup>16</sup> Typically, kidney transplant patients are older than the general population and are at increased risk of conventional cardiovascular risk factors, compounded by complications related to chronic kidney disease and maintenance immunosuppressive therapy.<sup>17</sup> Thus, both long-term kidney graft failure and the worryingly high rate of cardiovascular mortality represent major clinical challenges.

## mTOR inhibition: an alternative immunosuppressive strategy

Everolimus and sirolimus block the mTOR pathway initiated via the mTORC1/RAPTOR complex. By acting synergistically with CNI therapies, mTOR inhibitors can help avoid progressive loss of graft function by minimizing CNI exposure to attenuate chronic CNI-related nephrotoxicity.<sup>18</sup> As experience has grown, however, the impact of mTOR inhibitors on other post-transplant complications has become apparent. mTOR inhibitors block the phosphatidylinositol-3-kinase pathway, and sensitize tumor cells to apoptosis, exerting an antineoplastic effect<sup>19</sup> that has been convincingly demonstrated for everolimus in renal cell carcinoma.<sup>20</sup> Everolimus is licensed for the treatment of advanced renal carcinoma and for advanced breast cancer. More recently, interest in the role of mTOR inhibitors in improving long-term outcomes has been heightened by evidence concerning a possible cardioprotective role of this class of immunosuppressants,<sup>21</sup> which could represent an important opportunity to improve long-term outcomes after kidney transplantation.

## Preserving renal function

The nephrotoxic effect of long-term CNI therapy represents an important cause of histological damage and progressive loss of graft function<sup>22,23</sup> even at low doses.<sup>24,25</sup> Since renal output declines only late in the course of progressive glomerulosclerosis, after irreversible interstitial fibrosis and arteriolar hyalinosis have become established,<sup>26</sup> preservation of kidney function is best achieved by pre-emptive changes to the immunosuppressive regimen. Mycophenolic acid with complete CNI avoidance<sup>27</sup> or early CNI discontinuation<sup>28–30</sup> after kidney transplantation does not provide adequate protection against rejection. Similarly, entirely CNI-free therapy with an mTOR inhibitor appears inadvisable.<sup>31</sup> In contrast, mTOR inhibition with reduced CNI exposure or

early CNI withdrawal has been shown to offer effective immunosuppression. Several randomized trials have shown that immunosuppressive efficacy is maintained with low (or very low) exposure to CNI therapy from time of transplant in the presence of either everolimus or sirolimus.<sup>32–34</sup> However, a significant benefit in terms of renal function was not consistently observed in these trials, likely due to persistent CNI exposure with subsequent nephrotoxicity. A more marked advantage in terms of renal function has been achieved by switching patients from CNI therapy to an mTOR inhibitor, so long as this is undertaken within the first 6 months after kidney transplantation.<sup>18</sup> There is evidence that early CNI-free regimens may increase the risk of mild acute rejection, but such episodes are typically reversible and do not appear to adversely affect kidney function or survival. The primary analysis of these trials has typically been performed at one year post-transplant,<sup>35–39</sup> a point at which renal function predicts long-term graft survival.<sup>40,41</sup> Follow-up data to 5 years post-transplant from the ZEUS trial (in which conversion took place at month 4.5 post-transplant)<sup>42–44</sup> and 2-year results from the HERAKLES trial (conversion at month 3)<sup>44</sup> have confirmed that a renal benefit of switch to mTOR inhibition is maintained after the first year.

## Cardiac remodeling

In the last few years, various lines of evidence have pointed to a new area of potential benefit for mTOR inhibition after kidney transplantation, ie, cardioprotection. A major area of interest is cardiac remodeling. Experimental evidence has shown that inhibition of mTOR can lead to regression of cardiac hypertrophy induced by pressure overload<sup>45–47</sup> or after myocardial infarction,<sup>48</sup> an effect that is associated with reduced cardiac fibrosis<sup>47</sup> and increased autophagy.<sup>48</sup> These direct effects may be enhanced by reduced blood pressure under mTOR inhibition compared with CNI therapy, although the contribution of an antihypertensive effect remains to be confirmed. CNI agents exert a potent hypertensive effect by increasing oxidative stress and arteriolar vasoconstriction,<sup>16,49,50</sup> and CNI-free regimens with mTOR inhibitor immunosuppression are associated with either a numerical or significant reduction in blood pressure or use of antihypertensive medication.<sup>21</sup>

A small number of nonrandomized trials in kidney<sup>51</sup> and heart<sup>52–55</sup> transplantation and one substudy analysis of a randomized trial in kidney transplant patients<sup>56</sup> have measured left ventricular mass and other remodeling parameters under mTOR inhibition (Table 1). The limited available data in kidney transplantation are conflicting, while more consistent

benefits have been observed in heart transplant patients, typically with longer follow-up (Table 1).

## Antiatherosclerotic effect

mTOR inhibitors also exert a protective role by inhibiting atherogenesis.<sup>57</sup> Indeed, it has been proposed that mTOR inhibitors may be a promising intervention for the management of atherosclerosis aside from their immunosuppressive properties.<sup>58</sup> mTOR inhibitors decrease arterial neointimal proliferation, and possibly reduce levels of nitric oxide with an associated reduction in oxidative stress.<sup>59</sup> Experimental data have demonstrated that mTOR inhibition reduces transplant atherosclerosis,<sup>60,61</sup> restricting plaque size by blocking lipid accumulation triggered by the mTOR pathway.<sup>62–65</sup> Importantly, inhibition of the local inflammatory response and stimulation of macrophage clearance by mTOR inhibitors also lower the risk of plaque rupture.<sup>66–68</sup> These effects have led to widespread use of these agents in drug-eluting coronary stents during revascularization procedures to reduce stent stenosis.<sup>69</sup>

Clinical evidence of an antiatherogenic effect has been provided by intravascular ultrasound assessments in a subpopulation of 189 heart transplant patients in the recent A2310 study.<sup>70</sup> In this trial, 721 de novo heart transplant patients were randomized to receive everolimus with low-dose cyclosporine or mycophenolate mofetil with standard-exposure cyclosporine. Intravascular ultrasound measurements showed that the mean  $\pm$  standard deviation increase in maximal intimal thickness of the coronary arteries from baseline to month 12 post-transplant was significantly smaller with everolimus than with mycophenolate mofetil ( $0.03 \pm 0.05$  mm versus  $0.07 \pm 0.11$  mm,  $P < 0.001$ ). These results confirmed earlier findings from a large randomized trial of everolimus versus azathioprine<sup>71</sup> and from single-arm studies in which maintenance heart transplant patients were converted to sirolimus.<sup>53,72</sup>

## Arterial stiffness

There is evidence that mTOR inhibitors can ameliorate arterial stiffness,<sup>8,9</sup> which arises from structural changes in the arterial wall induced by age but is exacerbated by atherosclerosis and vascular calcification.<sup>73</sup> Both arterial stiffness and aortic calcification are known risk factors for cardiovascular events following kidney transplantation.<sup>74</sup> The gold standard for measuring arterial stiffness is pulse wave velocity.<sup>75</sup> Clinically, the effect of mTOR inhibitors on pulse wave velocity in kidney transplant recipients has been assessed only in one small randomized trial<sup>8</sup> and in a subpopulation analysis of the large randomized CONCEPT trial.<sup>9</sup> In both

**Table I** Studies of left ventricular mass in kidney or heart transplant patients receiving mTOR inhibitor therapy

Study	Study design	n	Population	Intervention	Follow-up	LVM	Comment
<b>Kidney transplantation</b>							
Murbaech et al <sup>56</sup>	Substudy of randomized trial (CENTRAL), prospective multicenter	44	De novo patients	Conversion from CNI to everolimus at week 7 post-transplant	1 year	No difference in LVM or left atrial volume	Significantly faster peak early mitral velocity with everolimus versus controls ( $P=0.02$ )
Paoletti et al <sup>51</sup>	Nonrandomized, controlled, prospective, single-center	13	Maintenance patients	Conversion from CNI to sirolimus	1 year	Significant decrease in LVM after conversion to sirolimus ( $P<0.001$ ) LVH regressed in 92.3% sirolimus patients versus 38.5% controls ( $P=0.002$ )	No change in blood pressure
<b>Heart transplantation</b>							
Hiemann et al <sup>52</sup>	Prospective, nonrandomized, single-center	132	De novo patients	Everolimus versus MMF	3 years	Negative vascular remodeling observed in 24% everolimus patients versus 76% MMF patients ( $P=0.053$ )	Significantly less endomyocardial fibrosis ( $P=0.049$ ) and scarring ( $P=0.017$ )
Topilsky et al <sup>53</sup>	Case-controlled, retrospective, single-center	45	Maintenance patients (mean 1.2 years post-transplant)	Conversion from CNI to sirolimus	Mean 3.1 years	Improved positive vascular remodeling after conversion to sirolimus versus controls ( $P=0.01$ )	Significantly fewer cardiac-related events after conversion to sirolimus versus controls (6.4% versus 83.1%, $P=0.002$ )
Raichlin et al <sup>54</sup>	Single-arm, retrospective, single-center	70	Maintenance patients (mean 5.8 years post-transplant)	Conversion from CNI to sirolimus	3 years	Significant benefit with sirolimus for: LVM ( $P=0.011$ ) LVM index ( $P=0.017$ ) Left atrium volume ( $P=0.002$ )	No change in blood pressure after conversion
Kushwaha et al <sup>55</sup>	Single-arm, retrospective, single-center	58	Maintenance patients	Conversion from CNI to sirolimus	1 year	Significant decrease in LVM ( $P=0.05$ ) and LVM index ( $P=0.031$ ) from baseline with sirolimus but not in controls Significant decrease in left atrial volume index from baseline ( $P=0.008$ ) with sirolimus and significant increase in controls ( $P=0.0012$ )	Difference between groups was independent of blood pressure

**Abbreviations:** CNI, calcineurin inhibitor; LVM, left ventricular mass; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin.

cases, CNI-free immunosuppression with everolimus or sirolimus either stabilized or improved pulse wave velocity, indicating a protective effect on the arterial wall. In addition to a direct effect of mTOR inhibitors on the arterial endothelium, avoidance of a CNI-related pathogenic effect on arterial stiffening<sup>76</sup> is likely to have contributed to the between-group differences that were observed.

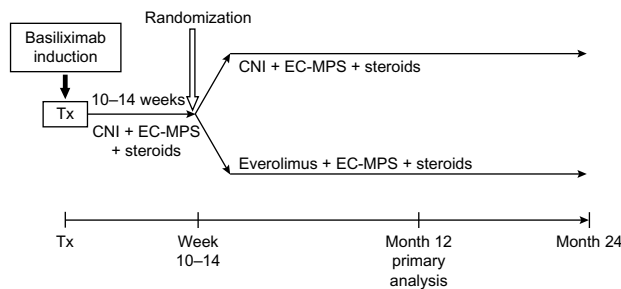
## ELEVATE: the study design

### Overview

ELEVATE is a 24-month, multicenter, open-label, randomized, controlled trial (NCT01114529) in which de novo kidney allograft recipients are randomized 10–14 weeks

post-transplant to convert from CNI therapy to everolimus or remain on a standard CNI therapy (Figure 1). All patients receive enteric-coated mycophenolate sodium and steroid therapy throughout the study. The study is designed to evaluate: the evolution of graft function after early conversion from CNI therapy to everolimus; the efficacy and safety of early conversion; and the impact of a CNI-free regimen on evolution of cardiovascular parameters in this setting.

A blinded trial design was not considered appropriate due to the small therapeutic window for the study drugs and the burden of placebo therapy to ensure blinding of three study medications. The timing of conversion (10–14 weeks after transplantation) is based on results from earlier trials of



**Figure 1** ELEVATE study design.

**Abbreviations:** CNI, calcineurin inhibitor; EC-MPS, enteric-coated mycophenolate sodium; Tx, transplantation.

conversion from CNI to mTOR inhibitor therapy at similar time points.<sup>37,39,44</sup>

## Study population

The study population comprises adult patients receiving a first or second kidney transplant from a deceased or living donor. Inclusion and exclusion criteria at the time of study entry are summarized in Table 2. At the time of randomization, patients are randomized if they are still receiving CNI therapy (tacrolimus or cyclosporine) with enteric-coated mycophenolate sodium and steroids and if they have acceptable renal function. Acceptable renal function is defined as serum creatinine  $<250 \mu\text{mol/L}$  and estimated glomerular filtration rate (eGFR)  $\geq 25 \text{ mL/min/1.73 m}^2$  based on the four-variable Modification of Diet in Renal Disease (MDRD4) formula.<sup>77</sup> Full exclusion criteria at the point of randomization are shown in Table 2.

## Randomization procedure

Randomization takes place between 10 and 14 weeks post-transplant. All eligible patients are randomized via an interactive voice response system or web system. Patients are stratified according to graft function, categorized as eGFR (MDRD4)  $<30$ , 30 to  $<60$ , 60 to  $<90$ , or  $\geq 90 \text{ mL/min/1.73 m}^2$  and whether or not they have previously experienced cardiovascular events, defined as myocardial infarction or percutaneous coronary intervention (eg, balloon angioplasty, stent angioplasty). Randomization also ensures a 2:1 ratio of tacrolimus versus cyclosporine therapy in the control arm.

## Immunosuppression and concomitant medication

All patients receive basiliximab induction (20 mg on days 0 and 4), with no other induction therapy. Immunosuppression up to the point of randomization comprises either tacrolimus (target  $C_0$  6–12 ng/mL) or cyclosporine (150–300 ng/mL), with enteric-coated mycophenolate sodium (1,080–1,440 mg/day)

and steroids administered according to local practice but at a minimum dose of 5 mg/day.

At randomization, patients in the everolimus group can be converted from CNI therapy either overnight or stepwise over one week (Figure 2). In both groups, the target concentration  $C_0$  for everolimus is 6–10 ng/mL to month 24. All patients randomized to everolimus are to be CNI-free by the end of week 16. Protocol-stipulated everolimus dose decreases or discontinuations are permitted in response to prespecified hematological or lipid abnormalities or other adverse events. In the control arm, tacrolimus or cyclosporine is continued (target  $C_0$  5–10 ng/mL for tacrolimus, 100–250 ng/mL for cyclosporine). In both treatment groups, enteric-coated mycophenolate sodium is continued at a dose of 1,080–1,440 mg/day, unless a dose reduction or discontinuation is required for tolerability reasons including hematological changes defined in the protocol. Steroids are maintained at a minimum dose of 5 mg/day until month 24. Rejection episodes are treated according to local practice, ie, there is no protocol-stipulated treatment regimen.

Details of concomitant medication and treatment of rejection episodes are summarized in Table S1.

## Study endpoints

Efficacy and safety endpoints are summarized in Table 3. The primary efficacy endpoint is the change in eGFR from randomization to month 12, calculated by MDRD4.<sup>77</sup> Key secondary efficacy endpoints are a composite endpoint of treated biopsy-proven acute rejection (Banff  $\geq \text{Ib}$ ), graft loss or death at month 12, and the change in left ventricular mass index from randomization to month 12 as measured by echocardiogram. Key secondary endpoints are treated biopsy-proven acute rejection (Banff  $\geq \text{Ib}$ ), graft loss or death at month 12, and change in left ventricular mass index from randomization to month 12 as measured by echocardiogram.

Cardiovascular events during the study are of particular interest. The endpoints of angina pectoris leading to hospitalization or intervention, myocardial infarction, or stroke from randomization to months 12 and 24 are captured on a specific case report form. At selected centers of excellence, the change in arterial pulse wave velocity from randomization to months 12 and 24 is measured by pulse wave blood pressure monitoring and 24-hour blood pressure monitoring is performed. Other safety endpoints are shown in Table 3.

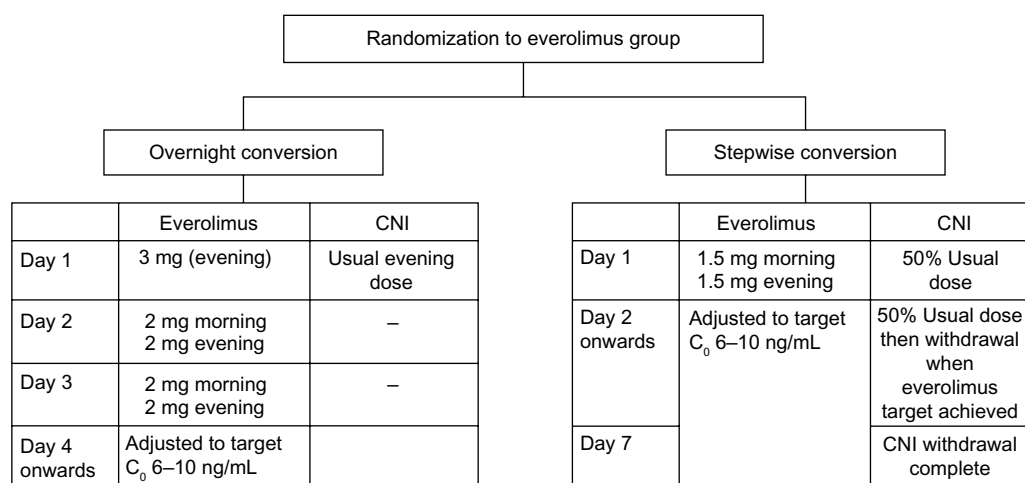
Serum samples for assessment of donor-specific antibodies are obtained at baseline, randomization, and at months 12 and 24, or in the event of a clinically indicated



**Table 2** Key inclusion and exclusion criteria for the ELEVATE study

Inclusion criteria	Exclusion criteria
<b>Baseline (day of transplantation)</b>	
<ul style="list-style-type: none"> <li>Male or female patients <math>\geq 18</math> years</li> <li>Recipients of a primary or secondary kidney transplant from a deceased, living-related, or living-unrelated donor</li> <li>Cold ischemia time <math>&lt; 24</math> hours</li> <li>Negative pregnancy test (female patients)</li> <li>Written informed consent to participate in the study</li> </ul>	<ul style="list-style-type: none"> <li>Multiple organ transplantation</li> <li>ABO incompatible allograft or positive cross-match</li> <li>Panel reactive antibodies level <math>\geq 30\%</math> within 3 months prior to baseline</li> <li>Positive for human immunodeficiency virus</li> <li>Donor positive for HBsAg or HCV</li> <li>HBsAg and/or HCV positivity with elevated liver function tests (ALT or AST <math>\geq 2.5</math> times upper limit of normal)</li> <li>Severe restrictive or obstructive pulmonary disorders</li> <li>Severe allergy requiring acute (within 4 weeks of baseline) or chronic treatment that would prevent patient from potential exposure to everolimus, or with hypersensitivity to drugs similar to everolimus (eg, macrolides)</li> <li>Severe uncontrolled hypercholesterolemia or hypertriglyceridemia</li> <li>White blood cell count <math>\leq 2,000/\text{mm}^3</math> or platelet count <math>\leq 50,000/\text{mm}^3</math></li> <li>History of malignancy (other than localized skin basal cell carcinoma), treated or untreated, within the past 5 years</li> </ul>
<b>Randomization (10–14 weeks post-transplant)</b>	
<ul style="list-style-type: none"> <li>Immunosuppression with CNI therapy (tacrolimus or CsA) with EC-MPS and steroids</li> <li>Serum creatinine <math>&lt; 250 \mu\text{mol/L}</math> and eGFR (MDRD4) <math>\geq 25 \text{ mL/min/1.73 m}^2</math> without renal replacement therapy</li> </ul>	<ul style="list-style-type: none"> <li>Graft loss</li> <li>Renal replacement therapy</li> <li>Severe humoral and/or cellular rejection (Banff <math>\geq \text{IIb}</math>)</li> <li><math>\geq 2</math> episodes of acute rejection or antibody treatment for rejection</li> <li>Ongoing or currently treated acute rejection within 2 weeks prior to randomization</li> <li>Proteinuria <math>&gt; 1 \text{ g/day}</math> (as calculated from the urinary protein-to-creatinine ratio)</li> <li>Focal segmental glomerulosclerosis</li> <li>White blood cell count <math>\leq 2,000/\text{mm}^3</math> or absolute neutrophil count <math>\leq 1,500/\text{mm}^3</math> with platelet count <math>\leq 50,000/\text{mm}^3</math></li> <li>Hemoglobin <math>&lt; 8 \text{ g/dL}</math></li> <li>AST, ALT or total bilirubin <math>&gt; 2.5</math> times upper limit of normal</li> <li>Current severe systemic infection requiring continued therapy that would interfere with the objectives of the study</li> <li>Severe uncontrolled hypercholesterolemia or hypertriglyceridemia</li> <li>Ongoing wound healing problems, clinically significant infection requiring continued therapy or other severe surgical complication</li> <li>Intractable immunosuppressant complications or side effects</li> <li>Anticoagulant therapy that prevents renal allograft biopsy</li> </ul>

**Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; CNI, calcineurin inhibitor; CsA, cyclosporine; EC-MPS, enteric-coated mycophenolate sodium; eGFR, estimated glomerular filtration rate; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; MDRD4, four-variable Modification of Diet in Renal Disease.

**Figure 2** Protocol for conversion to everolimus.

**Abbreviations:** CNI, calcineurin inhibitor;  $C_0$ , trough concentration.

**Table 3** ELEVATE study endpoints

Primary endpoint	Change of eGFR (MDRD4) from randomization to month 12
Key secondary endpoints	Treated BPAR (Banff $\geq$ Ib), graft loss, or death at month 12 <sup>a</sup> Change in left ventricular mass index from randomization to month 12 as measured by echocardiogram
Other secondary efficacy endpoints	Treated BPAR $\geq$ Ib (incidence, time to event, severity, requirement for antibody treatment), graft loss or death from randomization to months 12 and 24 eGFR increase $\geq$ 5 mL/min/1.73 m <sup>2</sup> from randomization to month 12 Evolution of serum creatinine Urinary protein/creatinine ratio (incidence of proteinuria $\geq$ 0.5–0.9, > 1.0–2.9, and > 3.0 g/day) Renal replacement therapy to months 12 and 24 Chronic allograft nephropathy defined as interstitial fibrosis and tubular atrophy grade I–III at 12 months on protocol biopsy <sup>b</sup> Cardiovascular events: angina pectoris leading to hospitalization or intervention, myocardial infarction <sup>c</sup> or stroke <sup>d</sup> from randomization to months 12 and 24 DSA at baseline, randomization, month 12 and 24, in the event of a clinically-indicated graft biopsy
Safety and tolerability endpoints	All adverse events and serious adverse events (including infections) Treatment-related adverse events Premature discontinuation of study medication or study Dose interruption or adjustment of study medication CNI reintroduction in the everolimus group Vital signs and laboratory data
Additional assessments at selected centers	Change in arterial pulse wave velocity from randomization to months 12 and 24 24-hour blood pressure monitoring Pharmacokinetics of EC-MPS with concomitant tacrolimus, CsA or everolimus at months 6 and 12

**Notes:** <sup>a</sup>Components of this composite efficacy endpoint were also assessed, comprising: incidence, time to event, and severity of treated BPAR  $\geq$  Ib; incidence of BPAR requiring antibody treatment; incidence of antibody-mediated (humoral) rejection; incidence of treated BPAR  $\geq$  Ib, graft loss, death, or loss to follow-up; incidence of graft loss; and patient survival. <sup>b</sup>Protocol biopsies were performed at time of transplantation and at months 12 and 24, and assessed centrally by an independent pathologist. <sup>c</sup>Categorized as segment elevation myocardial infarction (STEMI) or non-STEMI. <sup>d</sup>Defined as brain ischemia due to thrombosis, embolism, or systemic hypoperfusion.

**Abbreviations:** BPAR, biopsy-proven acute rejection; CNI, calcineurin inhibitor; CsA, cyclosporine; DSA, donor specific antibodies; EC-MPS, enteric-coated mycophenolate sodium; eGFR, estimated glomerular filtration rate; MDRD4, four-variable Modification of Diet in Renal Disease.

graft biopsy, and donor-specific antibody levels are measured centrally. Any patient who discontinues study medication prematurely is to be followed up at months 6, 12, and 24 to obtain clinical and laboratory data, with allograft biopsies performed at months 12 and 24.

## Statistical analysis

The primary endpoint, ie, change in eGFR (MDRD4) from randomization to month 12, will be compared between groups using analysis of covariance, with treatment, center (as a random effect) donor type, age (<50 versus  $\geq$  50 years) and cold ischemia time ( $\leq$  24 versus > 24 hours) as factors and eGFR at randomization as a covariate, based on least square means (two-sided type I error rate of 0.05). Patients with graft loss will be assigned a zero value for eGFR at month 12, with the last observation carried forward method applied in other cases of missing values.

Given that patients in the control group could receive either tacrolimus or cyclosporine, the homogeneity of the treatment effect of everolimus versus either CNI will be tested at the significance level of 0.15. If homogeneity is not shown, the primary endpoint will be compared between everolimus and tacrolimus or cyclosporine separately (two-sided type I

error rate of 0.025), with 97.5% confidence intervals for the between-group differences.

For the composite efficacy endpoint (treated biopsy-proven acute rejection  $\geq$  Ib, graft loss, or death), noninferiority of everolimus to CNI continuation will be assessed using a noninferiority margin of 10%, with Z-test-based two-sided 95% confidence intervals for the difference in incidence at 12 months. The everolimus group will be considered to have a noninferior efficacy failure rate if the upper limit of the confidence interval values is within 10%. The other key secondary endpoint, ie, left ventricular mass index at 12 months, will be compared between groups using analysis of covariance, with treatment, center (as a random effect), and donor type as factors and left ventricular mass index at randomization as covariate (two-sided type I error rate 0.05). Patients with no echocardiogram measurements available either centrally or locally at randomization or month 12 will be excluded from the analysis. Local measurements will be used only when central measurements are missing. To account for the fact that there are two secondary endpoints, a hierarchical fixed hypothesis testing procedure will be used to maintain the familywise type I error rate at 0.05. The statistical methodology for other endpoints is shown in Table 4.

**Table 4** Summary of statistical methods

Type of variable	Variable	Statistical test
Primary variable	Change in eGFR (MDRD4) from randomization to month 12	ANCOVA with treatment, center (as a random effect), donor type, age ( $<50$ versus $\geq 50$ years), and cold ischemia time ( $\leq 24$ versus $>24$ hours) as factors and eGFR at randomization as a covariate
Key secondary variables <sup>a</sup>	Composite efficacy endpoint at month 12 <sup>b</sup> Change in left ventricular mass index from randomization to month 12	Noninferiority testing with a margin of 10% (Z-test) ANCOVA with treatment, center (as a random effect), and donor type as factors and left ventricular mass index at randomization as covariate
Other efficacy variables	Treated BPAR (Banff $\geq 1b$ ) Graft loss Death eGFR improvement $\geq 5$ mL/min/1.73 m <sup>2</sup> from randomization to month 12 or month 24 Serum creatinine Urinary protein to creatinine ratio IFTA grade I–III Cardiovascular events <sup>c</sup>	Noninferiority testing with a margin of 10% (Z-test)   Fisher's exact test  Descriptive  Fisher's exact test Fisher's exact test
Safety and tolerability assessments	Adverse events Vital signs and laboratory data	Descriptive Descriptive (including incidence of data within clinically notable ranges)

**Notes:** <sup>a</sup>Accounts for the fact that there are two secondary endpoints, a hierarchical fixed hypothesis testing procedure will be used to maintain the familywise type I error rate at 0.05. <sup>b</sup>Treated BPAR (Banff  $\geq 1b$ ), graft loss, or death at month 12. <sup>c</sup>Angina pectoris leading to hospitalization or intervention, myocardial infarction, or stroke from randomization to months 12 and 24.

**Abbreviations:** ANCOVA, analysis of covariance; BPAR, biopsy-proven acute rejection; eGFR, estimated glomerular filtration rate; IFTA, interstitial fibrosis and tubular atrophy; MDRD4, four-variable Modification of Diet in Renal Disease.

The intent-to-treat population will include all randomized patients, other than patients in the everolimus group who do not achieve CNi withdrawal. The safety population will include all patients who receive at least one dose of randomized drug and provide at least one post-randomization safety assessment.

The sample size calculation, based on the primary efficacy variable, assumed a standard deviation of 20 mL/min/1.73 m<sup>2</sup> and equal randomization between the everolimus and CNi groups. Applying a two-sided type I level of 0.05, a sample size of 304 patients per group was estimated to provide 86% power to detect a difference of 5 mL/min/1.73 m<sup>2</sup> in change of eGFR (MDRD4) from randomization to month 12 between treatment groups. In total, randomization of 338 patients per group would be required to allow for a dropout rate of 10%. Assuming that approximately 65% of enrolled patients will be eligible for randomization, approximately 1,000–1,100 patients will be enrolled. A population of this size will have 86% power to show that the efficacy failure rate is not more than 10% worse in the everolimus group versus the CNi group, and 92% power to detect a difference of 5 g/m<sup>2.7</sup> in left ventricular mass index between treatment groups.

## Conclusion

Expansion of the immunosuppressive armamentarium and intensive research into optimal regimens has achieved good efficacy and safety after kidney transplantation in terms of

conventional outcome measures. The new challenge facing clinicians is to maintain these results while addressing the obstacles to long-term graft and patient survival. The ELEVATE study employs an innovative design which addresses the need for preservation of graft function and effective rejection prophylaxis, but includes new endpoints relating to the cardiovascular impact of everolimus versus CNi therapy. As the first trial to adopt this approach, its results are awaited with considerable interest.

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## Supplementary material

**Table S1** Concomitant medication and rejection therapy

Event	Therapy/recipients	Regimen
Cytomegalovirus	Prophylaxis Mandatory for donor-positive/recipient-negative transplants Administration is optional in other cases, according to local center practice, but consistent for all patients at each center	According to local center practice
<i>Pneumocystis carinii</i> pneumonia	Prophylaxis All patients	80 mg/sulfamethoxazole 400 mg daily or trimethoprim 160 mg/sulfamethoxazole 800 mg three times a week for $\geq 3$ months
Hyperlipidemia	Lipid-lowering therapy As per guidelines/local center practice	According to local center practice <sup>a</sup>
Hepatitis B virus recurrence	Prophylaxis At the discretion of the investigator	According to local center practice
Acute rejection	Antirejection therapy should be postponed until histological confirmation if possible	Intravenous methylprednisolone or antithymoglobulin <sup>b</sup>

**Notes:** <sup>a</sup>Statins concomitantly with fibrates should be avoided, due to the increased risk of myopathy and rhabdomyolysis in combination with cyclosporine. If combination therapy is needed, ezetimibe should be used in combination with statins. <sup>b</sup>Mild rejection episodes which are easily reversed with antirejection therapy can continue with randomized treatment after optimizing/increasing the doses of CNI, everolimus, and/or EC-MPS and steroids. For patients who experience severe acute rejection, recurrent rejection or do not respond adequately to treatment, discontinuation of study treatment may be considered.

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