The effect of everolimus initiation and calcineurin inhibitor elimination on cardiac allograft vasculopathy in *de-novo* heart transplant recipients– three year results of a Scandinavian randomized trial

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WHAT IS NEW ?

- The intravascular ultrasound results of the SCHEDULE trial demonstrate that everolimus initiation and total early cyclosporine elimination significantly reduces the progression of cardiac allograft vasculopathy (CAV) at 12 and 36 months compared to patients treated with standard cyclosporine therapy.
- The early use of everolimus with total elimination of cyclosporine is safe as demonstrated by similar cardiac function and low mortality in both treatment groups.
- Virtual Histology analysis confirmed that this immunosuppressive approach is not associated with any significant increase in inflammatory tissue components (calcified and necrotic tissue) or systemic inflammatory markers.

WHAT ARE THE CLINICAL IMPLICATIONS ?

- There is currently no effective medical therapy for CAV after HTx. Everolimus and total early cyclosporine elimination had a beneficial effect on CAV progression after HTx and such therapy could potentially improve long-term outcome after HTx.
- Everolimus initiation and total early cyclosporine elimination does not appear to have any detrimental effect on CAV morphology or immune marker activity. This immunosuppressive protocol also has an additional beneficial effect on renal function highlighting the advantage of considering such therapy early after HTx.

ABSTRACT

Background: Cardiac allograft vasculopathy (CAV) limits survival after heart transplantation (HTx) and the effect of different immunosuppressive regimens on CAV is not fully understood. The randomized SCHEDULE (Scandinavian heart transplant everolimus de-novo study with early calcineurin inhibitors avoidance) trial evaluated whether initiation of the proliferation signal inhibitor everolimus and early cyclosporine elimination can reduce CAV development.

Methods and Results: The SCHEDULE trial was a multicenter Scandinavian trial where 115 de-novo HTx recipients were randomized to everolimus with complete cyclosporine withdrawal 7-11 weeks after HTx or standard cyclosporine-based immunosuppression. 76 (66%) patients had matched intravascular ultrasound (IVUS) examinations at baseline, 12 months and 36 months. IVUS analysis evaluated maximal intimal thickness (MIT), percent atheroma volume (PAV) and total atheroma volume (TAV). Qualitative plaque analysis using Virtual Histology assessed fibrous, fibrofatty and calcified tissue as well as necrotic core. Serum inflammatory markers were measured in parallel.

The everolimus group (n=37) demonstrated significantly reduced CAV progression as compared to the cyclosporine group (n=39) at 36 months [ΔMIT 0.09±0.05 versus 0.15±0.16 mm (p=0.03), ΔPAV 5.3±2.8% versus 7.6±5.9 (p=0.03), ΔTAV 33.9±71.2 mm^3 versus 54.2±96.0 mm^3 (p=0.34), respectively]. At 36 months the number of everolimus patients with rejection graded ≥2R was 15 (41%) as compared to 5 (13%) in the cyclosporine group (p=0.01). Everolimus did not affect CAV morphology or immune marker activity during the follow-up period.

Conclusions: The SCHEDULE trial demonstrates that everolimus initiation and early cyclosporine elimination significantly reduces CAV progression at 12 months and this beneficial effect is clearly sustained at 36 months.

Clinical trial registration: ClinicalTrials.gov (NCT01266148) at http://clinicaltrials.gov/

INTRODUCTION

Cardiac allograft vasculopathy (CAV) is reported to affect 50% of heart transplant (HTx) recipients within 5 years after transplantation [1] and is the second leading cause of post-HTx mortality [1]. Prevention of CAV remains a significant clinical challenge as the widespread use of powerful immunosuppressive agents including calcineurin inhibitors (CNI), such as cyclosporine A (CsA) or tacrolimus, do not appear to ameliorate CAV development [2]. In fact, the use of CNI agents is associated with adverse effects including metabolic disturbances (e.g. new onset diabetes) and renal toxicity [3] which may negatively influence CAV development [4]. An alternative immunosuppressive protocol providing sufficient immune modulation without such adverse effects and with potential attenuating effects on CAV development is, therefore, an ideal but elusive goal in HTx.

During the last decade, immunosuppressive drugs inhibiting the mammalian target of rapamycin (mTOR) signaling pathway have received considerable attention. Everolimus is an mTOR inhibitor that arrests the cell cycle of lymphocytes and vascular smooth cells in the G1 phase [5] and this combined immunosuppressive and antiproliferative effect is relevant to CAV development. It has previously been demonstrated that everolimus initiation in *de-novo* HTx recipients with standard or reduced CNI therapy can attenuate intimal thickening of coronary arteries [6-8]. We demonstrated in the 12-month SCHEDULE study that *de-novo* everolimus therapy and early elimination of CNI therapy is also feasible and reduces the early progression (i.e. 12 months) of CAV [9]. Furthermore, such a CNI-free strategy is associated with improved renal function at 12 months [10] and a beneficial effect on renal function and coronary artery intimal thickness is maintained at 36 months[11].

The purpose of the current report of the SCHEDULE trial is to provide an in-depth evaluation of the effect of everolimus initiation and CNI elimination on CAV at 36 months by utilizing percent atheroma volume (PAV) and total atheroma volume (TAV) as alternative endpoints to MIT as well as angiographic data. Inflammatory marker measurement was also performed in parallel to evaluate underlying inflammatory pathways that could potentially

mediate any observed intimal changes associated with everolimus therapy. Finally, the current report also provides qualitative assessment of plaque morphology by Virtual Histology (VH) analysis at 12 and 36 months after randomization.

METHODS

Patient population

A detailed description of the SCHEDULE trial has been reported previously [10]). In brief, the SCHEDULE trial was a 12-month prospective, open-label, multicenter, randomized controlled study undertaken at five HTx centers in Scandinavia. Adult *de-novo* HTx recipients were randomized in a 1:1 ratio to: (i) low dose everolimus, low-dose cyclosporine, mycophenolate mofetil (MMF) and corticosteroids with elimination of cyclosporine and step up to full-dose everolimus after 7-11 weeks or (ii) conventional treatment with cyclosporine, MMF and corticosteroids. All patients received the first dose of everolimus, cyclosporine, MMF, and corticosteroids no later than the fifth postoperative day. *The current report is 36-month continuation of the original SCHEDULE study evaluating CAV progression.* The first SCHEDULE visit was performed in December 2009 and the final month 36 follow-up visit took place in November 2014. Written informed consent was obtained from all patients. The study was approved by the regional ethical authority in each country and was carried out in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, applicable local regulations and the Declaration of Helsinki and was registered with ClinicalTrials.gov (NCT01266148). Because of the sensitive nature of the data collected for this study, requests to access the dataset from qualified researchers trained in human subject confidentiality protocols may be sent to the corresponding author and will be subject to approval from the steering committee.

IMMUNOSUPPRESSIVE MEDICATION

All patients received induction treatment with antithymocyte globulin (ATG, Thymoglobulin®, Genzyme Corporation, Cambridge, MA) within 12 hours of HTx and this continued for up to five days. In the everolimus group, everolimus was initiated at a dose of 0.75 mg twice daily no later than the fifth postoperative day and cyclosporine elimination took place at week 7 unless there was ongoing rejection at that time, in which case discontinuation was allowed to

be postponed up to week 11. All patients received statin therapy and patients who were seronegative for cytomegalovirus (CMV) and received a graft from a CMV-positive donor were given prophylaxis with oral valganciclovir for at least three months according to the local protocol.

IVUS imaging

The SCHEDULE trial protocol specified IVUS examination of the same major epicardial coronary artery (preferentially the left-anterior descending coronary artery) and this was conducted while performing coronary angiography at week 7–11, 12 months and 36 months post-HTx using a 20 MHz, 2.9F, monorail electronic Eagle Eye Gold IVUS catheter (Volcano Corporation Inc, CA, USA). Patients with severe renal impairment (as judged by the principal investigator but generally considered as GFR < 30 ml/min/1.72 m^2) were to be excluded from IVUS study (due to the risk of angiographic contrast) but no patients had this level of renal impairment and, hence, this exclusion criterion did not apply to any of the randomized patients. IVUS images were acquired at a rate of 30 frames/sec and pullback speed of 0.5 mm/sec. Images were stored digitally for off-line analysis conducted after trial closure by a core laboratory (Oslo University Hospital, Rikshospitalet, Oslo, Norway) blinded to patient treatment. IVUS analysis was performed according to the guidelines for acquisition and analysis of IVUS images by the American College of Cardiology and European Society of Cardiology [12]. Precise matching of the IVUS recordings was performed followed by contour detection of both the lumen and external elastic membrane (EEM) at approximately 1 mm intervals using validated software (QIVUS, v.3.0, Medis medical imaging systems, Leiden, the Netherlands).

Greyscale IVUS analysis

Maximal intimal thickness (MIT) is an established predictor of all-cause mortality, myocardial infarction, and angiographic abnormalities amongst HTx recipients [13,14]. Hence, change in

MIT between matching segments at baseline and 12 and 36 months was utilized as the primary greyscale IVUS efficacy variable. Other secondary IVUS variables were: (i) percent atheroma volume (PAV) which expresses the summation of atheroma areas in proportion to the EEM area using the equation: PAV = $\sum$ (EEM_{area} – $Lumen_{area}$)/$\sum EEM_{area}$) x 100 (ii) normalized total atheroma volume (TAV) and (iii) incidence of CAV (defined as mean MIT ≥0.5 mm over the entire matched segment). The mean length of analyzed segments at baseline, 12 months and 36 months was 36.7±7.3 mm, 36.4±8.1 and 36.8±10.5 mm, respectively.

Angiographic assessment of CAV

Coronary artery angiogram data was evaluated by experienced local staff blinded to treatment and was reported according to the International Society for Heart and Lung Transplantation standardized nomenclature for CAV[15].

Virtual histology analysis

Virtual Histology (Volcano Corporation Inc, Rancho Cordova, CA) is a technological tool that utilizes backscatter radiofrequency data obtained during IVUS pullback for qualitative plaque assessment. VH-IVUS possesses 94–97% *ex-vivo* and 87–97% *in-vivo* accuracy for characterization of basic tissue components [16,17]. VH-IVUS data obtained at baseline, 12 and 36 months was analyzed with validated software (QIVUS, v.3.0, Medis medical imaging systems, Leiden, the Netherlands) that reconstructs tissue maps with four identifiable major components (fibrous, fibrofatty, dense calcified and necrotic core components) based on mathematical autoregressive spectral analysis of backscattered radiofrequency data. The various tissue components are expressed as a percentage of total intima and media area.

Inflammatory marker analysis

In total, 65 patients (from two centers), underwent plasma sampling by standard venipuncture at baseline, 12 and 36 months. Plasma levels of vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1(ICAM-1), the CXC chemokine CXCL16 and soluble tumor necrosis factor receptor (sTNFR)-1 were measured by enzyme immunoassays (EIA) (R&D Systems, Minneapolis, MN). Plasma levels of C-reactive protein (CRP) and von Willebrand factor (vWf) were measured by EIAs as previously described [18]. All intra-assay and inter-assay coefficients of variance were <10%.

Statistical analysis

Analyses were performed with the SPSS v 22.0 statistical software (SPSS Inc. Chicago, IL). Data is expressed as mean±SD and a two-tailed p-value <0.05 was considered statistically significant. Baseline characteristics were compared using Student's t-test, Mann-Whitney test and Pearson's chi-square test as appropriate. Pearsons' chi-square test was used to compare the incidence of acute rejection and Cochran–Mantel–Haenszel test was utilized to compare the 36-month incidence of CAV in the treatment groups (adjusted for baseline CAV). Changes in IVUS endpoints were compared between treatment groups by performing analysis of covariance (ANCOVA) with the baseline IVUS value included as a covariate and treatment group as a fixed factor. ANCOVA p-values represent between group contrast. Multivariate regression analysis was performed to identify predictors of CAV progression following an initial exploratory univariate analysis that selected potentially relevant covariates (p-value <0.05). Multivariable regression analysis was performed using the forward stepwise method with criteria for entry and exit at p <0.05 and <0.10, respectively. Change in inflammatory marker values were compared between treatment groups by ANCOVA analysis of log-transformed data.

RESULTS

PATIENT POPULATION

Of the 115 patients randomized in the SCHEDULE study, 76 (66%) patients had matching IVUS recordings at baseline, 12 and 36 months with a similar number between both treatment groups (39 everolimus versus 37 controls, Figure 1). Fifty-one (44%) patients had matching VH-IVUS recordings at these three timepoints. Overall, 10 (9%) patients died during the 36 month follow-up period. There were 7 deaths in the cyclosporine group with cause of death recorded as cerebrovascular accident, sepsis, cardiac arrest, sudden death, graft loss and 2 cases of malignancy. There were 3 deaths in the everolimus group with cause of death recorded as graft failure, pleural hemorrhage and malignancy.

Mean age of patients in the 36-month IVUS study population was 50.8±12.1 years and there was no significant difference in baseline characteristics between the treatment groups (Table 1). There was no significant difference in baseline characteristics when comparing the 76 IVUS patients with the total SCHEDULE population (n=115) (GFR in IVUS population was 59.7 mL/min/1.73 m^2 as compared to 60.7 mL/min/1.73 m^2 in the total SCHEDULE population (p=0.41) - remaining data not shown).

IMMUNOSUPPRESSION

All patients in the everolimus group discontinued cyclosporine at week 7–11 according to the trial protocol. The mean everolimus trough level at 12 and 36 months was 7.9±3.8 ng/mL and 6.9±2.1 ng/mL, respectively. In the everolimus group, 6 (16%) patients resumed low-dose cyclosporine in combination with everolimus while 6 (16%) patients discontinued everolimus permanently due to adverse effects. Among the 39 control patients, 5 (13%) patients were switched from cyclosporine to everolimus therapy due to deteriorating renal function. There were, therefore, 59 patients in the per-protocol population.

Acute rejection

The incidence of biopsy proven acute rejection (BPAR) during the first 12 months was 78.4% in the everolimus group versus 56.4% in the cyclosporine group (p=0.14). The vast majority of rejection episodes ≥2R occurred during the first 12 months with only 7 episodes occurring in the 12-36 month period. These episodes occurred in 6 patients of whom 5 had previously experienced similar rejection episodes (≥2R) during the first 12 months. Overall, at 36 months the number of patients with rejection graded ≥2R was 15 (41%) in the everolimus group as compared to 5 (13%) in the cyclosporine group (p=0.01). Rejection treatment consisted of corticosteroids in all cases without the need for cytolytic agents. No cases of humoral rejection or rejection with hemodynamic compromise were observed in the 36-month IVUS study population.

GREYSCALE IVUS OUTCOME

Only patients with exact matching IVUS recordings at baseline, 12 and 36 months (n=76) are considered in the current IVUS report. As reported previously, greyscale IVUS at 12 months demonstrated that everolimus patients had a mean increase in MIT of 0.02±0.05 as compared to 0.08±0.11 mm amongst cyclosporine patients (p<0.01) (Figure 2). This effect of everolimus persisted at 36 months with a total mean increase in MIT of 0.09±0.05 as compared to 0.15±0.16 mm amongst cyclosporine patients (p=0.03 (36-month values compared to baseline), Figure 2). In the current study, a similar pattern was seen for PAV with a significantly smaller mean increase amongst everolimus patients (1.2±2.0 and 5.3±2.8%) as compared to the cyclosporine group (3.7±4.1 and 7.6±5.9%) (12 [p<0.01] and 36 [p=0.03] months, respectively) (Figure 2). In contrast, when considering normalized TAV the differences between the everolimus and cyclosporine group did not reach statistical significance. Thus the increase in total atheroma burden in everolimus patients was -0.13±20.5 mm^3 and 33.9±71.2 mm^3 at 12 and 36 months, respectively, as compared to

11.1±27.6 mm^3 and 54.2.±96.0 mm^3 in the cyclosporine group (p=0.08 and p=0.34, respectively) (Figure 2).

The number of everolimus patients defined as having CAV (mean MIT ≥0.05 mm) increased from 12 (32%) at baseline to 16 (43.2%) at 36 months as compared to an increase from 10 (26%) to 21 (53.8%) patients in the cyclosporine arm (p=0.10 when analyzing differences in changes). Multivariable stepwise regression analysis was performed with the following five candidate variables: recipient age, donor age, everolimus, gender and diabetes mellitus. Of these variables recipient gender, donor age and treatment with everolimus were selected as independent predictors of CAV progression with everolimus having an independent adjusted treatment effect of *B* = -0.04 (95% CI -0.10-0.01) mm (p=0.03) (Table 2).

CMV infection/viremia did not influence CAV progression as evidenced by nearly identical disease progression in patients with and without CMV infection/viremia (ΔMIT, PAV and TAV at 36 months 0.12±0.13, 6.4±5.0 and 41.9±89.2 versus 0.12±0.13 mm, 6.5±5.0% and 59.2±65.4 mm3, respectively (all p-values >0.05).

Per protocol greyscale IVUS analysis

Analysis of greyscale IVUS recordings was also performed in the per protocol population, which excluded 17 patients (12 in the everolimus and 5 in cyclosporine arm) where treatment according to randomization arm was not followed. Mean increase in MIT at 36 months in the everolimus group was 0.09±0.05 mm as compared to 0.16±0.17 mm in the cyclosporine arm (p=0.04). ΔPAV and ΔTAV in everolimus patients was 4.9±2.8% and 15.9±47.3 mm^3 as compared to 8.0±6.0% (p=0.02) and 59.3±101.0 mm^3 (p=0.05) in the cyclosporine group, respectively.

Angiographic assessment of CAV

The number of everolimus patients with angiographic CAV increased from 4 (10.8%) (all CAV_1) to 10 (27.0%) (6, 3 and 1 patient with CAV_1, CAV_2 and CAV_3) as compared to an

increase from 5 (12.8%) at baseline (all CAV_1) to 8 (20.5%) at 36 months (5 and 3 patients with CAV_1 and CAV_2) in the cyclosporine arm (p=0.32).

CAV progression at 36 months according to presence of donor disease

When utilizing baseline mean MIT ≥ 0.50 mm as the threshold for defining donor disease [19] (i.e. atherosclerosis) we noted that 54 patients (25 everolimus, 29 cyclosporine) did not have donor disease. There was a beneficial effect of everolimus in this subgroup of patients and mean ΔMIT at 36 months was 0.08±0.05 and 0.15±0.16 mm in the everolimus and cyclosporine group, respectively (p=0.04) (Figure 3). The alternative endpoint PAV confirmed a significant beneficial between the two treatment arms (ΔPAV at 36 months 4.5±2.7 and 7.7±6.1 mm in the everolimus and cyclosporine group, respectively), whereas TAV did not show a significant difference between the two groups (Figure 3). Amongst 22 patients with donor disease (12 everolimus and 10 cyclosporine) the three independent IVUS endpoints MIT, PAV and TAV demonstrated similar disease progression in both treatment groups (Figure 3).

Virtual Histology analysis

Virtual histology analysis of matched VH-IVUS recordings at baseline and 12 and 36 months revealed no significant difference in change in plaque morphology according to treatment group. Overall, the increase in fibrotic, fibrofatty, calcified and necrotic tissue during the 36 month period everolimus group was -7.4±12.8%, 9.6±10.9%, 0.0±8.4% and -2.6±9.1% in the everolimus group, as compared to 0.3±12.9%, 7.4±14.7%, -3.5±8.3% and -4.5±9.8% in the cyclosporine group, respectively (all p-values >0.05; Figure 4).

Immune marker profile

There was a significant decline in levels of all measured inflammatory markers from baseline to 36 months, but there was no significant difference between the two treatment groups

(Table 3). A *post-hoc* analysis was performed to evaluate the change in inflammatory marker levels according to rapid CAV progression (Δ MIT ≥0.10 mm), but there was no evidence of a relationship between these biomarkers and increased CAV (data not shown). A separate *post-hoc* analysis found no significant difference in change in levels of inflammatory amongst everolimus patients with and without 2R rejection episodes (all p-values >0.05).

DISCUSSION

The current follow-up of *de-novo* HTx recipients in the SCHEDULE trial has demonstrated that everolimus initiation and early CNI elimination reduces CAV progression at 12 months and this beneficial effect is sustained at 36 months. This immunosuppressive approach is safe as demonstrated by similar cardiac function and low mortality in both treatment groups, despite the difference in biopsy verified acute rejection rate [10,11]. Furthermore, Virtual Histology analysis confirmed that everolimus initiation and CNI withdrawal is not associated with any significant increase in inflammatory tissue components (calcified and necrotic tissue) or systemic inflammatory markers.

CAV is an important complication following HTx. Although CNI therapy is a pillar of current immunosuppressive protocols, the effect of such agents on CAV is limited and may potentially have a detrimental effect on disease progression [20]. The mTOR inhibitor everolimus is an immunosuppressive agent with additional effects that could be of interest in relation to CAV (i.e., anti-proliferative and anti-fibrotic). Indeed, a beneficial effect of everolimus on CAV has been demonstrated in *de-novo* HTx recipients but not in maintenance recipients. The study by Eisen et al. [6] demonstrated that everolimus instead of azathioprine together with background CNI therapy reduces CAV amongst *de-novo* HTx recipients. Similarly, another trial evaluating CAV in *the de-novo* setting demonstrated a beneficial effect of everolimus and reduced CNI as compared to MMF and standard CNI therapy [7]. In contrast, the NOCTET trial [21] amongst maintenance HTx recipients failed to demonstrate a beneficial effect of everolimus and reduced CNI therapy on CAV, although a beneficial effect was seen in those who received azathioprine but not in those receiving MMF.

The SCHEDULE trial is the first to investigate the use of everolimus with *early* CNI elimination showing that this immunosuppressive approach significantly reduces CAV progression at 12 and 36 months. As reported previously, everolimus initiation and CNI elimination markedly improved renal function with a significant increase in measured GFR in

the everolimus group [10,11]. The association between early intimal thickening and adverse prognosis has previously been established amongst HTx recipients [13,14]. Similarly, there is sizeable data indicating that declining renal function following HTx is associated with increased morbidity and mortality [4,22]. Hence, everolimus initiation and early CNI elimination in *de-novo* HTx recipients seems to have a dual beneficial advantage on CAV and renal function that may have a positive and potential synergistic impact on long-term clinical outcome, although this remains to be demonstrated.

Our post-hoc analysis of CAV progression according to the presence of donor disease revealed that everolimus only attenuates intimal thickening in patients without underlying donor disease. Everolimus was unable to influence progression of CAV in the setting of pre-existing donor disease. However, it should be noted that our study had a relatively younger donor age as compared to previous studies[14] and, hence, this negative finding is based on a relatively small cohort of patients with donor disease. Despite the possibility of a type 2 error our results indicate the need to consider everolimus at the earliest possible timepoint following HTx. Our results suggest that a certain window of opportunity is likely to exist where everolimus has a beneficial effect on intimal thickening as supported by the NOCTET results [21] where everolimus did not influence CAV progression amongst maintenance recipients.

Everolimus is a mTOR inhibitor that has been shown to possess both anti-proliferative and anti-fibrotic effect in addition to its immunomodulatory properties [23] and, hence, may have a qualitative effect on intimal thickening. The current study, however, demonstrated that everolimus does not significantly influence intimal tissue composition as compared with traditional immunosuppression. The proportion of inflammatory tissue components remained unchanged in both treatment groups and a similar pattern was also seen in markers of systemic inflammation. This neutral finding is in contrast to the previous NOCTET reporting increased inflammatory tissue in maintenance recipients treated with everolimus [21]. Hence, the effect of everolimus on CAV in the current study is primarily manifested by the observed quantitative decrease in intimal tissue (measured by greyscale IVUS) without any significant

morphological changes (measured by Virtual Histology). Moreover, the beneficial effects of everolimus on CAV seem unrelated to its anti-inflammatory properties as a similar anti-inflammatory effect was seen during CNI treatment both within the lesion and systemically.

According to the SCHEDULE trial patients in the everolimus arm received both CNI and everolimus until week 7. We found no association between the measured systemic inflammatory markers and CAV progression. Nevertheless, it remains possible that the increased intensity of immunosuppression coupled with anti-lymphocyte induction during the early period had a positive influence on inflammation and CAV and should be explored further. The everolimus arm was noted to experience a significantly greater number of grade 2R rejections and this could potentially mitigate a reduction in systemic inflammation attributable to everolimus. With reference to this, a post-hoc analysis found no significant difference in change in levels of inflammatory amongst everolimus patients with and without 2R rejection episodes (all p-values >0.05) indicating that cellular rejection did not influence systemic markers of inflammation measured at 36 months.

The current immune marker results are in contrast to previously reported studies [24-27] demonstrating a clear association between these diverse makers and traditional atherosclerosis further supporting the notion that the pathophysiology of CAV is distinctly different from native atherosclerosis [28]. Nonetheless, the neutral effect of everolimus in this study (*de novo* recipients) as compared with the increased inflammatory effect in the NOCTET study (maintenance HTx) further suggest that early intervention with everolimus is preferable.

The present study has some limitations. Imaging of the left ascending artery was utilized used as a surrogate for all potential CAV that may be present. The number of patients was relatively small in certain sub-analyses including the evaluation of everolimus according to presence/absence of donor disease as well as assessment of systemic inflammatory markers particularly amongst patients with and without 2R rejection. Angiographic assessment of CAV did not reveal any benefit of everolimus but it should be noted that a 3-year follow-up may be too short a time to observe such a benefit.

The relative homogeneous study population in this Scandinavian cohort of predominantly male Caucasian patients should also be noted. Data regarding cause of death was available for all mortality cases but histology of the coronary arteries of these patients would have been beneficial. Despite these limitations, the authors believe the data is robust and particularly relevant to clinical practice as it provides the longest CAV follow-up period to date amongst *de-novo* HTx patients treated with everolimus.

In conclusion, this 36 month follow-up of the SCHEDULE trial has confirmed that the beneficial effect of everolimus on CAV at 12 months is maintained at 36 months. The benefit of everolimus was not evident amongst a small cohort of patients with established donor-transmitted disease suggesting that early use of this agent is likely to be more effective. There is no evidence of everolimus having a detrimental effect on CAV morphology or immune marker activity during this follow-up period even if the number of grade 2R rejections was increased in the everolimus group. Given these positive findings, coupled with the beneficial effect of everolimus on renal function during the same follow-up period, it appears that everolimus and early CNI withdrawal is an attractive alternative immunosuppressive protocol that may improve long-term outcome after HTx. The beneficial effect of everolimus on CAV may involve anti-fibrotic and anti-proliferative effect, but at present it mechanisms of action on CAV development seem elusive.

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FIGURE LEGENDS

Figure 1. Patient disposition for the 36-month IVUS analysis.

Figure 2. Progression of cardiac allograft vasculopathy in the two treatment arms as assessed by change in Maximal Intimal Thickness (MIT), Percent Atheroma Volume (PAV), and Total Atheroma Volume (TAV) at 12 and 36 months (values shown are change from baseline with statistical testing by ANCOVA analysis and p-value represents between group contrast).

Figure 3. Progression of cardiac allograft vasculopathy in the two treatment arms stratified according to absence or presence of donor disease defined as baseline Maximal Intimal Thickness ≥0.5 mm (values shown are change from baseline with statistical testing by ANCOVA analysis and p-value represents between group contrast).

Figure 4. Virtual histology tissue analysis with change in composition of fibrous-, fibrofatty-, calcified- and necrotic tissue in the two treatment arms at 12 and 36 months.

Table.1. Study population characteristics according to treatment group.

	Everolimus (n=37)	Controls (n=39)	p-value
Recipient characteristics			
Recipient age (years)	51.2±11.6	50.4±12.6	0.76
Female gender (%)	10 (26)	12 (32)	0.52
BMI (kg/m^2)	24.9±3.1	24.0±3.9	0.22
Systolic blood pressure (mmHg)	107.5±18.6	66.7±13.5	0.97
Diastolic blood pressure (mmHg)	107.3±20.8	66.5±12.6	0.94
Medical history			
Hypertension (%)	4 (10)	5 (14)	0.66
Diabetes mellitus (%)	9 (23)	4 (11)	0.16
Left ventricular assist device (%)	9 (23)	10 (27)	0.69
Previous smoking history (%)	21 (54)	19 (51)	0.83
Primary reason for HTx			
Idiopathic cardiomyopathy	30 (77)	27 (73)	0.69
Coronary artery disease	6 (15)	5 (14)	0.82
Donor Characteristics			
Donor age (years)	45.7±12.3	41.4±14.0	0.16
Female donor gender (%)	12 (31)	16 (43)	0.26
Cold ischemia time (min)	187.5±73.2	185.3±74.7	0.91
Recipient CMV negative/donor CMV positive (%)	8 (21)	3 (8)	0.12
Renal function and lipid profile*			
mGFR (mL/min)	61.8±15.0	64.7±14.9	0.39
Serum creatinine (mmol/L)	103.4±31.5	99.0±29.7	0.53
Total cholesterol (mmol/L)	3.5±2.0	3.3±1.7	0.64
HDL (mmol/L)	0.9±0.6	0.7±0.4	0.22
LDL (mmol/L)	2.0±1.3	2.0±1.2	0.89
Triglycerides (mmol/L)	1.3±1.3	1.1±0.7	0.38
Rejection episodes			
Number of patients with rejection ≥2R (%) within 12 months	5 (13)	14 (38)	0.01
Number of patients with rejection ≥2R (%) within 36 months	5 (13)	15 (41)	0.01
CMV infection/viremia	11 (28)	3 (8)	0.02
Baseline IVUS			
MIT (mm)	0.45±0.18	0.44±0.19	0.82
PAV (%)	20.1±7.0	20.2±8.0	0.94
TAV (mm^3)	133.2±107.9	144.6±113.1	0.66

Data reported as mean±SD or absolute number (percentage) as appropriate.*Data at time of transplantation (V1).
BMI = body mass index, LDL = low-density lipoprotein, HDL = high-density lipoprotein, mGFR = measured glomerular filtration rate, CMV = cytomegalovirus, LVAD = left ventricular assist device, MIT=maximal intimal thickness, PAV= percent atheroma volume, TAV=total atheroma volume.

Table 2. Regression analysis evaluating variables predicting progression of cardiac allograft vasculopathy at 36 months utilizing the endpoint maximal intimal thickness as a continuous variable.

Variable	**B**	**95% CI**	**β**	**p-value**
Recipient age	0.001	-0.002 ─ 0.003	0.06	0.61*
Donor age	0.002	0.000 ─ 0.004	0.23	0.05
Treatment with everolimus	-0.04	-0.10 ─ 0.01	-0.18	0.03
Male recipient	0.07	0.007 ─ 0.13	0.29	0.03
Diabetes mellitus	-0.04	-0.11 ─ 0.04	-0.11	0.32*

B=unstandardized coefficient. **β =**standardized coefficient

*variables significant on univariate analysis and selected as covariates for the multivariate analysis but excluded in final multivariate model (non-significant p-value)

Table 3. Change in inflammatory biomarker profile from baseline to 36 months in the two treatment groups (n=65).

Variable	Cyclosporine group	Everolimus group	p-value
C-reactive protein (mg/L)	-1.13 (-4.85 ─ 1.32)	-2.74 (-4.86 ─ 0.58)	0.92
von Willebrand factor (AU)	-4.78 (-7.35 ─ -0.12)	-2.16 (-7.34 ─ 2.26)	0.15
Vascular cell adhesion molecule (ng/mL)	-86.4 (-188.1 ─ 32.1)	-61.9 (-131.2 ─ 0.6)	0.58
Soluble tumor-necrosis factor receptor-1 (pg/L)	-0.61 (-1.34 ─ -0.12)	-0.36 (-1.21 ─ 0.14)	0.11
Intercellular adhesion molecule-1(pg/L)	-0.03 (-86.8 ─ 36.2)	-4.03 (-77.2 ─ 61.2)	0.17
Chemokine ligand 16 (ng/mL)	-0.16 (-0.56 ─ 0.18)	-0.23 (-0.59 ─ 0.12)	0.26

Values shown are median values with IQ range in parentheses.

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