Calculated cardiovascular risk after conversion from calcineurin inhibitor to belatacept in kidney transplant recipients: A randomized controlled trial

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Abstract

In renal transplant recipients (RTRs), a belatacept-based immunosuppressive regimen is associated with beneficial effects on cardiovascular (CV) risk factors compared with calcineurin inhibitor (CNI)-based regimens. The aim of this randomized, multi-national trial was to compare calculated CV risk between belatacept and CNI (predominantly tacrolimus) treatments using a valdidated model developed for RTRs. From 9 transplant centers, RTRs from 3 to 60 months post-transplantation were recruited to either continue treatment with a CNI-based regimen or switch to belatacept. We compared the change in estimated 7-year risk of major adverse cardiovascular events (MACE) and all-cause mortality after 12 months of treatment. In the 105 RTRs randomized, we found no differences between the treatment groups in predicted risk for MACE or mortality. Diastolic blood pressure was lower after belatacept treatment compared with CNI. The mean changes in traditional CV risk factors, including renal transplant function, were otherwise similar in both treatment groups. The belatacept group had four acute rejection episodes; two were severe rejections, of which one led to graft loss. In conclusion, we found no effects on calculated CV risk by switching to belatacept treatment.

1. Introduction

The risk of cardiovascular disease (CVD) in patients with renal failure is much higher than in the general population across all age groups [1,2]. While a successful transplant reduces this risk significantly, renal transplant recipients (RTRs) still have an annual cardiovascular (CV) event rate of 3.5–5% [3]. Accordingly, CVD remains one of the leading causes of death in RTRs [4,5]. Managing a transplanted patient should therefore include CV risk reduction measures to improve both graft and patient outcomes. Current guidelines for prevention of CVD are based upon data from the general population and from studies specifically targeting CVD in RTRs [6]. In addition to addressing traditional risk factors for CVD, such as lifestyle choices, hypertension, hyperlipidemia, and diabetes, RTRs present two potentially modifiable factors: renal graft function and type of immunosuppressive maintenance regimen.

First, evidence indicates that declining graft function and graft loss are potentially modifiable risk factors for CVD and all-cause mortality in this population, which make strategies for optimizing graft function important [7,8]. Second, among immunosuppressive drugs used for transplantation, both steroids and calcineurin inhibitors (CNIs) are associated with adverse CV side effects [9]. Therefore, attempts have been made to minimize or eliminate their use. While these have led to reasonably safe steroid-free regimens [10–12], CNIs are still the cornerstone of immunosuppression in modern solid organ transplantation. Early graft survival improved greatly after the introduction of cyclosporine (CsA) in the early 1980s [13], and tacrolimus (TAC) has been the CNI of choice since the 1990s [14]. Despite the benefits of CNIs in the early post-transplant period, they have dose-dependent side effects, including post-transplant diabetes mellitus (PTDM), hypertension, hypercholesterolemia, and nephrotoxicity, leading to progressive decline in renal graft function [15–19]. Therefore, there is an ongoing incentive for development of novel immunosuppressive agents without the side effects of CNIs.

Belatacept, a modified form of CTLA4-Ig, binds to CD80 and CD86 on antigen presenting cells, thus blocking CD28 mediated co-stimulation of T-cells. The BENEFIT trials have shown promise for belatacept as an option in designing a more favorable immunosuppressive regimen [20–24]. In brief, despite higher rates of early rejection, the relative risk of death or graft loss after 7 years was reduced by 43% in patients treated with belatacept versus CsA-treated patients, and eGFR in the belatacept-group was on average 22 ml/min/1.73m^2 higher than in the CsA-group. Furthermore, in a meta-analysis comparing belatacept with CNIs, treatment with belatacept was associated with lower blood pressure, lower incidence of diabetes and a more favourable lipid profile [25].

However, it is not yet proven whether these findings translate into overall CVD reduction. Soveri et al. have previously developed a risk calculator for CVD and all-cause mortality for use in RTRs [26]. The group later used the data of the BENEFIT and BENEFIT-EXT trials to calculate the potential benefit associated with belatacept treatment and found a substantial calculated 7-year risk reduction for major adverse cardiac endpoints (MACE) and mortality by converting from CsA to belatacept [27].

A shortcoming of belatacept that has hindered its implementation in kidney transplantation has been the relatively high rate of early rejection, as well as the lack of studies comparing its efficacy with low-dose TAC, the current standard of care in RTRs. In the present study, our aim was to investigate 1) the effects of conversion from a low-dose CNI-based therapy to belatacept on estimated risk of CVD and all-cause mortality using a previously validated calculator and 2) the changes in traditional markers of cardiovascular health, as well as measures of arterial stiffness.

2. Results

2.1 Study participants and characteristics

A total of 112 patients from 9 centers signed the patient informed consent form. Of these, one patient was a screen failure (history of rejection) and was never randomized. Of the 111 randomized patients, 6 withdrew consent before any study drug was given, 4 in the belatacept arm and 2 in the CNI arm. Thus, 105 patients were administered study medication: 54 in the belatacept arm and 51 in the CNI arm (defining our ITT population). In the belatacept-arm 5 patients were withdrawn from the study; 3 due to adverse events (AEs), 1 withdrew consent and 1 moved out of the country. Similarly, there were 2 withdrawals in the CNI arm; 1 due to AE and 1 withdrew consent. The remaining 49 patients in each treatment arm were defined as the per protocol (PP) population (Figure 1). As the difference between the PP population and the ITT population was quite small, we did not perform PP analyses to avoid the risk of type I error caused by multiple comparisons. The first patient was enrolled September 18th, 2014, and the last patient completed the study on September 13th, 2018. Baseline demographic data and clinical characteristics for each group are presented in Table 1.

Table 1
Baseline demographics and clinical characteristics (ITT population).

	Belatacept arm (n=54)	CNI arm (n=51)
Female	13 (24%)	13 (25%)
Age, years	55.0 (15.2)	54.2 (13.8)
BMI, kg/m^2	26.1 (4.1)	27.1 (4.1)
Renal replacement therapy		
Number of transplantations	1 (1 - 2)	1 (1 - 2)
Time since last transplantation, months	25.3 (3.7 - 59.6)	23.4 (3.1 - 58.8)
Total time on renal replacement therapy, months	35.6 (12.1 – 489.5)	36.8 (5.3 - 220.8)
Prior immunosuppressive therapy		
Tacrolimus	53 (98%)	48 (94%)
Cyclosporine	1 (2%)	3 (6%)
Steroids	50 (93%)	50 (98%)
Mycophenolate	50 (93%)	47 (92%)
mTOR inhibitor	3 (6%)	1 (2%)
Baseline immunosuppression trough levels		
Tacrolimus	5.8 (1.7)	5.7 (1.7)
Cyclosporine	94	89 (5.1)
Diabetes mellitus	12 (22%)	7 (14%)
Hypertension	30 (56%)	33 (65%)
Systolic blood pressure, mmHg	137 (17.2)	133 (18.4)
Diastolic blood pressure, mmHg	84 (9.7)	81 (11.2)
Smoking habits		
Current smoker	6 (11%)	8 (16%)
Previous smoker	19 (35%)	22 (43%)
Cardiovascular disease		
Peripheral vascular disease	8 (15%)	7 (14%)
Previous heart failure	2 (4%)	3 (6%)

	Belatacept arm (n=54)	CNI arm (n=51)
Previous coronary heart disease	4 (7%)	6 (12%)
Previous cerebrovascular disease	2 (4%)	4 (8%)
Plasma creatinine, µmol/L	135 (35.7)	125 (42.6)
eGFR (mL/min/1.73 m^2)	49.4 (14.8)	56.6 (19.1)
hs-CRP, mg/L	3.1 (4.1)	2.8 (2.8)
Plasma glucose, mmol/L	6.2 (1.8)	5.9 (1.7)
Total cholesterol, mmol/L	5.0 (1.0)	5.1 (1.0)
HDL-cholesterol, mmol/L	1.5 (0.5)	1.6 (0.6)
LDL-cholesterol, mmol/L	2.7 (0.9)	2.7 (0.9)
Triglycerides, mmol/L	1.9 (0.9)	1.9 (0.9)
Apolipoprotein B, g/L	1.0 (0.4)	1.0 (0.3)
Apolipoprotein A1, g/L	1.4 (0.3)	1.5 (0.4)
Data are presented as number (percentage) for categorical data, as mean value (standard deviation) for continuous variables and as median (min - max) for renal replacement therapy.		

2.2 Estimated risk of MACE and mortality

The primary endpoint was the estimated 7-year risk of MACE and all-cause mortality per the risk calculator developed by Soveri et al. (Figure 2). After 12 months of treatment, there was no statistically significant difference between the treatment groups in terms of change in predicted risk, neither for MACE nor for mortality (Table 2).

Table 2
Estimated 7-year risk of MACE and mortality.

		Belatacept arm		**CNI arm**		***Difference***
		Baseline	End of study	Baseline	End of study	
MACE	Mean (SD)	0.15 (0.13)	0.15 (0.15)	0.14 (0.14)	0.15 (0.15)	
Log mean risk change [95% CI]		-2.31 [-2.40, -2.23]		-2.25 [-2.33, -2.16]		**0.06 [-0.06, 0.14]**
Mortality	Mean (SD)	0.21 (0.19)	0.23 (0.20)	0.19 (0.18)	0.21 (0.19)	
Log mean risk change [95% CI]		-1.94 [-1.96, -1.91]		-1.92 [-1.94, -1.90]		**0.02 [-0.01, 0.05]**
MACE = major adverse cardiac event. CI = confidence interval						

In the belatacept-arm, mean (SD) estimated 7-year risk of MACE at baseline was 0.15 (0.13), and it remained unchanged after 1 year to 0.15 (0.15). Similarly, the risk estimation for the CNI continuation arm was 0.14 (0.14) at baseline and 0.15 (0.15) after 1 year. After applying the ANCOVA models and adjusting for hospital centers, the log mean risk prediction decreased by 2.31 (95% CI: 2.23, 2.40) for the belatacept-group, and 2.25 (95% CI: 2.16, 2.33) for the CNI group. The difference between interventions in log mean risk prediction for MACE was 0.06 (95% CI: -0.06, 0.14).

The estimated 7-year mortality risk in the belatacept-arm at baseline was 0.21 (0.19), which increased non-significantly to 0.23 (0.20) after 1 year. Correspondingly for the CNI continuation arm, the predicted risk of mortality was 0.19 (0.18) at baseline and increased non-significantly to 0.21 (0.19) after 1 year. After applying the ANCOVA models and adjusting for hospital centers, the log mean risk prediction decreased by 1.94 (95% CI: 1.91, 1.96) for the belatacept-group, and 1.92 (95% CI: 1.90, 1.94) for the CNI group. The difference between interventions in log mean risk prediction for mortality was 0.02 (95% CI: -0.01, 0.05). An overview of the variables used in the risk calculation is presented in Table 3.

Table 3
Overview of variables composing estimated cardiovascular risk.

		Belatacept arm		CNI arm	
Risk calculator composite	Variable	Baseline	End of study	Baseline	End of study
Common for MACE and mortality	Age, years	54.5 (15.2)	55.5 (15.2)	53.8 (13.7)	54.8 (13.7)
	Creatinine, µmol/L	135.1 (35.7)	132.2 (44.1)	124.7 (42.6)	119.1 (38.4)
	Diabetes mellitus	12 (22.2%)	12 (22.2%)	7 (13.7%)	7 (13.7%)
	Coronary HD	4 (7.4%)	4 (7.4%)	6 (11.8%)	6 (11.8%)
	Current smoker	6 (11.1%)	6 (11.1%)	8 (15.7%)	8 (15.7%)
	Previous smoker	19 (35.2%)	19 (35.2%)	22 (43.1%)	22 (43.1%)
MACE only	LDL-cholesterol, mmol/L	2.7 (0.9)	2.6 (1.0)	2.7 (0.9)	2.6 (0.8)
	Number of transplants: 1	51 (94.4%)	51 (94.4%)	48 (94.1%)	48 (94.1%)
	Number of transplants: 2	3 (5.6%)	3 (5.6%)	3 (5.9%)	3 (5.9%)
Mortality only	Total time RRT, months	51.4 (69.5)	62.9 (69.6)	45.1 (37.0)	56.9 (37.0)

Data are presented as number (percentage) for categorical data and mean value (standard deviation) for continuous variables. HD = Heart Disease. MACE = major adverse cardiac event. RRT = renal replacement therapy.

Subgroup analysis was also performed to investigate whether time since transplantation influenced the results in risk calculation. Treatment arms were divided upon the median time after transplantation, thus creating an early and late group (before and after 26 months). There was no difference between belatacept and CNI in calculated risk of MACE (p = 0.33) and mortality (p = 0.56) in the subgroups.

2.3 Traditional CVD risk factors

The changes in traditional CV biomarkers from baseline to end of study are presented in Table 4. The mean changes were similar between the treatment groups, except for a significant difference in diastolic blood pressure, with lower levels after belatacept treatment compared with CNI. Systolic blood pressure showed a similar reduction, but the difference was not statistically significant.

Table 4
Change from baseline for traditional CVD risk factors.

Risk factor	Belatacept arm	CNI arm	p-value
Systolic BP, mmHg	-3.3 (21.3)	2.9 (14.2)	0.09
Diastolic BP, mmHg	-2.6 (10.0)	2.8 (10.7)	0.01
hs-CRP, mg/L	4.64 (19.9)	0.81 (4.7)	0.19
Plasma glucose, mmol/L	0.04 (2.5)	-0.06 (2.0)	0.83
eGFR, mL/min/1.73 m^2	1.40 (7.9)	0.73 (7.7)	0.67
Total cholesterol, mmol/L	0.22 (2.8)	-0.09 (0.8)	0.45
HDL-cholesterol, mmol/L	-0.10 (0.3)	-0.02 (0.2)	0.08
LDL-cholesterol, mmol/L	-0.10 (0.7)	-0.05 (0.6)	0.71
Triglycerides, mmol/L	-0.06 (0.8)	-0.05 (0.7)	0.96
Apolipoprotein B, g/L	-0.09 (0.3)	-0.06 (0.2)	0.59
Apolipoprotein A1, g/L	0.02 (0.3)	0.01 (0.3)	0.88

Presented as mean (standard deviation). BP = blood pressure. hs-CRP = high-sensitivity C-reactive protein. eGFR = estimated glomerular filtration rate.

P-value results from two-sample t-tests.

2.4 Arterial stiffness

Arterial stiffness was measured at baseline and at end of study using the SphygmoCor® method. Compared with the CNI group, central diastolic pressure in patients of the belatacept group decreased by 6.55 mmHg (95%CI: 1.83, 11.27; p = 0.007) after one year of treatment. For central systolic pressures, the difference of 6.1 mmHg between study groups (95% CI: -0.11, 12.34; p = 0.054) was borderline significant. There were no differences between the treatment arms in central pulse pressure, pulse wave velocity and augmentation index (Table 5).

Table 5
Change from baseline in arterial stiffness variables.

Risk factor	Belatacept arm	CNI arm	p-value
Augmentation Index, %	-1.26 (10.6)	1.04 (10.9)	0.33
Pulse Wave Velocity, cm/sec	-0.44 (1.9)	0.1 (3.0)	0.34
Central systolic pressure, mmHg	-4.45 (15.3)	1.65 (13.7)	0.054
Central diastolic pressure, mmHg	-3.72 (12.1)	2.83 (9.6)	0.007
Central pulse pressure, mmHg	-0.60 (11.3)	-0.37 (10.1)	0.92
Presented as mean (standard deviation). P-value results from two-sample t-tests.			

2.5 Cardiovascular events and patient survival

During the one-year study period, there were no cardiovascular events observed (including cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, hospitalization due to congestive heart failure or angina pectoris, or coronary intervention) or deaths in the study population.

2.6 Safety evaluation

All patients in both study groups reported at least one AE during the duration of the study (Table 6). The majority of the events were of mild severity and considered unrelated to study drug. More patients in the belatacept-group (53.7% vs 21.6%) reported AEs that were considered possibly or probably related to the intervention. Three patients in the belatacept-group and one patient in the CNI continuation group reported AEs that led to withdrawal from the study. Serious Adverse Events (SAEs) were reported by 29.6% of the patients in the belatacept-group compared with 15.7% in the CNI group. Patients allocated to the belatacept-group had more infections (Table 7). There was 1 case of incident cancer (lung cancer), which occurred in the belatacept-group.

Table 6
Number and proportion of patients with adverse events

	Belatacept arm		CNI arm	
	n	%	n	%
Any adverse event	54	100	51	100
1 adverse event	10	18.5	21	41.2
>1 adverse events	44	81.5	30	58.8
Any possibly or probably intervention-related adverse events	29	53.7	11	21.6
Adverse events leading to withdrawal	3	5.6	1	2.0
Serious adverse events	16	29.6	8	15.7
- suspected acute rejection	7	13.0	1	2.0
- biopsy-proven acute rejection	4	7.4	1	2.0
- graft loss due to acute rejection	1	1.9	0	-
- cancer	1	1.9	0	-

Table 7
Adverse events reported by ≥5% of patients in either treatment group

Event	Belatacept arm (n=54)	CNI arm (n=51)
Urinary tract infection	35.3	7.8
Pyrexia	31.5	2.0
Abdominal pain/discomfort	18.6	2.0
Nasopharyngitis	18.5	15.7
Respiratory tract infection	14.9	9.8
Coughing	14.8	2.0
Oedema	9.4	6.0
Diarrhoea	9.3	3.9
Anemia	9.3	2.0
Fatigue	7.4	2.0
Headache	7.4	2.0
Dizziness	7.4	0
Arthralgia	3.7	5.9
Gastroenteritis	1.0	5.9
Nausea	5.6	2.0
Herpes zoster	5.6	2.0
Myalgia	5.6	2.0
Aphthous ulcer	5.6	0
Given as incidence rates (in %)		

During the study, 8 acute rejection episodes were suspected, and graft biopsies were obtained for further investigation. Acute rejection was confirmed in 4 of the 7 suspected cases in the belatacept-group, and in the single case in the CNI group. Three of the rejection episodes were considered severe (Banff grade IIA or higher): two in the belatacept-group and in the CNI-treated patient. One patient (belatacept) proved refractory despite anti-rejection treatment with methylprednisolone and T-cell depleting antibody. All other rejection episodes recovered upon treatment with corticosteroids or anti-thymocyte globulin as per local practices.

3. Discussion

In this randomized study, where stable renal transplant patients were converted from a CNI-based maintenance immunosuppressive regimen to belatacept, no difference in calculated 7-year risk of MACE or all-cause mortality could be demonstrated after 1 year of follow-up. We were unable to find a significant effect on any of the three modifiable cardiovascular risk factors which were used as input-variables in the risk calculator (serum LDL-cholesterol, diabetes-prevalence, and serum creatinine). The belatacept arm had significantly lower diastolic blood pressure, measured both centrally (SphygmoCor® method) and peripherally. We found a similar improvement for systolic pressure (Table 4, 5), but this difference was not statistically significant.

Of the three modifiable risk factors in the calculator, the expectations regarding effect on lipid profile were limited. While CsA has been implicated in dyslipidemia[28], TAC seems to be less detrimental to lipid status. In our study, 94% of the participants were on TAC before randomization. Ferguson et al. [29] compared three steroid-avoiding regimens of immunosuppression: belatacept with MMF vs belatacept with sirolimus vs TAC with MMF. Both belatacept-arms had lower LDL (23.9 mg/ml and 25.0 mg/ml vs 34.0 mg/ml for TAC with MMF) after one year, but the difference was non-significant, possibly related to the limited sample-size of the study. Another observational study focusing on the metabolic effects of conversion from TAC to belatacept found improvement in GFR and acid-base status, but not in blood lipids [30]. Our findings are in line with these reports, as we found no effect on LDL-cholesterol (Table 3).

However, we are surprised by the lack of effect on GFR, which is in contrast to the BENEFIT studies, as well as other conversion studies reported in the literature [24,31–33]. In those studies, there was a consistent improvement in graft function by converting to belatacept. One possible explanation for this was the predominant use of TAC by our study participants with relatively low trough levels (Table 1) at baseline. In the Symphony trial [34], the low-dose TAC group had an average trough-level of 6.7 ng/ml 1 year after transplantation and achieved an eGFR on average 5.7 ml/min/1.73m^2 higher than the other 3 comparator groups. A belatacept conversion study by Grinyo et al. [35] examined 173 patients with a mean time after transplantation to randomization of 19 months, baseline eGFR of 54 ml/min/1.73m^2, and a low immunologic risk profile, making the population reasonably comparable to ours. Belatacept patients in that study showed an average improvement in eGFR of 4.9 ml/m/1.73m^2 compared to CNI-patients. At baseline, patients using TAC (56%) had an average trough level of 7.2 ng/mL, while patients on CsA (44%) had an average trough level of 160.2 ng/mL. In our study, the mean trough levels of TAC (5.7 ng/ml) and CsA (91 ng/ml, 4 patients only) at the time of randomization were lower compared to both these studies [34,35]. The lower CNI trough levels may have already significantly decreased the nephrotoxic side-effects and explain why our belatacept patients only experienced a non-significant gain in eGFR of 0.7 ml/m/1.73m^2.

The third element of the calculator is the diabetes status. Multiple studies have corroborated the diabetogenicity of TAC in transplantation [36–38]. Furthermore, reversibility of beta cell dysfunction and of PTDM after TAC-withdrawal has been established in both animal studies and in clinical experience [39–42]. Thus, we expected to improve glycemic metabolism in converting from TAC to belatacept. However, no

subject in our study reversed diabetes mellitus or developed PTDM in either study arm (Table 3). Also, triglycerides, serum ApoB, and serum ApoA1 did not improve (Table 4), which is of interest, since all three of these parameters are mentioned as risk factor for developing PTDM. [43,44]

Beside trough levels, we also need to consider another bias. All patients were already treated with CNI for a median of 26 months since transplantation. Serious negative side effects of CNI-treatment could be less likely found in the control group, as patients suffering from these side effects could have been converted to alternative immunosuppression earlier on and thus not be eligible for this study.

The only positive effect that we found for belatacept was a significant improvement in diastolic blood pressure, measured both centrally (SphygmoCor® method) and peripherally. For systolic pressure, a similar improvement was found (Table 4), but it was not statistically significant ($p = 0.09$), most likely due to the relatively small sample size of this study. Although not included in the calculator, blood pressure is of course an established risk factor for cardiovascular disease. Moreover, high blood pressure is strongly associated with risk of graft failure and finding an improvement in this parameter could still indicate an advantage for belatacept-treatment [45].

Regarding safety, AEs occurred in both groups, but SAEs were reported almost twice as often in belatacept-treated patients (29.6% vs 15.7%), and the latter were more likely (5.6% vs 2.0%) to discontinue their study treatment than patients treated with CNI. Rejection was seen more often in the belatacept-patients. Four episodes of biopsy-proven acute rejections occurred in the belatacept-group vs one single episode in the CNI-group (7.4% vs 2.0%). Three patients showed signs of vascular inflammation in the biopsy, corresponding to Banff grade II, two of which were in the belatacept-group. All three patients were treated according to local protocol with high-dose steroids (4) and T-cell depleting antibodies (1), despite which one belatacept-patient suffered graft loss and re-initiated dialysis treatment. The other two recovered with treatment.

The rate of rejection in this study is in line with earlier reports. For example, in the trial by Grinyo et al. [35], 7.1% of belatacept-patients experienced rejection versus none in the CNI-group. In another trial by Adams et al. [46], 1-year rejection rates were around 50% when belatacept was used right after transplantation, declining to 33% when TAC was tapered off 3-5 months after transplantation. When TAC was tapered after 11 months, the rejection rates between TAC- and belatacept-treated patients were similar, around 16%. Other reports have described varying (0-11%) rates of rejection, but these are data from non-randomized ‘rescue’-settings after even longer time post-transplantation and are therefore not comparable with our results [47,48].

Beside rejection, urinary tract infections (UTIs), nasopharyngitis and other respiratory tract infections (RTIs) were more often seen in the belatacept arm (Table 7). The present study’s planned visits could have led to a bias in the reporting of uncomplicated infections, since a study visit was planned every month for belatacept-patients, instead of every 3 months for the CNI-continuation group.

Not a single case of pneumocystis-jirovecii pneumonia, cytomegalovirus- (CMV), polyoma- or EBV-associated disease was seen in the belatacept-patients. Three cases of CMV-infection were seen in CNI-patients. Previous reports have been inconclusive on opportunistic infections (OPIs) in belatacept-treatment. The follow-up study to the first belatacept-conversion trial noted a slightly higher incidence of viral infection (11% vs 14%) [35]. In a recent study by Bertrand e.a. 50 OPI's were noted in 453 patients treated with belatacept (9.8%) [49]. In a multivariate analysis of that study, the authors concluded that patients with low GFR (<25 ml/min) and patients converted early after transplantation (within six months) were more likely to develop OPIs.

There was one case of lung cancer in the belatacept group in the present study. Previous studies have not indicated a higher risk of malignancy in belatacept beyond post-transplant-lymphoproliferative disorder [24,35].

A major strength of the current study is the international multicenter-approach, making it representative for European transplantation practice. However, this study also has important limitations which must be taken into account. The study duration of 1 year was most likely too short to reveal a significant difference in renal function between the two study groups. We have overestimated the potential reduction in MACE and mortality for patients that use low-dose TAC instead of CsA. Another limitation was the heterogeneous time from transplantation to trial enrollment, and the small number of patients on CsA and the relatively large span of eGFR also contributed to the heterogeneity. Patients with severely diminished graft function were less likely to benefit from conversion.

In conclusion, we have shown no effect on calculated cardiovascular risk or renal function in this study comparing late conversion to belatacept with continuation of CNI-based immunosuppression. We did show a significant difference in diastolic blood pressure. We re-confirmed the increased chance of rejection when converting to belatacept. After more than 10 years of clinical experience, the place of belatacept in kidney transplantation is still not fully established, but it may be an attractive option when patients suffer from significant side effects of CNI, like nephrotoxicity or PTDM. However, it is hard to define a significant benefit of belatacept for patients that are doing well on low-dose TAC-based therapy without severe CNI-related side-effects. Further studies are needed to define the place of belatacept in kidney transplantation.

4. Methods

4.1 Study design

This was a prospective, randomized, open label, parallel group, investigator-initiated, international multi-center trial (EudraCT no. 2013-001178-20, registered at www.clinicaltrialsregister.eu on 29-10-2013). Patients were randomized in 1:1 ratio to either continue treatment with a CNI-based regimen or to switch to belatacept for a study duration of 12 months. An open design was chosen since CNIs were given as tablets daily and belatacept was administered as infusion every four weeks.

Patients were recruited from 9 transplant centers in Denmark, the Netherlands, Norway, and Sweden. RTRs aged 18–80 years with a stable graft function (estimated glomerular filtration rate [eGFR] >20 mL/min per 1.73 m^2), 3–60 months post-transplantation treated with TAC or CsA were eligible for inclusion. Patients were excluded if they were Epstein-Barr virus (EBV) IgG seronegative, had severe de novo or recurrent renal disease, had a history of vascular or antibody-mediated rejection in the present transplant or had a history of recent malignancy.

The study was approved by the Regional Ethical Review Board in Uppsala, Sweden, and subsequently by the local ethics committees of the other affiliated hospitals. Written informed consent was obtained from all patients, and the trial was performed in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice.

4.2 Study medication

For patients randomized to the study arm with belatacept, the previous CNI treatment (TAC or CsA) was tapered in the initial period as follows: 100% on day 1, to 70-80% on day 7, to 40-60% on day 15, 20-30% on day 23 and none on day 29 and beyond. Belatacept was dosed 5 mg/kg IV on day 1, 15, 29, 43, 57 and then every month thereafter in the 12-month study period (Figure 3). Patients randomized to the control group with continuation of CNI treatment were to maintain trough levels of CsA between 75 and 200 ng/ml and TAC between 5 and 10 ng/ml. Both groups were to continue their underlying immunosuppressive regimen, consisting of mycophenolate mofetil (MMF) or mammalian target of rapamycin inhibitor and corticosteroids. Any other concomitant medication necessary to maintain the patients' baseline condition or to treat a coexisting disease was permitted.

4.3 Efficacy assessment and procedures

The primary endpoint of this trial was estimated cardiovascular risk after 12 months, using a prediction model developed for RTRs by Soveri et al.[26]. The estimated 7-year risk of MACE and mortality in the two treatment groups were calculated as a linear combination of the following variables: age, previous coronary heart disease, previous smoker, current smoker, creatinine, diabetes mellitus, low-density lipoprotein (LDL), number of transplants and total time on renal replacement therapy (Figure 2). Secondary endpoints were arterial stiffness, traditional CVD risk factors in RTRs (blood pressure, lipid profiles and eGFR), acute rejections, allograft loss, CV events and patient survival. Blood samples were drawn at a fasting state in the morning at baseline and at end of study visits for measurement of renal function and CV biomarkers: creatinine, high-sensitivity C-reactive protein (hs-CRP), total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), LDL cholesterol, triglycerides, apolipoprotein B (ApoB) and apolipoprotein A1 (ApoA1). Arterial stiffness was assessed at the same time points by measuring central pulse pressure, pulse wave velocity and augmentation index using the SphygmoCor® method [50].

4.4 Sample size and randomization

We performed a power calculation hypothesizing that the intervention arm would decrease the risk of MACE by 30%. We came to that estimate by extrapolation of the reduction in calculated risk in the

previously mentioned paper by Soveri e.a.[27]; the calculated risk of MACE for BENEFIT-patients decreased by 31.2% (from 14.3–10.9%), and for mortality by 40% (17.5–12.5%). The corresponding risk reduction for BENEFIT-EXT-patients was 27.8% (22.5 to17.6%) and 22.6% (30.9–25.2%). For a two-sample t-test on a two-sided significance level of 0.05, assuming a standard deviation of 0.64 (on the natural logarithmic scale), a sample size of 51 per group was required to obtain a power of 0.8 (80%) to detect a 30% calculated risk reduction in MACE. The ANCOVA model was expected to have slightly greater power than the two-sample t-test, and therefore a sample size of 102 patients was seen as sufficient for this study. To account for 8% drop-out, a total of 110 patients, 55 per treatment arm were included in the study. Randomization to treatment arm was performed using a computerized procedure, stratified by center, in a 1:1 ratio.

4.5 Statistical analysis

The primary endpoint was a comparison of the estimated CV risk between treatment groups (CNI- vs. belatacept-based immunosuppression) at one year. For patients who discontinued the study before one year, the last available estimate of CV risk was used in the analysis of the intention-to-treat (ITT) population. Due to a skewed distribution, estimated CV risk was log-transformed. The primary analysis was performed using analysis of covariance (ANCOVA) with treatment as a group variable and baseline log CV risk for MACE and center as covariates. All other comparisons on primary and secondary endpoints were based on ITT comparisons of treatment groups using two-sample t-test, or ANCOVA with correction for baseline variables and/or center. A two-sided P value of <0.05 was considered statistically significant. Analyses were performed using SAS® version 9.4 (SAS Institute, Cary, NC).

Abbreviations

AE adverse event

ANCOVA analysis of covariance

ApoA1 apolipoprotein A1

ApoB apolipoprotein B

CNI calcineurin inhibitor

CsA cyclosporine

CV cardiovascular

CVD cardiovascular disease

EBV Epstein-Barr virus

eGFR estimated glomerular filtration rate

GFR glomerular filtration rate

HDL high-density lipoprotein

hs-CRP high-sensitivity C-reactive protein

ITT intention-to-treat

LDL low-density lipoprotein

MACE major adverse cardiovascular event

MMF mycophenolate mofetil

OPI opportunistic infection

PP per protocol

PTDM post-transplant diabetes mellitus

RTI respiratory tract infection

RTR renal transplant recipients

SAE serious adverse event

TAC tacrolimus

Declarations

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Study Steering committee:

Prime principal investigator of each participating center; Hallvard Holdaas, Oslo; Alan Jardine, Glasgow; Bengt Fellström, Uppsala, chairman of SSC.

Data and Safety Monitoring Board:

Anders Hartmann, Oslo; Josep M Grinyo, Barcelona.

Author contributions

OWB, JC First draft of manuscript

OWB, JC, MS, JWdF, KTS, AjvZ, BF Substantial revision and finalization of manuscript

OWB, MS, AB, HF, AH, HH, OH, LM, KS, SSS, BF Inclusion of patients and acquisition of data.

JG, AJ, BF Conception of study

JWdF, JMG, HH, AJ, IS, AJvZ, BF Study design

OWB, JC, MS, JWdF, KTS, AJvZ, BF Analysis and data preparation

Data availability statement.

The data used to support the findings are included in the manuscript. Any additional (raw) data are available from the author (OWB) and co-author (KTS) upon reasonable request. Restrictions to availability may apply due to privacy or ethical reasons.

Competing interests

The authors of this manuscript have the following conflicts of interest to disclose:

MS has received honoraria from Astellas.

KTS reports that his employer, Smerud Medical Research International AS, is a contract research organisation that delivered clinical trial management services to this study and was remunerated by Bristol Myers Squibb for that work.

IS has received honoraria from Sandoz, Vifor and Astra Zeneca

BF has received funding or honoraria from Astra Zeneca, Novartis, Astellas, Alexion, Bristol Myers Squibb and Calliditas Therapeutics.

The other authors have no conflict of interests to disclose.

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Figures

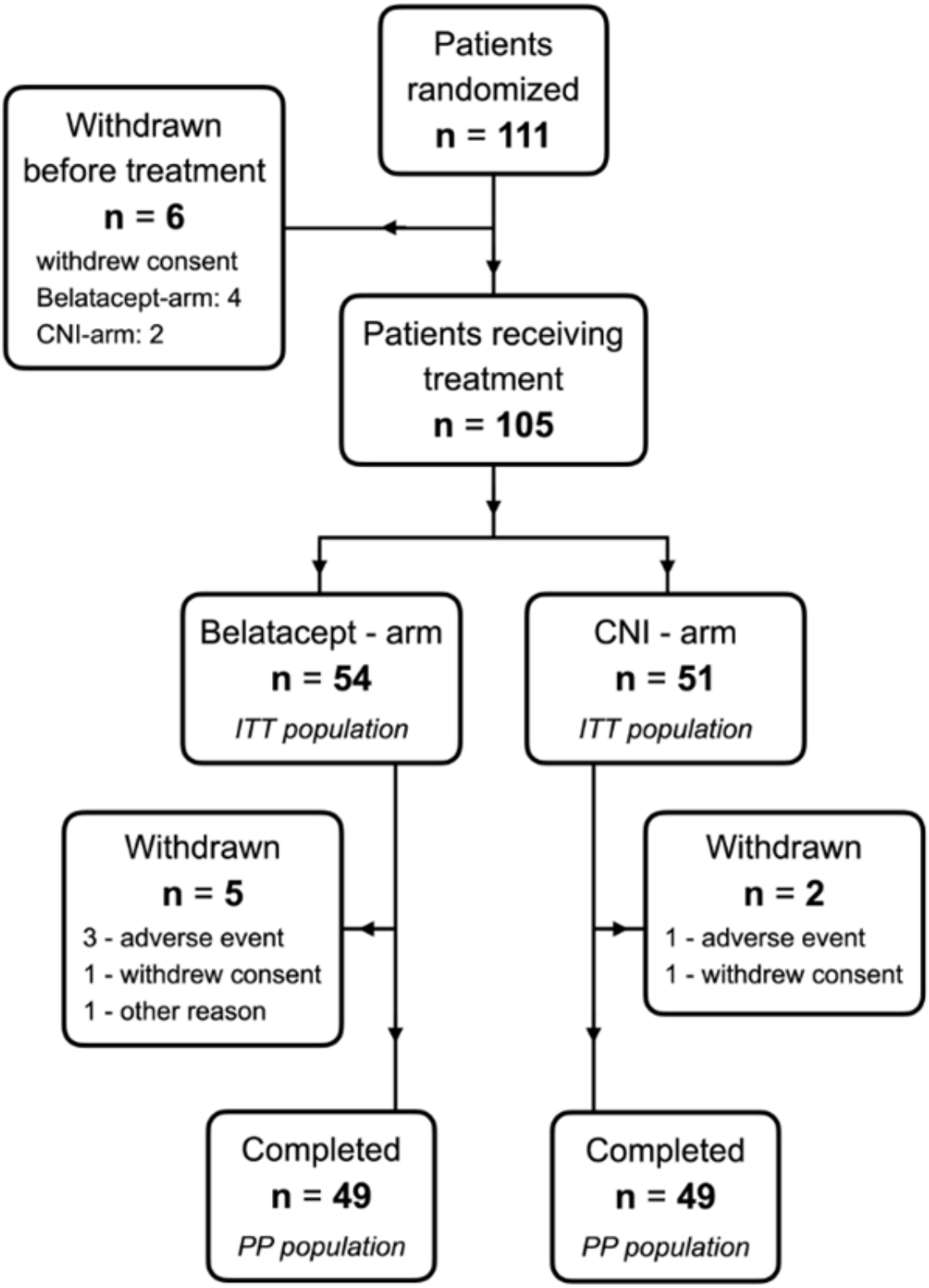

Figure 1

Study flow chart.

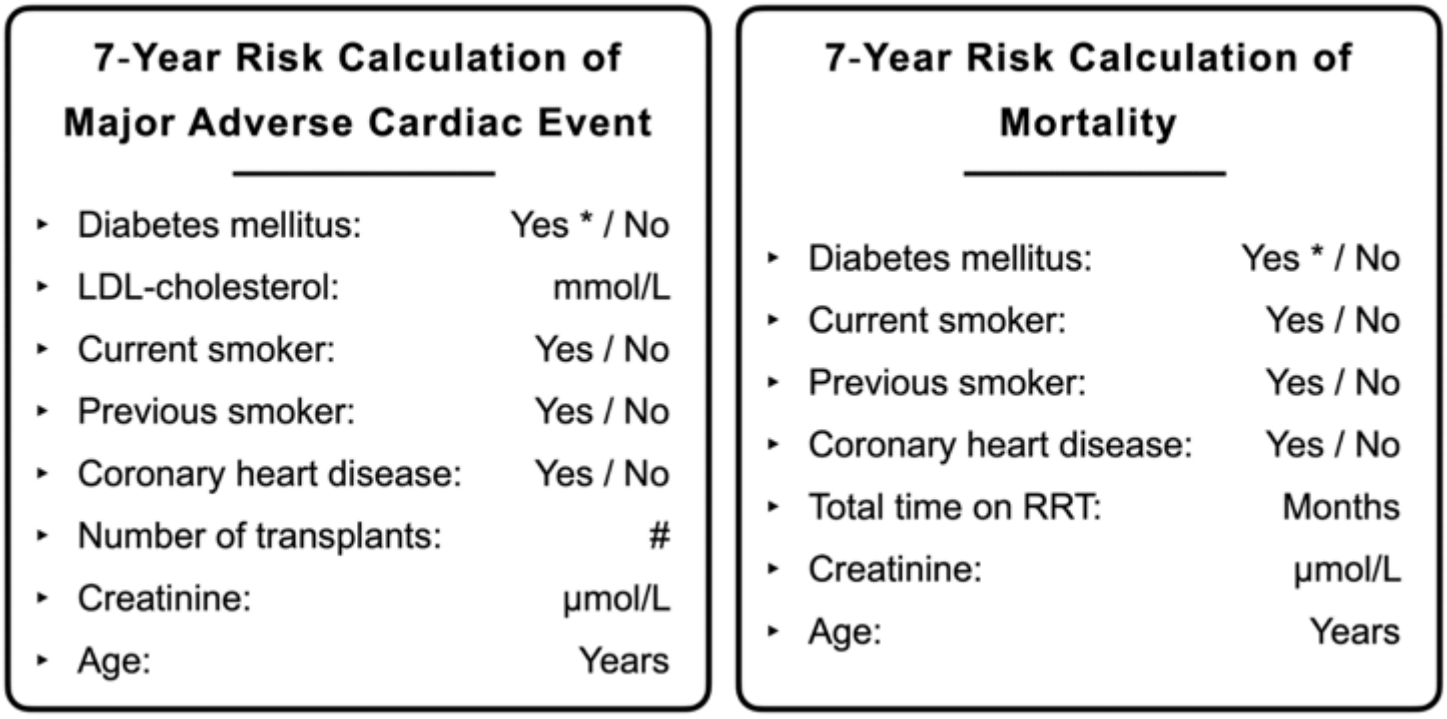

Figure 2

Cardiovascular risk calculator for renal transplant recipients (Soveri et al., 2012).

List of variables used in the cardiovascular risk calculator. *Includes post-transplant diabetes mellitus. RRT = renal replacement therapy (including dialysis and transplantation)

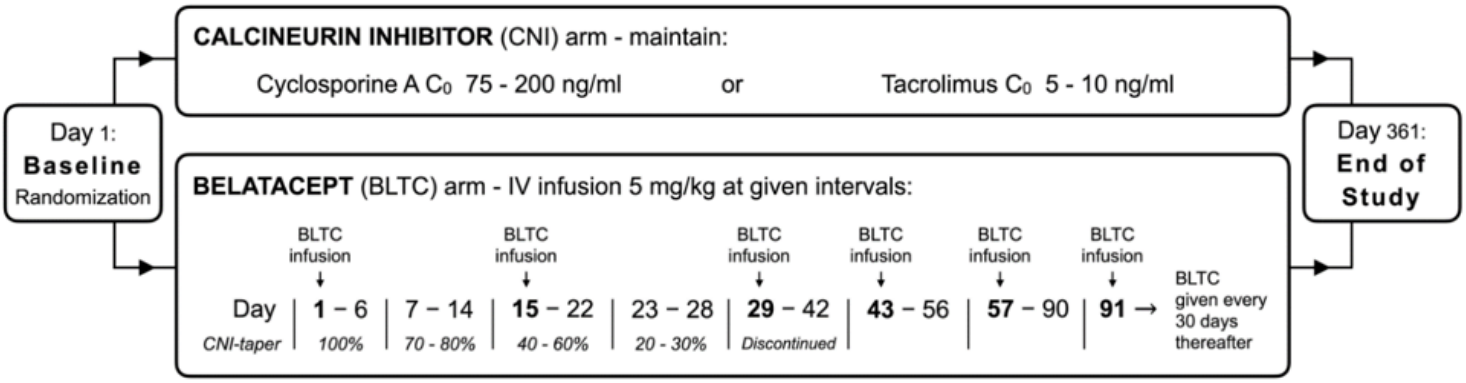

Figure 3

Conversion and dosing scheme.