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Sotrastaurin, a Novel Small Molecule Inhibiting Protein-Kinase C: Randomized Phase II Study in Renal Transplant Recipients

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Sotrastaurin, a selective protein-kinase-C inhibitor, blocks early T-cell activation through a calcineurin-independent mechanism. In this study, *de novo* renal transplant recipients with immediate graft function were randomized 1:2 to tacrolimus (control, n = 44) or sotrastaurin (300 mg b.i.d.; n = 81). All patients received basiliximab, mycophenolic acid (MPA) and steroids. The primary endpoint was the composite of treated biopsy-proven acute rejection (BPAR), graft loss, death or lost to follow-up at month 3. The main safety assessment was estimated glomerular filtration rate (eGFR); modification of diet in renal disease (MDRD) at month 3. Composite efficacy failure at month 3 was higher for the sotrastaurin versus control regimen (25.7% vs. 4.5%, p = 0.001), driven by higher BPAR rates (23.6% vs. 4.5%, p = 0.003), which led to early study termination. Median (± standard deviation [SD]) eGFR was higher for sotrastaurin versus control at all timepoints from day 7 (month 3: 59.0 ± 22.3 vs. 49.5 ± 17.7 mL/min/1.73 m^2, p = 0.006). The most common adverse events were gastrointestinal disorders (control: 63.6%; sotrastaurin: 88.9%) which led to study-medication discontinuation in two sotrastaurin patients. This study demonstrated a lower degree of efficacy but better renal function with the calcineurin-inhibitor-free regimen of sotrastaurin+MPA versus the tacrolimus-based control. Ongoing studies are evaluating alternative sotrastaurin regimens.

Key words: Allotransplantation, calcineurin inhibitor toxicity, drug development, efficacy, immunosuppression, mycophenolic acid, renal function, safety, tacrolimus, T-cell activation

Abbreviations: AEs, adverse events; AMR, antibody-mediated rejection; BPAR, biopsy-proven acute rejection; bpm, beats per minute; CIT, cold ischemic time; CMV, cytomegalovirus; CNIs, calcineurin inhibitors; DMC, data-monitoring committee; ECGs, electrocardiograms; eGFR, estimated glomerular filtration rate; HR, heart rate; MDRD, modification of diet in renal disease; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; PKC, protein kinase C; SAEs, serious adverse events; SD, standard deviation.

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Introduction

Although acute rejection rates following kidney transplantation have declined in recent years (1), current immunosuppressive agents have significant safety and tolerability shortcomings that hinder successful long-term outcomes in many patients. Calcineurin inhibitors (CNIs), the cornerstone of immunosuppression due to their efficacy, are nephrotoxic, neurotoxic and associated with an increased risk of cardiovascular events due to their diabetogenic

potential and increased risk of hypertension and dyslipidemia (2). Mycophenolic acid (MPA) is a valuable option for multidrug immunosuppressive regimens, but its efficacy in preventing acute rejection episodes is insufficient to avoid using CNIs in *de novo* patients (3,4). MPA also has side effects (including an increased risk of hematologic toxicity) that are partly due to its antiproliferative actions (5). Furthermore, the high-intensity immunosuppression provided by tacrolimus and MPA is associated with an increased risk of polyoma virus-associated nephropathy (6). CNI-free regimens based on a combination of MPA + mammalian target of rapamycin (mTOR) inhibitors (sirolimus/everolimus) appear to improve renal function in maintenance patients (7,8). However, in *de novo* renal recipients they have insufficient efficacy (9) and, for converted maintenance patients, the risk of proteinuria may increase (10). Recently, the pivotal program for belatacept demonstrated improved glomerular filtration rate (GFR) but significantly inferior efficacy versus cyclosporine. Hence, the need for new immunosuppressants with novel mechanisms of action, particularly calcineurin-independent mechanisms for blocking early T-cell activation, remains.

Protein kinase C (PKC) isoforms play key roles in T- and B-cell signaling (11–13) and therefore represent attractive therapeutic targets. Sotrastaurin is a small-molecular-weight immunosuppressant that blocks early T-cell activation through selective inhibition of PKCθ and PKCα, isoforms that are crucial for IL-2 (14) and interferon-gamma production (15), respectively. Sotrastaurin markedly prolonged graft survival times in experimental heart and kidney allotransplantation animal models both as monotherapy and in combination with other immunosuppressants (16–19). Preclinical and early clinical studies demonstrated no signs of nephrotoxicity, hepatotoxicity, or metabolic or blood-pressure effects at standard exposures (data on file; Novartis Pharma AG, Basel, Switzerland; 2004). Gastrointestinal effects were the dose-limiting toxicity in all species tested (data on file; Novartis Pharma AG, Basel, Switzerland; 2004). Initial concerns regarding the potential for QT-prolongation from *in vitro* tests (data on file; Novartis Pharma AG, Basel, Switzerland; 2004) have not been confirmed at therapeutic doses in healthy-volunteer studies (20), a thorough QT study (data on file; Novartis Pharma AG, Basel, Switzerland; 2009) or in clinical studies to date (21). A reversible increase in mean ventricular heart rate (HR) was observed following a single 500 mg dose of sotrastaurin with mean HRs in the upper part of the normal range in healthy-volunteer studies (20).

In six patients with moderate-to-severe plaque psoriasis, sotrastaurin (300 mg b.i.d.) resulted in a mean reduction from baseline of 69% for the Psoriasis Area and Severity Index score after 2 weeks' treatment (20), which is comparable to the efficacy observed with cyclosporine (22). Since psoriasis is a T-cell-mediated disease, the efficacy of sotrastaurin in this setting may predict its immunosuppressive activity in transplant recipients.

Because sotrastaurin blocks early T-cell activation through a calcineurin-independent mechanism, and based on encouraging results in animal models (19), it was tested in CNI-free immunosuppressive regimens in combination with current adjunctive therapies such as MPA and mTOR inhibitors. This phase II study evaluated the efficacy, safety and tolerability of oral sotrastaurin+MPA versus tacrolimus+MPA in *de novo* renal transplant recipients.

Material and Methods

Design and population

This study (ClinicalTrials.gov: NCT00492869) was designed as a 12-month, open-label, randomized, dose-finding, two-stage study. It was conducted between June 2007 and May 2008 in 26 centers in Europe, North America and Asia.

Male and female patients aged ≥18 years who were recipients of a primary kidney transplant from a deceased, living unrelated or non-HLA-identical living-related donor were eligible. Patients had to receive a kidney with a cold ischemic time (CIT) <24 h from a donor aged 10–65 years, and have a functional graft within 24 h after graft reperfusion (defined as urine output >250 mL/12 h for patients without residual urinary output from the native kidneys or a decrease in serum creatinine by ≥20% from pretransplant level). Important exclusion criteria included: multiorgan transplant recipients or patients who had previously received an organ transplant, recipients of an organ from a non-heart-beating donor, recipients of ABO-incompatible or cross-match-positive transplants, and patients with a significant cardiac history (e.g. long QT-syndrome; unstable angina, recent hospitalization for heart failure or significant and persistent left ventricular dysfunction during the previous 6 months). All patients gave written, informed consent and the study was conducted in accordance with the International Conference on Harmonized Tripartite Guidelines for Good Clinical Practice and local regulations and was reviewed by the Independent Ethics Committee/Institutional Review Board for each center. An independent data-monitoring committee (DMC) was established at study commencement.

All patients were scheduled to receive one dose of basiliximab 20 mg and MPA 720 mg prior to transplantation. If the graft showed immediate function, patients were randomized (stratified by donor source and center) within 24 h of transplant in a 1:2 ratio to receive either tacrolimus (initial dose 0.1mg/kg b.i.d., target trough levels: month 1: 8–15 ng/mL; month 2–3: 6–12 ng/mL; month 4–12: 5–10 ng/mL; control regimen) or sotrastaurin 300 mg b.i.d. (sotrastaurin regimen). The randomization list was produced by the study sponsor using a validated system that automated the random assignment of treatment arms to randomization numbers in the specified ratio. Each center received a set of treatment allocation cards on which the treatment group information was covered by a scratch-off label. Patients were given the lowest numbered card available; the scratch-off labels were then removed revealing designated treatment. All patients received a second 20 mg basiliximab dose on day 4 post-transplant and MPA 720 mg b.i.d. for study duration. The initial dose of corticosteroids was 500 mg at transplantation, 250 mg on day 2, 125 mg on day 3 and 0.5 mg/kg from day 4, tapered to 5–10 mg/day by month 1 and to ≥5 mg/day at day 45 until month 12.

Acute rejections were treated with steroids according to local practice. Patients were discontinued and converted to local standard of care if they experienced a rejection requiring the use of other antirejection therapy (i.e. antibodies), a biopsy-proven acute rejection (BPAR) Banff grade ≥2, more than one BPAR of any grade, or more than one treated acute rejection.

Efficacy and safety assessments were scheduled on days 1, 2, 3, 4, 8, 15 and 22, and then monthly for months 1–12. Patients who prematurely discontinued study drug remained in the study and were scheduled to undergo follow-up visits at months 3, 6 and 12.

Efficacy assessments

The primary efficacy endpoint was the composite of treated BPAR, graft loss, death or lost to follow-up at month 3 (day 104). Secondary efficacy variables included analyses of the components of the primary variable.

A graft core biopsy was to be performed within 48 h of all suspected rejection episodes, regardless of antirejection treatment. Biopsies were graded by the local pathologist using 2003 Banff criteria (23). A BPAR was defined as a clinically apparent rejection episode confirmed by a biopsy graded Banff IA or greater, or antibody-mediated rejection (AMR) treated with antirejection therapy. BPARs were treated according to local practice.

Safety assessments

The main safety assessment was renal function estimated by GFR (eGFR) using the abbreviated modification of diet in renal disease (MDRD) formula (24) at months 3 and 12. Other safety assessments included adverse events (AEs), discontinuations, hematology, standard laboratory measures, vital signs, electrocardiograms (ECGs) and physical examinations.

Statistical methods

Patient background information was summarized using descriptive statistics (n, mean, median, standard deviation [SD], minimum and maximum) for continuous variables and frequency distributions (percentages) for categorical variables. All efficacy analyses were based on the intent-to-treat analysis set, comprising all randomized patients. The safety analyses were performed on the same analysis set as all randomized patients were treated with the randomized study medication. The primary endpoint was evaluated for noninferiority by computing a Z-test-based 95% confidence interval to determine the difference in efficacy-failure probabilities for the sotrastaurin versus the control regimen. Efficacy-failure probabilities were obtained from Kaplan–Meier estimates considering efficacy events up to 7 days after discontinuation of study medication (on-treatment). The median eGFRs between treatment regimens were compared using a two-sided Wilcoxon rank sum test. No multiplicity adjustment was made for multiple treatment comparisons because the analyses were not of a confirmatory nature. No missing-data imputation was performed.

Sample size and power considerations

The study was designed to assess efficacy-failure rates for the sotrastaurin versus control arm. A sample size of 129 (sotrastaurin: n = 86; control: n = 43) was required to demonstrate that the upper bound of the two-sided 95% confidence interval for the difference in primary efficacy-failure rates (sotrastaurin-control arm) was ≤20% at month 3. A failure rate of 12% in both arms would have led to a power of approximately 90%. The margin of 20% was selected because the data were to be used for feasibility assessment and decision making about starting the dose-ranging second stage of the study, rather than hypothesis testing.

Results

Patients

A total of 125 patients were randomized (control: n = 44; sotrastaurin: n = 81; Figure 1). Both groups consisted predominantly of Caucasian patients (control: 90.9% and sotrastaurin: 82.7%) and were well matched for baseline characteristics, except that the control group included more males (72.7%, 32/44) than the sotrastaurin group (60.5%, 49/81; Table 1). Median follow-up times were 147 days for control and 120 for sotrastaurin.

Immunosuppressant dosing

Patients were exposed to study drug for a median duration of 110 (range: 2–228) versus 75 (range: 1–240) days with the control and sotrastaurin regimens, respectively. Median sotrastaurin trough levels remained between 604 and 807 ng/mL during the study. In the control group, the majority of patients were within the protocol-defined trough tacrolimus target ranges throughout the study, with median trough levels between 7.9 and 11.0 ng/mL. The control and sotrastaurin groups had similar mean daily MPA doses of 1361 and 1302 mg/day at month 1, and 1294 and 1211 mg/day at month 3, respectively. Median MPA trough levels at month 1 were 3613 ng/mL and 3160 ng/mL with the sotrastaurin and control regimens, respectively, and 4720 ng/mL and 3560 ng/mL at month 3. Both groups had mean oral steroid doses of 0.2 mg/kg at month 1 and 0.1 mg/kg at month 3.

Efficacy

During the first 4 weeks post-transplant, composite-endpoint rates were similar between treatment regimens. Beyond this, the event curves for the two regimens diverged, due to BPARs with the sotrastaurin regimen (Figure 2). By month 3, a higher proportion of sotrastaurin-regimen versus control-regimen patients had experienced primary efficacy failure (25.7% [n = 16] vs. 4.5% [n = 2]; p = 0.001; Table 2) and the study was terminated based on the DMC's recommendation. All patients were discontinued from study medication and converted to local standard-of-care treatment. Month-6, *post hoc* Kaplan–Meier estimates of efficacy endpoints are presented in Table 3.

At the time of study termination, 17 patients had experienced BPARs with the sotrastaurin regimen, including three patients who had their first BPAR after month 3. Banff grades of these 17 patients (most severe is stated where patients experienced more than one BPAR) were as follows: IA (one patient), IB (five), IIA (nine) and IIB (two). Complete resolution occurred in 10 patients, while 5 had residual renal dysfunction and 2 had no improvement. Ultimately, one patient with no improvement lost her graft 3 months after conversion to a different regimen, in a context of recurring and unresponsive rejection; histologically, signs of AMR became apparent 2 months after conversion. No other patient showed signs of AMR. One additional patient experienced a IB BPAR 12 days after discontinuation of study medication (off-treatment), which was therefore not considered in the primary analysis according to the prespecified rules. Two control-regimen patients experienced BPARs, one Banff IB (the outcome was residual renal dysfunction) and the other IIB (which resolved).

Other variables included in the composite endpoint were graft loss and death. Neither group had any graft losses

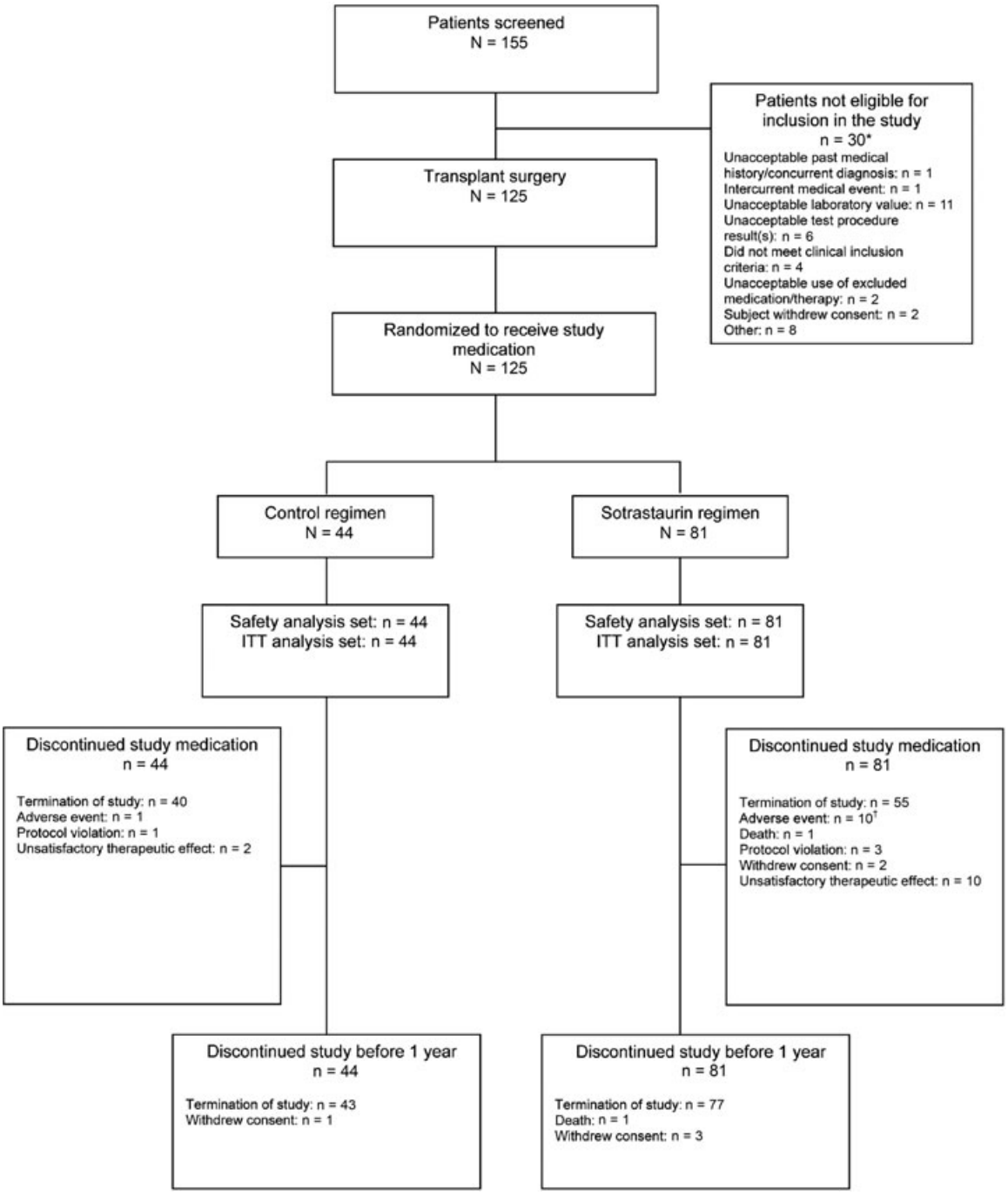

Figure 1: Flow of patients through the study.
*Patients could have ≥1 reason for screening failure, †includes two patients whose adverse event was acute rejection. Control regimen: tacrolimus + MPA; sotrastaurin regimen: sotrastaurin + MPA; ITT: intent-to-treat; MPA: mycophenolic acid.

but on day 27 post-transplant, one sotrastaurin-regimen patient died 1 day after discharge from wound-revision surgery. The clinical context suggests pulmonary embolism but no autopsy was performed. The cause of death was reported as a cardiac arrest and was not suspected by the investigator to be related to study drug. No control-regimen patients died during the study. At last observation, one sotrastaurin-regimen patient had died and one had lost their graft; no control-regimen patients had either died or lost their graft. One control-regimen patient and

Table 1: Baseline demographics and characteristics of donors and recipients

	Control N = 44	Sotrastaurin N = 81
Recipient		
Mean age, years (range)	48.3 (20–72)	46.4 (20–70)
Male, n (%)	32 (72.7)	49 (60.5)
Race, n (%)		
Caucasian	40 (90.9)	67 (82.7)
Black	2 (4.5)	5 (6.2)
Asian	1 (2.3)	5 (6.2)
Other	1 (2.3)	4 (4.9)
End-stage renal disease leading to transplant, n (%)		
Glomerular disease	10 (22.7)	20 (24.7)
Diabetes mellitus	7 (15.9)	6 (7.4)
Polycystic disease	5 (11.4)	13 (16.0)
Hypertension/nephrosclerosis	4 (9.1)	9 (11.1)
Unknown	3 (6.8)	7 (8.6)
Other	15 (34.1)	26 (32.1)
Pre-emptive transplantation, n (%)	10 (22.7)	18 (22.2)
Panel reactive antibodies >5%, n (%)	6 (13.6)	3 (3.7)
Number of HLA matches, mean (SD)	3.2 (2.0)	3.4 (1.7)
Zero HLA mismatches, n (%)	12 (27.3)	13 (16.0)
Cold ischemic time in hours, median (range)*	13.5 (4.8–22.8)	15.4 (5.2–25.3)
Donor		
Living related, n (%)	15 (34.1)	23 (28.4)
Living unrelated, n (%)	8 (18.2)	20 (24.7)
Deceased, n (%)	21 (47.7)	38 (46.9)
Donor		
Mean age, years (range)	43.5 (17.0–62.0)	42.7 (16.0–65.0)
Male, n (%)	16 (36.4)	48 (59.3)

*For deceased, heart-beating donors only.
Control regimen: tacrolimus + MPA; sotrastaurin regimen: sotrastaurin + MPA.
HLA = human leukocyte antigen; MPA = mycophenolic acid; SD = standard deviation.

three sotrastaurin-regimen patients withdrew consent during the study.

To explore exposure–efficacy associations for sotrastaurin, a time-weighted average trough level until month 3 or event was calculated for each patient, and patients were sorted to trough-level tertiles. In the lowest tertile, 27% of patients experienced a BPAR, versus 12% in the highest tertile. However, no reliable sotrastaurin therapeutic trough-level range for efficacy could be identified (Figure 3).

Safety

Renal function: Renal function assessed by eGFR (MDRD; Figure 4) and serum creatinine was significantly improved

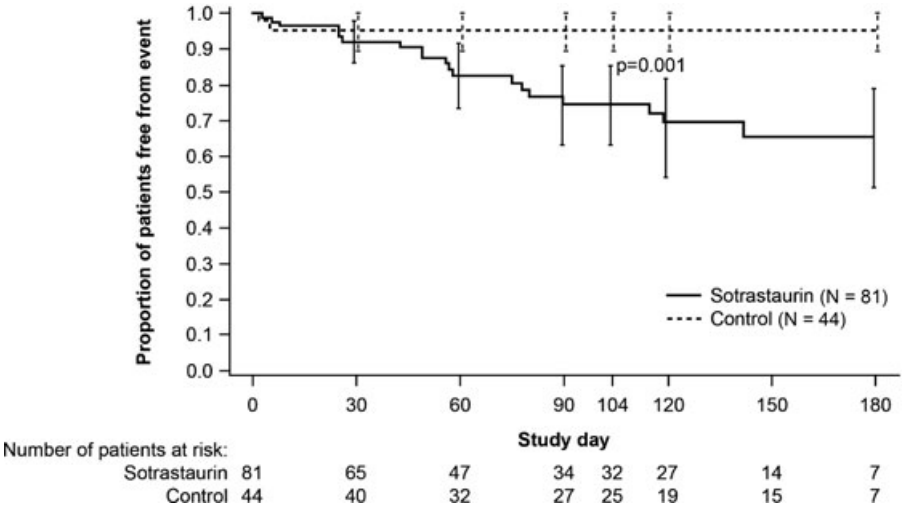

Figure 2: Kaplan–Meier plot of time to first on-treatment composite primary efficacy failure. Note that a Kaplan–Meier analysis is used to present the primary efficacy endpoint in line with the protocol although the study was terminated early. Control regimen: tacrolimus + MPA; sotrastaurin regimen: sotrastaurin + MPA.

Table 2: Kaplan–Meier estimates of efficacy endpoints at 3 months*

	Control N = 44, n (%)	Sotrastaurin N = 81, n (%)	95% CI of difference (%)	Difference in K–M estimate (p-Value)
Composite efficacy failure	2 (4.5)	16 (25.7)	(8.4, 34.0)	0.001
Treated BPAR	2 (4.5)	14 (23.6)	(6.4, 31.8)	0.003
Graft loss	0	0	–	–
Death	0	1 (1.4)	(−1.4, 4.3)	0.314
Lost to follow-up	0	1 (1.2)	(−1.2, 3.6)	0.314

BPAR = biopsy-proven acute rejection; CI = confidence interval; K–M = Kaplan–Meier.
Control regimen: tacrolimus + MPA; sotrastaurin regimen: sotrastaurin + MPA.
Composite efficacy failure: treated BPAR, death, graft loss, lost to follow-up; treated BPAR: clinical signs of acute rejection (confirmed by biopsy showing Banff ≥IA) and antirejection therapy given.
*Day 104 or events (except lost to follow-up) within 7 days of discontinuation of study drug were used as the cut-off for this analysis.
†Day 180 or events (except lost to follow-up) within 7 days of discontinuation of study drug were used as the cut-off for this *post hoc* analysis.

with the sotrastaurin versus the control regimen in both all-data and on-treatment analyses. The improvements in calculated creatinine clearance achieved with the sotrastaurin regimen did not reach statistical significance (Table 4). The differences in renal-function parameters were noted as early as postoperative day 4, achieved statistical significance at week 2, and remained significant until month 6. Of note, the benefit in eGFR with the

Table 3: Kaplan–Meier estimates of efficacy endpoints at 6 months†

	Control N = 44, n (%)	Sotrastaurin N = 81, n (%)	95% CI of difference n (%)	Difference in K–M estimate (p-Value)
Composite efficacy failure	2 (4.5)	19 (34.9)	(15.0, 45.7)	<0.001
Treated BPAR	2 (4.5)	17 (33.1)	(13.0, 44.0)	<0.001
Graft loss	0	0	–	–
Death	0	1 (1.4)	(−1.4, 4.3)	0.314
Lost to follow-up	0	1 (1.2)	(−1.2, 3.6)	0.314

BPAR = biopsy-proven acute rejection; CI = confidence interval; K–M = Kaplan–Meier.
Control regimen: tacrolimus + MPA; sotrastaurin regimen: sotrastaurin + MPA.
Composite efficacy failure: treated BPAR, death, graft loss, lost to follow-up; treated BPAR: clinical signs of acute rejection (confirmed by biopsy showing Banff ≥IA) and antirejection therapy given.
*Day 104 or events (except lost to follow-up) within 7 days of discontinuation of study drug were used as the cut-off for this analysis.
†Day 180 or events (except lost to follow-up) within 7 days of discontinuation of study drug were used as the cut-off for this *post hoc* analysis.

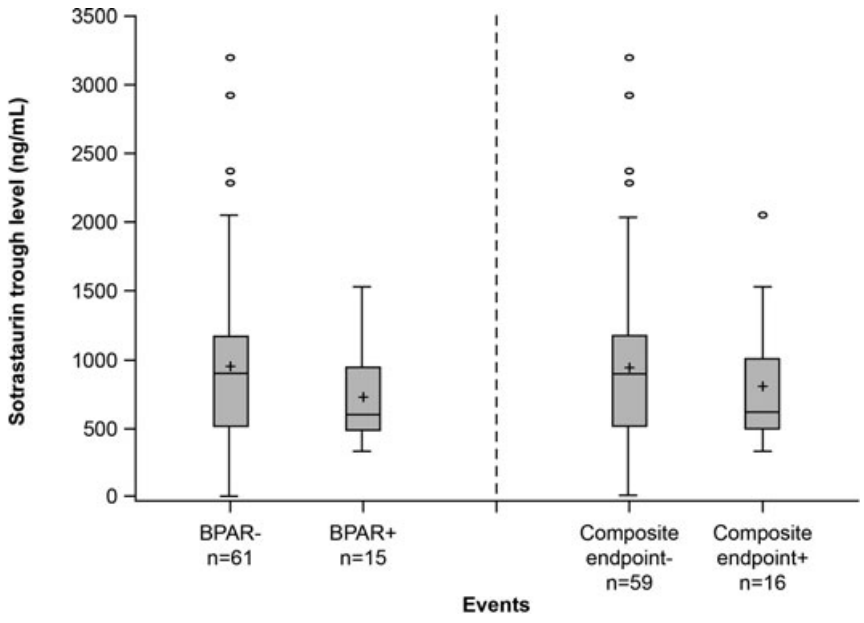

Figure 3: Exposure–efficacy analyses of sotrastaurin trough levels by biopsy-proven acute rejection and composite endpoint status. BPAR+: patients experiencing biopsy-proven acute rejection; BPAR−: patients not experiencing biopsy-proven acute rejection; composite endpoint+: patients experiencing any component of composite endpoint; composite endpoint−: patients not experiencing any component of composite endpoint.

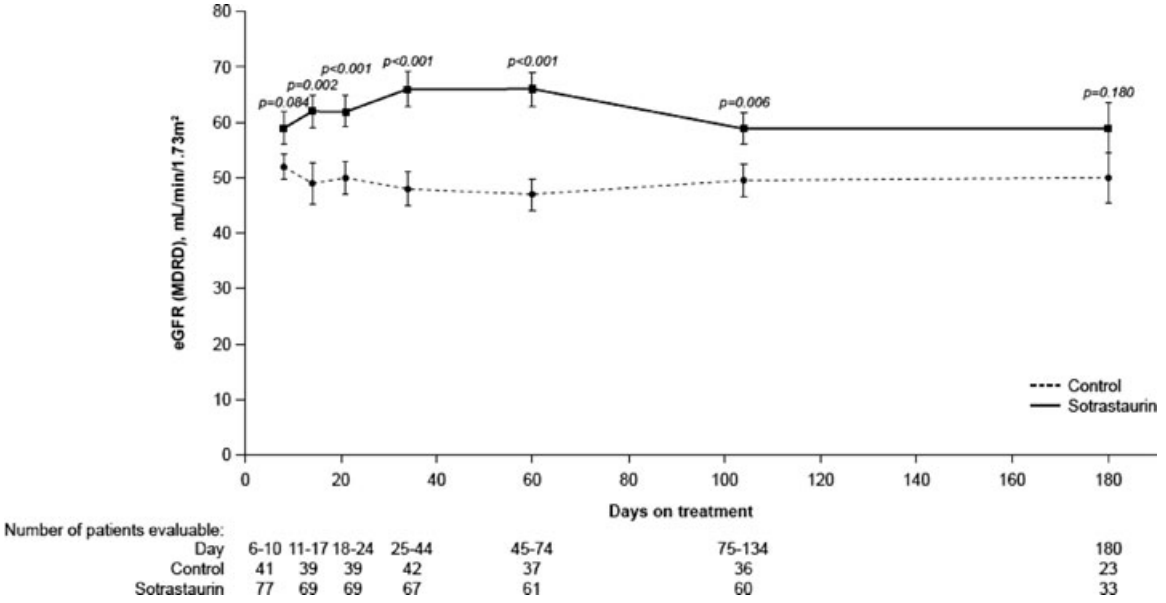

Figure 4: Median (±SE) estimated glomerular filtration rate by modification of diet in renal disease equation for safety analysis set. All data are included in this figure, i.e. including those collected after study-medication discontinuation. SE = nonparametric standard error = interquartile range/(1.075 * sqrt[n]); eGFR = estimated glomerular filtration rate; MDRD = modification of diet in renal disease formula.

sotrastaurin regimen remained even when patients who had experienced rejections and been converted to the local standard-of-care regimen were included in the analysis. Median (range) GFR for patients experiencing rejection at month 3 for whom data were available (n = 17) was 45 (18–95) mL/min/1.73 m^2. The low patient numbers beyond month 6 precluded meaningful analysis for later time points.

Table 4: Renal function at 3 and 6 months

	Control	Sotrastaurin	p-Value
At 3 months			
All data, median ± SD	N = 36	N = 61	
eGFR (MDRD) in mL/min/1.73 m^2	49.5 ± 17.7	59.0 ± 22.3*	0.006
Calculated creatinine clearance (Cockcroft–Gault) in mL/min	64.5 ± 20.5	75.5 ± 27.9*	0.080
Serum creatinine in μmol/L	137.5 ± 45.6	106.0 ± 27.0	< 0.001
On-treatment, median ± SD	N = 29	N = 38	
eGFR (MDRD) in mL/min/1.73 m^2	49.0 ± 14.9	59.0 ± 29.8†	0.040
Calculated creatinine clearance (Cockcroft–Gault) in mL/min	64.0 ± 17.7	74.0 ± 26.0	0.068
Serum creatinine in μmol/L	137.0 ± 41.9	103.5 ± 31.6	0.003
At 6 months			
All data, median ± SD	N = 23	N = 33	
eGFR (MDRD) in mL/min/1.73 m^2	52.0 ± 11.2	59.0 ± 26.0	0.180
Calculated creatinine clearance (Cockcroft–Gault) in mL/min	66.5 ± 14.9‡	68.0 ± 28.8	0.700
Serum creatinine in μmol/L	129.0 ± 26.0	109.0 ± 27.9	0.046
On-treatment, median ± SD	N = 14	N = 17	
eGFR (MDRD) in mL/min/1.73 m^2	51.0 ± 14.0	54.0 ± 19.1§	0.371
Calculated creatinine clearance (Cockcroft–Gault) in mL/min	59.0 ± 17.2**	64.0 ± 26.0	0.912
Serum creatinine in μmol/L	126.0 ± 23.3	106.0 ± 33.5	0.351

eGF = glomerular filtration rate; MDRD = modification of diet in renal disease; SD = nonparametric standard deviation = interquartile range/1.075.

Control regimen: tacrolimus + MPA; sotrastaurin regimen: sotrastaurin + MPA.

Both analyses were conducted on the intent-to-treat analysis set.

All-data analysis: all reported data were included; on-treatment analysis: data collected more than 2 days after discontinued study medication were not included.

*n = 60; †n = 37; ‡n = 20; §n = 16; **n = 12 (numbers differ owing to availability of data used in formulae).

Table 5: Incidence of adverse events (AEs) occurring in ≥10%* with either regimen

	Control N = 44, n (%)	Sotrastaurin N = 81, n (%)
At least one AE	43 (97.7)	79 (97.5)
Any SAE	13 (29.5)	38 (46.9)
Adverse events leading to discontinuation of study medication	2 (4.5)	18 (22.2)
Adverse events		
Blood and lymphatic system disorders		
Anemia	6 (13.6)	16 (19.8)
Cardiac disorders		
Tachycardia*	2 (4.5)	8 (9.9)
Gastrointestinal disorders		
Constipation	10 (22.7)	46 (56.8)
Diarrhea	14 (31.8)	33 (40.7)
Nausea	8 (18.2)	35 (43.2)
Vomiting	6 (13.6)	25 (30.9)
General disorders and administration site conditions		
Peripheral edema	9 (20.5)	12 (14.8)
Infections and infestations		
Urinary tract infection	10 (22.7)	16 (19.8)
Injury, poisoning and procedural complications		
Procedural pain	9 (20.5)	16 (19.8)
Investigations		
Increased blood creatinine	8 (18.2)	8 (9.9)
Metabolism and nutrition disorders		
Hyperglycemia	4 (9.1)	10 (12.3)
Hyperkalemia	6 (13.6)	7 (8.6)
Hypocalcemia	3 (6.8)	9 (11.1)
Hypokalemia	5 (11.4)	10 (12.3)
Hypomagnesemia	11 (25.0)	7 (8.6)
Hypophosphatemia	9 (20.5)	12 (14.8)
Musculoskeletal and connective tissue disorders		
Arthralgia	5 (11.4)	3 (3.7)
Nervous system disorders		
Dizziness	5 (11.4)	12 (14.8)
Dysgeusia	0	11 (13.6)
Headache	7 (15.9)	5 (6.2)
Tremor	9 (20.5)	6 (7.4)
Psychiatric disorders		
Insomnia	7 (15.9)	21 (25.9)
Renal and urinary disorders		
Dysuria	6 (13.6)	8 (9.9)
Vascular disorders		
Hypotension	5 (11.4)	6 (7.4)

*Tachycardia is included although the incidence was <10% with both treatment regimens as it was considered an important finding.

Adverse events: With both regimens, most patients experienced at least one AE (Table 5), the majority being mild-to-moderate in severity. The most frequent AEs were gastrointestinal disorders (control: 63.6%, 28/44; sotrastaurin: 88.9%, 72/81) and metabolism and nutrition disorders (control: 61.4%, 27/44; sotrastaurin: 59.3%, 48/81). Serious adverse events (SAEs) were reported in 29.5% (13/44) and 46.9% (38/81) of patients receiving the control and sotrastaurin regimen, respectively. SAEs were most frequently related to infections (three and nine patients, respectively) or hospitalizations secondary to an acute rejection. The majority of infections were a secondary complication of another AE such as wound dehiscence/infection, and duodenal ulcer perforation. Bacteria were identified as the cause for seven of the nine infection SAEs with the sotrastaurin regimen. AEs leading to study-drug discontinuation occurred in 4.5% (2/44) of patients receiving the control and 22.2% (18/81) receiving the sotrastaurin regimen. Half of the discontinuations with each regimen were a result of acute rejection.

Gastrointestinal adverse events

Constipation, diarrhea, nausea and vomiting were the most commonly reported gastrointestinal AEs for both regimens and occurred at a higher frequency with the sotrastaurin

versus the control regimen (Table 5). The majority of gastrointestinal AEs were single events, and were rated by the investigator as mild; two cases of diarrhea and one of nausea were rated as severe with the sotrastaurin regimen. Twenty-one gastrointestinal-related AEs required dosage adjustment/interruption with the sotrastaurin versus four with the control regimen. Two gastrointestinal AEs (nausea and intestinal perforation) required permanent study-drug discontinuation or hospitalization with the sotrastaurin regimen.

Dysgeusia: Dysgeusia was only reported with the sotrastaurin regimen (13.6%, 11/81). It was described as a bad or metallic taste, usually mild and reported by week 3. One event required a study-drug dosage adjustment.

Cardiac effects: The overall incidence of cardiac AEs was higher with the sotrastaurin (22.2%, 18/81) versus the control regimen (13.6%, 6/44). The only cardiac AE with an incidence >5% was tachycardia, which was reported more frequently with the sotrastaurin (9.9%, 8/81) versus the control regimen (4.5%, 2/44) (Table 5). Both tachycardia AEs in control patients and six of those in sotrastaurin-regimen patients occurred by week 3. All were rated as mild by the investigator and were managed with either concomitant medication or nondrug therapy, or no action was taken. The sotrastaurin regimen was associated with a numerically higher incidence of ECG-measured tachycardia (>25% increase in HR from baseline resulting in a HR of >100 beats per minute [bpm] at any time during the study) versus control (27.5%, 22/80 vs. 14.6%, 6/41; 95% confidence interval: −1.7, 27.5; calculated using data only from patients who had a measurement at baseline and any time postbaseline). No significant differences in the PR, QRS or QT intervals from centrally read ECGs were observed between the regimens (data not shown).

Diabetes: Diabetes mellitus AEs were reported more often in patients receiving the control (6.8%, 3/44) versus the sotrastaurin regimen (2.5%, 2/81). In addition, glycated hemoglobin elevation (>6.4%) occurred more frequently with control versus the sotrastaurin regimen (27.3%, 12/44 vs. 11.3%, 8/71, respectively) as did high blood glucose (>13.9 mmol/L: 15.9%, 7/44 vs. 7.4%, 6/81, respectively). Conversely, hyperglycemia was reported more frequently as an AE with the sotrastaurin regimen than with control (12.3%, 10/81 vs. 9.1%, 4/44, respectively; Table 5).

Infections: The incidence of infections was similar for the two regimens (Table 6). Urinary tract infection was the most frequent infection reported (control: 22.7%, 10/44; sotrastaurin: 19.8%, 16/81). Bacterial infections were the most frequent type occurring in 20.5% (9/44) and 29.6% (24/81) of patients receiving the control and sotrastaurin regimens, respectively. No patients developed cytomegalovirus (CMV) infection and both regimens had similar incidences of patients with BK-polyoma viremia, defined as a viral load $>10^3$ copies/mL, while on treatment (control: 6.8%, 3/44; sotrastaurin: 6.2%, 5/81). Histological signs of BK nephropathy were observed in one patient receiving the sotrastaurin regimen, and one after discontinuation of study therapy.

Table 6: Incidence of infections

	Control N = 44, n (%)	Sotrastaurin N = 81, n (%)
Infections		
Any infection	21 (47.7)	37 (45.7)
Any serious infection	3 (6.8)	9 (11.1)
Bacterial	9 (20.5)	24 (29.6)
Viral	2 (4.5)	4 (4.9)
Fungal	2 (4.5)	5 (6.2)
Unknown	14 (31.8)	20 (24.7)

Control regimen: tacrolimus + MPA; sotrastaurin regimen: sotrastaurin + MPA.
MPA = mycophenolic acid; SAE = serious adverse event.

It was impossible to determine whether a relationship existed between sotrastaurin trough levels and tolerability because of limited follow-up and the confounding effect of MPA (data not shown).

Discussion

Sotrastaurin blocks PKC-mediated early T-cell activation (20) providing a new approach for immunosuppression distinct from calcineurin inhibition. However, the efficacy results of this phase II study do not support the combination of sotrastaurin 300 mg b.i.d. + MPA as a CNI-free regimen. Despite showing comparable efficacy to the tacrolimus+MPA control regimen during the first 4 weeks after transplantation, efficacy of the CNI-free sotrastaurin regimen subsequently diminished. Although higher acute rejection rates may be an acceptable trade-off for improved renal function, and the observed rejections on treatment all appeared to be T-cell mediated, the improvements in renal function in patients randomized to the sotrastaurin+MPA regimen versus the control regimen in this study were considered insufficient to outweigh the higher acute rejection rate. The acute rejection rate was deemed unacceptably high and the study was terminated.

Several factors may have contributed to the inadequate efficacy associated with the sotrastaurin+MPA combination. Although the present study did not show a clear association between efficacy failure and exposure, patients with efficacy failure had a numerically lower sotrastaurin trough level. In theory, the immunosuppressive efficacy might be improved by increasing exposure levels, but this appears to be unfeasible for the sotrastaurin+MPA regimen due to dose-limiting gastrointestinal AEs. Reducing MPA exposure might reduce gastrointestinal side effects but this is unfeasible due to lack of efficacy with this regimen; combining sotrastaurin with other

immunosuppressants might improve gastrointestinal tolerability. No pharmacokinetic drug–drug interactions exist between sotrastaurin and MPA (25); therefore, efficacy failure from insufficient MPA exposure is unlikely.

The efficacy of CNIs demonstrates that early T-cell-activation inhibition is a key to immunosuppressive effectiveness; however, the long-term side effects of CNIs (cardiovascular events, nephrotoxicity, and neurotoxicity) (2) highlight the need for alternative methods of inhibiting early T-cell activation. While the use of an mTOR inhibitor+MPA regimen in the maintenance period appears to be feasible (7), *de novo* CNI-free immunosuppression is invariably associated with an increase in rejection rate (26). Similarly, the first large-scale study assessing CNI-free immunosuppression using intravenous costimulatory blockade demonstrated higher acute rejection rates in the belatacept versus the CNI arms (22% and 17% vs. 7% for the more-intensive-belatacept, less-intensive-belatacept and cyclosporine regimens, respectively) (27). Although the combination of sotrastaurin and MPA demonstrated insufficient efficacy in this study, CNI side effects were absent. As early as postoperative day 4, better renal function was observed in patients randomized to sotrastaurin+MPA versus the control regimen. Other expected benefits of a CNI-free regimen included a reduced incidence of HbA_{1c} elevation and high blood glucose levels, and a decreased incidence of tremor.

No significant safety concerns were documented with sotrastaurin in combination with MPA. Dysgeusia was generally mild, required no action by the investigator, and did not lead to discontinuation of sotrastaurin. Nausea, vomiting, constipation and diarrhea were the most commonly reported AEs with both regimens. Gastrointestinal AEs occurred more frequently with the sotrastaurin+MPA regimen, which was consistent with data from previous healthy-volunteer and psoriasis studies at sotrastaurin doses of >200 mg b.i.d. (20). The majority of gastrointestinal AEs were rated as mild but more required dose adjustment or interruption with the sotrastaurin versus the control regimen. It was not possible to draw conclusions about the relationship between sotrastaurin concentration and tolerability because of limited follow-up and the confounding effect of MPA.

Neither regimen showed signs of severe overimmunosuppression and the infections reported were typical of organ transplant recipients. Many of the infections were secondary to other conditions (e.g. sepsis following duodenal-ulcer perforation). BK-polyoma nephropathy and CMV infection were not observed, possibly due to the use of CMV prophylaxis and the short study duration.

A dose-dependent chronotropic effect has been observed in preclinical and phase I sotrastaurin studies (20); therefore the higher HR and tachycardia AEs observed with the sotrastaurin+MPA regimen were not unexpected. All tachycardia AEs were mild and managed conservatively, and the majority occurred soon after transplantation. Although an *in vitro* preclinical study suggested a potential for QT prolongation, no QT effect was observed in this study.

When interpreting the results, it should be considered that the majority of patients were Caucasian, the proportion with diabetes or hypertension as the cause of end-stage renal disease requiring transplantation was low and patients were immunologically low risk (recipients of a first transplant with CIT <24 h; exclusion of patients with delayed graft function or receiving a donor organ from a donor aged >65 years). In addition, the relatively small sample size, an unblinded protocol, multiple participating centers, local evaluation of renal biopsies and a relatively short follow-up due to the premature cessation of the trial might have had a negative impact on our ability to precisely determine the incidence and severity of biopsy-proven acute rejection episodes. These shortcomings are in part intrinsic to the nature of a phase II study whose main objective was to determine whether the sotrastaurin+MPA drug regimen should be further developed in larger clinical trials.

In conclusion, the CNI-free sotrastaurin+MPA regimen was associated with increased rejection rates from week 4 onwards. However, this regimen showed an acceptable safety profile, and evidence of potential benefits including improved renal function versus the control. These results indicate that further characterization of sotrastaurin as a component of multidrug immunosuppressive regimens that exploit its calcineurin-independent mechanism for inhibiting early T-cell activation is warranted. Studies evaluating the efficacy and safety of other sotrastaurin regimens, including dose-ranging studies combining sotrastaurin with both everolimus and tacrolimus, are ongoing and planned.

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References

1. Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. Am J Transplant 2004; 4: 378–383.
2. Halloran PF. Immunosuppressive drugs for kidney transplantation. N Engl J Med 2004; 351: 2715–2729.
3. Vincenti F, Ramos E, Brattstrom C et al. Multicenter trial exploring calcineurin inhibitors avoidance in renal transplantation. Transplantation 2001; 71: 1282–1287.
4. Ekberg H, Grinyo J, Nashan B et al. Cyclosporine sparing with mycophenolate mofetil, daclizumab and corticosteroids in renal allograft recipients: The CAESAR study. Am J Transplant 2007; 7: 560–570.
5. US Renal Transplant Mycophenolate Mofetil Study Group. Mycophenolate mofetil in cadaveric renal transplantation. Am J Kidney Dis 1999; 34: 296–303.
6. Prince O, Savic S, Dickenmann M, Steiger J, Bubendorf L, Mihatsch MJ. Risk factors for polyoma virus nephropathy. Nephrol Dial Transplant 2009; 24: 1024–1033.
7. Pietruck F, Klempnauer J, Arns W et al. Efficacy and Safety of Everolimus (RAD) / Enteric-coated Mycophenolate Sodium (EC-MPS) after Calcineurin inhibitor (CNI) withdrawal in de novo renal transplant patients: Final results of the ZEUS trial. Am J Transplant 2009; s2: 499.
8. Weir M, Mulgaonkar S, Pearson T et al. Mycophenolate mofetil/sirolimus maintenance therapy after calcineurin inhibitor withdrawal in renal transplant recipients: 2-year outcomes of the spare-the-nephron (STN) trial. Am J Transplant 2009; s2: 200.
9. Ekberg H, Tedesco-Silva H, Demirbas A et al. Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med 2007; 357: 2562–2575.
10. Buchler M, Caillard S, Barbier S et al. Sirolimus versus cyclosporine in kidney recipients receiving thymoglobulin, mycophenolate mofetil and a 6-month course of steroids. Am J Transplant 2007; 7: 2522–2531.
11. Spitaler M, Cantrell DA. Protein kinase C and beyond. Nat Immunol 2004; 5: 785–790.
12. Guo B, Su TT, Rawlings DJ. Protein kinase C family functions in B-cell activation. Curr Opin Immunol 2004; 16: 367–373.
13. Mecklenbrauker I, Saijo K, Zheng NY, Leitges M, Tarakhovsky A. Protein kinase Cdelta controls self-antigen-induced B-cell tolerance. Nature 2002; 416: 860–865.
14. Bauer B, Krumbock N, Ghaffari-Tabrizi N et al. T cell expressed PKC-theta demonstrates cell-type selective function. Eur J Immunol 2000; 30: 3645–3654.
15. Pfeifhofer C, Gruber T, Letschka T et al. Defective IgG2a/2b class switching in PKC alpha−/− mice. J Immunol 2006; 176: 6004–6011.
16. Wagner J, Evenou J-P, Zenke G et al. The first-in-class oral protein kinase C (PKC) inhibitor NVP-AEB071 (AEB) prolongs renal allograft survival in non-human primates (NHP) and suppresses lymphocyte proliferation at safe exposures in human proof-of-concept studies. Transplantation 2006; 82(Abstract 57): 86.
17. Bruns C, Pally C, Beerli C et al. NVP-AEB071 (AEB), a novel oral inhibitor of early T-cell activation, prolongs rat cardiac allograft survival when used alone and in combination with cyclosporine, everolimus or FTY720. Transplantation 2006; 1(Abstract 741): 316.
18. Bigaud M, Wieczorek G, Riesen S et al. NVP-AEB071 (AEB), a novel oral inhibitor of early T-cell activation, prolongs the survival of non-human primate (NHP) kidney allografts when used as monotherapy or at non-effective doses combined with a non-effective dose of cyclosporine (CsA). Transplantation 2006; 1(Abstract 546): 250.
19. Bigaud M, Wieczorek G, Preussing E et al. NVP-AEB071 (AEB), the novel oral inhibitor of protein kinase C (PKC) and early T-cell activation, prolongs non-human primates (NHP) kidney allograft

survival when combined with everolimus (RAD), ERL080 (ERL) or FTY720 (FTY) without calcineurin inhibitor (CNI). Transplantation 2006; 1(Abstract 550): 251–252.
20. Skvara H, Dawid M, Kleyn E et al. The PKC inhibitor AEB071 may be a therapeutic option for psoriasis. J Clin Invest 2008; 118: 3151–3159.
21. Budde K, Sommerer C, Becker T et al. Sotrastaurin, a novel small molecule inhibiting protein kinase C: First clinical results in renal-transplant recipients. Am J Transplant 2010; 10: 571–581.
22. Flytstrom I, Stenberg B, Svensson A, Bergbrant IM. Methotrexate vs. ciclosporin in psoriasis: Effectiveness, quality of life and safety. A randomized controlled trial. Br J Dermatol 2008; 158: 116–121.
23. Racusen LC, Colvin RB, Solez K et al. Antibody-mediated rejection criteria—an addition to the Banff 97 classification of renal allograft rejection. Am J Transplant 2003; 3: 708–714.
24. Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. Am J Kidney Dis 2003; 41: 1–12.
25. Slade A, Pfrunder A, Koelle EU, Seiberling M, Schmouder R. NVP-AEB071 pharmacokinetics: Combination with mycophenolate mofetil (Cellcept) is safe and does not result in an interaction. Transplantation 2006; 1(Abstract 2436): 866.
26. Srinivas TR, Meier-Kriesche HU. Minimizing immunosuppression, an alternative approach to reducing side effects: Objectives and interim result. Clin J Am Soc Nephrol 2008; 2: S101–S116.
27. Vincenti F, Charpentier B, Vanrenterghem Y et al. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). Am J Transplant 2010: 535–546.