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ORIGINAL ARTICLE

CD25 blockade in kidney transplant patients randomized to standard-dose or high-dose basiliximab with cyclosporine, or high-dose basiliximab in a calcineurin inhibitor-free regimen

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Conflicts of interest
The authors have the following conflict of interests to declare in relation to Novartis in the last 3 years: Gilles Thibault, Maxime Coulon and Gilles Paintaud received a research grant from Novartis, institution (Tours University Hospital), for this study. Gilles Paintaud received research grants from Roche Pharma, Institution (Tours University Hospital). Christophe Legendre received lecture fees, travel grants from Novartis, Alexion, CSL Behring. Pierre Merville received research grants and travel funding from Novartis. David Ternant, Elodie Chasseuil, and Antoine Durrbach declare no relevant conflict of interest. Lionel Rostaing received speaker's fees from Novartis and a member of Novartis

Summary

An increased basiliximab dose may saturate T-cell CD25 receptors in kidney transplant patients receiving calcineurin inhibitor (CNI)-free immunosuppression. In a 12-week study, 16 *de novo* kidney transplant patients were randomized to (i) 40 mg basiliximab with cyclosporine [$n = 3$] (controls), (ii) 80 mg basiliximab with cyclosporine [$n = 6$], or (iii) 80 mg basiliximab with everolimus (CNI-free) [$n = 7$], all with mycophenolic acid and steroids. Recruitment was stopped prematurely due to increased biopsy-proven acute rejection (BPAR) in the basiliximab 80 mg CNI-free group. BPAR occurred in 1/3, 1/6, and 4/7 patients in the three treatment groups, respectively. The primary endpoint, area under the effect curve of CD25 saturation to week 12, was 8.4(1.6) % × weeks in the control group, 11.1(1.1) % × weeks with basiliximab 80 mg + cyclosporine, and 9.7(0.7) % × weeks in the basiliximab 80 mg CNI-free group ($P = 0.020$ for basiliximab 80 mg + cyclosporine versus controls; $P = 0.119$ for basiliximab 80 mg CNI-free versus controls). Although small patient numbers prohibit robust conclusions, these results suggest that doubling the cumulative basiliximab dose to 80 mg does not provide adequate immunosuppression during the first 3 months after kidney transplantation in the absence of CNI therapy (ClinicalTrials.gov number: NCT01596062).

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Introduction

Interleukin-2 (IL-2) receptor antibodies provide effective induction therapy, especially in low-to-moderate risk kidney transplant patients [1,2]. Basiliximab, the only commercially available IL-2 receptor antibody, is effective in combination with calcineurin inhibitor (CNI) therapy in preventing acute rejection early after kidney transplantation [3] and is associated with a lower rate of adverse events than lymphocyte-depleting induction [4].

Basiliximab acts by blocking IL-2 binding to the CD25 antigen (the α-chain) of IL-2 receptors on activated T cells, causing selective inhibition of antigen-activated T cells [5]. At the standard cumulative dose of 40 mg, pharmacodynamic studies have shown that CD25-saturating concentrations of basiliximab (i.e., ≥0.2 μg/ml) were maintained for 4–6 weeks on average after kidney transplantation in patients receiving concomitant cyclosporine (CsA) [6–8]. High interindividual variability was observed; however, the minimum saturation duration was 2 weeks and half of the population (interquartile range) was suppressed for between 4 and 8 weeks [6–8]. Early dose-finding studies conducted in kidney transplant recipients reported that the duration of complete CD25 suppression is dose dependent within the range 20–60 mg [8,9] but pharmacodynamic data are lacking at higher doses of basiliximab. Basiliximab pharmacokinetics are also unexplored in patients not receiving CNI maintenance therapy. CNI agents block T-cell receptor-mediated production of IL-2 [10,11] and thus act synergistically with IL-2 receptor antibodies to limit IL-2-mediated T-cell proliferation [12,13]. Moreover, CD25 is upregulated by IL-2 [14,15] and CNI therapy inhibits activation marker expression on T cells including CD25 [16]. It is therefore conceivable that a higher dose of basiliximab may be required to achieve a similar level of CD25 saturation in the absence of concomitant CNI therapy.

A series of randomized trials has assessed the use of the mammalian target of rapamycin (mTOR) inhibitors in *de novo* kidney transplant patients [17,18]. Whereas the mTOR inhibitor everolimus combined with reduced-exposure CNI therapy from time of transplant maintains immunosuppressive efficacy [19–23], mTOR inhibition with entirely CNI-free regimen may be less effective. The SYMPHONY study showed an increased rate of biopsy-proven acute rejection (BPAR) in patients randomized to low-dose sirolimus and mycophenolate mofetil (MMF) with IL-2RA induction compared to standard CNI regimens [24], and even standard-exposure sirolimus with MMF and IL-2RA induction appears to be associated with a higher-than-expected rate of BPAR [25]. In this setting, more intensive short-term immunosuppression from IL-2RA induction may be a helpful contribution to the treatment regimen.

We hypothesized that an increased cumulative dose of basiliximab would achieve saturation of T-cell CD25 receptors in *de novo* kidney transplant recipients not receiving CNI therapy. A proof-of-concept study was performed to compare the saturation kinetics of the CD25 antigen of IL-2 receptors by basiliximab over the first 12 weeks post-transplant in low-risk kidney transplant patients randomized to a cumulative basiliximab dose of 40 or 80 mg with CsA maintenance therapy, or 80 mg in a CNI-free, everolimus-based regimen.

Methods

Study design and conduct

This was a phase II, prospective, multicenter, randomized, open-label study in which patients were recruited at three French centers during March 2012 to March 2013. The study comprised a 12-week pharmacokinetic and pharmacodynamic analysis of basiliximab, with a further 12-week follow-up phase during which clinical events were monitored. The study was conducted in accordance with good clinical practice and with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all patients.

Eligibility criteria

Adult (18–65 years) recipients of a primary kidney transplant from a deceased or living donor who required

basiliximab induction were eligible for enrollment. Cold ischemia time was required to be <30 h. Key exclusion criteria were as follows: multiorgan transplantation, previous kidney transplantation, nonheart-beating donor, ABO incompatible graft or positive T-cell cross-match, an expanded criteria donor (United Network for Organ Sharing [UNOS] definition), the presence of anti-HLA antibodies as detected by a solid-phase immunoassay (e.g., Luminex), primary focal and segmental hyalinosis as the primary disease, primary disease related to atypical hemolytic uremic syndrome, an Epstein–Barr virus (EBV)-positive donor with an EBV-negative recipient, severe pretransplant hyperlipidemia, thrombocytopenia, neutropenia or leukopenia, hemoglobin <8 g/dl, liver enzymes (aspartate transaminase, alanine transaminase, or total bilirubin) ≥3 time upper limit of normal, or body mass index ≥30 kg/m².

Intervention

Patients were randomized before transplantation in a 1:1:2 ratio, using a validated scratch card system, to one of three groups: (i) a cumulative 40 mg dose of basiliximab [20 mg on days 0 and 4] with CsA maintenance therapy [control group], (ii) a cumulative 80 mg dose of basiliximab [40 mg on days 0 and 4] with CsA maintenance therapy, or (iii) a cumulative 80 mg dose of basiliximab [40 mg on days 0 and 4] with everolimus.

CsA was initiated within 12 h of transplantation at an initial dose of 6–8 mg/kg/day, adjusted to target a trough concentration of 150–200 ng/ml to week 12 and then 120–180 ng/ml during weeks 12–24. The first dose of everolimus was to be given within 24 h of transplantation at a starting dose of 6 mg/day, adjusted to target a trough concentration of 6–10 ng/ml. Enteric-coated mycophenolate sodium (EC-MPS) was administered to all patients at a minimum dose of 2160 mg/day for a minimum of 2 weeks and a maximum of 4 weeks, to be reduced to 1440 mg/day until the end of the study. Intravenous steroids therapy could be administered according to local practice, consistently applied for all patients enrolled at the center. Oral steroids were initiated within the first week post-transplant in all patients (minimum 20 mg/day), tapered as per local practice but with a minimum dose of 5 mg/day throughout the study.

Study endpoints

The primary endpoint was the area under the effect (AUE) curve for saturation of the CD25 antigen of IL-2 receptors by basiliximab to week 12 post-transplant. Secondary endpoints included basiliximab binding to CD25 receptors, lymphocyte population phenotype, and basiliximab pharmacokinetics. Clinical secondary endpoints included BPAR, graft loss, death, renal function (assessed by the abbreviated modification of diet in renal disease (MDRD) formula [26]), adverse events, serious adverse events, infections, and discontinuation of the study or study treatment.

Evaluation

Pharmacokinetic and pharmacodynamic analyses were performed centrally in a blinded manner. Samples for pharmacokinetic analysis were obtained on day 0 (before and 2 h after the first basiliximab infusion), day 1 (24 ± 6 h after the first infusion), day 4 (before and 2 h after the second infusion), and day 6 (48 h after the second infusion), and at days 14, 21, 28, 56, and 84 post-transplant. For saturation analyses, one sample of blood was collected at the same time points as those for the pharmacokinetic analysis in a special EDTA tube (containing a preservative to prolong the viability of fresh cells) and sent for central analysis within 48 h. Another sample was collected on day 0 (before the first basiliximab infusion) and day 6 (48 h after the second infusion), and on days 42 and 84 post-transplant in an EDTA tube for lymphocyte phenotyping, and also sent to the central laboratory within 48 h. Basiliximab serum concentrations were measured centrally using a validated enzyme-linked immunosorbent assay (ELISA; lower limit of detection 0.02 μg/ml; lower limit of quantification 0.067 μg/ml). Saturation of CD25 receptors by basiliximab was evaluated on unwashed blood samples using Alexa Fluor 488-conjugated basiliximab (488-BAS, using the Alexa Fluor 488 protein labeling kit from Invitrogen [Life Technologies Ltd, Paisley, UK]). In brief, blood samples from patients were incubated with a saturating concentration of unlabeled basiliximab, and with PECy5-conjugated anti-CD3 mAb (clone UCHT1 from Beckman Coulter [Brea, CA, USA]) to identify T cells, 488-BAS to identify cells for which the basiliximab epitope is available (i.e., unsaturated cells), and a mouse PE-conjugated anti-CD25 mAb (clone M-A251 from BD Biosciences [San Jose, CA, USA] that did not interfere with the basiliximab epitope) to identify $CD25^{+}$ cells regardless of CD25 saturation by basiliximab. After red blood cell lysis, cells were analyzed by flow cytometry as described elsewhere [27]. Saturation was expressed as the percentage of T cells with saturated CD25 (i.e., $CD3^{pos}CD25^{pos}488\text{-}BAS^{neg}$ cells in the absence of unlabeled basiliximab). Binding of basiliximab was evaluated on blood samples washed three times to remove endogenous plasma IgG using PECy5-conjugated anti-CD3 mAb and FITC-conjugated goat anti-human IgG F(ab')2 (from Beckman Coulter). After red blood cell lysis, cells were analyzed by flow cytometry. T cells ($CD3^{pos}$), B cells (CD^{19pos}), $CD4^{pos}$ cells, $CD8^{pos}$ cells, and natural killer (NK) cells ($CD3^{neg}CD56^{pos}$) were enumerated using flow

cytometry as per published methodology [27]. Protocol biopsies were performed at week 12.

Statistical analysis

For this proof-of-concept study, no formal sample size calculation was performed, but it was planned to recruit 50 transplanted patients who received at least two doses of basiliximab.

The primary endpoint, saturation of CD25 receptors by basiliximab (i.e., percentage of T cells with saturated CD25), was expressed as % saturation × days (AUE) during the 12-week study. AUE was calculated using the trapezoidal rule. Missing data were imputed by the treatment group mean at the given time point. Patients who did not receive two basiliximab injections were excluded from the analysis. Equivalence testing was based upon a margin of 20%, corresponding to a difference of 2.4 weeks. AUE and 95% confidence interval (CI) values for each group and for the between-group differences were calculated. Values for the basiliximab 80 mg + CsA and basiliximab 80 mg CNI-free groups were considered equivalent to the control group if 95% CI for the difference was included in the equivalence interval (−2.4 weeks, 2.4 weeks).

The secondary endpoint of basiliximab binding, expressed as % × weeks of binding (AUE) to week 12, was calculated using a similar approach to the primary endpoint.

Pharmacokinetics data for basiliximab were analyzed using both noncompartmental and compartmental approaches. Noncompartmental analyses were used to calculate area under the concentration versus time curve from 0 to the time of last nonzero concentration ($AUC_{0\text{-}z}$) and peak concentrations observed in the first sample after the first and second basiliximab injections (C_{max1} and C_{max2}) were obtained. Compartmental analyses were performed based on a population approach, using the nonlinear mixed effects program Monolix® 4.2.2. (Lixoft®, Orsay, France). The best description of basiliximab serum concentrations was obtained using a two-compartment model with both first-order and zero-order elimination rates.

The intent-to-treat (ITT) population comprised all randomized, transplanted patients who received at least one dose of basiliximab. The population for pharmacokinetic and pharmacodynamic analyses comprised all ITT patients with at least one blood sample for analysis. The two populations in this study were identical. Recruitment in the study was stopped prematurely, resulting in a low number of assessable patients. All data are presented descriptively. Statistical analyses were exploratory and CIs and p values are for information only. Statistical analyses were performed with SAS® version 9.3 (SAS® Institute Inc., Cary, NC, USA).

Results

Study population

Recruitment to the study was stopped prematurely due to a high number of BPAR episodes in the basiliximab 80 mg CNI-free group. At the point recruitment was stopped, 16 patients had been enrolled: three in the control group (basiliximab 40 mg + CsA), six in the basiliximab 80 mg + CsA, and seven in the basiliximab 80 mg CNI-free. One patient in the control group discontinued the assigned study regimen before week 12. One patient in the basiliximab 80 mg + CsA group and three patients in the basiliximab 80 mg CNI-free group discontinued the assigned study regimen by week 12, respectively, and the remaining four patients in the latter group discontinued the assigned regimen by week 24 (Fig. 1).

Patient characteristics are summarized in Table 1. The majority of patients (75%) were male. The mean age was slightly lower in the basiliximab 80 mg CNI-free group. One patient in each of the basiliximab 80 mg groups was black. As per study protocol, no patient had anti-HLA class I or II antibodies at the time of transplant.

Immunosuppression

All patients in the control group and the basiliximab 80 mg + CsA group received basiliximab as per protocol. In the basiliximab 80 mg CNI-free group, one patient did not receive the second 40 mg dose on day 4. Mean (SD) CsA trough concentration in the control and basiliximab 80 mg + CsA arms was 195 (20) and 160 (47) ng/mL at week 12, respectively, and 182 (82) and 133 (53) ng/mL at week 24 (Fig. 2a). Everolimus trough concentration varied widely between patients in the basiliximab 80 mg CNI-free group during the study (Fig. 2b). Mean (SD) everolimus trough concentration was 11.6 (8.2) ng/mL at week 12 (no data were provided at week 24). The median EC-MPS dose was 2160 mg/day in all groups to day 14, then 1440 mg/day in all groups to week 12. Mean (SD) EC-MPS dose at week 12 was 1440 (0) mg/day, 1260 (441) mg/day, and 1286 (283) mg/day in the control, basiliximab 80 mg + CsA, and basiliximab 80 mg CNI-free groups, respectively. Oral steroid dosing was similar in all groups during the study except for day 56, when mean dose was higher in the control arm due to a dose of 90 mg/day in one patient to treat acute rejection.

Acute rejection episodes

Biopsy-proven acute rejection occurred in one of the three patients in the control group (33.3%) (Table 2). This was an antibody-mediated rejection episode on day 43 and was

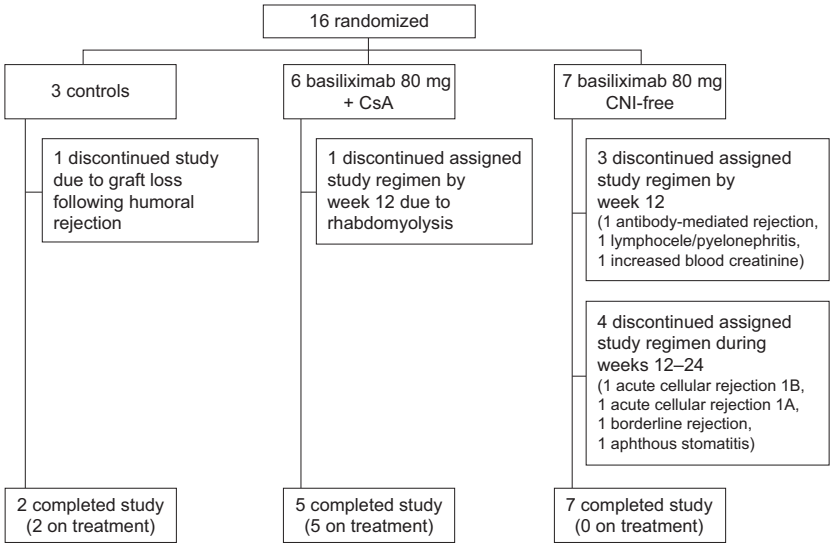

Figure 1 Patient disposition. CNI, calcineurin inhibitor; CsA, cyclosporine.

Table 1. Demographics and baseline characteristics.

	Control group (n = 3)	Basiliximab 80 mg + CsA (n = 6)	Basiliximab 80 mg CNI-free (n = 7)
Recipients			
Age, years	53.7 (8.7)	45.0 (11.4)	39.7 (12.2)
Male, *n* (%)	3 (100.0)	4 (66.7)	5 (71.4)
Caucasian, *n* (%)	3 (100.0)	5 (83.3)	6 (85.7)
Primary disease leading to transplantation, *n* (%)			
Glomerulonephropathy/glomerular disease/IgA	1 (33.3)	1 (16.7)	3 (42.9)
Polycystic disease	1 (33.3)	3 (50.0)	2 (28.6)
Other	1 (33.3)	2 (33.3)	2 (28.6)
Donors			
Age, years	51.3 (9.3)	45.3 (10.4)	44.6 (15.6)
Deceased donor, *n* (%)	3 (100.0)	3 (50.0)	4 (57.1)
Living donor, *n* (%)	0	3 (50.0)	3 (42.9)
Transplant			
Cold ischemia time, hours	11.1 (3.8)	7.3 (6.8)	8.9 (7.0)

Continuous variables are shown as mean (SD).
CNI, calcineurin inhibitor; CsA, cyclosporine.

followed on day 69 by unspecified humoral rejection and graft loss. One of the six patients (16.7%) in the basiliximab 80 mg + CsA group experienced Banff grade IB cellular rejection on day 81. Lastly, BPAR occurred in four of the seven patients (57.1%) in the basiliximab 80 mg CNI-free group. One patient had antibody-mediated rejection (day 3). The other three patients had cellular rejection: Banff IA (on day 94), Banff IB (on day 86), and Banff IIB (on day 40, followed by two episodes graded IB on days 68 and 131). In addition, borderline lesions were observed on protocol biopsy at week 12 in one patient in the basiliximab 80 mg + CsA group and two patients in the basiliximab 80 mg CNI-free group.

Other efficacy endpoints

There were no deaths during the study, including the follow-up phase, and no patients were lost to follow-up. Only one graft loss occurred, in a control patient following humoral rejection. Based on Kaplan–Meier estimates, the risk of treatment failure including borderline lesions (i.e., BPAR, borderline lesions, graft loss, death, or loss to follow-up) at last follow-up (week 24) was 33.3% in both the basiliximab 80 mg + CsA group and the control group, and 82.9% in the 80 mg CNI-free group. No statistically significant differences were observed between groups, as would be expected for such small patient populations.

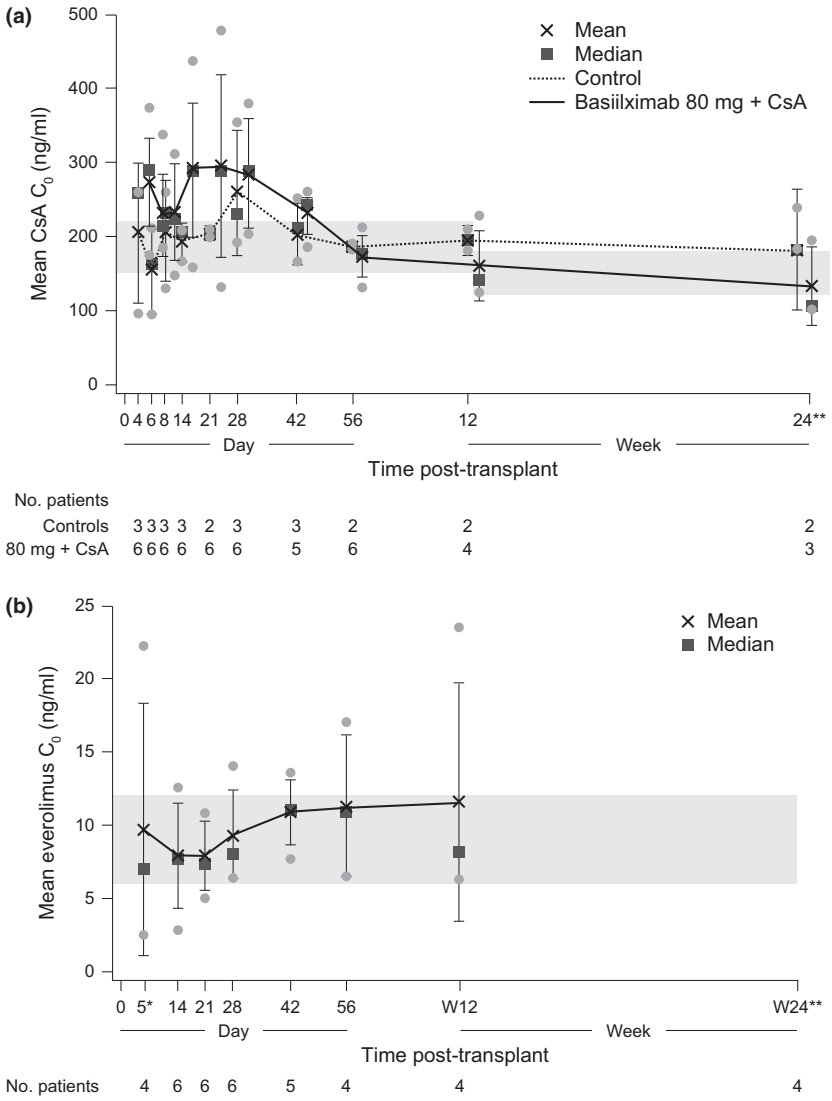

Figure 2 Trough concentration of (a) cyclosporine [CsA] and (b) everolimus. *5 days after first dose; **No data available. Vertical lines represent SD values. Circles represent maximum and minimum values.

Renal function

At day 8, mean (SD) estimated GFR (eGFR) was 35.0 (13.2), 53.8 (23.8), and 49.9 (18.5) ml/min/1.73 m^2 in the control group, basiliximab 80 mg + CsA group, and basiliximab 80 mg CNI-free group, respectively. Corresponding values were 50.6 (1.1), 57.0 (16.9), and 47.3 (12.5) ml/min/1.73 m^2 at week 12, respectively, and 47.9 (5.5), 55.7 (15.7), and 54.4 (10.5) ml/min/1.73 m^2 at week 24.

CD25 receptor saturation

The mean (SD) AUE of IL-2 CD25 saturation to week 12 was 8.4 (1.6) % × weeks in the control group, 11.1 (1.1) % × weeks in the basiliximab 80 mg + CsA group, and 9.7 (0.7) % × weeks in the basiliximab 80 mg CNI-free group. The difference in AUE versus the control group was significant for the basiliximab 80 mg + CsA group ($P = 0.020$) but not for the basiliximab 80 mg CNI-free group (Table 3). AUE to week 12 was significantly lower in the basiliximab 80 mg CNI-free group versus the basiliximab 80 mg CNI-group ($P = 0.028$).

Mean CD25 saturation was >90% in all three groups to week 4 post-transplant (Table 4). It decreased to 78.3% by week 6 and to 65% by week 12 in the control arm, but remained >90% in both 80 mg basiliximab groups until week 8 (Table 4). However, because of the small patient numbers, mean values at specific time points should be regarded with caution. Individual saturation curves are shown in Fig. 3. In the control arm, $CD25^+$ T cells from

Table 2. Efficacy endpoints.

	Control group (n = 3)	Basiliximab 80 mg + CsA (n = 6)	Basiliximab 80 mg CNI-free (n = 7)
Treatment failure*, *n* (%)	1 (33.3)	1 (16.7)	4 (57.1)
BPAR, *n* (%)			
Any	1 (33.3)	1 (16.7)	4 (57.1)
Antibody-mediated	1 (33.3) (day 43)	0	1 (14.3) (day 3)
Banff IA	0	0	1 (14.3) (day 94)
Banff IB	0	1 (day 81)	1 (14.3) (day 86)
Banff IIA	0	0	0
Banff IIB	0	0	1 (14.3) (day 40)
Borderline lesions, *n* (%)	0	1 (16.7)	2 (28.6)
BPAR or borderline lesions, *n* (%)	1 (33.3)	2 (33.3)	6 (86.7)
Graft loss, *n* (%)	1 (33.3)	0	0
Death, *n* (%)	0	0	0

The most severe episode of BPAR is shown for each patient. All events occurred by week 12, with no further events during weeks 12–24.
BPAR, biopsy-proven acute rejection; CNI, calcineurin inhibitor; CsA, cyclosporine.
*BPAR, graft loss, death, or loss to follow-up.

Table 3. CD25 antigen saturation and binding by basiliximab to week 12 post-transplant.

	Control group (n = 3)	Basiliximab 80 mg + CsA (n = 6)	Basiliximab 80 mg CNI-free (n = 6)
CD25 antigen saturation, % × weeks			
Mean AUE (SD)	8.4 (1.6)	11.1 (1.1)	9.7 (0.7)
95% CI	6.5; 10.2	10.2; 12.0	9.1; 10.2
Median AUE (range)	9.2 (6.5–9.4)	11.3 (9.5–12.1)	9.6 (8.8–10.8)
P value*			0.028†
Difference in AUE versus control group			
Mean, 95% CI	–	2.7 (0.7, 4.7)	1.3 (−0.6, 3.2)
P value*	–	0.020	0.119
CD25 antigen binding,% × weeks			
Mean AUE (SD)	7.0 (1.8)	9.9 (2.2)	8.4 (0.8)
Median AUE (range)	6.0 (6.0–9.1)	10.7 (6.8–12.0)	8.2 (7.4–9.5)
P value*		0.093‡	0.163†
			0.366‡

Missing data were imputed by the treatment group mean at the given time point. AUE was calculated only for patients who received two basiliximab injections.
AUE, area under the effect curve; CI, confidence interval; CNI, calcineurin inhibitor; CsA, cyclosporine; SD, standard deviation.
*Student's *t*-test.
†Versus basiliximab 80 mg + CsA.
‡Versus basiliximab 40 mg + CsA.

one patient became completely desaturated by day 56, and those from the two other patients by day 84. In the basiliximab 80 mg + CsA group, $CD25^+$ T cells from two of the five patients who provided data to the end of the 12-week study were completely desaturated by day 84: These two patients experienced borderline and Banff IB rejection. $CD25^+$ T cells from the three remaining patients were still completely saturated by day 84. In the basiliximab 80 mg CNI-free group, $CD25^+$ cells from three of the five patients who provided data to the end of the 12-week study were completely desaturated by day 84 (one patient experienced Banff IA rejection, one experienced Banff IIB rejection, and one had no rejection). $CD25^+$ T cells from the two remaining patients were saturated by ≤50% at that time.

When data from all three groups were pooled, mean CD25 saturation at the measurement prior to BPAR (47.3%) was similar to the final value recorded in patients without BPAR (47.4%, n = 10) (P = 0.468).

Basiliximab binding to CD25 receptors

The mean (SD) AUE of basiliximab binding to CD25 receptors was 7.0 (1.8) % × weeks in the control group, 9.9 (2.2) % × weeks in the basiliximab 80 mg + CsA

Table 4. Mean (SD) percentage of CD25 saturation at different points during the follow-up.

	Control group (n = 3)	Basiliximab 80 mg + CsA (n = 6)	Basiliximab 80 mg CNI-free (n = 7)
Week 4	95.3 (4.2)	100.0 (0)	92.7 (9.3%)
Week 6	78.3 (9.1)	100.0 (0)	99.2 (1.8)
Week 8	65.0 (56.4)	93.3 (13.2)	94.2 (13.0)
Week 12	0.0 (0)	67.5 (48.6)	14.2 (20.9)

CNI, calcineurin inhibitor; CsA, cyclosporine.

group, and 8.4 (0.8) % × weeks in the basiliximab 80 mg CNI-free group. Mean (SD) values for percentage of $CD25^+$ T cells with detectable basiliximab binding at week 12 were 6.7 (6.1)%, 57.7 (43.8)%, and 15.8 (9.7)% in the three groups, respectively. At week 12, the three patients in the control arm showed 0%, 8%, and 12% binding; patients in the basiliximab 80 mg + CsA group showed 0%, 13%, 50%, 83%, and 100% binding; and patients in the basiliximab 80 mg CNI-free group showed 10%, 11%, 11%, 14%, and 33% binding.

Lymphocytes

Cell counts for T-cell subpopulations ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD56^+$) remained relatively stable over time in the three treatment groups. There were no marked differences between treatment groups in terms of the proportion of lymphocyte subpopulations at week 12 (Table S1).

Pharmacokinetics of basiliximab

Twelve blood samples were assessed per patient, except in one patient for whom 11 blood samples were available. Mean values for basiliximab peak concentration and $AUC_{0\text{-}z}$ were approximately threefold higher in patients receiving a cumulative dose of 80 mg vs. 40 mg (Table 5). Basiliximab concentrations were best described when a nonlinear, zero-order elimination rate (k_0) was integrated into the pharmacokinetic model. Using the parameters of the linear component of basiliximab elimination, mean distribution and elimination half-lives of 0.118 and 25.6 days, respectively, were estimated.

Adverse events

All patients experienced one or more adverse event. The most frequent adverse events were hypertension (two patients, one patient, and four patients in the control, basiliximab 80 mg + CsA, and basiliximab 80 mg CNI-free groups, respectively), anemia (one patient, two patients, and two patients), and peripheral edema (three patients, one patient, and one patient). One adverse event (a case of BK virus infection in the basiliximab 80 mg CNI-free group) was considered by the investigator to be related to basiliximab. Infections occurred in three of the basiliximab 80 mg + CsA patients (50.0%) and five of the basiliximab 80 mg CNI-free patients (71.4%).

Serious adverse events were reported in two control patients (postprocedural hematuria and graft loss, and femur fracture), two patients in the basiliximab 80 mg + CsA group (cough, and diabetes mellitus with gastritis and increased blood creatinine), and five patients in the basiliximab 80 mg CNI-free group (epididymitis/lymphocele/pyelonephritis; acute renal failure/acute cellular rejection; increased blood creatinine; acute renal failure/acute cellular rejection and peritoneal hemorrhage; and acute cellular rejection).

The assigned study regimen was discontinued due to adverse events in four patients in the basiliximab 80 mg CNI-free group (lymphocele/pyelonephritis, increased blood creatinine, acute cellular rejection, and aphthous stomatitis), one patient in the basiliximab 80 mg + CsA group (rhabdomyolysis), and one patient in the control group (acute humoral rejection) (Fig. 1).

Discussion

This randomized trial was designed to examine the effect of a higher cumulative dose of basiliximab on T-cell CD25 saturation in *de novo* kidney transplant patients receiving CNI-free immunosuppression. An increased rate of BPAR in the CNI-free treatment arm, however, led to recruitment being stopped prematurely. Instead of the planned 50 patients, only 16 patients were enrolled, and results should thus be interpreted with caution. Nevertheless, the study provides detailed information about the pharmacodynamics and pharmacokinetics of basiliximab at the higher cumulative dose of 80 mg.

The timing of BPAR (days 40–94 post-transplant) in patients receiving a 80 mg dose of basiliximab with a CNI-free regimen was somewhat later than the typical clustering around 3 weeks after kidney transplantation. Among the five patients in this group who provided data to the end of the 12-week study, T-cell CD25 saturation was total in four cases (and 71% in the remaining case) at day 56 but was below 50% in all patients by day 84. Despite this extended saturation period as compared to previous pharmacodynamic studies [6–8], an entirely CNI-free regimen with basiliximab induction at an increased cumulative dose of 80 mg did not appear to prevent early acute rejection adequately. The increased rate of acute rejection observed in the CNI-free regimen is in agreement with the absence of synergistic benefit described between T-cell receptor-mediated production of IL-2 blocked by CNI agents and the effect of IL-2 receptor antibodies to limit IL-2 mediated

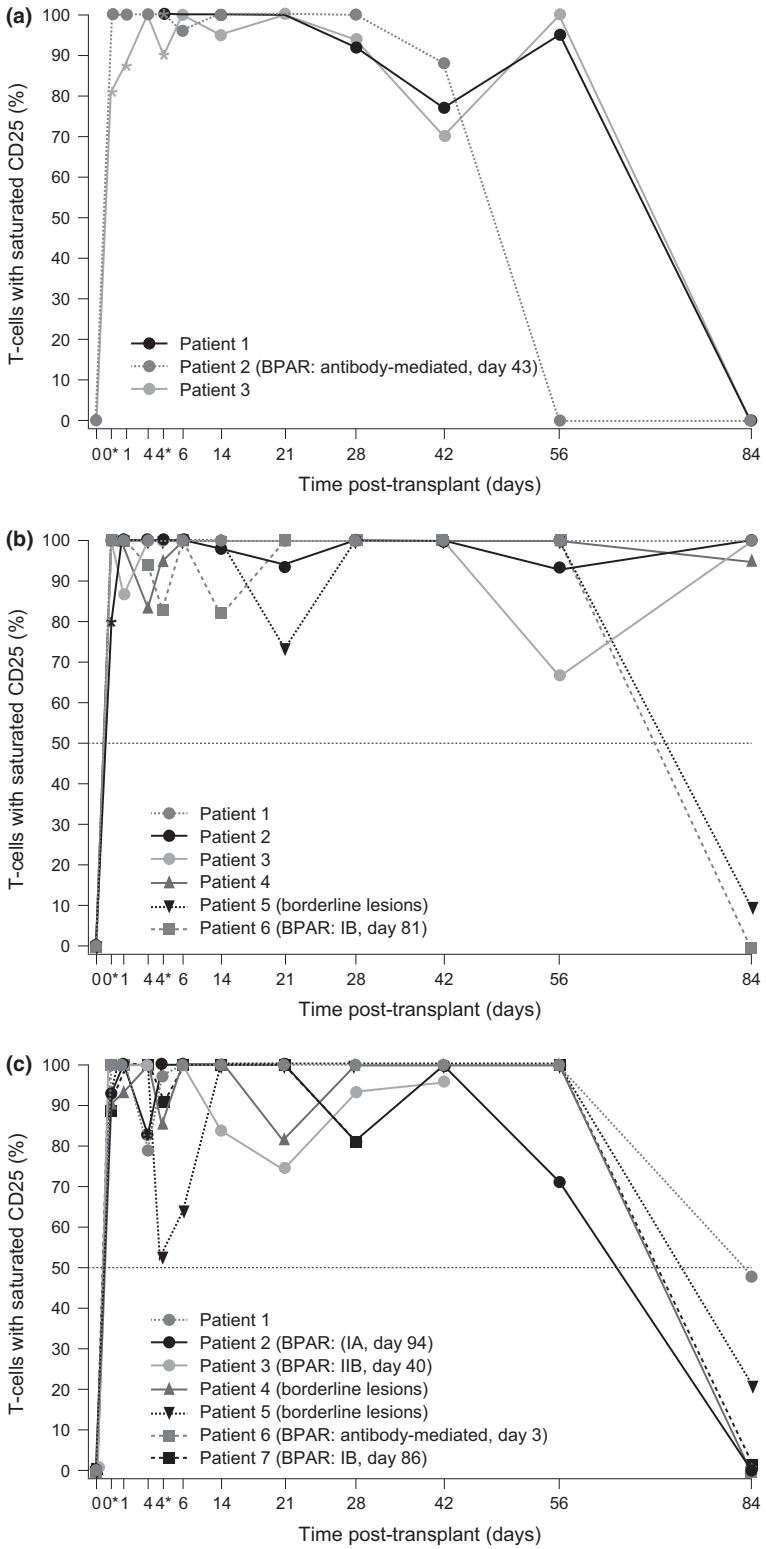

Figure 3 CD25 saturation curves for individual patients in (a) control group (b) basiliximab 80 mg + CsA group (c) basiliximab 80 mg CNI-free group. D0* and D4* represent 2 h after injection of basiliximab on day 0 and day 4, respectively. Asterisks indicate limit values, that is, values obtained from a low number of $CD25^+$ cells or morphologically unaltered cells, or from samples with shipment time >72 h. BPAR, biopsy-proven acute rejection; CNI, calcineurin inhibitor; CsA, cyclosporine.

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Table 5. Basiliximab pharmacokinetics.

	Noncompartmental analysis		Compartmental analysis	
	Basiliximab 40 mg (n = 3)	Basiliximab 80 mg (n = 13)	Estimate	Relative Standard Error (%)
$AUC_{0\text{-}tz}$ (mg.d/l)	78 (33)	234 (65)		
C_{max1} (mg/l)	3.4 (1.2)	10.7 (3.3)		
C_{max2} (mg/l)	5.2 (1.4)	14.5 (3.4)		
V_1 (L)			4.01 (0.29)	8
CL (L/day)			0.25 (0.17)	11
k_{12} (day^{-1})			0.27 (0)	8
k_{21} (day^{-1})			0.24 (0.28)	13
k_0 (mg/L/day)			0.32 (0.59)	29

Values are shown as mean (SD). Relative standard error (RSE) quantifies the precision of parameter estimation.
AUC, area under the concentration versus time curve; C_{max1}, the peak concentration observed in the first sample after the first injection; C_{max2}, the peak concentration observed in the second basiliximab injection; V_1, volume of distribution of the central compartment; CL, clearance; k_{12}, constant of transfer between central and peripheral compartments; k_{21}, constant of transfer between peripheral and central compartments; k_0, zero-order rate constant corresponding to the nonlinear component of basiliximab elimination.

T-cell proliferation. Although immunological effects of IL-2 receptor antagonists others than the blockade of IL-2-mediated T-cell proliferation (signal 3) have been described [28], as mTOR inhibitors exert their immunosuppressive effect downstream of IL-2 receptor signaling, this study suggests that "double-blocking" of signal 3 is not as efficient as a combination of signal 1 and 3 inhibition. The occurrence of cellular rejection in three patients and antibody-mediated rejection in one patient in the group randomized to 80 mg basiliximab and no CNI therapy was remarkably high, and higher than in the CNI-free patients of the SYMPHONY study who were given IL-2 receptor induction with MPA and steroids [24]. It is possible that wide variation in everolimus exposure may have contributed, but no firm conclusions are possible for such a small series of patients.

All three treatment groups showed more than 90% CD25 saturation at day 28, with a marked decline in saturation levels only after week 8. This is consistent with published data reporting that CD25-saturating concentrations of basiliximab (i.e., ≥0.2 μg/ml) were maintained for ~25–43 days after kidney transplantation in half of the patients receiving 40 mg basiliximab and CsA [6,7,9]. The mean AUE for CD25 saturation tended to be higher in patients receiving a cumulative dose of 80 mg compared to 40 mg, regardless of concomitant medication, mirrored in the percentage of basiliximab binding to CD25 receptors. This is as expected, given previous evidence for a dose-dependent effect of basiliximab in the range 20–60 mg [8]. CD25 desaturation seemed to occur earlier when basiliximab 80 mg was administered in a CNI-free regimen versus a CsA-containing regimen. This observation has not been described before. As CNI agents, but not mTOR inhibitors, inhibit CD25 expression on activated T cells [16] and suppress IL-2 production [10,11], which can in turn upregulate CD25 expression [14,15], this difference may be genuine but with such small numbers it is not possible to draw firm conclusions. IL-2 levels were not measured in the study, so any effect of differing IL-2 production between the two treatment groups could not be examined.

There was considerable variability in basiliximab concentration versus time between patients, as described by other authors [5,8,9]. A nonlinear relation was apparent between dose and AUC: Doubling the dose of basiliximab led to approximately a threefold increase in AUC, a finding that has not been observed previously at lower doses (up to 60 mg in total) [5,9]. Clearance, that is, the linear component of basiliximab elimination, was lower in the current study (0.245L/day) than in previous reports (0.4–1.1L/day) [6–9], and the elimination half-life ($t_{1/2\beta}$) was 25.6 days compared to 6.5–14.4 days in other pharmacokinetic trials in kidney transplant patients [5–9]. These findings could be accounted for by different analytical techniques or by the observed nonlinear elimination. Basiliximab is eliminated both by nonspecific endogenous mechanisms and after binding to its target antigen CD25. The first-order elimination rate (expressed here as the clearance [CL]) describes the endogenous elimination of basiliximab, whereas k_0 (the zero-order elimination rate) may be interpreted as target-mediated elimination. Elimination by the target antigen is saturable because of the finite availability of CD25, explaining why the rate of elimination increases at low basiliximab concentrations and therefore for low doses. We recognize the small size of the study population, particularly in the control arm. Additionally, in view of the multiple functions of IL-2 and its receptor in the immune response [29], future studies could usefully assess its immunomodulatory properties, for example, the effect on regulatory T lymphocytes. Nevertheless, these are the first data to explore the use of an increased basiliximab dose to preserve immunosuppressive efficacy during the early phase after kidney transplantation in patients receiving a CNI-free regimen. The findings indicate even doubling the cumulative basiliximab dose to 80 mg does not provide adequate immunosuppression during the first 3 months post-transplant in the absence of CNI therapy.

Authorship

GT and GP: established the pharmacokinetic and pharmacodynamic measurement techniques and collected data.

YL, CL, and PM: recruited patients and collected data. MC: performed pharmacodynamic analyses. EC and DT: performed the pharmacokinetic analysis, and FdG provided medical input to the study and contributed to the study protocol. All authors provided critical input to the study design and interpretation of the results, contributed to the manuscript, and approved the final version for publication.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Lymphocyte subpopulations at week 12. Data are shown as the mean (SD) percentages (reported to total lymphocytes) at week 12.

References

1. Adu D, Cockwell P, Ives NJ, Shaw J, Wheatley K. Interleukin-2 receptor monoclonal antibodies in renal transplantation: meta-analysis of randomised trials. *Br Med J* 2003; **326**: 789.
2. Webster AC, Ruster LP, McGee R, *et al.* Interleukin 2 receptor antagonists for kidney transplant recipients. *Cochrane Database Syst Rev* 2010; (1): CD003897.
3. Nashan B, Moore R, Amlot P, Schmidt AG, Abeywickrama K, Soulillou JP. Randomised trial of basiliximab versus placebo for control of acute cellular rejection in renal allograft recipients. CHIB 201 International Study Group. *Lancet* 1997; **350**: 1193.
4. Lebranchu Y, Bridoux F, Büchler M, *et al.* Immunoprophylaxis with basiliximab compared with antithymocyte globulin in renal transplant patients receiving MMF-containing triple therapy. *Am J Transplant* 2002; **2**: 48.
5. Amlot PL, Rawlings E, Fernando ON, *et al.* Prolonged action of a chimeric interleukin-2 receptor (CD25) monoclonal antibody used in cadaveric renal transplantation. *Transplantation* 1995; **60**: 748.
6. Kovarik JM, Moore R, Wolf P, *et al.* Screening for basiliximab exposure-response relationships in renal allotransplantation. *Clin Transplant* 1999; **13**(1 Pt 1): 32.
7. Kovarik JM, Kahan BD, Rajagopalan PR, *et al.* Population pharmacokinetics and exposure-response relationships for basiliximab in kidney transplantation. The U.S. Simulect Renal Transplant Study Group. *Transplantation* 1999; **68**: 1288.
8. Kovarik JM, Rawlings E, Sweny P, *et al.* Pharmacokinetics and immunodynamics of chimeric IL-2 receptor monoclonal antibody SDZ CHI 621 in renal allograft recipients. *Transpl Int* 1996; **9**(Suppl 1): S32.
9. Kovarik J, Wolf P, Cisterne JM, *et al.* Disposition of basiliximab, an interleukin-2 receptor monoclonal antibody, in recipients of mismatched cadaver renal allografts. *Transplantation* 1997; **64**: 1701.
10. Heidt S, Roelen DL, Eijsink C, *et al.* Calcineurin inhibitors affect B cell antibody responses indirectly by interfering with T cell help. *Clin Exp Immunol* 2010; **159**: 199.
11. Hamawy MM. Molecular actions of calcineurin inhibitors. *Drug News Perspect* 2003; **16**: 277.
12. Ueda H, Cheung YC, Masetti P, *et al.* Synergy between cyclosporine and anti-IL-2 receptor monoclonal antibodies in rats. Functional studies of heart and kidney allografts. *Transplantation* 1991; **52**: 437.
13. Ueda H, Hancock WW, Cheung YC, Diamantstein T, Tilney NL, Kupiec-Weglinski JW. The mechanism of synergistic interaction between anti-interleukin 2 receptor monoclonal antibody and cyclosporine therapy in rat recipients of organ allografts. *Transplantation* 1990; **50**: 545.
14. Reem GH, Yeh NH. Interleukin 2 regulates expression of its receptor and synthesis of gamma interferon by human T lymphocytes. *Science* 1984; **225**: 429.
15. Welte K, Andreeff M, Platzer E, *et al.* Interleukin 2 regulates the expression of Tac antigen on peripheral blood T lymphocytes. *J Exp Med* 1984; **160**: 1390.
16. Johannisson A, Thuvander A, Gadhasson IL. Activation markers and cell proliferation as indicators of toxicity: a flow cytometric approach. *Cell Biol Toxicol* 1995; **11**: 355.
17. Gurk-Turner C, Manitpisitkul W, Cooper M. A comprehensive review of everolimus clinical reports: a new mammalian target of rapamycin inhibitor. *Transplantation* 2012; **94**: 659.
18. Lebranchu Y, Snanoudj R, Toupance O, *et al.* Five-year results of a randomized trial comparing *de novo* sirolimus and cyclosporine in renal transplantation: the SPIESSER study. *Am J Transplant* 2012; **12**: 1801.
19. Langer RM, Hené R, Vitko S, *et al.* Everolimus plus early tacrolimus minimization: a phase III, randomized, open-label, multicentre trial in renal transplantation. *Transpl Int* 2012; **25**: 592.
20. Vitko S, Tedesco H, Eris J, *et al.* Everolimus with optimized cyclosporine dosing in renal transplant recipients: 6-month safety and efficacy results of two randomized studies. *Am J Transplant* 2004; **4**: 626.
21. Tedesco-Silva H Jr, Vitko S, Pascual J, *et al.* 12-month safety and efficacy of everolimus with reduced exposure cyclosporine in *de novo* renal transplant recipients. *Transpl Int* 2007; **20**: 27.
22. Chan L, Greenstein S, Hardy MA, *et al.* Multicenter, randomized study of the use of everolimus with tacrolimus after renal transplantation demonstrates its effectiveness. *Transplantation* 2008; **85**: 821.
23. Albano L, Berthoux F, Moal MC, *et al.* Incidence of delayed graft function and wound healing complications

after deceased-donor kidney transplantation is not affected by *de novo* everolimus. *Transplantation* 2009; **88**: 69.
24. Ekberg H, Tedesco-Silva H, Demirbas A, *et al.* Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.
25. Flechner SM, Glyda M, Cockfield S, *et al.* The ORION study: comparison of two sirolimus-based regimens versus tacrolimus and mycophenolate mofetil in renal allograft recipients. *Am J Transplant* 2011; **11**: 1633.
26. Levey AS, Bosch JP, Lewis JB, *et al.* A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med* 1999; **130**: 461.
27. Mélet J, Mulleman D, Goupille P, Ribourtout B, Watier H, Thibault G. Rituximab-induced T cell depletion in patients with rheumatoid arthritis: association with clinical response. *Arthritis Rheum* 2013; **65**: 2783.
28. Herr F, Lemoine R, Gouilleux F, *et al.* IL-2 phosphorylates STAT5 to drive IFN-γ production and activation of human dendritic cells. *J Immunol* 2014; **192**: 5660.
29. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. *Nat Rev Immunol* 2012; **12**: 180.