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Switching renal transplant recipients to belatacept therapy: results of a real-life gradual conversion protocol

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ABSTRACT

Conversion to belatacept immunosuppression is a therapeutic option for renal-transplant recipients with calcineurin inhibitors (CNI) toxicity, but it associates with high risk of acute rejection. Gradual conversion and serial immune monitoring with urinary chemokine CXCL9 may allow increasing safety of this maneuver.

We converted kidney transplant recipients with signs of toxicity to CNI or other immunosuppressive drugs to belatacept over a 2-month period. We monitored renal function, metabolic profile, and circulating lymphocyte subsets. We also quantified urinary CXCL9 over a 12-month follow-up period.

Between September 2016 and March 2017, 35 patients were successfully switched to belatacept immunosuppression at 3.3 (1.3–7.2) years after transplant. Two patients had a reversible rise in serum creatinine, associated with acute rejection in one case. Urinary CXCL9 increased before serum creatinine. After conversion, blood pressure and HbA1c significantly declined while eGFR and proteinuria remained stable. The percentage of circulating effector T cells and memory B cells significantly declined.

Conversion from CNI to belatacept, in this setting, was feasible and safe, provided it was performed over a 2-month time-period. Monitoring urinary CXCL9 may further increase safety through earlier identification of patients at risk for acute rejection. The procedure associates with improved blood pressure, metabolic profile, and reduced circulating effector T and B cells.

1. Introduction

The use of calcineurin inhibitor (CNI)-based maintenance immunosuppression has dramatically reduced the rates of acute cellular rejection after kidney transplantation [1,2], but long-term graft survival has not improved significantly over the past decades [3]. Several studies have correlated the long-term use of CNIs with renal interstitial fibrosis and tubular atrophy [4–6] and have prompted attempts to transition patients to other immunosuppressive agents. Mammalian target of rapamycin inhibitor (mTORi)-based regimens resulted in more frequent rejection episodes than tacrolimus and comparable chronic arteriolar toxicity [7]. Furthermore, CNI-free mTORi-based regimens have been associated with an increased risk of de novo donor-specific antihuman leukocyte antibodies (DSA) [7,8]. More recently, belatacept, a selective T-cell inhibitor of CD28-CD80/86 co-stimulation, in combination with mycophenolate mofetil (MMF) and steroids, preserved long-term graft function in de novo kidney-transplant recipients better than cyclosporine-receiving counterparts, and reduced the incidence of DSA development [9,10]. Despite these promising results, belatacept treated patients experienced significantly higher rates of acute rejection. Rejection events occurred mainly during the first-year post-transplantation, suggesting that selective co-stimulation blockade may not be sufficient to inhibit alloreactive memory T cells (less dependent on CD28-CD80/86 signaling for activation) in early phase post-transplantation when the alloimmune response is strongest [11,12].

In an attempt to limit long-term nephrotoxicity while maintaining

Abbreviations: BPAR, biopsy proven acute rejection; CNI, calcineurin inhibitors; CXCL9, chemokine (C-X-C motif) ligand 9; DSA, donor-specific antihuman leukocyte antibodies; GFR, glomerular filtration rate; HbA1c, Hemoglobin A1c; HLA, human leukocyte antigen; MMF, mycophenolate mofetil; MFI, mean fluorescence intensity; mTORi, Mammalian target of rapamycin inhibitor

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the beneficial peri-transplant immunosuppressive effects of CNIs, several groups have conducted trials in which kidney-transplant recipients were switched from CNIs to belatacept maintenance immunotherapy at 6 to 24 months post-transplantation [13–15]. Although these studies demonstrated an improved or stable glomerular filtration rate (GFR) while receiving belatacept, acute-rejection episodes were common during conversion [13]. One potential explanation for the high rates of acute rejections may rely in the relatively fast conversion, that generally occurred in 1 month or less [13,15,29]. A longer time period may be needed to allow the immune system to "adapt" to the different targets of the new immunosuppressive agent that often also imply changes in cell metabolism and epigenetic profile.

Moreover, the ability to identify patients at risk of acute rejection prior to graft damage during conversion to belatacept would improve the safety of this strategy. Urinary chemokine (C-X-C motif) ligand 9 (CXCL9), an interferon-γ-induced T-cell chemoattractant chemokine released by monocytes/macrophages, endothelial cells, and renal parenchymal cells [16], can non-invasively detect clinical and subclinical acute cellular rejection with a high negative predictive value [17–22]. Data from the Clinical Trial in Organ Transplantation 09 (CTOT-09), a study where kidney transplant recipients underwent tacrolimus withdrawal, show that urinary CXCL9 at a positivity threshold of ≥200 pg/ml can detect acute rejection 3 to 30 days before clinical presentation [22]. Data from a case-series indicate that urinary CXCL9 can also be used to monitor response to therapy for acute rejection [23]. Therefore, serial measurements of urinary CXCL9 could allow to identify early patients at risk for acute cellular rejection during conversion from CNI to belatacept and to treat them promptly.

Based on this background, we developed a strategy of gradual (over a 2-month time period) conversion from CNI to belatacept to reduce the risk of acute rejection. In the present study, we tested the safety and feasibility of such approach in the real-life setting of a large transplant center and we tested the association between changes in urinary CXCL9 and acute rejection episodes during conversion. We also performed serial measurement of main circulating immune B and T cell subsets to monitor immune changes associated with conversion to belatacept therapy.

2. Materials and methods

2.1. Design

This was a retrospective study performed at the Grenoble University Hospital (Grenoble, France), where deceased or living kidney-transplant recipients on chronic CNI immunosuppressive therapy underwent conversion to belatacept immunosuppression. Inclusion criteria were: time from transplant > 3 months, signs of CNI nephrotoxicity (eGFR decline without donor specific antibodies and compatible allograft biopsy findings), CNI neurotoxicity, or toxicity related to any other immunosuppressive drug, eGFR < 35 ml/min/1.73 m^2, or metabolic disorders (e.g. metabolic syndrome or new onset of diabetes after transplant). Patients with negative EBV serology were excluded. Urine samples were collected on the day of the first belatacept infusion (before infusion) and at 1, 3, 6, and 12 months thereafter. All patients provided their written informed consent for urine collection. All medical data were collected from the Grenoble University Hospital database [CNIL (French national committee for data protection) approval number 1987785v0].

2.2. Conversion to belatacept

All included patients started conversion by receiving 5 mg/kg of belatacept on day 1, 15, 28 and then monthly thereafter. During the first month following initiation of belatacept, CNI dose was unchanged; then, at month 2 after initiation of belatacept, CNI dose was halved and then stopped at the beginning of month 3. For one patient, CNI was stopped at 6 months after initiation of belatacept because of a prescription error. The doses of mycophenolate mofetil and/or corticosteroids remained unchanged in all the participants. The two subjects that were on combined tacrolimus and everolimus therapy had everolimus replaced by mycophenolic acid at conversion.

2.3. Clinical and immunological follow-up

Participants were examined at baseline (before first belatacept administration) and before each subsequent belatacept infusion. We also measured systolic and diastolic blood pressure in a seated position after 10 min of rest using vital signs monitor (DYNAMAP PRO 300 V2, GE Healthcare). Patients had serum creatinine and proteinuria measured on the day of the first infusion of belatacept and at 1, 3, 6, and 12 months thereafter. GFR was estimated using the CKD EPI equation [25]. Complete blood count and total $CD4^+$ and $CD8^+$ T cells, B cells, NK cells and T and B cells subset were measured at the same time points.

For patients with diabetes, HbA1c was measured on the day of the first infusion of belatacept and at 3, 6, 12 months after starting conversion. For the others, HbA1c was assessed before conversion and 1-year later. Hemoglobin A1C (HbA1c) dosage was performed by High-Performance Liquid Chromatography (HPLC). Patients had total cholesterol, LDL-c, and triglycerides measured before conversion and 1-year after the conversion to belatacept. All these analyses were performed at the central lab of the Grenoble University Hospital.

Anti-donor human leukocyte antigen (HLA) antibodies were measured at the time of first belatacept infusion, at the end of the 12-month follow-up period, and at the time of any suspected rejection episode by Luminex (Immucor®). Identification of anti-HLA specificity was performed by single antigen assays if screening test was positive. Assessment of anti-HLA antibodies was performed at the Etablissement Français du Sang –EFS- at Grenoble University Hospital.

2.4. Measurement of urinary CXCL9

Urinary samples for CXCL9 analyses were centrifuged at 2000*g* for 30 min at 4 °C within 4 h from collection. The supernatant was divided into aliquots and frozen at −80 °C.

Urinary CXCL9 was measured in batches at the end of the study. We strictly followed the protocol defined by a multicenter validation study for CXCL9 measurement [21] and reported the data as pg/ml without creatinine correction accordingly. We used the same previously defined threshold for positivity of 200 pg/ml [21]. Frozen aliquots of urine supernatants were diluted (1:1) in 0.05% Tween 20/0.4% bovine serum albumin in phosphate-buffered saline (pH 7.2–7.4) and tested by ELISA for CXCL9 (R&D Systems, Minneapolis, MN), as previously reported [21].

2.5. Flow cytometric peripheral blood lymphocyte analysis

Cell staining was performed on whole blood samples using a direct immunofluorescence method with erythrocytes lysis and washing, then lymphocyte subsets were identified using a 3-laser, 8-colour BD FACSCanto-II TM flow cytometer and FACSDiva software (BD Biosciences, San José, CA). The absolute numbers of subsets were calculated by multiplying their percentage by the total lymphocyte number obtained from an ABX MICROS 60 device (HORIBA ABX SAS, Montpellier, France).

Three combinations of anti-human antibodies were analyzed i) FITC-CD3 (Sk7 clone, BD), PE-CD16 (B73.1 clone, BD), PE-CD56 (NCAM16.2 clone, BD), PerCP-CD45 (2D1 HLE-1, BD), PE-Cy7-CD4 (SK3 clone, BD), APC-CD19 (SJ25C1 clone, BD), APC-Cy7-CD8 (SK1 clone, BD), BV450-HLA-DR (L243 clone, BD), ii) PE-CD8 (B9.11 clone, BD), PerCP-Cy5.5-CD4 (SK3 clone, BD), PE-Cy7-CD3 (UCHT1 clone, BD), APC-CD45RA (HI100 clone, BD), BV421-CD197 (CCR7) (150,503

clone, BD) and iii) FITC-IgD (F0189 clone, AGILENT (DAKO)), PE-CD10 (ALB1 clone, Beckman Coulter (BC)), PerCP-Cy5.5-CD38 (HIT2, BD), PE-Cy7-CD27 (1A4CD27 clone, BC), APC-IgM (G20–127 clone, BD), and APC-H7– CD19 (SJ2SC1 clone, BD). The first combination allowed the identification of whole B-cells ($CD19^+$), NK-cells ($CD16/CD56^+CD3^-$) and T-cells ($CD3^+$), as well as $CD4^+$ and $CD8^+$ T-cell subsets. $CD3^+HLA\text{-}DR^+$ evaluated activated T-cells. The second combination discriminated naive ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), and effector memory ($CD45RA^{+/-}CCR7^-$) $CD4^+$ and $CD8^+$ T-cell subsets [26]. The last combination distinguished naive ($CD19^+IgD^+CD27^-$), natural memory ($CD19^+IgD^+CD27^+$), post-germinal center ($CD19^+IgD^-CD27^+$) B-cell subsets [27], and plasma cell precursors ($CD19^+IgD^-CD27^{high}CD38^{high}$).

2.6. Statistical analyses

Baseline characteristics were compared by Wilcoxon rank sum, χ^2, or Fisher's exact test as appropriate. eGFR slopes were calculated using a single slope linear model for each patient who had at least three eGFR values. eGFR slopes before vs. after belatacept initiation were compared using a paired *t*-test, as appropriate. Statistical analyses were performed with GraphPad Prism 5.0 software using paired Wilcoxon rank tests to compare parameters with baseline values. Two-sided significance tests were used throughout. $P < .05$ was considered statistically significant.

3. Results

3.1. Patients

Between September 2016 and March 2017, the study included 35 kidney transplant recipients (Table 1). The median time between transplant and conversion to belatacept was 3.3 years (interquartile interval: 1.3–7.2). Four patients were recipients of a second transplant. Mean age at conversion was 55.5 ± 12.5 years. Seven (20%) subjects had circulating anti-HLA antibodies and two subjects had low level (MFI <2000) class I DSA. No one had class II DSA. All patients received induction therapy (32 with antithymocyte globulins and 3 with basiliximab). Before belatacept conversion, 31 patients were receiving tacrolimus, 3 were on cyclosporine, and one was receiving azathioprine (this patient was switched from azathioprine to belatacept, due to recurrent squamous cell carcinomas [24]. Thirty-one patients took mycophenolic acid in association with the CNIs, 2 patients received everolimus, and 11 patients received low doses of steroids. The reasons for the conversion are specified in Table 2.

Table 1
Patient characteristics.

Patients	*N* = 35
Donors	
Deceased, n (%)	26 (71.4)
Living, n (%)	9 (25.7)
Recipients	
Male, n (%)	27 (77.1)
Age, years	55.5 ± 12.5
Median time between transplant and conversion, years (IQ)	3.3 (1.3–7.1)
2nd transplantation, n (%)	4 (11.4)
DSA, n (%)	2 (5.7)
Anti-HLA class I antibodies, n (%)	4 (14.8)
Anti-HLA class II antibodies, n (%)	2 (5.7)
Anti-class I and class II antibodies, n (%)	2 (5.7)
Immunosuppression	
Induction	
ATG, n (%)	32 (91.4)
Basiliximab, n (%)	3 (8.6)
Maintenance	
Tacrolimus + MMF, n (%)	18 (51.4)
Tacrolimus + MMF + steroids, n (%)	11 (31)
Cyclosporine + MMF, n (%)	3 (8.6)
Tacrolimus + everolimus, n (%)	2 (5.7)
Azathioprine + steroids, n (%)	1 (2.9)

Data refer to the time of conversion and are expressed as mean ± SD or median (interquartile range – IQ). ATG: anti-thymocyte globulin, DSA: donor-specific antihuman leukocyte antibodies MMF: mycophenolate mofetil.

Table 2
Reasons for conversion from CNI to belatacept.

Chronic allograft dysfunction	23 (65.7)
Chronic allograft dysfunction + diabetes	1 (2.9)
Nephroprotection without allograft dysfunction	2 (5.7)
Peripheral neuropathy	2 (5.7)
Diabetes	2 (5.7)
Dyslipidemia	1 (2.9)
CNI intolerance	2 (5.7)
Non adherence	1 (2.9)
Recurrent squamous cell carcinoma	1 (2.9)

Data are number (percentage).

No patient had BK virus nephropathy previous to or at the time of conversion. BKV viremia was performed only in those patients that had kidney graft dysfunction. None of these patients developed viremia during the first year following conversion. No patient developed CMV disease.

3.2. Clinical outcomes and safety

All patients withdrew CNI and were converted to belatacept over a 2-month time period except for one patient who stayed with halved CNI doses from month 2 to month 6 after study entry because of a prescription error (starting from month 3, his tacrolimus trough levels were below detection limit at our lab of 1 ng/ml).

Patient- and graft-survival rates at 1-year post-conversion were 100%. Kidney function measured by eGFR (Fig. 1) and proteinuria was stable over the 12-month follow-up: mean delta between conversion and 1 year later was 0.11 ± 10.5 ml/min/1.73m^2 ($p = .448$) for eGFR and 0.02 ± 0.3 g/24 h ($p = .987$) for proteinuria. For patients with kidney transplantation performed more than 1 year before conversion, the 12-month slope of eGFR before conversion showed faster worsening of graft function the year before conversion compared to the one after conversion, but this difference did not reach statistical significance (median difference: −6.2 vs −1.8 ml/min/1.73m^2 for eGFR slope before vs. after conversion, respectively; $p = .213$). When we stratified patients according to eGFR at study entry, we found that during the 12-month follow-up period, eGFR was stable across all levels of renal function (Supplemental Fig. 1). Changes in serum creatinine, eGFR, and proteinuria remained significant when we repeated the analyses after excluding the patient that continued CNI for 6 months.

Two patients underwent per-cause graft biopsy due to acute rise in serum creatinine following belatacept conversion (at 4.5 months and 3.5 months after first belatacept infusion). In one patient, the graft biopsy showed mild acute rejection (IA), while the biopsy of the second patient did not show any infiltrate reaching criteria for acute rejection. Both patients received steroid pulses followed by rapid dose reduction and creatinine values returned to baseline following treatment. After steroid pulses, both patients were maintained on belatacept.

Conversion was overall well tolerated, including belatacept infusions. Adverse events are listed in Table 3 and none of them was associated with belatacept therapy. No major infection requiring hospitalization occurred. No patient stopped belatacept during the study. Two patients were converted to belatacept for neuropathy: one patient felt a significant improvement after 1 year, while the second did not.

3.3. Blood pressure and metabolic profile

Mean systolic arterial blood pressure significantly decreased

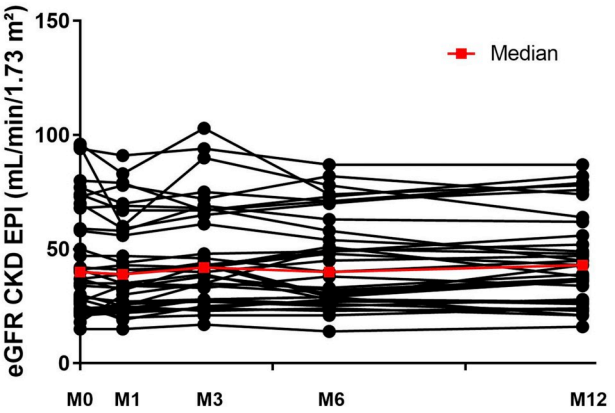

Fig. 1. eGFR during the 12-month follow-up period. Each black line represents a single patient. Red line represents the median values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Patients with adverse events.

Serious	
Cardiovascular	
Cerebral Stroke	1
Myocardial infarction	1
Arteriovenous fistula thrombosis	1
Infectious	
Viral pneumonia	2
Pyelonephritis	2
Non serious	
Cardiovascular	
Superficial thrombophlebitis	1
Infectious	
Cystitis	1

between baseline (before conversion) and 12 months post-conversion (146.3 ± 18.8 vs. 137.5 ± 16.1 mmHg; $p = .03$), while mean diastolic blood pressure did not significantly change (86.5 ± 12.3 at baseline vs, 84.1 ± 13.5 at 12 months post-conversion; $p = .36$) (Fig. 2A). Twenty-three patients reduced or did not change the dosage of their antihypertensive drugs, while 12 patients had their doses increased. In patients with diabetes mellitus history ($n = 10$), the mean HbA1c also significantly declined after conversion (7.1 ± 1.2%, 6.4 ± 1.4%, 6.3 ± 1.1% at baseline, 6 and 12 months after first belatacept administration; $p < .05$ for both time points vs. baseline) (Fig. 2B). None of these patients had their antidiabetic drugs increased and in 4 patients their doses were reduced.

Total cholesterol, LDL cholesterol, and triglyceride levels did not significantly change between the baseline levels and those at the end of the study (not shown).

4. Circulating immune globulin and lymphocyte subsets

Concentrations of various immunoglobulin subclasses did not significantly change from baseline to 12 months post-conversion (IgG: 9.0 ± 2.3 vs. 9.3 ± 2.4 g/l; $p = .44$, IgA: 2.1 ± 1.1 vs. 2.0 ± 1.0 g/l; $p = .72$, and IgM: 0.8 ± 0.6 vs. 0.8 ± 0.6 g/l; $p = .80$). No patient developed de novo DSA during the follow-up period.

Absolute numbers and percentages of circulating total leukocytes, T cells, B cells, and NK cells were stable across the study period. Percentages of central memory $CD4^+$ and effector $CD8^+$ T cells and memory and post-germinative B cells significantly declined between the day of first belatacept infusion and 12 months thereafter (Fig. 3 and Table 4). The other subsets did not vary significantly.

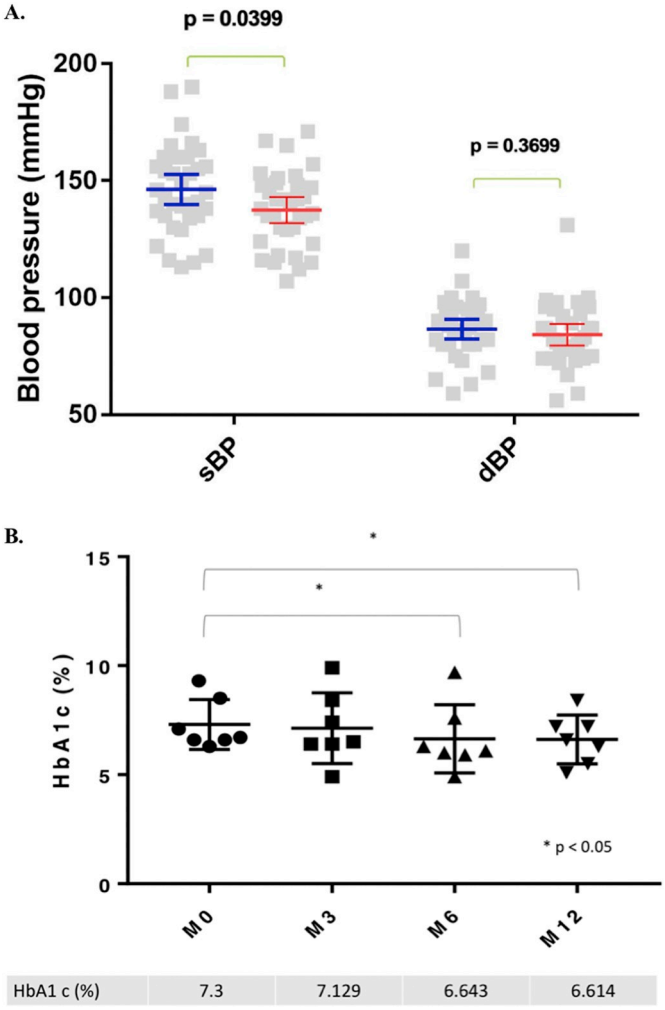

Fig. 2. Blood pressure and glycemic control. (A) Systolic (sBP) and diastolic (dBP) blood pressure at baseline (before conversion, blue) and at the end of the 12-month follow-up period (red). (B) Glycosylated hemoglobin (HbA1c) at baseline (month 0), 3, 6 and 12 months after belatacept initiation in subjects with diabetes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.1. Urinary CXCL9

Urinary CXCL9 levels were below the positivity threshold of 200 pg/ml for all but 3 patients, who showed elevated values at the time of first belatacept administration ($n = 1$) or during follow-up ($n = 2$) (Fig. 4A).

The subject with high baseline CXCL9 value was on tacrolimus and everolimus at the time of first belatacept administration. Tacrolimus was stopped at month 2 after belatacept initiation, but 2 months later (4 months after starting conversion) the creatinine-level rose, and a graft biopsy showed acute cellular rejection (grade IA). Following steroid pulses, serum creatinine returned to baseline values (Fig. 4B).

In a second patient, CXCL9 was normal at baseline and increased to > 250 pg/ml at 3 months after conversion. This increase happened 3 weeks before the rise in serum creatinine, which prompted a kidney graft biopsy. The histology showed interstitial inflammation, although it did not meet the Banff criteria for acute rejection. The patient received steroid pulses that lowered serum creatinine and urinary CXCL9 levels (Fig. 4C).

In the third patient, urinary CXCL9 was normal at baseline and at 1-month post-conversion, but became positivity threshold and then remained elevated. Creatinine level fluctuated but remained within ± 40 μmol/l from baseline, therefore no biopsy was performed

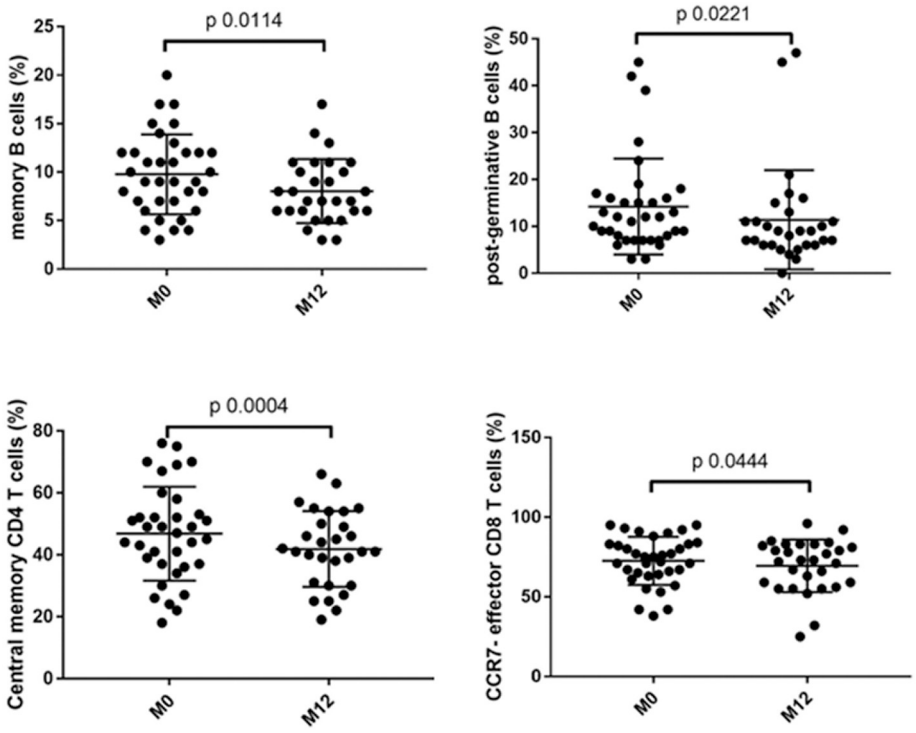

Fig. 3. Changes in circulating T and B lymphocyte subsets. Here are shown the subsets that showed significant changes between baseline and 1 year after, expressed as percentages of total circulating B cells, $CD4^+$, or $CD8^+$ T cells.

Table 4
Changes in circulating lymphocyte subsets.

	Month 0	Month 12	p
Total $CD4^+$ T cells ($\times 10^6$/dl)	0.4 ± 0.3	0.4 ± 0.3	0.1899
Naive $CD4^+$ T cells (%)	28.8 ± 18.8	34.7 ± 17.5	0.0001
Central memory $CD4^+$ T cells (%)	46.8 ± 15.2	41.9 ± 12.3	0.0004
Total $CD8^+$ T cells ($\times 10^6$/dl)	0.4 ± 0.4	0.5 ± 0.5	0.0001
Naive $CD8^+$ T cells (%)	22.2 ± 13.1	25.1 ± 15.5	0.0003
Effector $CD8^+$ T cells (%)	72.6 ± 15.1	69.5 ± 16.5	0.0444
Total B cells ($\times 10^6$/dl)	0.1 ± 0 0.1	0.1 ± 0.1	0.5488
Naive B cells (%)	59.8 ± 13.9	63.2 ± 15.1	0.0261
Post-germinal center B cells (%)	14.2 ± 10.2	11.4 ± 10.6	0.0221

Month 0 represents the time of conversion. Data are expressed as mean ± SD of absolute numbers or percentages.

(Fig. 4D). At 12 months after conversion, the patient's serum creatinine level was lower than baseline (131 μmol/l vs. 119 μmol/l).

None of the other patients with stable negative urinary CXCL9 values experienced rises in serum creatinine that could be interpreted as clinical rejection episodes.

5. Discussion

We demonstrated that conversion of kidney transplant recipients from CNI to belatacept can be performed safely in the real-life setting of a large transplant center, provided that it is performed gradually and > 3 months post-transplant. Conversion was associated with improved blood pressure control, better metabolic profile, and stable graft function. Circulating T and B cells with effector or memory phenotype significantly declined after conversion to belatacept, which may suggest a lower level of immune activation. Serial urinary CXCL9 measurements were able to detect the few patients who developed intragraft infiltrates or graft dysfunction during conversion prior to an increase in serum creatinine, providing an important tool to monitor safety of the maneuver.

In contrast to the high rate of acute rejection reported by others, i.e. 8.2% to 20% [13,15,28,29], only one patient developed biopsy proven acute rejection (BPAR) in our series. This low-rate of acute rejections could be explained by the CNI weaning strategy that we adopted: CNIs and belatacept were overlapped for 1 month and then CNIs were fully tapered off over a 2-month period, which exceeds the 1-month conversion time reported in most of the previous studies [15,29]. Importantly, since previous studies showed that most BPAR events develop within 1 year of belatacept initiation, the extent of our follow-up period was enough to reasonably exclude that our conversion strategy simply delayed their occurrence and that we did not capture them.

No patient developed de novo DSA in our study, which is consistent with previous studies showing that de novo DSA is a rare event in belatacept-treated patients [30,31]. This result is supported by experimental evidence indicating that costimulatory blockade inhibits T follicular helper cell differentiation, B cell clonal expansion, germinal center reaction and DSA formation [32,33,35]. Consistently, our data indicate a significant reduction in circulating $CD4^+$ and $CD8^+$ effector T cells and post-germinal center and memory B cells after conversion to belatacept. Albeit small, these significant changes in peripheral cell subsets may actually have a biological effect, as previously described by others in other kidney transplant recipient cohorts [36,37].

Belatacept conversion showed clinical benefit. Patients' kidney function did not deteriorate and, although not statistically significant, the rate of GFR decline was reduced after conversion to belatacept. CNI interruption also resulted in a significant lowering of HbA1c in diabetic patients despite no change in sugar-lowering drugs. This may infer that tacrolimus, which is a well-known diabetogenic drug, may enable hyperglycemia late after its introduction. [38]

Importantly, conversion to belatacept improved blood pressure and metabolic profile and stabilized graft function. The patient on azathioprine had numerous recurrences of squamous cell carcinomas and has been free from recurrence since conversion to belatacept. One of the two patients with CNI-induced neuropathy reported significant improvement of the symptoms. Following conversion, all patients reported satisfaction with the new intravenous regimen and none asked to return to CNI.

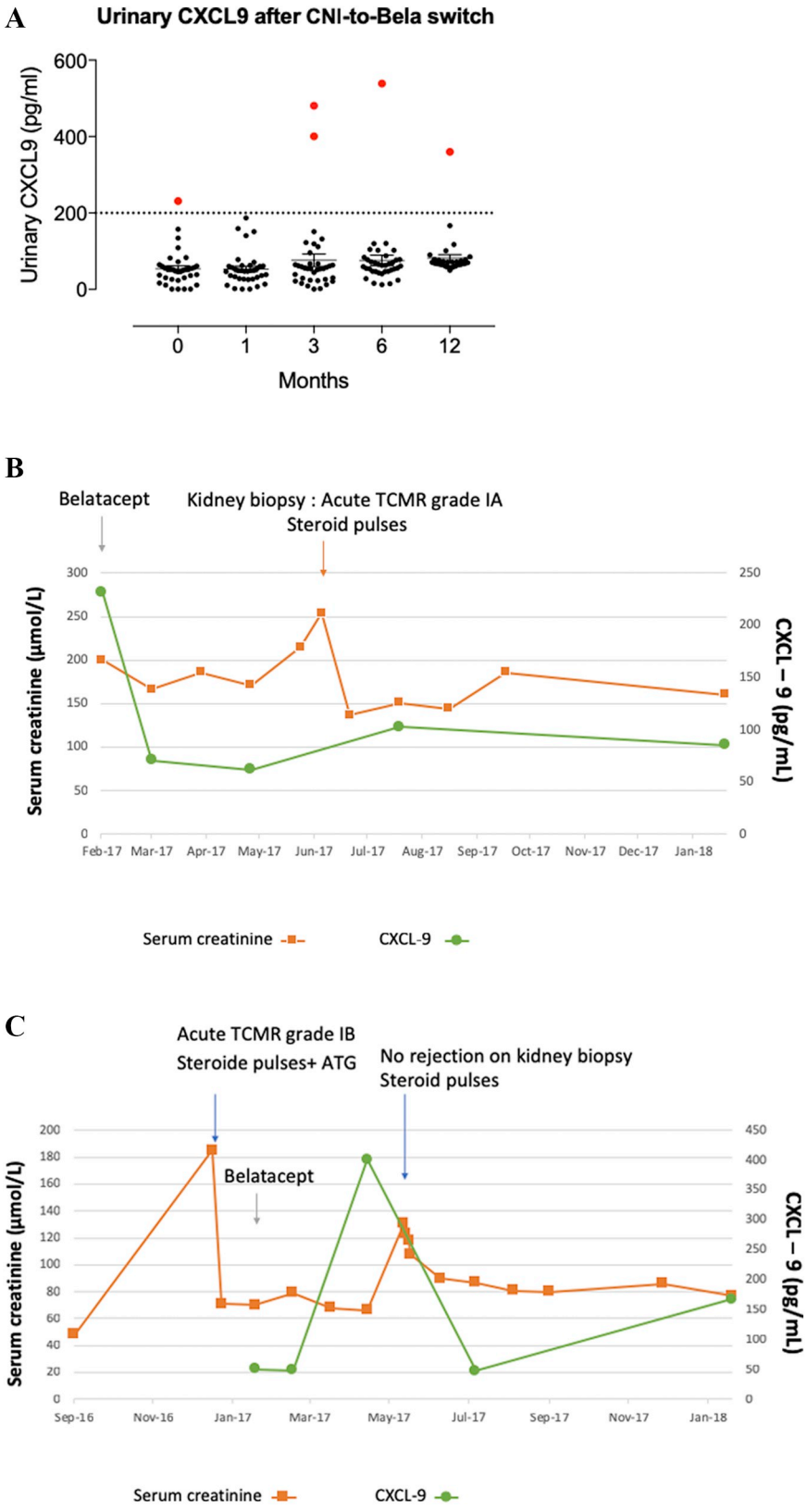

Fig. 4. Urinary CXCL9. (A) Urinary CXCL9 before and at various time points after belatacept initiation in the whole study cohort. Dotted line represents the positivity threshold of 200 pg/ml. In red are depicted the positive values. (B–D) Changes in serum creatinine, and urinary CXCL9 in the three patients who displayed at least one CXCL9 value above the positivity threshold. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

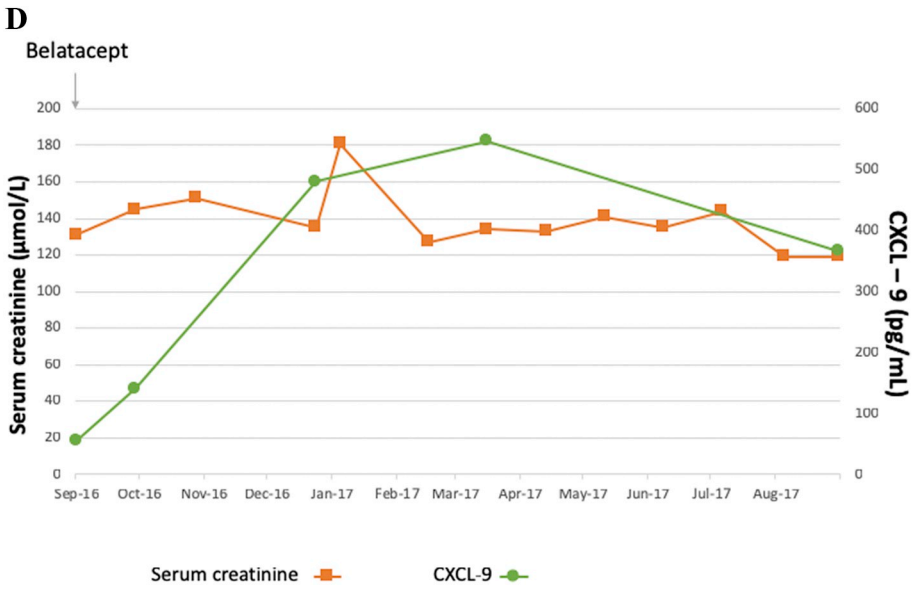

Fig. 4. (*continued*)

Our data also support the favorable safety profile of belatacept. Belatacept infusions were well tolerated, the adverse events were limited in number, and in no case were any adverse events attributable to belatacept. All included patients were EBV positive, which reduced the risk for post-transplant lymphoproliferative disorder (PTLD) previously associated with belatacept therapy [39].

In the present study, urinary CXCL9 levels were within the normal range in most of the measurements. The three patients with elevated CXCL9 levels were the only ones who eventually developed kidney dysfunction, possibly mediated by an immunological reaction. Because the CXCL9 measurements were done post hoc, this information did not bias the clinical actions of the treating physicians, thus explaining why patients did not receive a kidney-graft biopsy despite the high CXCL9 levels. However, these data provide rationale for future interventional studies testing the hypothesis that serial measurements of urinary CXCL9 can lead to early identification of patients at risk for acute rejection, for whom CNI tapering should be avoided or performed cautiously to improve safety. On the other hand, low CXCL9 levels could reassure transplant physicians and lead to less frequent surveillance graft biopsies. Because histological diagnoses in kidney transplantation are subject to sampling bias [40,41] and inter-reader variation [42], and serum creatinine is an insensitive measurement of graft function [43], the present data support the testable hypothesis that elevated urinary CXCL9 measurements are better indicators of ongoing pathological inflammation than serum creatinine or histological evaluation.

Previous studies showed that urinary tract infections and BKV increase urinary CXCL9 [34]. While no patient in our study had either of these conditions when CXCL9 was tested in the urine, routine BKV monitoring would be important to increase specificity of positive CXCL9 results for acute rejection.

Herein, urinary CXCL9 was measured by ELISA. The 12- to 24-h requisite turnaround time for ELISAs is short but not ideal for "real-time" implementation of therapeutic changes based on assay results. However, urinary CXCL9 can be also detected by biolayer interferometry [44], an assay that requires < 1 h, making it potentially implementable in clinical transplantation practice.

Our study has limitations, including the single-center, retrospective design. However, the fact that these data were generated in the "real-life" setting of a large transplant center underlines the potential of using this strategy of conversion together with urinary CXCL9 to minimize the risk of rejection and early treat the subjects that develop such complication. Lack of a control group is another limitation. However, with the limitation of juxtapositions across different studies, our results compare well with previous conversion trials from CNI to belatacept in terms of acute rejection rate and metabolic improvement [13,15,28,29].

Overall, our data support the use of our strategy of conversion of kidney transplant recipients from CNI to belatacept and provide the rationale for a randomized control trial testing the hypothesis that CXCL9 driven management of patients improves the safety/efficacy profile of this procedure over standard serum creatinine monitoring.

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Authorship

PM, LR, PC: participated in study design, interpretation of data and writing of the manuscript. CF, PC: performed urinary CXCL9 measurements, GR: participated in collecting the data, MCJ, TR: performed the flow cytometric peripheral blood lymphocyte analysis, PM, TJ, LR, and BJ: were in charge of clinical follow-up of study participants. All the authors reviewed the manuscript.

Disclosure

The authors declare no conflicts of interest.

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