TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

REVIEW

Immunosuppressive minimization with mTOR inhibitors and belatacept

Fritz Diekmann

Department of Nephrology and Kidney Transplantation, Hospital Clínic, Barcelona, Spain

Keywords
clinical immunosuppression, costimulation blockade, mTOR inhibition.

Correspondence
Fritz Diekmann MD, Department of Nephrology and Kidney Transplantation, Hospital Clínic, Villarroel, 170, 08036 Barcelona, Spain.
Tel.: +34 93 227 5400 ext. 4410;
fax: +34 93 227 5498;
e-mail: fdiekman@clinic.ub.es

Conflicts of interest
The author declares no conflict of interest.

Received: 2 November 2014
Revision requested: 17 December 2014
Accepted: 2 May 2015
Published online: 8 June 2015

doi:10.1111/tri.12603

Summary

Immunosuppressive therapy after kidney transplantation consists of a calcineurin inhibitor (CNI)-based therapy in combination with mycophenolic acid and steroids in most cases. In spite of low acute rejection rates and excellent graft survival, it is associated with major long-term complications, such as cardiovascular events, malignancy, and nephrotoxicity, and does not favor tolerogenic processes. Mammalian target of rapamycin (mTOR) inhibitors in combination with low-dose CNI offer good rejection rates and acceptable allograft function; however, *de novo* mTOR inhitibor-based treatment in combination with mycophenolate is not widely used due to higher acute rejection rates. Early conversion from a CNI to an mTOR inhibitor is a feasible option in selected patients with a slightly higher acute rejection rate, but equal or better GFR. Costimulation blockade has been proven to facilitate antirejection prophylaxis without CNI-associated side effects. So far, belatacept has been approved in combination with mycophenolate and steroids with better graft function, however, a slightly higher acute rejection rate. Recently, the combination of an mTOR inhibitor and belatacept with lymphocyte-depleting antibody induction and without maintenance steroids has been explored in two pilot studies with very low acute rejection rates, very good graft function, and an acceptable side effect profile.

Introduction

Current immunosuppressive medication after kidney transplantation

Calcineurin inhibitors (CNIs) are the mainstay of immunosuppression after kidney transplantation. They have been proven safe and effective for prevention of rejection. Moreover, in combination with induction therapy with nonlymphocyte-depleting antibodies or with lymphocyte-depleting antibodies, mycophenolate, and steroids, 1-year acute rejection rates below 15% and 1-year graft survival rates of more than 90% are the benchmark [1].

However, calcineurin inhibitors are associated with long-term adverse effects such as cardiovascular events and malignancies and thus may also contribute to increased morbidity and mortality. Moreover, CNIs contribute to renal and nonrenal toxicities that limit allograft function and contribute to late graft failure. According to Nankivell and co-workers, CNIs cause chronic nephrotoxicity and contribute to interstitial fibrosis and tubular atrophy and finally to slowly deteriorating graft function and ultimately graft failure [2]. On the other hand, antibody-mediated chronic allograft damage as a consequence of inadequate immunosuppression has been identified as an important cause of late kidney graft loss [3]. In addition, CNIs can contribute to hypertension, dyslipidemia, and new-onset diabetes [4–7]. Similarly, corticosteroids are associated with adverse metabolic and lipid effects of particular concern in a population already at increased risk for cardiovascular mortality, including weight gain and diabetes [8].

Worldwide most of the kidney transplant patients are initially treated with a calcineurin inhibitor (in North America and Europe preferentially tacrolimus), an antimetabolite (preferentially mycophenolate in the aforementioned regions), and steroids, in many cases with additional induction with basiliximab. These drug combinations yield

good short-term results with excellent 1-year patient and graft survival; however, long-term patient and graft survival has not convincingly improved over the last two decades, and severe patient morbidity caused by cardiovascular events and malignancies is the rule and not the exception.

Possible future immunosuppressive regimens

Therefore, according to the author's opinion, there is an urgent need for potent immunosuppressive regimens that favor long-term patient and graft survival and at the same time minimize the impact of the immunosuppressive medication on cardiovascular morbidity and post-transplant malignancy. So far, only calcineurin inhibitor-based regimens seem to be potent enough to maintain long-term rejection prophylaxis in routine practice. However, ideally, the immunosuppressive maintenance regimen should not contain CNIs or steroids due to their unfavorable side effect profile. Moreover, future immunosuppressive regimens should have some tolerogenic potential.

mTOR inhibitors

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase belonging to the family of phosphatidylinositol-3 kinase (PI3K)-related kinases. mTOR is a central regulator of cell metabolism and proliferation. Moreover, it is a target of the so-called mTOR inhibitors. Sirolimus and everolimus are mTOR inhibitors approved in kidney transplantation. The mTOR inhibitor rapamycin or sirolimus was originally developed as an antifungal drug. However, later its immunosuppressive properties were discovered and sirolimus and everolimus were developed for rejection prophylaxis in solid organ transplantation. In the 1980s, antitumor activity of the mTOR inhibitors was discovered leading to the development of the mentioned mTOR inhibitors as anticancer drugs [9].

The mTOR-I sirolimus (SRL) was first used in combination with cyclosporine A (CsA) with at the time remarkably low rejection rates, but a high incidence of side effects due to very high exposure to SRL and synergistic nephrotoxicity of SRL and CSA [10].

Belatacept

Belatacept is a costimulation blocker preventing T-lymphocyte activation. It may facilitate immunosuppressive regimens that reduce reliance on CNIs and corticosteroids. It is an immunoglobulin (Ig) fusion protein containing a modified form of the CD28 homolog cytotoxic T.lymphocyte-associated antigen 4 (CTLA4) and was developed from CTLA4-Ig and specifically selected to bind to CD80 and CD86 with higher avidity and slower dissociation than CTLA4-Ig [11]. Antigen-presenting cells (APCs) capture and display antigens to T lymphocytes. The response of antigen-specific T cells to protein antigen requires the participation of APCs.

Antigen peptide that is bound to APCs interacts with the T-cell receptor (TCR)/CD3. This interaction mediates activation of T cells. However, this interaction of APC–antigen peptide with TCR/CD3 is not sufficient to activate T cells. Another signal (Signal 2) is provided by interaction of costimulatory molecules with their ligands [12].

The CD28 receptor lowers the threshold for T-cell activation and leads to interleukin (IL)-2 mRNA stabilization and T-cell proliferation, and to the ligands CD80 and CD86 on APCs [13]. CD86 is expressed in APCs at a low level and is rapidly upregulated, whereas CD80 is inducible and expressed later in the response. However, whereas the immune response is going on, the expression of CD152 ((CTLA4) cytotoxic T.lymphocyte-associated antigen 4) is upregulated by T cells. CD152 also is a ligand for CD80 and CD86; however, its affinity is up to 20 times greater than that of CD28. Thus, the interaction between CD152 and CD80 and CD86 is as a negative costimulatory signal. CD152 can lead to T-cell anergy attenuating the immune response by competing with CD28 for ligation of CD80/CD86. This was the basis of the development of CTLA4-Ig consisting of the extracellular domain of CTLA4 bound to the Fc of IgG1. The CTLA4-Ig binds to CD80 and CD86 and inhibits their ligation by CD28 [14].

Belatacept and T regulatory cells

Tang and colleagues could show that the costimulation pathway of CD28/B7 was critical in the thymic development and peripheral homeostasis of T_{reg} [15–17]. Bestard and co-authors have observed that costimulation blockade and sirolimus may promote T_{reg} cell number or function [18], whereas *ex vivo* effects have suggested a more nuanced effect dependent on duration of exposure [19]. So far, it is not clear why the results of the different studies differ. However, differences between immunologically naive mice on the one hand and large animals on the other in the concomitant drugs and dosing regimens might in part be responsible for this. In a nonhuman primate renal allograft model, Lo *et al.* [20] observed a twofold decrease in the absolute number of peripheral $CD4^+$ $CD25^{hi}FoxP3^+T_{reg}$ cells during belatacept treatment. In their study, T_{reg} reemerged after discontinuation of belatacept. The authors speculated that the deprivation of CD28 costimulatory signaling was detrimental to peripheral T_{reg} survival; however, apparently, this phenomenon was not important enough to provoke rejection. Lo *et al.* also suggest that T_{reg} cells behave like effector memory T cells in terms of their sensitivity to immunosuppressive drugs.

Calcineurin inhibitor avoidance or minimization with mTOR inhibitors

Several trials elucidated the potential of mTOR-I to substitute CNI in the *de novo* setting [21]. However, in a large, international multicenter trial, the mTOR-I sirolimus in a CNI-avoiding regimen was associated with a high incidence of acute rejection and worse 1-year graft function compared with the tacrolimus and mycophenolate regimen [22]. Also, in another multicenter trial, early sirolimus-based immunosuppression did not yield convincing results in terms of prevention of acute rejection in comparison with a tacrolimus-based immunosuppression [1]. Therefore, mTOR inhibition as a non-nephrotoxic alternative to substitute CNI did not convince.

Peddi *et al.* [23] reviewed calcineurin inhibitor minimization therapy in combination with an mTOR inhibitor. They identified and evaluated 21 relevant studies and concluded that immunosuppressive regimens including an mTOR inhibitor and tacrolimus minimization better preserve renal function versus standard-dose tacrolimus, without significant changes in patient survival or graft rejection rates. Rates of infection and malignancies were low. Other adverse events were more commonly reported including dyslipidemia/hyperlipidemia in up to two-thirds of patients, new-onset diabetes mellitus in up to 38%, wound complications in up to 22%, and hypertension in up to 17%.

Budde and colleagues performed a trial of early conversion from cyclosporine A to everolimus 4.5 months after transplantation [24]. Of the 503 included patients, 300 (60%) were randomized to receive everolimus or continue standard cyclosporine-based treatment both in combination with mycophenolate. Everolimus was associated with a significant improvement in GFR (71.8 ml/min vs. 61.9 ml/min), respectively; mean difference 9.8 ml/min per 1.73 m^2, 95% CI -12.2 to -7.5). However, rates of biopsy-proven acute rejection were higher in the everolimus group after randomization (10% vs. 3%). Some of the patients participating in the ZEUS trial as well as the HERAKLES trial were evaluated in a separate analysis with the detection of a higher incidence of *de novo* donor-specific antibodies [25]. Higher mean lipid concentrations, slightly increased urinary protein excretion, and lower hemoglobin concentrations were observed with the everolimus regimen.

Lebranchu *et al.* [26] performed a similar study of conversion from cyclosporine-based treatment to SRL at 3 months post-transplantation in combination with mycophenolate and oral steroids, planned to be discontinued at month 8. A total of 192 of 237 patients were converted. Cockcroft-Gault clearance at 1 year was significantly better in the SRL group (68.9 vs. 64.4 ml/min). Patient and graft survival was not statistically different. The incidence of acute rejection episodes, mainly occurring after withdrawal of steroids, was numerically but not statistically higher in the SRL group (17% vs. 8%, $P = 0.071$). Sixteen patients discontinued SRL, mainly for adverse events ($n = 11$), and seven patients discontinued CsA for renal failure or acute rejection. Significantly, more patients in the SRL group reported aphthous, diarrhea, acne, and high triglyceride levels.

Weir and colleagues evaluated early calcineurin inhibitor withdrawal and introduction of SRL in combination with mycophenolate [27]. After 1 year, the mean percentage change from baseline in measured GFR was significantly higher in the Mycophenolate mofetil (MMF)/SRL group compared with the MMF/CNI group; however, at 2 years, the change was indistinguishable. Calculated creatinine clearance and GFR were significantly greater with MMF/SRL at 2 years. Biopsy-proven acute rejection occurred in 14 MMF/SRL-treated patients (three graft losses) and in 17 receiving MMF/CNI (six graft losses). No patients receiving MMF/SRL, but five treated with MMF/CNI, died.

In general, *de novo* treatment with an mTOR inhibitor and minimized CNI exposure is associated with equivalent GFR and acute rejection rates compared with CNI, and mycophenolate treatment, however, allows a reduced CNI exposure. Early conversion from a calcineurin inhibitor to an mTOR inhibitor is associated with better GFR short term and midterm. However, a slightly higher proteinuria and an increased risk of acute rejection were detected in some studies.

Belatacept in combination with mycophenolate as an alternative to Calcineurin inhibitor-based treatment

Belatacept-based treatment *de novo*

Results from belatacept studies in kidney transplantation suggest that belatacept-based regimens may provide effective immunosuppression. At the same time, belatacept-based immunosuppression can offer better allograft function and improved cardiovascular and metabolic risk profiles compared to cyclosporine-based regimens [28–30].

So far, Belatacept has been used mostly with mycophenolate and steroids, and induction with basiliximab. However, belatacept-based therapy was associated with higher grades of acute rejection in both phase III studies [29,30]. Moreover, an increased risk for post-transplant lymphoproliferative disease (PTLD) involving the central nervous system was detected in patients who were negative for Epstein–Barr virus and who were taking a more intensive regimen of belatacept.

In the phase III BENEFIT study, kidney allograft recipients were randomized to cyclosporine A or to a more or less intensive belatacept maintenance regimen [30]. In spite

of a higher acute rejection rate in belatacept patients (22% and 17% for the more- and less intensive regimens, respectively, compared with 7% for cyclosporine A), significantly fewer belatacept patients met the criteria for renal impairment (measured GFR <60 ml/min/1.73 m^2 at month 12, or a decrease in measured GFR <10 ml/min/1.73 m^2 from month 3 to month 12) at 12 months (54–55% vs. 78% with cyclosporine A; $p < 0.001$). Moreover, kidney function in terms of measured GFR at month 12 was significantly higher with belatacept (63–65 ml/min/1.73 m^2) than with cyclosporine A (50 ml/min/1.73 m^2; $P < 0.001$). Furthermore, the same effect was sustained to 36 months (65–66 ml/min/1.73 m^2 vs. 44 ml/min/1.73 m^2) [31]. Five-year follow-up data for belatacept in renal transplantation are also available from an open-label extension to a phase II study: GFR remained stable, and the incidences of death, graft loss, and acute rejection were low [32]. The most common adverse events occurring during the BENEFIT study were anemia, urinary tract infection, hypertension, constipation, diarrhea, nausea, and peripheral edema which did not differ between the treatment arms [30]. Five patients receiving belatacept (more intensive regimen, $n = 3$; less intensive regimen, $n = 2$) and one patient receiving cyclosporine A developed PTLD; two cases in the more intensive belatacept arm involved the central nervous system (CNS) [31]. Of these six patients, four presented pretransplant negative serology for Epstein–Barr virus and/or had received T-cell-depleting therapy [30].

The design of the BENEFIT-EXT study was similar to that of the BENEFIT study, involving exclusively recipients of ECD kidneys [29]. One-year acute rejection rate was 18% in both of the belatacept arms and 14% in the cyclosporine A arm. Graft survival was similar in all arms (belatacept more intensive 86%, less intensive 89%, CsA 85%). In addition, fewer belatacept patients (more intensive regimen, 71%; less intensive regimen, 77%) reached the composite renal impairment endpoint (measured GFR <60 ml/min/1.73 m^2 at month 12, or a decrease in measured GFR <10 ml/min/1.73 m^2 from month 3 to month 12) compared with CsA (85%; $P = 0.002$ vs. the more intensive belatacept regimen). Mean measured GFR was 4–7 ml/min/1.73 m^2 higher in belatacept patients. Furthermore belatacept was associated with a better overall cardiometabolic profile. At 3 years, survival with a functioning graft was 80–82% in each treatment arm, and calculated GFR was 10–11 ml/min/1.73 m^2 higher in belatacept patients [33]. The most frequent adverse events in the BENEFIT-EXT study were anemia, graft dysfunction, constipation, and diarrhea, with no difference between the three treatment arms [29]. Seven patients developed PTLD by 3 years ($n = 2$ in the more intensive, $n = 4$ in the less intensive belatacept arm, $n = 1$ in the CsA arm) [29]. Five of the PTLD cases involved the CNS, and three were known to have negative EBV serology before transplantation. One of the drawbacks of the BENEFIT and BENEFIT-EXT studies is the fact that the control arm is based on cyclosporine A and not on the currently used tacrolimus.

Belatacept has been approved for kidney transplantation in combination with mycophenolate and steroids, however, so far has not been adopted for routine use. The high cost and, thus, the reluctance of healthcare payers to reimburse might be an important issue.

Conversion to belatacept-based treatment

Rostaing and colleagues performed a phase II trial of conversion from CNI-based to belatacept-based treatment in stable renal transplant patients. A total of 84 (belatacept) were compared with 89 (CNI) patients. Six patients in the belatacept group presented an acute rejection episode versus none in the CNI group. One patient in the CNI group died with a functioning graft due to a myocardial infarction. No other graft losses occurred. At month 12, the mean change from baseline in cGFR was higher in the belatacept group. The mean cGFR values after 1 year were 60.5 ml/min in the belatacept group and 56.5 ml/min in the CNI group [34].

Grinyo *et al.* published the 2-year results of the same trial. Of the 173 originally randomized patients, 162 completed the 12-month study and entered the extension. One patient in each group lost his graft between years 1 and 2. After 2 years, mean cGFR was 62.0 ml/min (belatacept) vs. 55.4 ml/min (CNI). The mean change in cGFR from baseline was +8.8 ml/min (belatacept) and +0.3 ml/min (CNI). Higher cGFR was observed in patients switched from either cyclosporine (+7.8 ml/min) or tacrolimus (+8.9 ml/min). No differences in acute rejection rates between the groups were observed. All acute rejection episodes occurred during Year 1 in the belatacept patients and during Year 2 in the CNI group [35].

Costimulation blockade and mTOR inhibition in clinical trials

A combination of SRL and belatacept has been used in two clinical trials in kidney transplant recipients [36,37]. In the first trial, 89 *de novo* patients received belatacept either in combination with MMF ($n = 33$), or with SRL ($n = 26$), or received tacrolimus (TAC) and MMF ($n = 30$). All patients received antithymocyte globulin as induction treatment. The protocol included steroid treatment for the first 4 days and then abrupt steroid withdrawal. The patient population was comprised of low-to-moderate immunologic risk adult recipients of both living donor and standard criteria-deceased donor kidney transplant recipients who underwent induction with thymoglobulin. The primary endpoint

was acute rejection rate by month six. Secondary endpoints included graft loss, steroid-free status, and eGFR. Thus, the 6-month and 12-month results from this study for belatacept/MMF, belatacept/SRL, and TAC/MMF were as follows: 12-month AR rates were 15%, 4%, and 3%, respectively; the 6-month delayed graft function rates were 18%, 15%, and 7%, respectively; 12-month patient survival rates were 91%, 100%, and 100%, respectively; 12-month graft survival rates were 91%, 92%, and 100%, respectively; and 12-month steroid-free status rates were 73%, 77%, and 93%, respectively. Mean 12-month GFR and 6-month protein/creatinine ratios were 64, 62, and 54 ml/mn and 0.3/0.5/0.3 for belatacept/MMF, belatacept/SRL, and TAC/MMF groups, respectively. By 6 months, for belatacept/MMF, belatacept/SRL, and TAC/MMF groups, the corresponding figures for serious infection rate were 18%, 8%, and 13%, respectively; viral infection rates were 6%, 0%, and 10%, respectively; and malignancy rates were 0%, 4%, and 0%, respectively.

In the second clinical trial, Kirk and colleagues applied alemtuzumab induction, monthly belatacept in combination with daily sirolimus in 20 live donor kidney transplant recipients without maintenance steroids [37]. Patients were randomized 1:1 to receive unfractionated donor bone marrow. After 1 year, patients were allowed to wean from oral therapy. Surveillance biopsies were performed. Mean creatinine (estimated GFR) was 1.10 $\pm$ 0.07 mg/dl (89 $\pm$ 3.56 ml/min) and 1.13 $\pm$ 0.07 mg/dl (and 88 $\pm$ 3.48 ml/min) at 12 and 36 months, respectively. Bone marrow infusion did not have a clear effect. Of 10 patients who elected oral immunosuppressant weaning, seven remained rejection-free on belatacept monotherapy. Those failing to wean were successfully maintained on belatacept-based regimens supplemented by oral immunosuppression. Seven patients declined immunosuppressant weaning, three patients were denied weaning for associated medical conditions, and all remained rejection-free. The authors could show that the combination of belatacept and sirolimus was an effective rejection prophylaxis. Moreover, it was well tolerated. EBV viremia was detected in five patients and resolved spontaneously. CMV viremia occurred in one patient and was cleared after intensification of valganciclovir treatment. BK viremia was detected in 10 patients, however, remained without clinical manifestation and resolved with reduction of the sirolimus dose. In the study performed by Ferguson *et al.*, BK virus occurred in one patient in the Belatacept/SRL group and in one patient in the tacrolimus/MMF group.

Kirk and colleagues evaluated the repopulation of the lymphocyte subpopulations after depletion with alemtuzumab. CD4-positive T cells showed a repertoire that was not distinguishable from the pretransplant situation. CD8-positive cells enriched for naïve phenotype T cells expressing CD28. At the same time, there was a proportionate decrease in CD28-negative effector or memory T cells. B-cell repopulation was more rapid reaching and even exceeding baseline levels long term. They were predominantly naïve with a reduced number of B memory cells. They concluded that the final lymphocyte repertoire was characterized by more naïve T and B lymphocytes, fewer differentiated effector cells, and increased expression of CD28, which is targeted by belatacept.

In both trials, patients were treated with depleting antibody induction, either thymoglobulin or alemtuzumab. Both types of depleting antibodies seemed to be safe in combination with belatacept. Induction with basiliximab might be a disadvantage compared with thymoglobulin or alemtuzumab, because basiliximab might have a depleting effect on T regulatory cells [38]. On the other hand, this effect has not been demonstrated with thymoglobulin or with alemtuzumab. The additional immunosuppressive potency through induction with thymoglobulin or with alemtuzumab seems to further enable steroid-free immunosuppression in the belatacept and mTOR-I combination.

The reports on the above-mentioned trials only include a very limited number of patients. Furthermore, as these two trials were conducted recently, long-term data are missing. Therefore, the results need to be interpreted as short term to midterm results and need confirmation in further trials.

Conclusion

Costimulation blockade using belatacept in combination with mTOR inhibition permits potent CNI-free and steroid-free immunosuppression in kidney transplantation with excellent clinical results and an acceptable side effect profile in two pilot studies. This combination has the potential to achieve acute rejection prophylaxis comparable with the so far standard of care consisting of antibody induction, tacrolimus, mycophenolate, and steroids on the one hand and to be free of side effects typically associated with steroid and CNI treatment. Therefore, the association between belatacept and mTOR inhibition as long-term maintenance therapy in kidney transplant recipients deserves further study.

Funding

The authors have declared no funding.

References

1. Ekberg H, Tedesco-Silva H, Demirbas A, *et al.* Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.

2. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. *N Engl J Med* 2003; **349**: 2326.
3. Einecke G, Sis B, Reeve J, *et al.* Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant* 2009; **9**: 2520.
4. Vincenti F, Friman S, Scheuermann E, *et al.* Results of an international, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. *Am J Transplant* 2007; **7**: 1506.
5. Kasiske BL, Anjum S, Shah R, *et al.* Hypertension after kidney transplantation. *Am J Kidney Dis* 2004; **43**: 1071.
6. Hjelmesaeth J, Hartmann A, Kofstad J, Egeland T, Stenstrom J, Fauchald P. Tapering off prednisolone and cyclosporin the first year after renal transplantation: the effect on glucose tolerance. *Nephrol Dial Transplant* 2001; **16**: 829.
7. Hjelmesaeth J, Hartmann A, Midtvedt K, *et al.* Metabolic cardiovascular syndrome after renal transplantation. *Nephrol Dial Transplant* 2001; **16**: 1047.
8. Veenstra D, Best JH, Hornberger J, Sullivan SD, Hricik DE. Incidence and long-term cost of steroid-related side effects after renal transplantation. *Am J Kidney Dis* 1999; **33**: 829.
9. Strimpakos AS, Karapanagiotou EM, Saif MW, Syrigos KN. The role of mTOR in the management of solid tumors: an overview. *Cancer Treat Rev* 2009; **35**: 148.
10. Kahan BD. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. The Rapamune US Study Group. *Lancet* 2000; **356**: 194.
11. Larsen CP, Pearson TC, Adams AB, *et al.* Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. *Am J Transplant* 2005; **5**: 443.
12. Janeway CA, Bottomly K. Signals and signs for lymphocyte responses. *Cell* 1994; **76**: 275.
13. June CH, Bluestone JA, Nadler LM, Thompson CB. The B7 and CD28 receptor families. *Immunol Today* 1994; **15**: 321.
14. Walunas TL, Bakker CY, Bluestone JA. CTLA-4 ligation blocks CD28-dependent T cell activation. *J Exp Med* 1996; **183**: 2541.
15. Tang Q, Henriksen KJ, Boden EK, *et al.* Cutting edge: CD28 controls peripheral homeostasis of CD4 + CD25 + regulatory T cells. *J Immunol* 2003; **171**: 3348.
16. Salomon B, Lenschow DJ, Rhee L, *et al.* B7/CD28 costimulation is essential for the homeostasis of the CD4 + CD25 + immunoregulatory T cells that control autoimmune diabetes. *Immunity* 2000; **12**: 431.
17. Bour-Jordan H, Bluestone JA. Regulating the regulators: costimulatory signals control the homeostasis and function of regulatory T cells. *Immunol Rev* 2009; **229**: 41.
18. Bestard O, Cassis L, Cruzado JM, *et al.* Costimulatory blockade with mTor inhibition abrogates effector T-cell responses allowing regulatory T-cell survival in renal transplantation. *Transpl Int* 2011; **24**: 451.
19. Singh K, Kozyr N, Stempora L, *et al.* Regulatory T cells exhibit decreased proliferation but enhanced suppression after pulsing with sirolimus. *Am J Transplant* 2012; **12**: 1441.
20. Lo DJ, Anderson DJ, Weaver TA, *et al.* Belatacept and sirolimus prolong nonhuman primate renal allograft survival without a requirement for memory T cell depletion. *Am J Transplant* 2013; **13**: 320.
21. Flechner SM, Kurian SM, Solez K, *et al. De novo* kidney transplantation without use of calcineurin inhibitors preserves renal structure and function at two years. *Am J Transplant* 2004; **4**: 1776.
22. 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.
23. Peddi VR, Wiseman A, Chavin K, Slakey D. Review of combination therapy with mTOR inhibitors and tacrolimus minimization after transplantation. *Transplant Rev (Orlando)* 2013; **27**: 97.
24. Budde K, Becker T, Arns W, *et al.* Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. *Lancet* 2011; **377**: 837.
25. Liefeldt L, Brakemeier S, Glander P, *et al.* Donor-specific HLA antibodies in a cohort comparing everolimus with cyclosporine after kidney transplantation. *Am J Transplant* 2012; **12**: 1192.
26. Lebranchu Y, Thierry A, Toupance O, *et al.* Efficacy on renal function of early conversion from cyclosporine to sirolimus 3 months after renal transplantation: concept study. *Am J Transplant* 2009; **9**: 1115.
27. Weir MR, Mulgaonkar S, Chan L, *et al.* Mycophenolate mofetil-based immunosuppression with sirolimus in renal transplantation: a randomized, controlled Spare-the-Nephron trial. *Kidney Int* 2011; **79**: 897.
28. Vincenti F, Larsen C, Durrbach A, *et al.* Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 2005; **353**: 770.
29. Durrbach A, Pestana JM, Pearson T, *et al.* A phase III study of belatacept versus cyclosporine in kidney transplants from extended criteria donors (BENEFIT-EXT study). *Am J Transplant* 2010; **10**: 547.
30. 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; **10**: 535.
31. Vincenti F, Larsen CP, Alberu J, *et al.* Three-year outcomes from BENEFIT, a randomized, active-controlled, parallel-group study in adult kidney transplant recipients. *Am J Transplant* 2012; **12**: 210.
32. Vincenti F, Blancho G, Durrbach A, *et al.* Five-year safety and efficacy of belatacept in renal transplantation. *J Am Soc Nephrol* 2010; **21**: 1587.
33. Pestana JO, Grinyo JM, Vanrenterghem Y, *et al.* Three-year outcomes from BENEFIT-EXT: a phase III study of

belatacept versus cyclosporine in recipients of extended criteria donor kidneys. *Am J Transplant* 2012; **12**: 630.
34. Rostaing L, Massari P, Garcia VD, *et al.* Switching from calcineurin inhibitor-based regimens to a belatacept-based regimen in renal transplant recipients: a randomized phase II study. *Clin J Am Soc Nephrol* 2011; **6**: 430.
35. Grinyo J, Alberu J, Contieri FL, *et al.* Improvement in renal function in kidney transplant recipients switched from cyclosporine or tacrolimus to belatacept: 2-year results from the long-term extension of a phase II study. *Transpl Int* 2012; **25**: 1059.
36. Ferguson R, Grinyo J, Vincenti F, *et al.* Immunosuppression with belatacept-based, corticosteroid-avoiding regimens in *de novo* kidney transplant recipients. *Am J Transplant* 2011; **11**: 66.
37. Kirk AD, Guasch A, Xu H, *et al.* Renal transplantation using belatacept without maintenance steroids or calcineurin inhibitors. *Am J Transplant* 2014; **14**: 1142.
38. Bluestone JA, Liu W, Yabu JM, *et al.* The effect of costimulatory and interleukin 2 receptor blockade on regulatory T cells in renal transplantation. *Am J Transplant* 2008; **8**: 2086.