

Kidney transplantation: into the future with belatacept

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Allogenic organ transplantations are limited by drug-associated toxicity and the occurrence of antibody-mediated rejection or chronic rejection. The development of immunosuppressants that have minimal adverse and nephrotoxic effects is important to improve outcomes after organ transplantation. Several promising new compounds, based on our improved understanding of the molecular mechanisms of rejection, have been or are being developed to prevent acute and chronic transplant rejection. However, these new molecules need to be evaluated for their safety and to ensure they do not increase the risk of developing infections or tumors in transplant patients. Among them, belatacept (LEA29Y) is a new CD28 pathway-blocking reagent that has been developed as an alternative to calcineurin inhibitors. Belatacept is a recombinant and modified molecule (CTLA4–Ig) that interferes with the second activation signal of T lymphocytes, thereby causing a CD28–CD80/86 blockade. In this paper, we review the recently published results on belatacept-based regimens in renal transplant recipients.

Keywords: acute rejection • antibody-mediated rejection • belatacept • chronic rejection • co-signal blockers • immunosuppressant • kidney • renal function • renal transplantation

Over the last decade, 1-year graft survival after renal transplantation has greatly improved. However, long-term outcome is impaired by the occurrence of death due to cardiovascular events, the development of calcineurin inhibitor (CNI)-associated nephrotoxicity and/or the development of chronic antibody-mediated rejection. The development of immunosuppressants that can overcome these events are necessary.

In the 1970s, immunosuppressants were not nephrotoxic (i.e., azathioprine, steroids, antilymphocyte globulins). However, the 1980s saw the introduction of a new generation of cyclic molecules that had anticalcineurin activity: cyclosporine (CsA) and tacrolimus (TAC), which have strong inhibitory properties against T-cell activation but were known to be nephrotoxic. These drugs, used in combination with others immunosuppressive molecules, reduced acute allograft-rejection rates and markedly increased overall graft survival. However, they are associated with a similar rate of chronic dysfunction. Although they decrease immunological chronic allograft rejection, they are also associated with increased risk of nonimmunological renal injury development (intrinsic nephrotoxic effects, high blood pressure, dyslipidemia, new onset diabetes) both in kidney from both standard- and extended-criteria donors, which can lead to irreversible graft failure, and higher mortality and morbidity due to cardiac events. In addition, and because of the increasing use of extended-criteria kidneys that are more vulnerable to immune and nonimmune injury, components that do not induce the decline of the renal function would be optimal.

Therefore, new immunosuppressants are needed. Ideally, these should be free of nephrotoxic effects, able to act on T- and B-cell activation pathways, be able

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to interfere with the immunological components of chronic graft nephropathy, to prevent or treat antibody-mediated rejection, and have no adverse effects such as cardiovascular/infectious complications, and cancer occurrence. Ideally they have to be evaluated for their efficacy and ability to improve the long-term survival of organ grafted and transplanted patients. However, because long-term outcome is a multifactorial issue, earlier surrogate markers such as early improvements in renal function or reduction of CV risk factors that correlate with the long-term outcome have to be taken into account.

Second-signal activation of T cells

Second-signal activation has an important role in the activation of T cells. The second-signal family is composed of two subfamilies: CD28 and tumor necrosis-factor receptors [1,2]. These subfamilies induce positive and negative signals in activated T cells, in both naive and memory T cells.

The CD28 pathway has been extensively studied, and its blockade has been evaluated in human transplantation [3]. CD28 is a disulfide-bound molecule that belongs to the immunoglobulin superfamily, and is constitutively expressed on T cells. CD28 interacts with B7.1 (CD80) and B7.2 (CD86) molecules expressed on the surface of antigen-presenting cells and induces, concomitantly with T-cell activation receptor, a full T-cell activation [2]. CD28 shares 20% of its sequence identity with the inhibitory receptor CTLA4 (CD152). Both CTLA4 and CD28 bind to CD80⁺ and CD86⁺ molecules, although CTLA4 has a higher affinity for their receptors (CD80 and CD86) [4]. While interacting with CD80 and CD86, CD28 transmits a positive signal (signal 2) in T cells leading to a complex T-cell activation, whereas CTLA4 exerts a negative effect on T cells, leading to decreased signal transmission by T-cell receptors. The expressions of these two molecules are regulated separately, allowing tight control of the activation of T cells: CD28 is constitutively expressed on T cells and will allow a prompt activation of T cells, while CTLA expression is regulated and inducible by the activation of T cells, CTLA acting as a modulator of the activation of T cells by limiting the action of CD28 during the activation process.

Preclinical studies of second-signal inhibition

It has been proposed to dissociate the interaction of CD28 and its receptors by developing a recombinant molecule composed of the extracellular domain of CTLA4 fused with a portion of Fc domain of human IgG1 (CTLA4-immunoglobulin). This molecule CTLA4-Ig, so called Abatacept, has been

demonstrated to dissociate CD28 from CD80⁺ and CD86⁺ molecules and to impair T-cell activation. This effect can also modify the activation status of target cells.

In addition to its ability to block CD28-CD80/86 interaction, CTLA4-Ig induces tryptophan catabolism by stimulating indoleamine 2,3-dioxygenase in the target cell (antigen-presenting cell), which degrades tryptophan and, therefore, impairs T-cell proliferation and promotes extension of CD4⁺, CD25⁺, FOXP3⁺ and T regulatory (T_{REG}) cells in animals, but not in humans [5-7]. This might be explained *in vivo* by the need of T_{REG} cells (CD4⁺, CD25⁺, FOXP3⁺), which express CTLA4, to interact with B7 on antigen-presenting cells. Therefore, the use of a molecule that impairs the interaction between CTLA4 (expressed on T_{REG}) with CD80/86 will reduce the T_{REG} expansion. In allotransplantation, the inhibitory effect of abatacept needs to be re-enforced by the development of a molecule that has greater affinity for the receptors CD80/86, such as belatacept, which was obtained through the introduction of two point mutations in the extracellular domain of CTLA4. These mutations increase the avidity of the fusion molecule for its receptors without impairing its immunological properties. In addition, in human allogeneic transplantation conditions, the binding of belatacept favors the secretion of the regulatory molecule HLA G by antigen-presenting cells, which will participate in the immunoregulatory properties of belatacept [8].

Prolonged graft survival and donor-specific tolerance have been induced by CTLA4-Ig in human pancreatic islet cell xenografts in rats, in a heterotopic cardiac-allograft models in rats and mice, in a rat model of renal allograft, and in a skin-allograft mouse model [9-13]. In addition, CTLA4-Ig reduces the incidence of graft-versus-host disease and its related mortality induced by allogeneic bone marrow transplantation [13].

Regarding the co-stimulation blockade, adolescent rhesus monkeys [14] displayed longer periods of kidney-graft survival (up to 6 months) with humanized anti-CD80 and -CD86 antibodies than untreated monkeys [11,15]. However, all monkeys developed donor-specific antibodies and had renal infiltrates, which indicates that the CD28 blockade has to be used in combination with other immunosuppressive drugs. Preclinical data show that belatacept, combined with blocking anti-CD25 antibodies or mycophenolate acid therapy, is associated with significant graft survival in renal-transplant monkeys [14,16].

Initial development of belatacept in renal transplantation

Belatacept has been evaluated in a Phase II, partially blinded, multicenter clinical trial on human adults receiving a non-HLA-identical kidney from a living or deceased donor [3]. In this study, 218 adults were randomly assigned to receive a more intensive regimen of belatacept without CsA (more intensive [MI]: 10 mg/kg on days 1, 5, 15, 43, 57, 71, 85, 113, 141 and 169 followed by 5 mg/kg every 4 or 8 weeks), a less-intensive (LI) regimen of belatacept without CsA (LI: 10 mg/kg on days 1, 15, 29, 57 and 85, and then 5 mg/kg every 4 or 8 weeks), or CsA alone adapted according to trough levels (control). All patients received a basiliximab-based induction therapy, mycophenolate mofetil, and steroids. Very interestingly, glomerular filtration rate at 12 months was significantly better in patients who received belatacept compared with those just receiving CsA (66.3 and 62.1 ml/min/1.73 m² in patients receiving MI and LI belatacept, respectively, vs 53.5 ml/min/1.73 m² for CsA patients). The long-term extension of this study confirmed that the improved renal function for these patients was maintained during a longer follow up.

The incidence of acute rejection at 6 months was similar in all three groups (7% for the MI-belatacept group, 6% for the LI-belatacept therapy, and 8% for CsA-alone group). Subclinical rejection at 12 months (as assessed by a routine biopsy) tended to be more common in the LI group (20%) than in the MI (9%) or CsA (11%) groups but the incidence of chronic allograft nephropathy was lower in both belatacept groups (29% for the MI and 20% for LI groups) compared with the CsA control group (44%). Both patient and graft survival were similar in belatacept-treated patients and those receiving CsA and were over 98 and 95%, respectively at 1 year. The incidence of infections or cancers did not differ between groups despite a higher proportion of patients developing post-transplant lymphoma (PTLD) in the belatacept groups.

Two Phase III studies have been reported with living and standard donors (BENEFIT study; n = 666) or with donors with extended criteria (BENEFIT-EXT study; n = 543) [17,18], including patients with extended-criteria donor defined as donors ≥60 years old, or donors ≥50 years old who had at least two other risk factors (cerebrovascular accident, hypertension or serum creatinine >1.5 mg/dl) or an anticipated cold ischemia time of ≥24 h or donation after cardiac death [17]. These trials showed that, compared with CsA, belatacept was associated with improved renal function, a similar graft and patient survival and decreased cardiovascular-risk factors in recipients. The benefits, in terms of renal function, determined by cGFR, were observed very early after transplantation and were maintained during the 3-year

follow up [18]. In terms of gain of renal function, the strongest benefit was observed in the BENEFIT trial (standard criteria donors) with a mean increase of +25 ml/min at 3 years compared with CsA treated patients. The gain of function in the BENEFIT-EXT (extended criteria donor) was significant but less dramatic when compared with the CsA treatment (+11 ml/min). The increase in renal function for patients treated with belatacept compared with CsA in the BENEFIT-EXT study was observed mostly in the patients who received a kidney from a cardiac death donor or from a donor with cardiovascular risk factor. For the two groups that were treated with belatacept (LI and MI belatacept), in Phase III studies (BENEFIT and BENEFIT-EXT trials), there was less decline in renal function [18–20], reinforcing the fact that belatacept is not nephrotoxic. In addition, renal function continued to improve after 3 years in belatacept-treated patients in the BENEFIT study. Overall, the estimated half-life of grafts was increased by 2 years compared with patients receiving CsA in a risk prediction model [18]. Renal biopsies, realized at 1 year, showed that belatacept-treated patients had less chronic allograft nephropathies compared with CsA patients [18]. Additional studies are required to confirm this benefit compared with the current standard of care in low-risk populations. A trend towards more acute rejections, however, was seen in patients receiving belatacept compared with those receiving CsA, especially in the BENEFIT study in patients who received a high dosage of belatacept. Acute rejection occurred mostly during the initial 6 months post transplantation [18–21]. A higher incidence of grade II acute rejection was observed in belatacept-treated patients. However, the evolution was good despite a decrease in renal function that occurred (~10 ml/min) for patients exhibiting acute rejection independently of the treatment. However, interestingly, the incidence of donor-specific antibodies was lower in the groups treated with belatacept compared with CsA-treated groups. Cardiovascular and metabolic end points from these two Phase III studies were assessed at months 12, 24 and 36 [22]. A total of 1209 patients were randomized and received a transplant in these two studies. Mean systolic blood pressure was 6–9 mmHg lower, and mean diastolic blood pressure was 3–4 mmHg lower, in the MI and LI groups versus the CsA group ($p \leq 0.002$) across both studies, by month 12. Non-HDL cholesterol was lower in the belatacept groups versus the CsA group ($p < 0.01$; MI or LI vs CsA in each study). Serum triglycerides were lower in the belatacept groups versus the CsA group ($p < 0.02$; MI or LI vs CsA in each study). New-onset diabetes mellitus after transplantation tended to

occur less often in the belatacept groups versus the CsA group, in prespecified pooled analysis. Thus, it appears that, by month 12, the belatacept regimens were associated with better cardiovascular and metabolic risk profiles, with lower blood pressure and serum lipids, and less new-onset diabetes mellitus after transplantation, versus the CsA group.

The safety profiles for these studies on kidney transplant recipients has been reported [19]. Belatacept-based regimens were generally safe for a period of at least 4 years. However there was a greater risk of PTLD, specifically CNS PTLD, in the belatacept groups versus the CsA group, especially in Epstein-Barr virus (EBV) patients, and those receiving the MI dose. In EBV-positive patients at the date of transplantation, the incidence of PTLD was not different in the three groups even with a long-term follow-up period of analysis (4 years). Deaths and serious infections were lower in the LI regimen versus the MI and CsA regimens. Thus, the overall safety profile was better for the LI regimen over the MI, and therefore the LI regimen will be recommended.

Development of new combinations with belatacept-based therapies

Based on belatacept's mechanisms of action, several other drug combinations could be explored. The results of a 1 year, randomized, controlled, open-label, exploratory study, which assessed two belatacept-based steroid-avoiding regimens (compared with a TAC-based, steroid-avoiding regimen), have recently been reported. Recipients of living or deceased standard-criteria donor renal allografts were randomized to receive belatacept–mycophenolate mofetil (MMF), belatacept–sirolimus (SRL), or TAC–MMF. The belatacept administration was similar to that administered in the MI regimens of Phase III studies. All patients received an induction therapy of four doses of thymoglobulin (cumulative dose of 6 mg/kg maximum) and an associated short course of corticosteroids. Acute rejection occurred at a lower rate in the belatacept–SRL group (4%) and TAC–MMF groups (3%), compared with the belatacept–MMF group (12%) by month 6 [23]. Mean calculated glomerular filtration rate was 8–10 ml/min higher in both belatacept regimens compared with the TAC–MMF group. Thus, primary immunosuppression with belatacept may enable simultaneous avoidance of CNIs and corticosteroids in recipients of living or deceased standard-criteria donor kidneys, while providing acceptable rates of acute rejection and improved renal function relative to a TAC-based regimen. However, a relatively large number of patients receiving the SRL therapy

needed discontinuation of SRL due to adverse events. Further investigations are required to improve our understanding of these side effects.

After *de novo* use of belatacept, switch strategies were explored in stable renal-transplant patients treated with CNIs (BMS 103010) [21]. Both conversions were stepwise, from a CsA- or TAC-based regimen to a belatacept-based regimen, and were associated with a high rate of patient graft survival despite a slightly higher risk of rejection. These conversions may improve renal function in patients with stable graft function currently treated with CNIs, and avoid the harmful consequences of long-term exposure to CNIs. Benefits, in terms of renal function, were observed earlier after conversion from a CsA-based regimen compared with a TAC-based therapy, and were greater in nondiabetic patients; however, at 2 years a similar improvement of renal function was observed in patients treated with TAC or CsA, when they have been converted from a CNI-based regimen to belatacept.

Belatacept mostly impairs the activation of naive T cells. All studies of belatacept to date have been carried out in patients with a low risk of acute rejection. In patients with a high immunologic risk, the use of belatacept is not recommended at this time. In addition, strategies with belatacept must be defined in immunosuppression protocols using, for example, early phase using 'classical' treatments with CNI followed by a late phase treatments with belatacept, with or without CNI withdrawal.

Conclusion

The current aim in renal transplantation is to reduce or avoid drugs that have toxic renal effects and to lessen the risk of immunological chronic allograft rejection. New molecules are being developed to inhibit specific pathways activated during the allogenic response and antibody production. These compounds have been evaluated to complement current validated strategies. For naive patients who have been in contact with EBV (EBV-positive) before transplantation, a combination of belatacept with steroids and acid mycophenolic is efficient and well tolerated. The LI-belatacept regimen seems to also be preferable because of its better benefit/risk ratio compared with a MI regimen. However, further strategies need to be explored to test combinations with mTOR inhibitors, or sequential CNI regimens, for example. Some of these strategies could be dedicated to patients with a higher immunological risk. Belatacept also appears to be safe for conversion strategies, however may be more effective in patients who still have good renal function. However, long-term studies must be performed in order to prove that

longer term outcomes may be better as suggested by improved shorter term surrogate outcomes. Therefore, a new area of therapeutic evaluation has appeared with the use of belatacept.

Future perspective

CNIs in association with inosine monophosphate dehydrogenase improve the early outcome of organ transplantation and reduce the rate of acute rejection. However, CNIs are associated with renal toxicity and increase cardiovascular risk factors. For 20 years, research has focused on overcoming these side effects. Belatacept is a novel concept of recombinant protein developed to replace CNIs. It has been demonstrated

to prevent acute rejection, to improve renal function – which correlates with the long-term function of transplanted kidneys – and to reduce cardiovascular risk factors in this population. In addition, belatacept is associated with a reduction in the occurrence of anti-HLA antibodies, which are associated with acute or chronic antibody-mediated rejections when compared with CsA. This promising medicine is associated with an increasing number of acute rejection and a higher risk of developing PTLD in EBV-negative recipients. Therefore, other combinations of immunosuppressants have to be tested to improve the benefit of belatacept. Belatacept is a recombinant protein that has to be given by intravenous injections. Despite a

Executive summary

- Combination of belatacept/cellcept/steroid is an effective therapy for the prevention of acute rejection in nonsensitized kidney transplants.
- Belatacept in combination with cellcept and steroids is not recommended for Epstein-Barr virus-negative patients before transplantation.
- Belatacept-based regimens improve renal function both in standard-criteria donors and extended-criteria donors including those with cardiac death.
- Belatacept-based regimens decrease cardiovascular risk factors as compared with conventional therapies but long-term additional trials are needed to demonstrate their benefit on patients mortality and morbidity.
- Belatacept-based regimens have demonstrated their benefit on renal function in both *de novo* treated patients and after conversion from a calcineurin-based regimen.

very good tolerance and acceptance by patients, novel formulations have to be tested to simplify its administration for long-term administration in patients.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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References

- 1 Bromley SK, Burack WR, Johnson KG *et al*. The immunological synapse. *Annu. Rev. Immunol.* 19, 375 (2001).
- 2 Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu. Rev. Immunol.* 23, 515 (2005).
- 3 Vincenti F, Larsen C, Durrbach A *et al*.
- 4 Linsley PS, Greene JL, Brady W, Bajorath J, Ledbetter JA, Peach R. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. *Immunity* 1(9), 793 (1994).
- 5 Finger EB, Bluestone JA. When ligand becomes receptor – tolerance via B7 signaling on DCs. *Nat. Immunol.* 3(11), 1056 (2002).
- 6 Grohmann U, Orabona C, Fallarino F *et al*. CTLA-4-Ig regulates tryptophan catabolism *in vivo*. *Nat. Immunol.* 3(11), 1097 (2002).
- 7 Chavez H, Beaudreuil S, Abbed K *et al*. Absence of CD4CD25 regulatory T cell expansion in renal transplanted patients treated *in vivo* with Belatacept mediated CD28-CD80/86 blockade. *Transpl. Immunol.* 17(4), 243 (2007).
- 8 Bahri R, Naji A, Menier C *et al*. Dendritic cells secrete the immunosuppressive HLA-G molecule upon CTLA4-Ig treatment: implication in human renal transplant acceptance. *J. Immunol.* 183(11), 7054 (2009).
- 9 Akalin E, Chandraker A, Russell ME,
- 10 Turka LA, Hancock WW, Sayegh MH. CD28-B7 T cell costimulatory blockade by CTLA4Ig in the rat renal allograft model: inhibition of cell-mediated and humoral immune responses *in vivo*. *Transplantation* 62(12), 1942 (1996).
- 11 Larsen CP, Elwood ET, Alexander DZ *et al*. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature* 381(6581), 434 (1996).
- 12 Lenschow DJ, Zeng Y, Thistlethwaite JR *et al*. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig. *Science* 257(5071), 789 (1992).
- 13 Pearson TC, Alexander DZ, Winn KJ, Linsley PS, Lowry RP, Larsen CP. Transplantation tolerance induced by CTLA4-Ig. *Transplantation* 57(12), 1701 (1994).
- 14 Turka LA, Linsley PS, Lin H *et al*. T-cell activation by the CD28 ligand B7 is required for cardiac allograft rejection *in vivo*. *Proc. Natl Acad. Sci. USA* 89(22), 11102 (1992).
- 15 Kirk AD, Harlan DM, Armstrong NN *et al*. CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc. Natl Acad. Sci. USA* 94(16), 8789 (1997).

- Induction therapy with monoclonal antibodies specific for CD80 and CD86 delays the onset of acute renal allograft rejection in non-human primates. *Transplantation* 72(3), 377 (2001).
- 16 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.* 5(3), 443 (2005).
- 17 Durrbach A, Medina Pestana J, Pearson T *et al.* A Phase III Study of Belatacept vs Cyclosporine in Kidney Transplants from Extended Criteria Donors (BENEFIT-EXT Study). *Am. J. Transplant.* 10(3), 547 (2010).
- 18 Vincenti F, Charpentier B, Vanrenterghem Y *et al.* A Phase III study of – belatacept-based immunosuppression regimens vs cyclosporine in renal transplant recipients (BENEFIT Study). *Am. J. Transplant.* 10(3), 535 (2010).
- 19 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.* 12(3), 630 (2011).
- 20 Vincenti F, Blanco G, Durrbach A *et al.* Five-year safety and efficacy of belatacept in renal transplantation. *J. Am. Soc. Nephrol.* 21(9), 1587–1596 (2010).
- 21 Grinyo J, Charpentier B, Pestana JM *et al.* An integrated safety profile analysis of belatacept in kidney transplant recipients. *Transplantation* 90(12), 1521–1527 (2010).
- 22 Vanrenterghem Y, Bresnahan B, Campistol J *et al.* Belatacept-based regimens are associated with improved cardiovascular and metabolic risk factors compared with cyclosporine in kidney transplant recipients (BENEFIT and BENEFIT-EXT studies). *Transplantation* 91(9), 976–983 (2011).
- 23 Ferguson, R, Grinyo, J, Vincenti *et al.* Immunosuppression with belatacept-based, corticosteroid-avoiding regimens in *de novo* kidney transplant recipients. *Am. J. Transplant.* 11, 66–76 (2011).