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REVIEW

Belatacept: from rational design to clinical application

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Summary

Gradually improved immunosuppression has contributed significantly to the progress achieved in transplantation medicine so far. Nevertheless, current drug regimens are associated with late graft loss – in particular as a result of immunologic damage or drug toxicity – and substantial morbidity. Recently, the costimulation blocker belatacept (marketed under the name Nulojix®) has been approved for immunosuppression in renal transplantation. Belatacept (a mutated version of CTLA4Ig) is a fusion protein rationally designed to block CD28, a critical activating receptor on T cells, by binding and saturating its ligands B7-1 and B7-2. In phase II and III trials, belatacept was compared with cyclosporine (in combination with basiliximab, MMF, and steroids). Advantages observed with belatacept include superior graft function, preservation of renal structure and improved cardiovascular risk profile. Concerns associated with belatacept are a higher frequency of cellular rejection episodes and more post-transplant lymphoproliferative disorder (PTLD) cases especially in EBV seronegative patients, who should be excluded from belatacept-based regimens. Thus, after almost three decades of calcineurin inhibitors as mainstay of immunosuppression, belatacept offers a potential alternative. In this article, we will provide an overview of belatacept's preclinical development and will discuss the available evidence from clinical trials.

Preclinical development of belatacept

The concept of T cell costimulation

For a T cell to become fully activated, a signal through its T cell receptor (TCR) binding to antigen presented by MHC is insufficient [1]. Only if the T cell receives additional, so-called costimulatory signals, is it activated to a degree enabling effector functions [2]. It was noted that, if these costimulatory signals are interrupted experimentally, the T cells do not just fail to respond to their antigen, but that they are rendered anergic, entering a state of antigen-specific tolerance [3]. As T cells are indispensible for graft rejection [4], this *in vitro* phenomenon of T cell tolerance as a result of costimulation blockade quickly attracted the interest of transplant immunologists looking for better ways to modulate the alloresponse [5,6].

The CD28/B7 costimulation pathway

CD28, found in 1980 [7], was identified as the most important activating costimulation receptor of T cells [8–10]. CD28, a member of the immunoglobulin

superfamily, is constitutively expressed on the surface of T cells as disulfide-linked homodimeric glycoprotein and is further upregulated upon T cell activation [9]. While virtually all mouse T cells express CD28, a substantial fraction of human T cells is CD28 negative (11,12; implications for the efficacy of belatacept are discussed elsewhere in the article). CD28 signals are critical for T cell survival and proliferation. Apoptosis is prevented by upregulation of bcl-x_L, production of numerous cytokines is enhanced (including IL-1, IL-2, IL-4, IL-5, TNF, and IFN-γ) and proliferation and cell growth are induced (in particular through downregulation of $p27^{kip1}$) [11]. At least for naïve T cells, CD28 serves as 'master switch' of activation.

B7-1 (CD80) and B7-2 (CD86) [13–15] are ligands of CD28 and are mainly found on the surface of antigen presenting cells (APCs; B cells, dendritic cells, macrophages) [12]. B7-2 is expressed constitutively at low levels and is rapidly upregulated upon APC activation, whereas B7-1 is expressed only inducibly (later than B7-2). Notably, B7-1 and B7-2 are also expressed on activated T cells. While B7-1 and B7-2 share structural similarities and overlapping functions, differing expression patterns and binding properties lead to some distinct functions [16,17].

Subsequently, another T cell surface receptor that binds the same ligands as CD28 was identified and was termed cytotoxic T lymphocyte antigen-4 (CTLA-4; CD152) [18,19]. CTLA-4 is not expressed on resting T cells, but rather is rapidly upregulated upon T cell activation. CTLA-4 is a low-density receptor, with maximum protein expression estimated at approximately 2–3% of CD28 [20]. Notably, CTLA-4 binds to B7-1/2 with many-fold higher avidity than CD28 (approximately 2,500-fold avidity for B7-1 and 500-fold avidity for B7-2) [21].

Rational drug design of CTLA-4Ig and belatacept

Intuitively, the development of anti-CD28 monoclonal antibodies (mAb) would seem to be the most straightforward approach to blocking CD28. However, purely antagonistic/blocking mAbs are difficult to design, with most anti-CD28 mAbs displaying some intrinsic agonistic activity, i.e., they trigger a signal through CD28 [22,23]. Targeting B7-1 – which was the sole ligand of CD28 identified at the time since B7-2 was cloned only some years later [15] – with mAbs was only partially effective [24]. Moreover, the fusion protein of CD28-immunoglobulin (Ig; binding to B7-1/2) also turned out to be quite ineffective in preventing B7 binding to CD28 [22,25]. Therefore, another strategy was chosen. The extracellular portion of (human) CTLA-4 – which was known at the time to bind the same ligands as CD28, but with higher affinity – was fused to the Fc portion of human IgG1 [18]. Importantly, the Fc portion was intentionally mutated in a manner so that it lost its complement binding and (virtually all) Fc receptor-binding capabilities and thus no longer mediates complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity [18,25,26]. Thereby, a soluble recombinant fusion protein – CTLA-4Ig (abatacept) – was generated with a molecular weight of ≈100 kDa (notably, CTLA-4Ig behaves as dimer *in vitro*) [18]. Subsequently, upon the identification of B7-2 the combined use of anti-B7-1 and anti-B7-2 mAbs was explored and found to be effective as short-term induction therapy [27,28]. However, the necessity of administering two instead of one biologic in addition to the challenge of achieving sufficient affinity are drawbacks of this approach.

CTLA-4Ig is approximately 100 times less potent at inhibiting B7-2 mediated T cell responses [29]. Therefore, a modified version of CTLA-4Ig – belatacept (formerly known as LEA29Y) – with increased B7-2 binding avidity was developed through mutagenesis [30]. Belatacept differs from CTLA-4Ig by two point mutations in the high avidity binding region of CTLA-4 (Fig. 1). Thereby, avidity for B7-2 increased approximately fourfold and for B7-1 approximately twofold, resulting in a approximately 10-fold higher overall *in vitro* potency [30].

Mechanism(s) of action of belatacept

In recent years, it has been recognized that T cell costimulation is substantially more complex than initially anticipated [31]. Numerous pathways have been identified that transmit either activating or inhibiting signals to T cells or APCs. These pathways are in part redundant, but

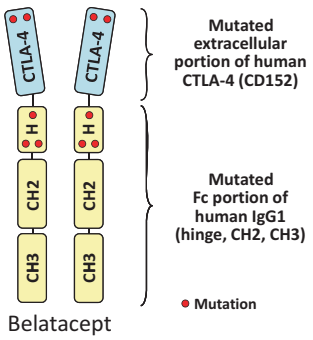

Figure 1 Structure of belatacept. CTLA-4Ig (abatacept), the parent compound of belatacept, was generated by fusing the extracellular portion of human CTLA-4 (CD152) to the Fc portion of human IgG1. The Fc portion used was intentionally mutated at three sites (cysteine to serine substitutions) to eliminate effector functions of the Fc part. Belatacept was generated by inserting two mutations in the CTLA-4 part of abatacept which increased its avidity to B7-1 and B7-2. Mutations are symbolically depicted as red dots.

also have distinct roles in distinct types of immune responses. The understanding of the CD 28/B7 costimulation pathway has also evolved dramatically. It involves more receptors and ligands and more functions than originally thought.

Belatacept was rationally designed as CD28 blocker. By binding to B7-1 and B7-2 with higher affinity than CD28, belatacept prevents CD28 to be triggered by its physiological ligands (Fig. 2a and b). The TCR signal is not directly affected by belatacept. In renal transplant patients, it has been confirmed that belatacept is binding to B7-2 with receptor saturation estimated at approximately 80% at the time of trough levels and 94% at the time of peak exposure (measured during maintenance therapy with infusions every 4 weeks) [32].

At the time when CTLA-4Ig was designed, the physiological function of CTLA-4 was unknown. It soon became clear, however, that CTLA-4 is an inhibitory surface

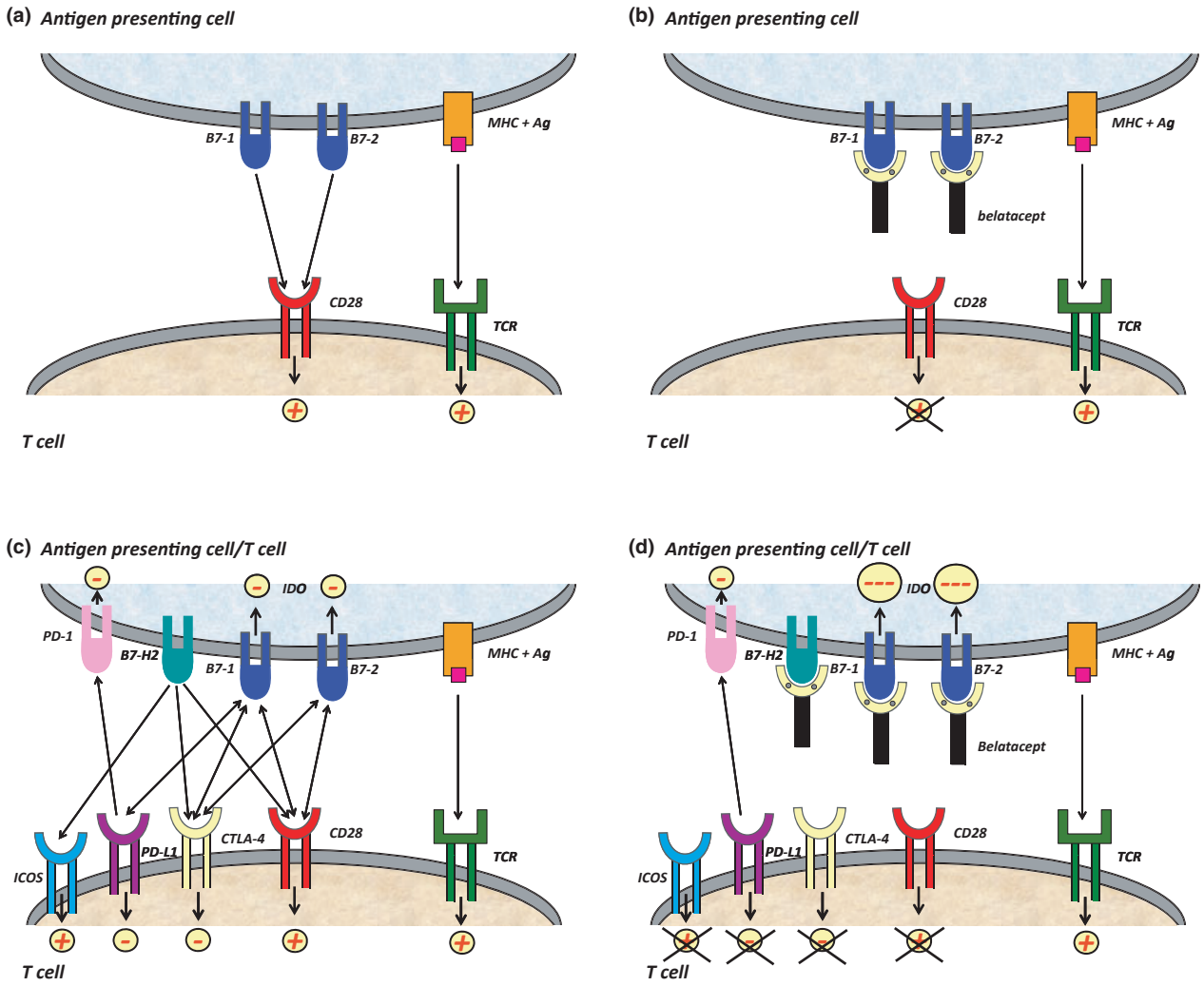

Figure 2 Mechanism(s) of action of belatacept. (a) CD28 is a cell surface receptor which transmits the arguably most important activating costimulation signal to T cells when it is triggered by binding to one of its ligands B7-1 or B7-2 expressed on antigen presenting cells. (b) By binding to B7-1 and B7-2 and saturating these ligands, belatacept precludes CD28 from engaging B7-1/2 and thus indirectly blocks CD28. The signal that the T cell receptor receives from antigen presented by MHC is left intact by belatacept. (c) Recent evidence has revealed that the CD28/B7 pathway is considerably more complex than originally thought, with several additional ligands and receptors having been identified. CTLA-4 (CD152) is an inhibitory receptor on T cells, which shares the same ligands with CD28. In addition to PD-1, PD-L1 also interacts with B7-1 transmitting a bidirectional inhibitory signal. B7-H2 – which was known to bind to ICOS – has recently been proposed as a third ligand for both CD28 and CTLA-4. B7-1 and B7-2 act not only as ligands but also transmit a signal, which is linked to upregulation of indoleamine-2,3-dioxygenase (IDO) in the APC. (d) In view of the expanded understanding of the CD28/B7 pathway, belatacept might have additional mechanisms of action by interfering with several signals when binding to B7-1, B7-2, and B7-H2. These putative mechanisms of action include interference with CTLA-4, PD-L1, and ICOS signals. ⊕denotes a positive/activating signal, ⊖denotes a negative/inhibitory signal, crossed out signals denote mechanisms of action of belatacept.

receptor [20,33,34]. Upon upregulation in activated T cells, CTLA-4 has a major role in downregulating the immune response, counter-acting many of the actions of CD28 [35]. Experimentally, lack of CTLA-4 function leads to break-down of self-tolerance [36,37]. The immunostimulatory effect of blocking the CTLA-4 signal is exploited therapeutically with anti-CTLA-4 monoclonal antibodies [35], one of which (ipilimumab) has recently been approved for the treatment of melanoma [38]. The precise mechanism(s) how CTLA-4 mediates its inhibitory function is still a matter of debate, with evidence for both cell-extrinsic and -intrinsic mechanisms having been published. CTLA-4 is important for the function of regulatory T cells (Tregs) on which it is constitutively expressed [39] and is involved in inducing a specific form of anergy [40,41]. Recently, it has been proposed that CTLA-4's main function is to remove B7-1/2 from the cell surface of APCs through trans-endocytosis [42]. Consequently, APCs cannot stimulate CD28 anymore.

As belatacept binds to ligands (i.e., B7-1 and B7-2) that are shared by both CD28 and CTLA-4 it can be assumed that it blocks the inhibitory signal of CTLA-4 to some – undetermined – degree as well. Though direct proof is lacking in the clinical setting, the nonlinear dose-response effects observed with belatacept in renal transplantation might be an indication that belatacept at higher doses inhibits inhibitory signals. Because of its higher affinity, CTLA-4 has a competitive advantage over CD28 in ligating unoccupied B7-1/2. At high belatacept doses, saturation of B7-1/2 might reach a level that leaves insufficient numbers of B7-1/2 molecules available for CTLA-4. This unintended effect of belatacept on the CTLA-4 signal is the major driving force behind efforts to develop biologicals achieving selective inhibition of CD28 (without interfering with other signals) [23,43,44,45].

A few years ago, programed cell death ligand 1 (PD-L1) was identified as another ligand of B7-1 (but not B7-2), mediating a (possibly bidirectional) inhibitory T cell – T cell interaction [46,47]. Thus, belatacept might block a second inhibitory signal by binding to B7-1. Recently, the existence of a third ligand for CD28 and CTLA-4 was proposed [48], further increasing the complexities of the CD28/B7 pathway. B7-H2 (also known as ICOSL) provides an activating signal to T cells via CD28 [48]. As binding of CTLA4Ig to B7-H2 was demonstrated [48], it appears likely that belatacept also binds to this target. Finally, the notion that B7-1 and B7-2 serve merely as ligands has also been challenged. Several *in vitro* studies conclude that B7-1 and B7-2 transmit a signal to the APC upon ligation, leading to the upregulation of indoleamine-2,3-dioxygenase (IDO), a tryptophan-catabolizing, immunomodulatory enzyme [49,50]. Of note, several 'self-made' CTLA-4Ig constructs have been generated for experimental research whose properties are distinct from abatacept, as in particular their Fc portions differ from the mutated human IgG1 of abatacept [49,51]. Results obtained might thus vary depending on which version of CTLA-4Ig is administered and thus should be interpreted with appropriate caution. Current evidence suggests, however, that IDO induction is not a critical mechanism of action of belatacept in the clinical setting [52].

Over the last decade, the crucial role of Tregs in modulating alloreactivity has been increasingly appreciated [53]. Originally designed to inhibit effector T cells, it now became of interest whether CTLA-4Ig/belatacept influence Tregs as well. In mice, CD28 signals are important for the development and homeostasis of Tregs [54,55], and administration of CTLA-4Ig leads to a reversible decline in the frequency of Tregs [54,56]. Moreover, CTLA-4 (CD152) – which is unintentionally also blocked by CTLA-4Ig/belatacept – has a critical role in Treg function [39,57]. Three studies have looked at the fate of Tregs in renal transplant recipients treated with belatacept [58–60]. Firm conclusions are complicated by the fact that all patients received combination therapies, which by themselves might affect Tregs and that only blood and biopsy samples were accessible for analysis. The two studies evaluating patients receiving basiliximab as induction therapy (and MMF) did not observe any striking effects of belatacept on Tregs in comparison to cyclosporine (CsA) [58,59]. Notably though, belatacept patients treated with thymoglobulin induction and sirolimus had a favorable Treg/memory T cell profile [60] (discussed in detail elsewhere in the article).

Thus, belatacept interferes with a complex pathway that regulates T cell activation and T cell downregulation (Fig. 2c and d). While designed to selectively inhibit CD28, it affects other signals with both activating and inhibitory functions as well. Whether and if so, how these additional mechanisms of action affect the alloresponse in transplant recipients, remains to be elucidated.

Efficacy of CTLA-4Ig (abatacept) and belatacept in experimental models of organ transplantation

CTLA-4Ig potently prolongs heart, kidney and islet graft survival in rodent models [51,61–64]. On its own, it does not prolong skin graft survival [65]; however, and leads to permanent heart graft survival in stringent strain combinations only when combined with donor-specific transfusion or anti-CD40L mAb [61,65]. Intriguingly, CLTA-4Ig is more effective if treatment is started 2 days after transplantation than when it is started at the day of transplantation [63]. The mechanism underlying this phenomenon has not been established, but the delayed administration might allow time for CTLA-4 to be upregulated and engaged on alloreactive T cells before its signal

is blocked by CTLA-4Ig [66]. CTLA-4Ig also suppresses T cell-dependent antibody responses in mice [64] and humans [67]. With regard to the mechanisms of action of CTLA-4Ig, anergy, regulation and clonal deletion have all been observed in various rodent models [66]. Recently, memory T cells have been recognized as a critical barrier to both graft acceptance achieved with immunosuppression and (experimental) tolerance induction. Importantly, alloreactive memory T cells are generated not only through direct contact to foreign HLA molecules, but also through exposure to pathogens (in particular viral antigens). The ensuing 'heterologous immunity' is a common source of donor-reactive T memory cells in experimental (tolerance) models and in the clinical setting [68–70]. Importantly, memory T cells are less dependent on CD28 and thus less susceptible to CTLA-4Ig treatment [71]. This is, however, not a black-and-white phenomenon, as important modulating effects of CTLA-4Ig on memory T cell responses have been observed [72–76]. While abatacept (human CTLA-4 fused with mutated human IgG1) is known to bind murine B7-1/2 effectively [64,77], belatacept does not, and is therefore not used in rodents.

Costimulation blockade with CTLA-4Ig, together with anti-CD40L mAb, is particularly potent in experimental bone marrow transplantation models inducing mixed chimerism and tolerance [78]. It promotes engraftment of fully allogeneic bone marrow even in the presence of an intact recipient T cell repertoire [79,80], allowing development of the mildest conditioning regimens reported so far [81–85]. Translation of these mixed chimerism protocols to the nonhuman primate setting has been difficult, however, with achieved efficacy being substantially lower than in rodents [86,87].

Encouraged by its potency in rodents, CTLA-4Ig was tested as immunosuppressant in nonhuman primates. Overall, results achieved in renal and islet transplantation were disappointing [30,88,89]. Given its efficacy in attenuating autoimmunity [90], CTLA-4Ig (abatacept, Orencia®), has since been successfully developed and approved for the treatment of rheumatoid arthritis [91]. The relative lack of potency of CTLA-4Ig monotherapy in inhibiting alloresponses in nonhuman primates appears related to the presence of $CD28^{neg}$ T cells. Unlike the case in mice, a substantial fraction of nonhuman primate and human T cells does not express CD28 and thus is not targeted by CTLA-4Ig [11,12]. In particular, CD8 T cells progressively lose CD28 expression upon activation. Recently, alefacept (LFA-3Ig, Amevive®) was found to effectively target those $CD28^{neg}$ effector/memory CD8 T cells as they upregulate CD2 (alefacept is a fusion protein binding to CD2) [92,93].

To increase potency to a level sufficient for the transplant setting, belatacept, a second generation CTLA-4Ig with increased affinity was developed. In their seminal nonhuman primate study, Larsen and colleagues tested belatacept as monotherapy and in combination with either basiliximab or MMF and steroids [30]. Belatacept monotherapy was superior to CTLA-4Ig monotherapy in maintaining renal allograft survival, demonstrating that improved binding affinity translated into augmented immunosuppressive activity. However, renal function declined during ongoing belatacept treatment, indicating that adjunctive therapies are warranted for clinical translation. Previous nonhuman primate studies found that conventional immunosuppressants can abrogate the therapeutic effect of another costimulation blocker, anti-CD40L mAb [94]. Therefore, it was important that the combination of belatacept with basiliximab induction and its combination with MMF and steroids was shown to be not only safe but also that it indeed led to increased efficacy [30]. The results achieved with these drug combinations directly led to the design of the phase II trial of belatacept in renal transplantation.

Moreover, belatacept – together with blockade of the CD40 pathway, sirolimus and basiliximab induction – also markedly prolongs the survival of allogeneic and xenogeneic islet grafts in nonhuman primates [95–97]. These promising results rejuvenated enthusiasm in clinical islet transplantation and led to the initiation of the first trials with belatacept for this indication (NCT00468403, NCT00501709).

Clinical development of belatacept

Belatacept phase II trial in *de novo* kidney transplant recipients

Because of the promising results on the use of belatacept in preclinical models of renal transplantation in nonhuman primates, this agent entered in a phase II, multicentre study. A therapy with CsA, mycophenolate mofetil (MMF), steroids plus basiliximab was compared with belatacept in conjunction with MMF, steroids, and basiliximab. Belatacept was given intravenously in two distinct dosage regimens, the so-called more intensive (MI) as a result of the frequency of administration early after transplantation of 10 mg/kg and its prolongation to 6 months, and the less intensive regimen (LI) with a reduced frequency and shorter duration of the high dose. Both belatacept regimens included an early phase (10 mg of belatacept per kilogram of body weight) and a late phase (5 mg of belatacept per kilogram at 4- or 8-week intervals). Of note, no serious infusion reactions were observed in any of the phase II and III trials. Two hundred and eighteen patients were enrolled to belatacept MI or LI or CsA [98]. The primary endpoint was noninferiority in the acute rejection (AR) rate at 6 months.

Secondary endpoints included measured glomerular filtration rate (mGFR) at 1, 6, and 12 months, calculated GFR (cGFR), and incidence of chronic allograft nephropathy (CAN) in 1-year protocol biopsies. At 12 months, the AR rates were similar between groups (7% MI, 6% LI, and 8% for CsA), and mGFR was significantly higher in patients receiving belatacept MI and LI versus CsA (66.3, 62.1, and 53.5 ml/min per 1.73 m2, respectively; $P = 0.01$) [98]. Protocol biopsies at 1 year showed a lower incidence of CAN with belatacept LI or MI compared with CsA (29%, 20%, and 40%, respectively). The efficacy of belatacept at 1 year was supported at 5-year follow-up [99]. Seventy-eight patients receiving belatacept and 16 patients receiving CsA completed a 5-year trial extension. GFR remained stable in patients who were receiving belatacept for 5 years, and the incidences of death/graft loss or AR were low. The frequencies of serious infections were 16% for belatacept and 27% for CsA, and neoplasms occurred in 12% of each group. No patients who were treated with belatacept and one patient who was treated with CsA developed post-transplant lymphoproliferative disorder (PTLD) beyond 12 months during the 5-year extension period. A pharmacokinetic analysis performed in a subset of patients enrolled in the trial extension revealed a mean serum half-life of belatacept of 8 days [99].

Belatacept phase III pivotal trials

The encouraging results of phase II trial on the amelioration of renal function and less chronic allograft damage were the basis for two pivotal trials. Because no clear conclusions could be made of the advantages of two belatacept regimens explored in the phase II trial, MI and LI, these regimens were employed again in the phase III pivotal trials. These studies were conducted in kidney transplant recipients of organs from conventional donors (BENEFIT) [100] and extended criteria donors (BENEFIT-EXT) [101]. The primary objective of the BENEFIT study assessed each belatacept-based regimen compared with the CsA-based regimen on three coprimary outcomes at 12 months: (i) composite patient and graft survival, (ii) composite renal impairment endpoint, and (iii) incidence of AR. The primary objectives of the BENEFIT-EXT study were to assess each belatacept-based regimen compared with the CsA-based regimen on two primary outcomes at 12 months: (i) composite patient and graft survival, and (ii) composite renal impairment.

In the BENEFIT study, at month 12, both belatacept regimens had similar patient/graft survival versus CsA (MI: 95%, LI: 97%, and CsA: 93%), and were associated with superior renal function as measured by the composite renal impairment endpoint (MI: 55%; LI: 54% and CsA: 78%, $P < 0.001$ MI or LI versus CsA) and by the GFR (65, 63, and 50 ml/min for MI, LI and CsA; $P < 0.001$ MI or LI versus CsA). Belatacept patients experienced a higher incidence (MI: 22%, LI: 17%, and CsA: 7%) and grade of AR episodes, and only the LI arm met the predefined 20% noninferiority margin for AR versus CsA. The high incidence of rejection seen with belatacept was unexpected (and had not been observed in the phase II trial [98]). These results raise the question whether basiliximab is similarly effective when combined with belatacept (as opposed to CNIs). Basiliximab might negatively interfere with Treg function, which hypothetically might be more important under belatacept – although such a relationship remains highly speculative at the present time and no evidence pointing to an 'antagonistic' effect of basiliximab was seen in nonhuman primate studies [30]. Cellular rejection under belatacept might be mediated by T memory cells, which are relatively costimulation blockade-resistant and in particular $CD28^{neg}$ T cells that are not targeted by belatacept at all. Combining belatacept with agents that affect these T cell subsets (e.g., alefacept) holds potential of improving belatacept-based immunosuppressive regimens [92]. Moreover, a numerically higher incidence of rejection seen with a higher dose of an immunosuppressant (MI versus LI) is surprising. Potentially, belatacept has a more pronounced effect on negative/downmodulating pathways (i.e. CTLA-4, PD-L1 and Treg function) when given at higher doses. At any rate, these data point to a nonlinear dose-response relationship of belatacept, which was also seen in early studies with CTLA4Ig/abatacept [67].

In the BENEFIT-EXT study, patient/graft survival with belatacept was similar to CsA (86% MI, 89% LI, and 85% CsA) at 12 months. Fewer belatacept patients reached the composite renal impairment endpoint versus CsA (71% MI, 77% LI, and 85% CsA; $P = 0.002$ MI versus CsA; $P = 0.06$ LI versus CsA). The incidence of AR was similar between groups and both belatacept arms met the 20% noninferiority margin versus CsA. The mean measured GFR was 4–7 ml/min higher on belatacept versus CsA ($P = 0.008$ MI versus CsA; $P = 0.1039$ LI versus CsA; rejection rates and graft function of the phase II and III trials are summarized in Table 1). The 1-year efficacy results of BENEFIT and BENEFIT-EXT were supported for up to 3 years of treatment [102–104]. AR episodes beyond 1 year after transplantation were scarce, and not associated with the development of anti-human leukocyte antigen (HLA) antibodies. Despite higher rates or grades of AR episodes in BENEFIT, few belatacept patients experienced graft loss as a result of AR, with little impact of individual AR occurrences on overall patient/graft survival [102]. In addition, belatacept was associated with superior renal function and reduced CAN, despite a higher rate

Table 1. Overview of rejection rates and graft function observed with belatacept in trials of *de novo* kidney transplant recipients.

	bela MI	bela LI	CyA	References
Phase II				
Rejection (%)	7	6	8	[98]
GFR (ml/min/1.73 m^2)	66.3*	62.1*	53.5	
Phase III BENEFIT				
Rejection (%)	22**	17	7	[100]
GFR (ml/min/1.73 m^2)	65.0*	63.4*	50.4	
Phase III BENEFIT-EXT				
Rejection (%)	18	18	14	[101]
GFR (ml/min/1.73 m^2)	52.1*	49.5	45.2	

Basiliximab induction, MMF and steroids were part of the treatment regimens of all groups. For details of belatacept dosing please refer to the respective references. Frequencies of clinically-suspected, biopsy-proven rejection episodes at 12 months as defined in the respective study protocols are presented. Mean measured GFR is shown at 12 months. LI, denotes less intensive; MI, more intensive; bela, belatacept; CyA, cyclosporine A; GFR, glomerular filtration rate. *$P < 0.05$; **does not meet noninferiority criteria.

of early AR [100]. The prevalence of CAN in protocol biopsies was 18%, 24% and 32% in the MI, LI and CsA groups, respectively ($P = 0.001$) in the BENEFIT study, and 45%, 46% and 52% in the corresponding groups, respectively ($P = 0.22$) in the BENEFIT-EXT. The differences between the two studies concerning the prevalence of CAN probably reflect the higher incidence of pre-existing lesions in kidneys from extended criteria donors in the BENEFIT-EXT study. The renal function in belatacept-treated patients evaluated by estimated GFR showed a positive slope through year 3 in contrast with a negative slope in patients receiving CsA in the BENEFIT study. In the BENEFIT-EXT, the three therapeutic arms displayed a negative slope through year 3, but attenuated in belatacept patients in comparison with those under CsA. The better preservation of renal function and parenchyma might have a positive impact on long-term graft survival, which should be assessed in extended follow-up. Whether the renal benefits of belatacept observed in these pivotal trials are as a result of its immunomodulatory properties or the mere avoidance of CNI-related nephrotoxicity is a question that remains open.

Another potential benefit of avoiding the use of CNI immunosuppressants in maintenance immunosuppression with belatacept might be the amelioration of cardiovascular risk profile of renal transplant recipients. In a pooled analysis of BENEFIT and BENEFIT-EXT studies including 1209 patients, cardiovascular and metabolic endpoints were assessed at 12 months after transplantation [105]. Across both studies, fewer patients in the belatacept regimens used three or more antihypertensive medications. In BENEFIT, 29% (MI), 26% (LI), and 35% (CsA) of patients used three or more antihypertensive medications. Both the belatacept MI and LI regimens were associated with a 30% reduction in the odds for requiring a higher number of antihypertensive medications at month 12 ($P = 0.02$ LI versus CsA). In BENEFIT-EXT, 43% (MI), 39% (LI), and 52% (CsA) of patients used three or more antihypertensive medications. The belatacept regimens were associated with a 30% (MI) and 40% (LI) reduction in the odds for requiring a higher number of antihypertensive medications at month 12 ($P = 0.011$; LI versus CsA) and 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 CsA ($P \leq 0.002$). The lipidic profile was also better under belatacept. Non-HDL cholesterol was lower in the belatacept groups versus CsA ($P < 0.01$ MI or LI versus CsA in each study) and serum triglycerides were lower in the belatacept groups versus CsA ($P < 0.02$ MI or LI versus CsA in each study). New onset diabetes after transplantation (NODAT) occurred less often in the belatacept groups versus CsA in a prespecified pooled analysis ($P < 0.05$ MI or LI versus CsA). These 1-year data showing improvement in cardiovascular risk profile with a better control of blood pressure, lower increments in atherogenic lipids and less NODAT may reduce the poly-pharmacy usually given to renal transplant patients and might potentially reduce cardiovascular morbidity/mortality in the long-term.

The use for the first time of a costimulatory blocker in induction and maintenance immunusuppression may raise concerns about the safety of this immunosuppressive strategy. The safety profile of belatacept-based immunosuppression was addressed in a pooled safety analysis including patients from the two pivotal trials and those patients recruited in the phase II trial [106]. This analysis included 1425 patients (MI: 477, LI: 472, and CsA: 476) with a median follow-up of approximately 2.4 years. The conclusions of this analysis were that belatacept was generally well tolerated and that the frequency of deaths (MI: 7%, LI: 5%, and CsA: 7%) and serious infections (MI: 37%, LI: 32%, and CsA: 36%) were lower in the LI group versus CsA. The frequency of malignancies was 10%, 6%, and 7% in the MI, LI, and CsA groups, respectively, but more PTLD was observed in belatacept groups. Sixteen cases of PTLD occurred ($n = 8$ MI, $n = 6$ LI, and $n = 2$ CsA), including nine cases involving the central nervous system (CNS; $n = 6$ MI, and $n = 3$ LI). The risk of PTLD was highest in Epstein-Barr virus negative recipients and more CNS PTLD cases were reported in the MI group and one case of progressive multifocal leukoencephalopathy (PML) was also reported in the MI group. These safety data indicate that EBV serostatus should be routinely checked in patients on the waiting list and that belatacept should be avoided in EBV seronegative patients or with unknown EBV serology, which might contraindi-

cate this biologic agent in approximately 10% of adult transplant recipients [107].

In summary, data from these two pivotal trials have led to the approval by EMA and FDA of the use of belatacept in renal transplantation for the LI regimen taking into account the similar efficacy to the MI regimen and a better safety profile. The advantages of belatacept-based immunosuppression over CNIs are the superior renal function, preservation of renal structure, attenuated humoral responses, and improved cardiovascular risk profile, which might result in improved long-term outcomes. The concerns raised are a higher incidence of manageable cellular rejection and more PTLD especially in EBV seronegative patients, who should be excluded from belatacept-based regimens. In view of the increasing number of generically available CNIs, the annual costs of belatacept-based immunosuppression will be an additional factor influencing the frequency of its use.

Other potential uses of belatacept

The prolonged use of CNI-based maintenance immunosuppression is considered one of the contributors to the development of chronic renal allograft damage. In this regard, several strategies have been attempted to treat established patients with non-nephrotoxic regimens. Prolonged treatment with belatacept is not usually associated with late AR episodes, which suggests that this agent might replace CNI in the long-term. The feasibility of this approach was assayed in a phase II trial [108,109]. One hundred and eighty seven patients with stable renal functions who were at ≥6 months, but ≤36 months after transplantation and receiving a CNI-based regimen were randomized to either switch to belatacept or continue CNI treatment. All patients received background maintenance immunosuppression. The primary end point was the change in calculated GFR from baseline to month 12. Patients continuing with CNI (CsA or tacrolimus) received these drugs to reach conventional trough levels. Patients converted to belatacept received 5 mg/kg every 2 weeks for 2 months and then every 28 days. CNI were withdrawn gradually over 4 weeks. At month 12, the mean change from baseline in GFR was higher in the belatacept versus the CNI group. Six patients receiving belatacept experienced AR within the first 6 months, which resolved without graft loss. The overall safety profile was similar between groups. Study follow-up demonstrated that renal function improved over time; at year 2, mean GFR was 62.0 ml/min with belatacept and 55.4 ml/min with CNI. The mean change in GFR from baseline was +8.8 ml/min versus +0.3 ml/min, respectively. The results of this study may open the door to another non-nephrotoxic immunosuppression in established renal transplant patients.

The so-called low-toxicity regimens usually address CNI or steroid sparing, and are difficult to conciliate both in a single immunosuppressive protocol. In this regard, the combination of T cell depletion with polyclonal ATG under costimulatory blockade with belatacept was used for exploring the feasibility of CNI and steroid-free therapies in a single regimen [110]. A randomized, controlled, open-label, exploratory study assessed two belatacept-based regimens compared with a tacrolimus-based, steroid-avoiding regimen. Eighty-nine EBV seropositive recipients of living and deceased donor renal allografts were randomized to receive belatacept-MMF, belatacept-sirolimus, or tacrolimus-MMF. Both macrolide immunosuppressants were dosed to reach conventional levels. All patients received induction with four doses of thymoglobulin (6 mg/kg maximum cumulative dose) and an associated short course of corticosteroids the first week after surgery. AR occurred in 4, 1 and 1 patient in the belatacept-MMF, belatacept-SRL and tacrolimus-MMF groups, respectively, and most AR occurred in the first 3 months. Interestingly, approximately two thirds of patients in the belatacept groups remained on CNI- and steroid-free regimens at 12 months (73% in the MMF group and 69% in the sirolimus group) and the GFR was 8–10 ml/min higher with either belatacept regimen than with tacrolimus-MMF. Overall safety was comparable between groups and no cases of PTLD were observed. This exploratory trial suggests that primary immunosuppression with belatacept may enable the simultaneous avoidance of both CNIs and corticosteroids without an increased rate of AR. However, despite the promising results of this exploratory study, data should be interpreted with caution because of the small number of patients recruited in this trial. Despite the small number of patients, the very low incidence of AR in the sirolimus arm is noteworthy. This might suggest potential immunosuppressive mechanisms of the drug combination enhancing graft acceptance. Recently, we have studied in small groups of patients, the 1-year evolution of memory/effectors and regulatory T cells and assessed the donor-specific T cell alloimmune responses [60]. We compared the patients treated with a CNI-based (rATG/tacrolimus/MMF), and three other belatacept-based regimens (rATG/belatacept/MMF, rATG/belatacept/sirolimus and basiliximab/belatacept/MMF/steroids). During the first year after transplantation, patients receiving rATG/belatacept/SRL had a significantly higher percentage of Tregs upon the memory T cell compartment and showed a potent antidonor suppressive activity. In an *in vitro* naive and memory/effector T cell co-culture, the combination of costimulation blockade and sirolimus could abrogate both antigen-specific T cell responses as efficiently as using a CNI drug. The combination of T cell depletion, costimulation blockade and

mTOR inhibition seems to be able to allow Treg survival and inhibit donor-specific alloreactive effector immune responses after kidney transplantation in humans, which might explain the very low incidence of AR in the exploratory trial. Moreover, experimental data in a stringent transplant model have shown graft survival prolongation and expansion of regulation with an increased Tregs/Tem ratio with the association of ATG, sirolimus and CTLA4-Ig [111], providing a rational basis for further exploring the utility of this immunosuppressive combination in the clinical setting.

Conclusion

Belatacept is an anti-B7 compound with a complex and incompletely understood mechanism of action. Efficacy results from registration trials support its approval as primary immunosuppressant in EBV-positive renal transplant recipients. Optimal dosing and drug combinations remain to be delineated and possible effects on long-term graft survival to be determined. Only then can the full potential be assessed. In the meantime, maintenance therapy with a costimulation blocking biologic signals a paradigm shift in transplantation medicine, which holds promise to improve the care of transplant patients.

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