REVIEW ARTICLE

Costimulation Blockade in Kidney Transplant Recipients

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Abstract

Costimulation between T cells and antigen-presenting cells is essential for the regulation of an effective alloimmune response and is not targeted with the conventional immunosuppressive therapy after kidney transplantation. Costimulation blockade therapy with biologicals allows precise targeting of the immune response but without non-immune adverse events. Multiple costimulation blockade approaches have been developed that inhibit the alloimmune response in kidney transplant recipients with varying degrees of success. Belatacept, an immunosuppressive drug that selectively targets the CD28-CD80/CD86 pathway, is the only costimulation blockade therapy that is currently approved for kidney transplant recipients. In the last decade, belatacept therapy has been shown to be a promising therapy in subgroups of kidney transplant recipients; however, the widespread use of belatacept has been tempered by an increased risk of acute kidney transplant rejection. The purpose of this review is to provide an overview of the costimulation blockade therapies that are currently in use or being developed for kidney transplant indications.

1 Introduction

Kidney transplant recipients (KTRs) require lifelong immunosuppressive therapy to prevent acute kidney transplant rejection (AR). Currently, the standard immunosuppressive regimen consists of induction therapy (either a T cell-depleting agent or basiliximab, an antibody directed against the interleukin [IL]-2 receptor), followed by maintenance therapy consisting of a calcineurin inhibitor (CNI; either tacrolimus or ciclosporin) and mycophenolic acid (MPA) with or without glucocorticoids [1–4]. Although transplantation is a success story of modern medicine, the long-term allograft and patient survival are influenced by the toxicity of CNIs, which include infections, malignancies, metabolic adverse effects, nephrotoxicity, and neurotoxicity [5–7]. Another limitation of current immunosuppression is that it

is a 'one size fits all' therapy and is not tailored to the individual needs of a KTR. Therefore, novel and personalized therapeutic strategies have to be developed.

Several approaches have been investigated to limit the adverse effects of CNIs, including monitoring of CNI concentrations to guide dosing, and CNI-sparing regimens. Examples of the latter are CNI minimization, CNI withdrawal, CNI conversion to alternative immunosuppressive agents, and, lastly, CNI avoidance from the time of the transplantation with substitution of an alternative immunosuppressive drug [8]. However, many such trials failed because they resulted in unacceptably high incidences of AR and toxicity, or an increased incidence of infections associated with the alternative immunosuppressants [9–15].

Costimulation is essential for the regulation of an effective alloimmune response. The costimulatory pathway is not targeted with the conventional immunosuppressive therapy. Biologicals that intervene with the costimulatory pathway may allow more precise targeting of the immune response without causing non-immune adverse events. Belatacept, a fusion protein composed of a crystallizable fragment (Fc) of immunoglobulin (Ig) G1 and the extracellular domain of cytotoxic T lymphocyte protein 4 (CTLA4), is the only costimulation blockade therapy that is currently approved for the prevention of rejection after kidney transplantation [16, 17]. Belatacept is well-tolerated and its use is associated with an improved allograft function compared with CNI in certain subgroups of KTRs [18, 19]; however, belatacept

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may not be the game changer it was hoped to be due to a high risk of AR [20]. In this review, the current applications of biologicals that target costimulation pathways in kidney transplantation are discussed, including the current status and future strategies of belatacept therapy.

2 Costimulation

The process of T-cell activation is a complex cascade consisting of three signals. First, alloantigens from the allograft are taken up by antigen-presenting cells (APCs; dendritic cells, macrophages, and B cells), which then home to the draining lymph nodes. In the lymph nodes, the alloantigens are presented on the surface of APCs by human leucocyte antigen (HLA) molecules. In humans, the T-cell receptor (TCR) on naive T cells is activated after interaction with the alloantigen/HLA complex, which is also known as signal 1 (Fig. 1). A costimulatory signal (signal 2) is necessary to achieve full activation of T cells. Several cell-surface proteins (costimulatory ligands) on APCs interact with their complementary receptors on naive T cells (Fig. 1). Signal 2 represents a combination of positive and negative signals that regulate the outcome of the HLA/TCR. Without this signal, naive T cells will undergo apoptotic cell death [21–23].

Two costimulatory pathways are critical for T-cell activation: (1) the Ig superfamily (e.g. CD28 [T-cell-specific surface glycoprotein CD28] family), the CD2/signaling lymphocytic activation molecule (SLAM) family, and the

T-cell/transmembrane, Ig, and mucin (TIM) family; and (2) the TNF (tumor necrosis factor)/TNF receptor superfamily (Fig. 1) [21].

Signal 3 is formed by cytokines and the (increased) expression of cytokine receptors, such as the IL-2 receptor α-chain (CD25) (Fig. 1). Activation of CD25 will activate intracellular signaling pathways downstream of the TCR, including the mitogen-activated protein kinase (MAPK), calcineurin, and PI3 K pathways, followed by the activation of transcription factors that regulate the production of several cytokines (i.e. IL-2 and interferon [IFN]-γ) [24]. These and other cytokines promote T-cell proliferation of diverse effector CD4$^+$ T-cell subsets and cytotoxic CD8$^+$ T cells [25].

3 Belatacept Therapy in Kidney Transplantation

3.1 Development of Belatacept

Belatacept targets the CD28-CD80/CD86 pathway. The costimulation molecule CD28 is a surface receptor that is constitutively expressed on T cells (Fig. 1). The inhibitory receptor CTLA4 is localized in intracellular vesicles in resting T cells and is expressed on the cell surface 48–72 h after T-cell activation. CTLA4 binds to CD80 and CD86 with a higher affinity than CD28 [21]. Therefore, the binding of CTLA4 to CD80/CD86 dampens the activation of T cells [26]. At birth, almost all human T cells express CD28 [27]. Aging, continuous antigenic stimulation (which can be caused by, for example, end-stage renal disease, human immunodeficiency virus infection, and auto-immune disease) and cytomegalovirus infection lead to loss of CD28 expression of T cells [27–29]. These CD28$^-$ effector memory T cells have reduced costimulatory requirements and an impaired proliferative capacity, but are highly proinflammatory [27, 30]. These cells rapidly secrete effector cytokines (i.e. TNFα and IFNγ) upon restimulation.

One of the first biologics that was designed to target the CD28-CD80/CD86 superfamily was abatacept (Fig. 1), a fusion protein composed of an Fc of IgG1 and the extracellular domain of CTLA4 [31]. Because CTLA4 binds with a higher affinity to CD80/CD86 than CD28, it was hypothesized that T-cell activation could be inhibited with such a CTLA4 construct. Abatacept is approved for the treatment of rheumatoid arthritis (Fig. 2) [32], and has been tested in non-human primates transplanted with a kidney or pancreatic islets; however, alloreactivity appeared to be inhibited insufficiently [33, 34]. Therefore, the development of abatacept therapy for transplantation was discontinued and a new CTLA4-Ig construct (belatacept) was developed with increased avidity for CD80 and CD86 by changing two amino acids (L104E and A29Y) (Figs. 1 and 2) [16].
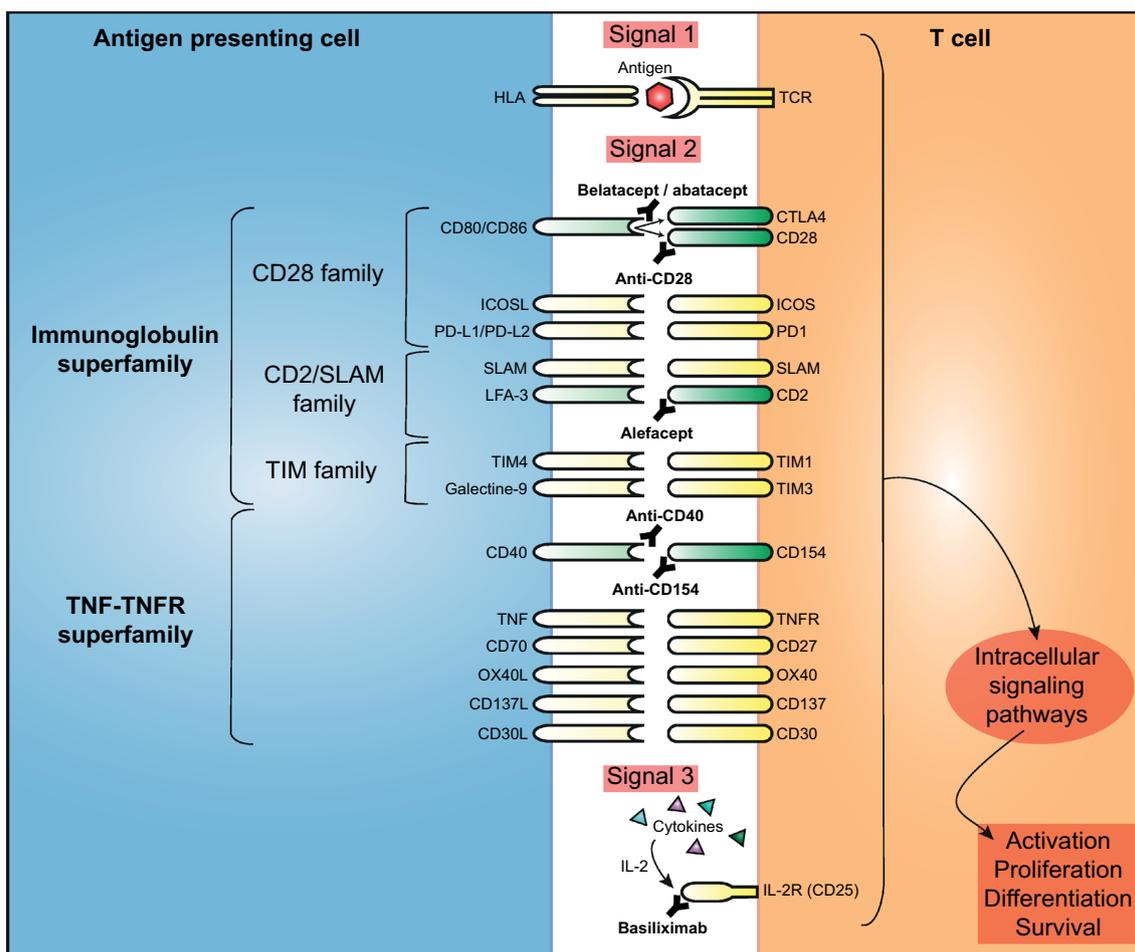
Fig. 1 Costimulation between T cells and antigen-presenting cells. Schematic overview of signal 1, 2 and 3 of T-cell activation. During signal 2, costimulatory molecules on T cells and antigen-presenting cells interact to activate or inhibit T cells after alloantigen recognition. Two important groups of costimulatory molecules are presented: the immunoglobulin superfamily and the TNF/TNFR superfamily. The costimulatory molecules discussed in this review are green and the costimulatory molecules that are not discussed are yellow. Sev-

eral biologicals are developed that interfere with the costimulatory molecules on T cells and antigen-presenting cells. *CTLA 4* cytotoxic T lymphocyte protein 4, *HLA* human leucocyte antigen, *ICOS* inducible T-cell costimulator, *PD* programmed death, *SLAM* signaling lymphocytic activation molecule, *TCR* T-cell receptor, *TIM* T cell/transmembrane, immunoglobulin, and mucin, *TNF* tumor necrosis factor, *TNFR* tumor necrosis factor receptor

Belatacept was found to have a fourfold higher binding affinity for CD86 and a twofold higher binding affinity for CD80 compared with abatacept [16]. Although the development of abatacept in transplantation was stopped, abatacept was recently used as rescue therapy in nine KTRs with an intolerance to CNI, because belatacept was temporarily unavailable due to manufacturing problems [35, 36]. None of the allografts were lost after a median period of 115 months and one patient experienced AR [35].

Belatacept was approved as treatment for the prevention of AR by the European Medicines Agency (EMA) and the US FDA in 2011 based on the results of two large randomized, controlled, multicenter, phase III trials (Fig. 2): The Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression (BENEFIT) study (with

standard criteria donors) and the BENEFIT-extended criteria donors (BENEFIT-EXT) study [17, 37, 38]. In these trials, 1264 KTRs were treated with either ciclosporin or belatacept as first-line treatment in combination with MPA and glucocorticoids. The main findings of the BENEFIT and BENEFIT-EXT studies were that the 1-year patient and allograft survival of patients treated with belatacept were similar to patients treated with ciclosporin [37, 38]. Although the incidence of acute T-cell-mediated rejection (aTCMR) was increased in belatacept-treated patients, the kidney function was better in these patients compared with ciclosporin-treated patients [37, 38]. In addition, the use of belatacept was associated with an increased risk for post-transplant lymphoproliferative disease, mostly in Epstein–Barr virus seronegative KTRs [37–39].
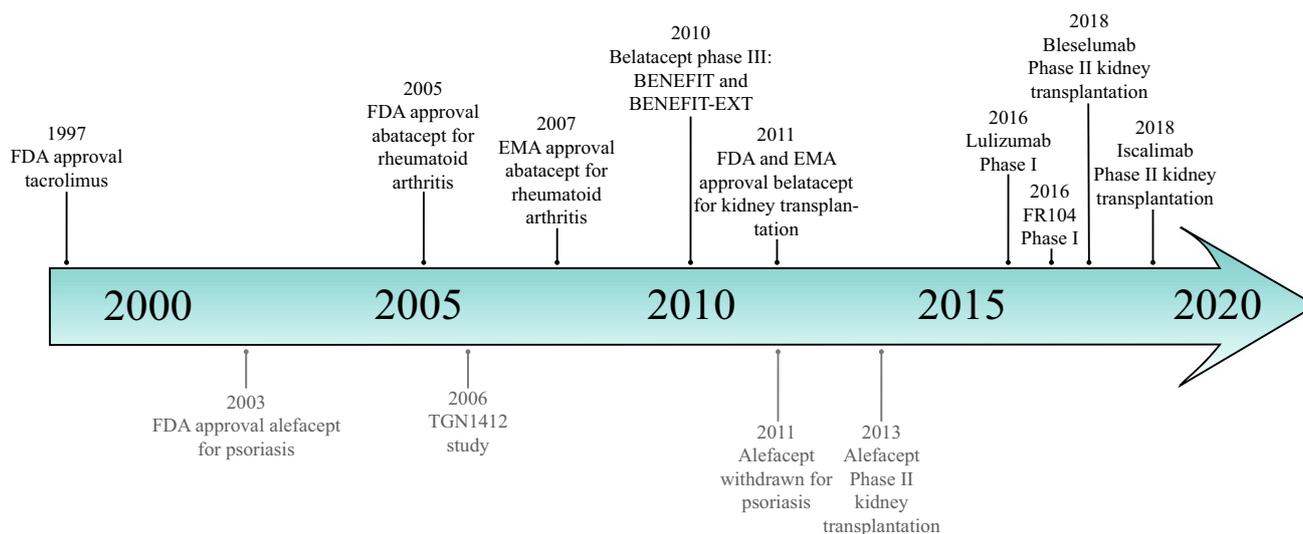
Fig. 2 Timeline of the development of costimulation blockade. The costimulation blockade drugs that are currently used or tested in kidney transplant recipients are shown in black, whereas the costimulation blockade drugs that are no longer being used or have not devel-oped for kidney transplantation are shown in grey. *EMA* European Medicines Agency, *FDA* United States Food and Drug Administration, *FR104* pegylated monoclonal antibody fragment antagonist of CD28, *TGN1412* CD28 humanized antibody

The safety and efficacy of belatacept were also tested in a phase II, randomized, controlled, multicenter trial in liver transplant recipients [40]. This trial randomized 260 patients between therapy with belatacept (three different belatacept regimens) or tacrolimus (two different tacrolimus regimens). The primary composite endpoint consisted of incidence of acute liver transplant rejection, graft loss, and death at 6 months after transplantation. The occurrence of the composite endpoint was higher in the belatacept groups (42–48%) than in the tacrolimus groups (15–38%) [40]. The results of this study were reason to discontinue further development of belatacept for liver transplantation. However, the mean estimated glomerular filtration rate (eGFR) was 15–34 mL/min/1.73 m^2 higher in the liver transplant recipients treated with belatacept [40]. Therefore, liver transplant recipients with an impaired renal function could benefit from belatacept therapy. Proper selection of patients and an adjusted treatment protocol can possibly improve the results of belatacept in liver transplantation in the future [41].

3.2 Clinical Outcomes of De Novo Use of Belatacept in Kidney Transplant Recipients

A systematic review with meta-analysis was performed of five studies that compared treatment with belatacept with CNIs (including the BENEFIT and BENEFIT-EXT studies) in 1535 KTRs [42]. Of the 521 patients treated with a CNI, 478 patients used ciclosporin and 43 patients were treated with tacrolimus. After 3 years of treatment, no difference was seen between patients treated with either belatacept or a CNI regarding the risk of death (relative risk 0.75, 95%

confidence interval [CI] 0.39–1.44; $p = 0.39$), allograft loss (relative risk 0.91, 95% CI 0.61–1.38; $p = 0.67$), and incidence of aTCMR (RR 1.56, 95% CI 0.85–2.86; $p = 0.15$) [42]. However, the kidney allograft function was better in patients treated with belatacept (eGFR mean difference of 9.96 mL/min/1.73 m^2, 95% CI 3.28–16.64; $p = 0.0035$). Furthermore, the use of belatacept was associated with a reduced incidence of post-transplant diabetes mellitus, better blood pressure, and a better lipid profile 1 year after therapy with belatacept [42].

In 2016, the 7-year follow-up results of the BENEFIT and BENEFIT-EXT studies were published. In these studies, the risks of death and graft loss in KTRs treated with belatacept were similar to those in KTRs treated with ciclosporin [18, 19]. Although, the risk of aTCMR was higher in belatacept-treated patients compared with ciclosporin-treated patients, their kidney function after 7 years was better. An explanation for the better kidney function may be that belatacept is associated with less interstitial inflammation and tubular atrophy compared with CNIs. Vitalone et al. compared the 1-year protocol biopsies of KTRs treated with belatacept or ciclosporin [43], and found that the biopsies of patients treated with belatacept contained less interstitial inflammation, interstitial fibrosis, and tubular atrophy, and gene expression analysis revealed a lower expression of genes involved in fibrosis and tubulointerstitial damage compared with the biopsies of patients treated with ciclosporin [43]. In another study, 10-year protocol biopsies of 23 clinically stable KTRs treated with belatacept and 10 KTRs treated with CNI (seven taking ciclosporin and three taking tacrolimus) were analyzed [44]. The biopsies of belatacept-treated

patients contained less interstitial inflammation and tubular atrophy, less interstitial inflammation, and less hyalinosis [44].

The 7-year follow-up studies also showed that the formation of de novo donor-specific anti-HLA antibodies (DSAs) was reduced in the belatacept-treated patients compared with the patients treated with ciclosporin [18, 19]. A possible explanation for this observation may be that costimulation blockade with belatacept leads to more effective prevention of DSA formation by B cells and that drug adherence is better in patients treated with belatacept because of intravenous administration. The occurrence of post-transplant diabetes mellitus, blood pressure, and lipid profile were not discussed in the long-term follow-up studies of belatacept.

To conclude, these long-term outcomes demonstrate that belatacept therapy is a safe therapy for KTRs and is associated with a better kidney function and a reduced incidence of de novo DSA. Whether long-term belatacept therapy leads to a better metabolic profile than CNI therapy is not known [18, 19, 39].

A limitation of the BENEFIT studies is that belatacept therapy was compared with ciclosporin therapy. Currently, the CNI of choice in most transplant centers is tacrolimus [1, 45]. No large, head-to-head, randomized controlled trials have been performed that compared the outcomes of patients treated with either belatacept or tacrolimus. In our center, a trial was performed that included 40 KTRs who were randomized between first-line therapy consisting of tacrolimus or belatacept, in combination with MPA and glucocorticoids [46]. The AR incidence in the first year after transplantation was higher in belatacept-treated patients (55% vs. 10%; $p = 0.006$) [46]. Another randomized controlled trial compared three treatment regimens in KTRs: alemtuzumab induction with tacrolimus, alemtuzumab induction with belatacept, and basiliximab induction with belatacept and a 3-month course of tacrolimus [47]. This study was halted prematurely after the inclusion of 19 patients, due to a high rate of serious adverse events in belatacept-treated patients, including thrombotic complications and aTCMR [47].

The comparison between belatacept and tacrolimus therapy has also been investigated in three indirect studies [48–50]. In a single-center, retrospective analysis, the outcomes of KTRs treated with belatacept ($n = 97$) were compared with a historical cohort of patients treated with tacrolimus ($n = 205$) [48]. An increased rate of aTCMR was noted in patients treated with belatacept compared with patients treated with tacrolimus (50.5% vs. 20.5%) [48]. In a retrospective propensity score-matched cohort study, the outcomes of KTRs treated with either tacrolimus or belatacept were compared [49]. The risk of AR was higher in the first post-transplant year in patients treated with belatacept (odds ratio 3.12, 95% CI 2.13–4.57; $p < 0.001$) but no difference

was seen in the risk of death (hazard ratio [HR] 0.84, 95% CI 0.61–1.15; $p = 0.28$) or allograft loss (HR 0.83, 95% CI 0.62–1.11; $p = 0.20$) [49]. Muduma et al. performed a systematic review and meta-analysis with an indirect treatment comparison analysis between tacrolimus (both immediate-release and prolonged-release formulations) and belatacept [50]. The AR rate was reduced in patients treated with tacrolimus compared with patients treated with belatacept (risk ratio 0.22 [95% CI 0.13–0.39] to 0.44 [95% CI 0.20–0.99]) [50]. The risks of allograft loss and death were similar between both treatments.

One of the reasons for the high risk of aTCMR after belatacept may be that the immunosuppressive function of regulatory T cells (Tregs) is impaired [51–55]. Tregs are dependent on signaling via CTLA4, and binding of belatacept to CD80/86 interferes with CTLA4. Therefore, the combination of belatacept with therapies that preserve Treg functionality, such as T-cell-depleting antibodies and mammalian target of rapamycin (mTOR) inhibitors could possibly lead to a reduced incidence of AR [4, 56, 57].

The combination of induction therapy with T-cell-depleting drugs and belatacept has been tested in several studies, with various outcomes. In one study, alemtuzumab induction followed by tacrolimus or belatacept led to a similar incidence of AR [58]. In another study, patients treated with T-cell-depleting induction therapy (either rabbit antithymocyte globulin [rATG] or alemtuzumab) followed by belatacept were compared with patients treated with rATG induction followed by tacrolimus [59]. In all patients, glucocorticoids were withdrawn early. The AR incidence was higher in patients treated with belatacept, but the allograft and patient survival were similar [59]. In a third study (described above), alemtuzumab induction with belatacept in KTRs resulted in a high rate of serious adverse events and the study was halted prematurely [47]. T-cell-depleting induction therapy has also been tested in KTRs treated with belatacept in combination with mTOR inhibitors (sirolimus or everolimus). The AR rate in patients treated with this combination of drugs is low, and a significant increase in Tregs is seen [60–63].

Possible explanations for a lower rate of acute rejection after T-cell-depleting induction therapy compared with basiliximab induction therapy were (1) after T-cell-depletion therapy an increased repopulation of Tregs is seen, and (2) repopulated memory T cells in rATG-treated KTRs showed impaired cytokine responsiveness compared with those of basiliximab-treated KTRs [57, 64].

To conclude, although the studies that compared belatacept therapy with tacrolimus therapy have their limitations (limited number of patients or indirect comparison), belatacept is associated with an increased risk of aTCMR.

3.3 Clinical Outcomes After Conversion to Belatacept in Kidney Transplant Recipients

Although the use of belatacept is associated with an increased risk of aTCMR, it has been shown to be a good alternative in KTRs with a contraindication to CNIs. Multiple studies have reported successful conversion to belatacept in KTRs with CNI-induced nephrotoxicity, impaired allograft function, delayed graft function, CNI-mediated thrombotic microangiopathy, or atypical hemolytic uremic syndrome [65–82]. Furthermore, KTRs with poorly controlled diabetes mellitus while receiving CNI therapy may benefit from belatacept [83, 84]. In addition, since belatacept must be administered intravenously, it has the potential advantage of providing better compliance, for instance in adolescent KTRs [85].

Several approaches for conversion to belatacept have been evaluated, such as early or late conversion [77, 86–89], belatacept combined with a short period of tacrolimus therapy [48], and non-invasive screening for AR after conversion to belatacept to detect AR at an early stage [90]. In a phase II, prospective, randomized trial, KTRs with a stable kidney function were randomized, 6–36 months after transplantation, to maintenance therapy with either belatacept ($n = 84$) or CNIs ($n = 89$) [88]. Three years after randomization, kidney function was better in the belatacept group [89]. The rate of acute rejection was higher in the belatacept group (8.4%) compared with the CNI-treated patients (3.6%), but this difference was not statistically significant ($p = 0.2$) [89]. In retrospective studies, a beneficial effect on kidney allograft function was seen in patients with early conversion to belatacept (within 3 months post-transplantation) and patients with low-grade proteinuria [77, 86, 87]. The combination of belatacept with 9 months of tacrolimus reduced the risk of aTCMR in a retrospective, single-center study (the 1-year aTCMR rate of belatacept therapy, tacrolimus therapy, and belatacept plus 9 months of tacrolimus was 50%, 20.5%, and 16%, respectively), without an increased incidence of infections [48]. Malvezzi et al. also examined a strategy to safely convert KTRs to belatacept [90]. After the start of belatacept, the dose of tacrolimus was gradually reduced and withdrawn after 2 months. Serial measurements (at 1-, 3-, 6-, and 12-month time points) of urine chemokine (C-X-C motif) ligand 9 (CXCL9) were used to screen for AR non-invasively. In this study, 35 KTRs with a contraindication for CNIs were converted to belatacept after a median of 3.3 years (interquartile range 1.3–7.2) after transplantation [90]. Only one patient had a biopsy-proven AR that responded well to glucocorticoid pulse therapy [90]. The urinary CXCL9 concentration was elevated during AR. In addition to CXCL9, other potential minimally invasive biomarkers in the urine and blood of KTRs, such as cell-free DNA and extracellular vesicles, may assist clinicians to identify AR at an early stage [91, 92].

Currently, two studies are actively recruiting KTRs for conversion to belatacept; one study will investigate the effect of conversion to belatacept on proteinuria (ClinicalTrials.gov identifier NCT02327403), while the other study will examine the outcomes of conversion to belatacept 3 months after transplantation (ClinicalTrials.gov identifier NCT02213068).

3.4 Belatacept Therapy in Sensitized Kidney Transplant Recipients

Initially, most studies that investigated the effectiveness of belatacept included only immunological low-risk KTRs [37, 42, 46]. However, because belatacept therapy is associated with a reduced incidence of de novo DSA production, a growing number of studies on the application of belatacept (de novo and conversion) in sensitized KTRs are available [18, 19].

In the BELACOR study, 49 KTRs with preformed DSAs (maximal mean fluorescence intensity between 500 and 3000) were treated with induction therapy of rATG followed by de novo belatacept maintenance therapy plus MPA and glucocorticoids [93]. The outcomes were compared with a retrospective control group of patients treated with CNIs. After 1 year of follow-up, no patients in the belatacept group experienced antibody-mediated rejection, while aTCMR occurred significantly more often in the belatacept-treated patients. Complete disappearance of class II DSAs was seen significantly more often in belatacept-treated patients [93].

In a retrospective study, the efficacy of belatacept in reducing anti-HLA antibodies in highly sensitized kidney transplant (current panel reactive antibodies ≥ 98–100%) was investigated [94]. Sixty highly sensitized KTRs were treated with belatacept de novo, glucocorticoids, MPA, and low-dose tacrolimus (targeted to predose concentrations 5–8 ng/mL in the first 6 months, 3–5 ng/mL in months 6–9, followed by tapering and discontinuation at months 9–11 post-transplantation). The control group existed of 44 highly sensitized KTRs treated with the current standard-of-care therapy (tacrolimus, MPA, and glucocorticoids). In the KTRs treated with belatacept, a decrease in the breadth and strength of HLA class I antibodies and current panel reactive antibodies was observed compared with the control group [94].

In another retrospective, single-center study, 29 DSA-positive KTRs with a contraindication for CNI therapy were converted to belatacept after a median of 444 days [95]. The control group consisted of 44 non-immunized belatacept-treated KTRs. After a median follow-up of 308 days, one belatacept-treated patient experienced AR and two rejections were diagnosed in the CNI-treated patients. The eGFR

improved from 32 to 41 mL/min/1.73 m^2 after conversion to belatacept [95]. In a smaller retrospective study, similar results were reported in six immunized KTRs (panel reactive antigen > 80%, or positive flow cytometry crossmatch) who were converted from tacrolimus to belatacept (median 4 months after transplantation) [96].

3.5 Biomarkers Predicting Belatacept-Resistant Rejection

Because of the increased risk of aTCMR, belatacept may not be the game changer it was hoped to be [20]. Possibly, the drug should be reserved for KTRs with a low risk of belatacept-resistant AR. Quantification of an individual KTR's risk of AR prior to transplantation is essential to identify those who might benefit from belatacept-based immunosuppressive therapy. Clinical tests to reliably predict the risk of belatacept-resistant AR are not yet available. The risk of AR is currently estimated with pretransplant assessment of DSAs, and HLA mismatch; however, alloreactive memory T-cell responses are not measured with these assays. The presence of alloreactive T cells pretransplantation can lead to rapid recognition of alloantigens after transplantation, and early AR [97, 98]. These alloreactive T cells can be measured with pretransplantation functional assays (e.g. measurement donor-reactive immune cells with enzyme-linked immunosorbent spot [ELISpot]) [97, 99].

Several studies have been performed to elucidate the pathogenesis of AR after belatacept therapy. An immunomic analysis of biopsies with AR of KTRs treated with tacrolimus or belatacept showed no difference in the intragraft gene expression and immunohistochemistry of markers that are involved in AR [100]. This implies a final common pathway of AR that is independent of the immunosuppressive regimen.

Apart from the effect of belatacept on Tregs [51–55], other T cells have been associated with belatacept-resistant AR, such as highly cytotoxic CD28$^-$ memory T cells, CD4$^+$CD28$^+$ effector memory T cells, CD4$^+$CD57$^+$Programmed Death-1$^-$ T cells, and T helper (Th) 17 memory cells [46, 98, 101–105]. However, conflicting data have been reported regarding the possibility of predicting belatacept-resistant AR by measuring some of these T-cell subsets [46, 98, 101–105], and currently none are clinically reliable for AR risk.

Another reason that may contribute to the increased incidence of AR is that belatacept therapy does not inhibit the T-cell activation pathway downstream of the TCR, in contrast to tacrolimus therapy [106]. In a study of 20 belatacept-treated KTRs, no inhibition of the phosphorylation of three important signaling molecules (p38MAPK, extracellular signal-regulated kinases [ERK] 1 and 2, and AKT8 virus oncogene cellular homolog [Akt]) was noted after treatment with belatacept [106]. Furthermore, the phosphorylation of ERK was increased in belatacept-treated patients on days 4 and 90 in patients with an AR compared with patients without an AR [106].

Prediction of AR was not possible with a targeted proteomic analysis of pre-rejection serum samples of KTRs treated with belatacept [107]. In an assay with 92 inflammation-related proteins, no difference was seen in the proteomic profile between the pre-rejection samples and samples of patients without AR [107].

To conclude, there are several explanations for the increased risk of AR associated with belatacept therapy. At present, no specific tests (besides pretransplant screening for degree of sensitization) are available that can predict the risk for belatacept-resistant AR.

4 Alternative Approaches of Costimulation Blockade

4.1 CD28 Antibodies

Selective targeting of the CD28 antigen on T cells might be a superior immunosuppressive therapy compared with belatacept since this blockade leaves the inhibitory signal of CTLA-4 intact and may preserve Treg function (Fig. 1) [108, 109]; however, blockade of CD28 has been challenging. Most anti-CD28 antibodies bind to an epitope lying in the basolateral C''D domain of CD28. Crosslinking of this epitope with an anti-CD28 antibody results in receptor clusterization, which leads to activation of the CD28 receptor instead of inhibition. In 2006, a CD28 humanized antibody (TGN1412) was tested in a phase I study (Fig. 2) [110]. This antibody was developed to cause activation and proliferation of Tregs independent of signals received from the TCR. In studies in cynomolgus macaques, TGN1412 revealed no toxic effects; however, in humans, infusion of TGN1412 led to life-threatening massive cytokine release in six healthy volunteers, and all of them had to be transferred to the intensive care unit [110]. CD4$^+$ effector memory T cells appeared to be responsible for the massive cytokine release [111]. The reason that preclinical testing failed to predict this dramatic adverse effect was that CD4$^+$ effector memory T cells of cynomolgus macaques do not express CD28, therefore these cells cannot be stimulated with TGN1412 [111].

Currently, two monovalent antibodies with only antagonistic action to CD28 are in clinical development—FR104 and lulizumab-pegol (Fig. 2) [112, 113]. In non-human primates transplanted with a kidney allograft, FR104 in combination with rapamycin, a low dose of tacrolimus, or 1 month of low-dose tacrolimus, induced long-term allograft survival [114, 115]. Lulizumab-pegol was tested in non-human primates and showed inhibition of T-cell-dependent antibody

responses and cytokine production [116]. In humans, both drugs have been evaluated in phase I clinical studies and were safe and well tolerated (Fig. 2) [113]. At present, a prospective multicenter study has been started to investigate the efficacy of lulizumab in combination with rATG, glucocorticoids, belatacept, tocilizumab, and everolimus in KTRs (ClinicalTrials.gov identifier NCT04066114).

4.2 CD2/SLAM Family Antibodies

An antibody that interferes with the CD2/SLAM family is alefacept (Fig. 1). This is a fusion protein of lymphocyte function-associated antigen (LFA)-3 and the Fc part of IgG1 [117]. LFA-3 is expressed on APCs and is the ligand of CD2 on T cells. CD2 is expressed on all T cells, but memory T cells express the highest levels [118]. Alefacept binds to CD2 on T cells and blocks the interaction between CD2 and LFA-3. It was approved by the FDA in 2003 for the treatment of psoriasis, and administration of alefacept leads to the depletion of memory T cells (Fig. 2) [117]. In a phase II, randomized, controlled, double-blind, multicenter study, maintenance treatment with alefacept was compared with placebo in KTRs [119]. Both patient groups were also treated with tacrolimus, MPA, and glucocorticoids. There was no significant difference in the rate of biopsy-proven AR (alefacept 11% vs. placebo 7%; $p = 0.3$). Furthermore, malignancy occurred more often in patients treated with alefacept (5.7%) compared with placebo (0.9%; $p = 0.06$) [119]. In 2011, the manufacturer decided to stop the development of alefacept [120].

4.3 CD40/CD154 (CD40 Ligand) Antibodies

The CD40/CD154 pathway is a promising target for immunosuppressive therapy in KTRs. CD40 (TNF receptor superfamily 5 [TNFRSF5]) is constitutively expressed on the surface of APCs, including B cells, macrophages and dendritic cells, and T cells (mainly CD8$^+$) [121]. CD154 is the ligand of CD40 and is expressed on activated T cells and subsets of natural killer cells, eosinophils, and activated thrombocytes (Fig. 1) [121]. Ligation of CD40 with CD154 leads to T-cell-dependent B-cell activation and proliferation, germinal center formation, Ig production, and isotype class switching. Furthermore, stimulation of CD40 provides macrophage effector function and promotes CD28-mediated costimulation through upregulation of CD80/CD86 and HLA molecules on APCs [122, 123].

Multiple antibodies that target the CD40/CD154 pathway have been developed and tested in patients with autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis, inflammatory bowel disease, and idiopathic thrombocytic purpura [124]. Several CD154 monoclonal antibodies (e.g. hu5C8, IDEC-131, ABI793, and

H106) were tested in non-human primates and showed prolonged kidney allograft survival [33, 125–128]. However, in humans, administration of anti-CD154 antibodies led to an unanticipated, higher incidence of thrombotic complications, possibly because of activation of the coagulation cascade through CD154 activation on thrombocytes [129]. Therefore, the clinical development of anti-CD154 antibodies was terminated. Since CD40 is not expressed on thrombocytes, antagonistic anti-CD40 antibodies might not evoke thrombotic events. At present, many antagonistic anti-CD40 antibodies (e.g. ASKP1240, CFZ533, HCD122, Chi220, 3A8, 2C10R1, 2C10R4, BI-655064, FFP104, ch5D12) are under investigation [124, 130]. Two of these anti-CD40 antibodies have been tested in KTRs, namely CFZ533 (iscalimab) and ASKP1240 (bleselumab).

Bleselumab is a fully humanized, non-depleting, anti-CD40 IgG4 antibody. In non-human primates, bleselumab prolonged kidney allograft survival [131]. In a phase Ib study in KTRs, bleselumab was well tolerated and no thrombotic events occurred [132]. A more extensive (phase II) trial examined the efficacy and safety of bleselumab in KTRs (ClinicalTrials.gov identifier NCT01780844) (Fig. 2). Preliminary data presented at a conference showed that the incidence of AR 3 years of therapy was 13% in KTRs treated with standard therapy (tacrolimus), 11% in patients treated with bleselumab with low-dose tacrolimus ($p = 1.00$ vs. standard therapy), and 41% in patients treated with bleselumab ($p = 0.02$ vs. standard therapy) [133]. Furthermore, an increased incidence of cytomegalovirus and BK virus infections was seen in patients treated with bleselumab [132, 133].

Iscalimab is a non-B-cell-depleting anti-CD40 antibody that induced prolonged survival and function of kidney allografts in cynomolgus monkeys [134]. Furthermore, iscalimab led to complete absence of splenic germinal center formation, and formation of de novo DSA [134]. It is currently being tested in 325 KTRs in a phase II trial (ClinicalTrials.gov identifier NCT02217410) comparing standard-of-care therapy (tacrolimus, MPA, and glucocorticoids) with subcutaneous iscalimab every 2 weeks in combination with MPA and glucocorticoids (Fig. 2). Data from a proof-of-concept trial performed in 2016–2017 demonstrated comparable efficacy on the composite endpoint of AR, graft loss, or death (21.2% vs. 22.2%), better kidney function (55.8 vs. 45.5 mL/min/1.73 m^2), and a reduced incidence of post-transplant diabetes mellitus (14.7 vs. 38.9%) in patients treated with iscalimab compared with tacrolimus after 6 months of treatment [135].

5 Future Directions

Modulation of the costimulation pathway with biologicals remains a promising strategy for the prevention of AR because it is more specific than traditional pharmacologic

immunosuppression and appears to have the advantage of having only limited non-immune toxicity. To date, belatacept is the only costimulation blockade therapy approved for the prevention of rejection. Although belatacept has been shown to be a promising therapy in subgroups of patients, its widespread use has been limited because of (1) the increased risk of aTCMR compared with tacrolimus; (2) concerns regarding its safety (increased risk of post-transplant lymphoproliferative disorder); and (3) logistical aspects, including the need for regular infusions and its temporary unavailability due to production shortages.
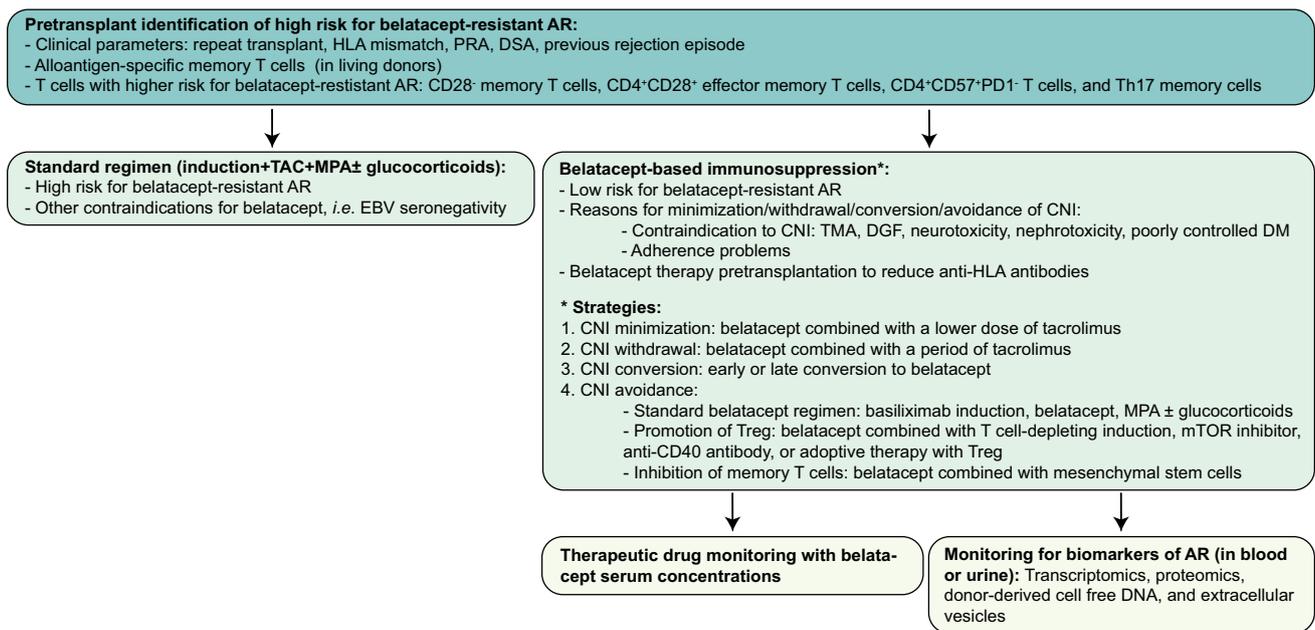
The use of belatacept could be expanded in the future, when it is possible to reliably identify patients who are at a low risk for belatacept-resistant AR (Fig. 3). Belatacept-based therapy may be administered to these low-risk patients, while the standard of care (tacrolimus-based therapy) should be offered to KTRs with a high risk for belatacept-resistant AR (Fig. 3). Belatacept may also be an attractive alternative for patients with contraindications for CNIs (Fig. 3).

Another strategy to expand the use of belatacept is to combine belatacept with tacrolimus or other immunosuppressive therapies (Fig. 3). The risk of belatacept-resistant AR is reduced when it is used in combination with a short period, or low-dose, of tacrolimus [48, 90]. Belatacept therapy influences the immunosuppressive function of Tregs

[51–55]; therefore, the combination of belatacept with therapies that preserve Treg functionality, such as mTOR inhibitors, T-cell-depletion therapy, anti-CD40 antibodies, and adoptive therapy with Tregs could possibly lead to a more precise control of alloimmunity (Fig. 3) [56]. The combination of CTLA4/Ig and blockade of CD40/CD154 has not yet been tested in humans; however, in several animal transplant models, this combination produced long-term allograft survival [136–140].

CD28⁻ memory T cells are insensitive to belatacept therapy; therefore, belatacept should be avoided in KTRs with a high number of these cells, or should be combined with drugs that effectively control CD28⁻ memory T-cell immunity (Fig. 3). Mesenchymal stem cell therapy has immunomodulatory properties and, in vitro, these cells are shown to inhibit CD28⁻ memory T cells [141, 142]. The combination of alemtuzumab induction followed by infusion with mesenchymal stem cells, belatacept, and sirolimus is currently being tested (ClinicalTrials.gov identifier NCT03504241).

More intensive monitoring of KTRs after conversion to belatacept might lead to better prevention or earlier recognition of AR. Belatacept dosing is administered at fixed intervals and is based on the body weight of the patient. According to the manufacturer of belatacept, it is not advisable to perform therapeutic drug monitoring of belatacept [143].

Pretransplant identification of high risk for belatacept-resistant AR:
- Clinical parameters: repeat transplant, HLA mismatch, PRA, DSA, previous rejection episode
- Alloantigen-specific memory T cells (in living donors)
- T cells with higher risk for belatacept-restistant AR: CD28⁻ memory T cells, CD4⁺CD28⁺ effector memory T cells, CD4⁺CD57⁺PD1⁻ T cells, and Th17 memory cells

Standard regimen (induction+TAC+MPA± glucocorticoids):
- High risk for belatacept-resistant AR
- Other contraindications for belatacept, *i.e.* EBV seronegativity

Belatacept-based immunosuppression*:
- Low risk for belatacept-resistant AR
- Reasons for minimization/withdrawal/conversion/avoidance of CNI:
  - Contraindication to CNI: TMA, DGF, neurotoxicity, nephrotoxicity, poorly controlled DM
  - Adherence problems
- Belatacept therapy pretransplantation to reduce anti-HLA antibodies

* Strategies:
1. CNI minimization: belatacept combined with a lower dose of tacrolimus
2. CNI withdrawal: belatacept combined with a period of tacrolimus
3. CNI conversion: early or late conversion to belatacept
4. CNI avoidance:
   - Standard belatacept regimen: basiliximab induction, belatacept, MPA ± glucocorticoids
   - Promotion of Treg: belatacept combined with T cell-depleting induction, mTOR inhibitor, anti-CD40 antibody, or adoptive therapy with Treg
   - Inhibition of memory T cells: belatacept combined with mesenchymal stem cells

Therapeutic drug monitoring with belatacept serum concentrations

Monitoring for biomarkers of AR (in blood or urine): Transcriptomics, proteomics, donor-derived cell free DNA, and extracellular vesicles

Fig. 3 Future directions for belatacept treatment in kidney transplant recipients. A more tailored approach in the selection, treatment strategy, and postconversion monitoring might be a way to expand the use of belatacept in kidney transplant recipients. *AR* acute kidney transplant rejection, *CNI* calcineurin inhibitors, *DGF* delayed graft function, *DM* diabetes mellitus, *DSA* donor-specific anti-HLA antibodies, *EBV* Epstein–Barr virus, *HLA* human leucocyte antigen, *MPA* mycophenolic acid, *mTOR* mammalian target of rapamycin, *PD-1* programmed death-1, *PRA* panel reactive antigens, *TAC* tacrolimus, *Th17* T helper 17, *TMA* thrombotic microangiopathy, *Treg* regulatory T cells

However, in the BENEFIT studies, a more intensive regimen was associated with an increased incidence of malignancies and infections, without an increase in efficacy [37, 38]. An automated assay to determine belatacept serum concentrations was recently developed [144]. The authors found reduced peak concentrations of belatacept in patients with a lower bodyweight [144]; however, whether this reduced exposure to belatacept leads to an increased risk of AR is unknown, but is certainly a reason for further investigation (Fig. 3).

Belatacept is currently administered every 4 weeks in the maintenance phase. In a 10-year follow-up study, the belatacept 4-weekly regimen was compared with administration of belatacept every 8 weeks [145]. After 10 years, kidney function and the risk of allograft loss or death was similar between the two groups; however, the risk of AR was higher in patients who received belatacept every 8 weeks [2]. With further investigation, such as the above-mentioned measurement of belatacept serum concentrations, the 8-weekly dosing regimen could offer logistical advantages in subgroups of KTRs in the future.

Early recognition and treatment of AR leads to less allograft damage; therefore, minimally invasive screening for (preclinical) AR in KTRs could lead to better allograft survival (Fig. 3). Potential biomarkers for minimally invasive screening of AR in blood or urine are now entering the clinic [91, 107, 146]. Applying these minimally invasive screening tools to belatacept-treated patients could be a way to expand the use of belatacept.

To conclude, targeting the costimulation pathway is a complex but exciting task. Belatacept is a promising immunosuppressive therapy for KTRs, but a more tailored approach in the selection of patients, treatment protocol, and post-transplant monitoring is necessary to expand the use of belatacept.

Compliance with Ethical Standards

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