Costimulation Blockade in Kidney Transplantation: An Update

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Abstract: In the setting of solid-organ transplantation, calcineurin inhibitor (CNI)-based therapy remains the cornerstone of immunosuppression. However, long-term use of CNIs is associated with some degree of nephrotoxicity. This has led to exploring the blockade of some costimulation pathways as an efficient immunosuppressive tool instead of using CNIs. The only agent already in clinical use and approved by the health authorities for kidney transplant patients is belatacept (Nulojix), a fusion protein that interferes with cytotoxic T lymphocyte-associated protein 4. Belatacept has been demonstrated to be as efficient as cyclosporine-based immunosuppression and is associated with significantly better renal function, that is, no nephrotoxicity. However, in the immediate posttransplant period, significantly more mild/moderate episodes of acute rejection have been reported, favored by the fact that cytotoxic T lymphocyte-associated protein pathway has an inhibitory effect on the alloimmune response; thereby its inhibition is detrimental in this regard. This has led to the development of antibodies that target CD28. The most advanced is FR104, it has shown promise in nonhuman primate models of autoimmune diseases and allotransplantation. In addition, research into blocking the CD40-CD154 pathway is underway. A phase II study testing ASK1240, that is, anti-CD40 antibody has been completed, and the results are pending.
CTLA-4 (CD152), may prevent maturation of deleterious effectors while also preserving regulatory T (Treg) cell function. Recent data from nonhuman primates indicate this. Finally, the blockade of the CD40/CD40L pathway may also be a useful approach, although few data are available from humans.

**Targeting Costimulation Pathways**

Linsley et al\(^{10}\) described, in 1990, the CD28 molecule on T lymphocytes (T cells) and its corresponding ligand on antigen-presenting cells (APCs), that is, CD80/CD86. The CTLA-4 was identified in 1987 on activated cytotoxic T lymphocytes,\(^{11}\) but it was not until 1991 that it was shown that both CD28 and CTLA-4 share the same ligand on APCs\(^{12}\); however, CTLA-4 binds to its ligand with a much higher avidity than does CD28. In 1995, it was shown that CTLA-4 had a negative regulatory effect on T cell activation.\(^{13}\)

The CD28 molecule is constitutively expressed on naive T cells and provides, besides T cell receptor-generated signal 1, a costimulation signal that is crucial for T cell proliferation via IL-2 secretion and for survival via Bcl2-Bclx. In addition, CD28 lowers the T cell activation threshold, that is, the number of interactions between the T cell receptor and the major histocompatibility complex–bound presented peptides are lowered to activate T cells.\(^{14}\) Upon T cell activation, CTLA-4 becomes rapidly expressed on T cell surfaces, delivering its inhibitory signal and thereby decreasing membranous expression of CD28, which ultimately modulates the immune response.\(^{15}\) However, this very simplistic model has been found to be more complex because of the many other costimulatory pathways between molecules on the surface of T cells and their ligands on APCs, resulting in the production of stimulating and/or inhibitory transducing signals (the cell surface signaling molecules).\(^{16,17}\) The expression of these molecules on both sides of the immunological synapse varies according to the type/subtype of cells, to their degree of activation, to their location within the immune system, and their intertwined regulation loops.\(^{16}\) Thus, with regard to the costimulatory pathways, the importance of a balance between CD28/CD80-86/CTLA-4 for all given cells varies according to subtype, to polarity, and the degree of differentiation. It also depends on integration of all the intracellular signals after interactions between the numerous cell surface signaling molecules, without taking into account soluble factors, such as cytokines.

However, CD28/CD80-86/CTLA-4 may interfere with other cell surface signaling molecules. Recently, Freeman\(^ {18}\) demonstrated that there was a bidirectional interaction between PD-L1, that is, a ligand of PD1, which is (apart from CTLA-4) the principal inhibitory molecule induced after T cell activation, and CD80, with both occurring in humans\(^ {19}\) and mice.\(^ {20}\) PD-L1’s affinity for CD80 is in between that of CD28 and that of CTLA-4. This functional interaction results in decreasing both cell proliferation and cytokine production.\(^ {21}\) In nonobese diabetic mice, its specific blockade exacerbates diabetes, when already present.\(^ {22}\) In humans, another interaction has been shown, that is, that of inducible costimulator-ligand (ICOS-L) with CD28 and CTLA-4.\(^ {23}\) ICOS is the main costimulation molecule induced after activation of T cells. In vitro, ICOS-L’s interaction with CD28 is functional, that is, this interaction is essential for costimulation of human T cells’ primary response to allogeenic antigens and memory-recall responses.\(^ {23}\) Therefore, when one takes into account the costimulation blockade, we have 5 players to deal with, that is, CD28, CD80/86, CTLA-4, PD-1L, and ICOS-L (see Figure 1). Apart from these 5 main players in the costimulation pathway, we also have to take into account the CD40-CD40L (CD154) pathway.

In this review, we first examine the blockade of the CTLA-4/CD80/86 pathway, then that of CD28/CD80-86, and finally that of CD40–CD40L.

**Blockade of CTLA-4/CD80/86**

CTLA-4 binds to its ligands with much higher avidity than does CD28.\(^ {24}\) Experimental data suggest that CTLA-4-Ig (abatacept), which is formed from a fusion protein composed of a Fc fragment of human IgG1 immunoglobulin linked to
an extracellular domain of CTLA-4, does not completely block the B-7-mediated response in vivo. The IgG Fc portion is mutated to prevent activation of the complement, and so prevents subsequent cell death upon binding. Subsequently, a high-avidity mutant for CD80 and CD86 (LEAY29; belatacept) was produced, which differs by only 2 amino acids from abatacept, but results in 10-fold more potent inhibition of T cell activation in vitro.

Belatacept, along with its precursor abatacept, is the only costimulation blocking molecules that have received approval for clinical use.

Abatacept was initially tested in clinical psoriasis studies and was then tested to treat many autoimmune diseases; it was finally approved for the treatment of rheumatoid arthritis and juvenile arthritis. In transplantation, it has proved less effective in nonhuman primate models, probably because of its undercapacity to completely block CD86. Belatacept was thus developed to further reinforce the immunosuppressive properties of the CTLA4-Ig construct (see Figure 2).

Experimental work with these molecules in animal models of transplantation has been conducted with the hope that they result in tolerance, the holy grail of all transplant clinicians. Short courses of CTLA-4-Ig have induced long-term survival of human pancreatic islets in mice, prolonged cardiac allograft survival in rats and, when combined with low-dose cyclosporine, they have induced indefinite engraftment of kidney allografts in rats.

Some key facts have emerged from these and other experiments: (i) CTLA-4 expression on T cell surfaces needs cell activation, thus CTLA-4-Ig is more effective if its administration is delayed until after transplantation; (ii) blocking either CD80 or CD86 selectively does not achieve immunosuppression; and (iii) CTLA-4-Ig is incapable of reversing acute rejection episodes. These and other published results have encouraged scientists and clinicians to develop clinical trials in the transplant field that involve these novel molecules.

The first phase II study involving belatacept and kidney transplantation was published in 2005. Patients were randomized to receive either a more intensive belatacept therapy, a less intensive belatacept, or a standard cyclosporine therapy. All groups received a basiliximab induction therapy, plus mycophenolate mofetil and steroids. The results were impressive because, for the first time, an immunosuppressive regimen without CNIs achieved the same results in terms of graft and patient survival rates, kidney function, and biopsy-proven acute rejection rate when compared with a classical cyclosporine-based treatment.

Five years later, the first year results from phase III studies were published: the BENEFIT study compared regimens (belatacept less intensive [LI]; belatacept more intensive [MI], and cyclosporine) in immunological low-risk patients that had received a living or a standard criteria donor kidney. The BENEFIT-EXT study did the same, but patients received extended-criteria donor kidneys. Both clinical trials demonstrated better kidney function in the arms treated by belatacept compared with cyclosporine. This phenomenon remained true even though there was a higher incidence of biopsy-proven acute rejection in belatacept-treated patients from the BENEFIT study, particularly in the belatacept MI arm. An important side effect was at this point recognized, that is, an increased number of posttransplant lymphoproliferative disorders, some of which affecting the brain. This leads in some cases to patient death. It was finally realized that almost all of the fatal posttransplant lymphoproliferative disorder cases were of patients whose pretransplant Epstein-Barr virus (EBV) serology was negative, whereas the donor had been EBV-seropositive, and that the disease corresponded to a primary EBV infection. For this reason, belatacept use is not approved in EBV-negative kidney transplant patients.

![FIGURE 2. Interaction of belatacept with costimulation molecules.](image)
The BENEFIT and BENEFIT-EXT cohorts were followed up until recently, for as long as 7 years (86 months) posttransplantation, such a long-term follow-up is never the case with phase III studies in organ transplantation. The recently published results of the BENEFIT follow-up study are quite impressive. Strikingly, the BENEFIT study showed significantly better graft and patient survival rates for those who were treated with belatacept, whereas, within the BENEFIT-EXT study, patient and graft survival rates were similar across the 3 groups. However, more interestingly, kidney function (estimated glomerular filtration rate [GFR]) was improved over time in the 2 studies. It was particularly striking in the BENEFIT study, that is, +0.20 mL per min per 1.73 m\(^2\) per year in the MI regimen, +0.38 mL per min per 1.73 m\(^2\) per year in the LI regimen, but −1.92 mL per min per 1.73 m\(^2\) per year in cyclosporine regimen. There is no clear explanation for that, this might be related to some extent to a certain growth of the transplanted kidney.

The other positive finding was the very low incidence of donor-specific antibodies (DSAs) in belatacept-treated patients: only 4.6% in the LI group, 1.9% in the MI group, and 17.8%, in the cyclosporine group at 86 months.

Although these are promising results, they must be viewed in perspective. First of all, belatacept was compared with a cyclosporine regimen and not the more modern low-dose tacrolimus regimen that was highlighted as being the most effective in the ELITE-Symphony study. No convincing data are available that have compared belatacept with tacrolimus, but it must also be said that no tacrolimus-based regimen has ever shown time-based improvement of estimated GFR.

Whereas belatacept is administered intravenously on a monthly basis, cyclosporine is administered orally on a daily basis. Thus, treatment nonadherence is easily discovered in belatacept patients, but can only be presumed for those on cyclosporine. This could explain the lower incidence of DSAs in belatacept groups, because nonadherence has been associated with de novo DSA development. Conversely, by inhibiting costimulatory pathways, belatacept might prevent T cell-mediated B cell help, thereby preventing DSA formation.

Belatacept has been used in steroid avoidance protocol in de novo kidney transplant recipients. In a 1-year, randomized, controlled, open-label, exploratory study, 2 belatacept-based regimens were compared with a tacrolimus (TAC)-based, steroid-avoiding regimen. Eighty-nine recipients were randomized 1:1:1 to receive belatacept-mycophenolate mofetil (MMF), belatacept-sirolimus, or TAC-MMF. All patients received induction with 4 doses of thymoglobulin (6 mg/kg maximum) and an associated short course of corticosteroids. Acute rejection rates were similar across the 3 groups. More than two thirds of patients in the belatacept groups remained on CNI- and steroid-free regimens at 12 months and the calculated GFR was 8 to 10 mL/min higher with either belatacept regimen than with TAC-MMF. Overall safety was comparable between groups.

Recent single-center studies have reported on the usage of belatacept in higher risk patients. Gupta et al reported their experience with high immunological risk recipients who had been switched from tacrolimus to belatacept. Four of their 6 patients had DSAs at the time of transplantation, the other 2 had greater than 80% panel-reactive antibodies. Although all had biopsy-proven acute rejection, none developed de novo DSAs after the switch, and all had improved kidney function. Although these are small single-center experiences, they raise hope for the use of belatacept to improve kidney function and prolong kidney graft survival, even in this high-risk population.

Switching from CNI regimens to belatacept has also been partially explored. A phase II trial was published in 2011 in which patients that had undergone kidney transplantation at 6 to 36 months earlier were randomized to receive either belatacept or were maintained on CNI-based immunosuppression. The results showed a significant benefit to kidney function in the belatacept group at 1 year after the switch. Six of the 84 belatacept-treated patients experienced an acute rejection, whereas there were none within the CNI group, however, these episodes were moderate and, at 1 year, no patient had experienced a chronic rejection or lost their kidney. A phase IIB study with the same design is underway (EudraCT number: 2012-001314-42).

In another recently published French study, patients were switched from CNI to belatacept during the first 6 months posttransplantation because of either prolonged delayed graft function, suspected CNI toxicity, or clinical intolerance to CNI. This retrospective descriptive observational study involved 25 patients whose renal function had deteriorated. At 1 year posttransplantation, 20 patients still had a functioning graft, thus showing that the switch probably helped maintain kidney function while also avoiding potentially nephrotoxic doses of CNI.

Another single-center study tried to demonstrate the feasibility of maintenance belatacept as a monotherapy. This strategy could be extremely useful for patients that have treatment adherence problems, because a monthly intravenous injection is easily traceable and widely accepted by patients. Twenty living-donor kidney transplant recipients were enrolled and were initially induced with alemtuzumab. Belatacept and sirolimus were then introduced, with an attempt to wean off sirolimus at 1 year posttransplantation in 10 of these patients (of the other 10, 7 declined and 3 were considered not clinically fit). Of the 10 who attempted stopping sirolimus, this strategy was successful in 7, demonstrating the feasibility of this approach in selected patients. No patients received steroids in this trial.

In 2014, Masson et al published a systematic review on the efficacy of belatacept in kidney transplant patients. They concluded that there was no evidence of any difference in the effectiveness of belatacept and CNI in preventing acute rejection, graft loss, and death, but treatment with belatacept was associated with less chronic kidney scarring and better kidney transplant function. Treatment with belatacept was also associated with better blood pressure and lipid profile and a lower incidence of diabetes versus treatment with a CNI. They concluded that “longer-term, fully reported and published studies comparing belatacept versus tacrolimus are needed to help clinicians decide which patients might benefit most from using belatacept.” However, the 7-year results of the BENEFIT trial challenge this conclusion.

To summarize, belatacept-based immunosuppression in de novo kidney transplant patients is very efficient in the long-term and even superior to cyclosporine-based immunosuppression, even though there can be relatively high numbers of early episodes of acute but easily reversible cellular
rejections. In addition, this approach is not nephrotoxic and may prevent DSA formation. Very limited data also show that belatacept-based immunosuppression can be used in highly sensitized patients and in steroid-free protocols. A phase IIIb trial on the conversion from CNI-based immunosuppression to belatacept-based immunosuppression is underway.

Selective Blockade of CD28

It has been difficult to generate monoclonal antibodies against CD28 that lack some level of agonist activity, whether it is by acting in concert with TCR activation or, in the most extreme case, an antibody that can stimulate T cells directly, as was the case with the CD28 super-agonist TGN1412 (see below).

Anti-CD28 Divalent Antibodies

One of the drawbacks of CTLA-4 Ig is that it also blocks CTLA-4 as well as CD28. Thus, there is major interest in exclusively blocking CD28, thus leaving CTLA-4 functionally active. The major problem of antagonizing CD28 selectively is derived from the characteristics of the anti-CD28 divergent antibodies, because they activate T cells with or without necessitating T cell receptor activation.

Anti-CD28 Super-Agonist Antibodies

The potential interest in these anti-CD28 super-agonistic (SA) antibodies is because they may help in vivo and in vitro Treg cell proliferation/expansion, with potential implications for autoimmune diseases. In murine models, this property helps prevent acute rejection as well as controlling autoimmunity: the SA CD28 antibody is able to induce donor-specific tolerance in rat renal allografts, and can ameliorate crescentic glomerulonephritis in Wistar-Kyoto rats.

An SA monoclonal antibody specific to rat CD28 (JJ316) expands and activates Treg cells in vivo and also in short term in in vitro culture. Very low dosages of this CD28 super-agonist given to normal Lewis rats was sufficient to induce Treg cell expansion in vivo without the generalized lymphocytosis observed with high dosages of JJ316. A single intravenous administration of low-dose CD28 SA into Dark Agouti rats or Lewis rats that suffered from experimental autoimmune encephalomyelitis was highly and as equally efficacious as a high-dose treatment. In humans, TGN1412, a SA anti-CD28 monoclonal antibody that directly stimulates T cells, has been developed.

In a phase 1 trial (6 volunteers), within 90 minutes of receiving a single intravenous dose of TGN1412, all had a systemic inflammatory response characterized by rapid induction of proinflammatory cytokines accompanied by headache, myalgia, nausea, diarrhea, erythema, vasodilatation, and hypotension. Within 12 to 16 hours after infusion, they became critically ill, with pulmonary infiltrates and lung injury (resulting in prolonged cardiovascular shock and acute respiratory distress syndrome in 2 patients), renal failure, and disseminated intravascular coagulation. In addition, severe and unexpected depletion of lymphocytes and monocytes occurred within 24 hours after infusion. After receiving intensive cardiopulmonary support (including dialysis), high-dose methylprednisolone, and an anti-IL-2 receptor antagonist antibody, they all survived. The severity of the adverse response to TGN1412 correlates with the level of IL-2 release. These side effects had not been anticipated in the preclinical primate model because of the differences in the threshold activations of primate and human lymphocytes. This is related to the loss, within the human evolutionary process, of inhibitory signaling molecules, that is, CD-33-related Siglecs, which are expressed on most immune cells and downregulates the cellular activation pathways via the cytosolic immunoreceptor tyrosine-based inhibitory motifs. The specific loss of human T cell Siglec expression thereby resulted in T cell hyperactivity.

Conventional Anti-CD28 Antibodies

The agonist properties of conventional divalent anti-CD28 antibodies are related to dimerization (crosslinking) of CD28 molecules at the lymphocyte surface. In the hypothesis where activation is dependent on Fc-receptor engagement, some “silent” anti-CD28 antibodies (ie, vis-à-vis the Fc receptor) have been developed; however, they retain (at a lower level) their agonist properties: this is the case for FK734, a humanized version of a mouse antihuman CD28 monoclonal antibody. In vivo, in rodent models, one of these antibodies has demonstrated that it may prevent graft-versus-host disease (GVHD). In a rat model, the use of the murine antirat Jj319 anti-CD28 antibody had modulatory properties: in vivo, it induced decreased expression of CD28 at the T cell surfaces, that is, it acted as a functional antagonist. However, so far, no antihuman anti-CD28 antibody has been found to have these properties.

Monovalent Anti-CD28 Antibodies

Because of the abovementioned hurdles regarding divalent anti-CD28 antibodies, monovalent anti-CD28 antibodies have been developed, that is, to block CD28. These monovalent anti-CD28 antibodies are monovalent Fab fragments of conventional monoclonal anti-CD28 antibodies. They do not crosslink with the CD28 molecule and are capable, in vitro, of inducing anergy. The major problem with these molecules, in vivo, is their very short half-lives. In a murine model of autoimmune encephalitis, Fab fragments from the PV1 clone anti-CD28 antibody were demonstrated to be efficient. Recently, the team of Vanhove et al developed a fusion molecule (sc28AT), consisting of a monovalent nonactivating human CD28-specific single-chain Fv antibody fragment from a high-affinity anti-human CD28 antibody (CD28.3) and human α1-antitrypsine to increase half-life. This sc28AT antibody is a CD28 antagonist: it inhibits T cell proliferation and T cell cytokine secretion. In suppressive tests, sc28AT did not interfere with Treg cell activity, as opposed to anti-CTLA-4: thus, selective CD28 blockade preserves CTLA-4–induced suppressive activity at the Treg cell level.

In a baboon kidney transplant model, sc28AT, given as a monotherapy, had marginal effects compared with the control therapy. Conversely, when sc28AT was associated with tacrolimus (as compared with tacrolimus as a monotherapy), it efficiently prevented acute rejection, even after treatments were stopped. In this model with regards to Treg cells, their numbers were significantly increased in the peripheral blood of the sc28AT/tacrolimus group compared to the controls. In addition, at 1 and 3 months posttransplantation, in kidney biopsies, the percentage of Foxp3+ CD3+ cells was significantly increased in the sc28AT group. In a model of heart allografts, sc28AT plus cyclosporine A was able to prevent chronic vasculopathy.
Similar results have been obtained with a nonactivating single-chain Fv-based reagent (α28scFv; antimouse anti-CD28) in a heart allograft model; when α28sc-Fv was used as a monotherapy for 2 weeks, median graft survival was 27 days compared with 9 days with a placebo. When α28scFv was associated with cyclosporine A or anti-CD154 (MR1), graft survival was indefinite (>100 days), and there was also a lower incidence and lower severity of allograft vasculopathy compared with controls. Moreover, in these 2 models, at postoperative day 10, the percentages of circulating CD4+ Foxp3+ cells were significantly increased when compared with the controls.

To improve the pharmacokinetic profile of sc28AT, Vanhove’s team has developed a new molecule, FR104, which consists of Fab’ (VH and VL variable domains of humanized CD28.3) and a polyethylene glycol fraction. This pegylation does not alter the binding capacities of CD28 but, conversely, it significantly increases its half-life, that is, 33.6 versus 1.5 hours. In vitro, FR104 inhibits T cell proliferation in a mixed lymphocyte reaction, and also IL-2 secretion, with both occurring in a dose-dependent manner. FR104 has also been evaluated in a murine model of GVHD. Without treatment, GVHD occurred within a week; when FR104 was administrated twice a week from day 0 to day 25, there was indefinite prevention of GVHD. In that model, coadministration of anti–CTLA-4 completely abrogated the effect of FR104. Weekly belatacept administration was partially efficient; in contrast, when belatacept was administrated twice a week, it had no effect on preventing GVHD.

FR104 has been used with success in rhesus monkey in a model of collagen-induced arthritis with potential applications in humans to treat rheumatoid arthritis. In addition, Haanstra et al have shown that FR104 could protect rhesus monkeys against acute fatal experimental autoimmune encephalomyelitis. Finally, Poirier et al showed that FR104 prevented alloimmunization and allowed minimization of CN1 therapy in a nonhuman primate renal allograft. FR104 reinforces immunosuppression in protocols that are low or free of CNIs, without the need for steroids. Accumulation of intragraft Treg cells suggests the promotion of immunoregulatory mechanisms.

Recently, another murine anti-CD28 antagonist antibody has been developed: it was compared with CTLA4-Ig in a murine skin-allograft model. In the setting of incompatibility of the major histocompatibility complex, this antibody prolonged skin allograft survival to longer than 30 days compared with a median survival of 30 days with the anti-CD40L antibody plus CTLA-4 Ig. When this antibody was used in the setting of a minor incompatibility of the major histocompatibility complex, it prolonged skin allograft survival to longer than 100 days compared with 32 days for CTLA4-Ig. In the graft cell infiltrates, specific CD4+ T cells and CD8+ T cells were less numerous and less differentiated in the anti-CD28 group compared with the controls.

An analog molecule specific of human CD28 has been developed, that is, anti-CD28 receptor antagonist V6L L chains. It has been combined with a 40-kDa branched pegylation to improve the pharmacokinetics. Both in vitro and in vivo, it has been demonstrated that this pegylated anti-CD28 antibody had no agonistic properties but, conversely, did have antagonistic properties, both in vitro and in vivo, in a macaque model of keyhole limpet hemocyanin immunization. In summary, the blockade of CD28 pathway with superagonistic antibodies resulted in T cell activation and cytokine release. Conversely, the use of monovalent anti-CD28 antibodies is safe, that is, it only has CD28 antagonistic properties. The most advanced antibody is FR104, and a phase I trial is underway. Phase II trials may target patients with autoimmune diseases, for example, rheumatoid arthritis and/or kidney transplant recipients.

**CD40-CD40L Pathway**

CD40 and CD40 ligands (CD40L, also known as CD154) are members of the TNF-related pathways. On CD40 ligation by CD40L, intracellular activation of transcription factors occurs through various TNF receptor-associated factors. The canonical signal is mediated by NF-κB, through TNF receptor-associated factors 1 and 2. CD40 is found on APCs, whereas CD154 is found on T lymphocytes. However, T cells also express CD40, especially CD8+ T cells.

In B cells, CD40 ligation leads to B cell clonal expansion, affinity maturation toward the B cell-specific antigen, and the generation of long-lived plasma cells. Dendritic cells also receive an activation signal via CD154, from helper T cells through CD40, which allows upregulation of CD80-86 expressions on their surfaces. These activated dendritic cells can, in turn, present an antigen with a costimulation signal, leading to rejection. CD8 and T lymphocytes (T-CD8) were also shown to express CD40. T-CD8 is cytotoxic and can destroy cells that present their cognate antigen by secreting perforin; thus, helper T cells also interact with T-CD8 through the CD40/CD154 costimulation pathway.

The importance of the CD40/CD154 pathway within the immune response has led to research into the various CD40 and CD154 inhibitors. The rationale behind these inhibitors is immunomodulation, immunosuppression, or even induction of tolerance.

The CD40L antibody’s ability to block its effects on APC is the focus of intense research. Two main antibodies have been the foci of most of this research, namely, hu5C8 and IDEC-131.

hu5C8 is a human antibody that targets the 5C8 complementary determining region of CD154. It has been used in combination with CTLA4-Ig to prevent kidney allograft rejection in a nonhuman primate model (rhesus monkeys). An induction treatment with these drugs prolonged graft survival with a synergistic effect from both drugs. Interestingly, using this combined therapy of CTLA4-Ig and hu5C8 after a rejection episode also restored normal graft function. In the same rhesus model, hu5C8, used as a monotherapy, also improved graft survival, but did not prevent the development of antibodies directed toward the transplanted organ; however, this was 10 months after discontinuing the drug. Of note, the use of tacrolimus or steroids prevented the development of this antirejection effect.

IDEC-131 is another anti-CD154 antibody. In a rhesus monkey model of skin transplantation, a triple therapy of IDEC-131, sirolimus, and a pretransplant donor-specific transfusion induced long-term allograft survival, but failed to prevent the development of donor-specific antibodies. In the same experimental model, this triple therapy prevented kidney rejection (during therapy) in all tested animals. It also induced operational tolerance in 3 of the 5 tested animals.
This tolerance was powerful enough to allow acceptance of a donor-specific skin graft.

However, thromboembolic events caused by expression of CD40L on platelets has limited research on these CD40L antibodies, although these thromboembolic events could be prevented by the usual anticoagulation therapies. Another concern with using the CD154 blockade is the failure to develop a proper antibody response against viral antigens.

In contrast, targeting CD40 to prevent APC costimulation also leads to immunomodulatory effects without the reported thromboembolic events. Various approaches are under investigation. In vitro–synthesized inhibitory CD40 antibodies are under development, with ASKP1240,6–8 ASKP84–86 2C10R4,87 and chi220.88–90 Targeting CD40 can also be achieved with small interfering RNA directed toward CD40 mRNA.91,92 Finally, gene transfer of CD40Ig fusion proteins is also being explored, with contrasting results.93,94

ASKP1240 (also known as 4D11) is an inhibitory CD40 antibody developed by Astellas: a phase II trial is completed, but results are not yet available. It is a fully human IgG4 monoclonal antibody. In a nonhuman primate model of kidney transplantation, a 10-week induction course of ASKP1240 increased graft survival without a dose-dependent effect (graft survival >100 days in the treatment group vs 6 days mean survival in the control group). A 4-week induction course gave the same survival results. B cells were depleted to one third of preoperative values.76 Subsequently, the same team compared a 2-week induction course of ASKP1240 with the same induction therapy plus 6 months of maintenance therapy. In both cases, ASKP1240 dose ranged from 1 to 20 mg/kg. Both schemes increased graft survival from a mean survival of 6 days in the control group to a mean of greater than 100 days in the treatment groups. Donor-specific antibodies appeared in most animals in the induction-only group, whereas a dose of 10 or 20 mg/kg in the maintenance group prevented DSA development. Histological analysis showed borderline changes only, without rejection, in all animals in the maintenance group that received ASKP1240 at doses of 10 mg/kg or greater. However, no long-term tolerance was achieved using these protocols, and all grafts eventually failed.77

In a nonhuman primate model of liver transplantation, the same authors then compared a 2-week induction therapy (with 10 mg/kg ASKP1240) with the same induction plus 6 months of maintenance therapy. Both schemes increased graft survival when compared with the controls, with greater survival in the maintenance group. T cell depletion was significant. ASKP1240 prevented DSA development during the course of treatment.78 Similar results have been obtained for pancreatic islet allografts in nonhuman primates.79 The association of ASKP1240 with tacrolimus or mycophenolate mofetil further increased kidney allograft survival in the same nonhuman primate model.80 In addition, good correlations were found between the pharmacokinetic and pharmacodynamic parameters.81 Specific monitoring for potential prothrombotic effects, both in vitro and in vivo, showed no thromboembolic complications using ASKP1240.82 A phase I clinical trial showed good tolerance and no thromboembolic events in healthy human subjects.83 Taken together, these results make ASKP1240 the best CD40 antibody candidate for clinical use within the near future.

3A8 is a CD40 mouse IgG2b monoclonal antibody. In a nonhuman primate bone marrow chimerism induction model, it showed prolonged engraftment when 3A8 was used together with CTLA4-Ig and sirolimus.84 It was then tested in a nonhuman primate model of pancreatic islet transplantation. 3A8 was given at a dose of 3 mg/kg for the first 35 days posttransplantation. 3A8 inhibited T cell alloreactivity but, importantly, it did not deplete B cells. 3A8 alone, or basiliximab and sirolimus alone, did not increase graft survival. However, the association of 3A8 with basiliximab and sirolimus did increase graft survival well beyond the first 35 days until sirolimus removal.85 The same team then compared the same triple immunosuppressive regimen with or without CTLA4-Ig. The addition of CTLA4-Ig prevented DSA formation but did not significantly change allograft survival (there was a >1-year follow-up).86 2C10R4 is another CD40 monoclonal antibody. It was used in a nonhuman primate model of antibody-mediated kidney rejection. In the longer than 6-month follow-up, 2C10R4 showed similar results in terms of graft survival and DSA development when compared with CTLA4-Ig therapy. Investigations into the mechanism of action of 2C10R4 showed regulation of follicular T cells, which inhibited the B cell isotype switch.87

These antibodies have varying degrees of depleting capacity: some induce profound B cell depletion, whereas others do not impact on B cell number. Because they all offer some immunosuppressive capacity, B cell depletion is not the only pathway through which CD40 antibodies work.

To summarize, with regard to CD40-CD40L pathway blockade, those antibodies that target CD40L have been abandoned due to the occurrence of thromboembolic complications. Conversely, many anti-CD40 antibodies have been developed. The phase II results of one of these (ASPP1240) are awaited.

In conclusion, in the clinic for kidney transplant patients, we have belatacept, a fusion protein with the capacity to block CD28/CTLA-4/CD80-86 pathways efficiently, resulting in very good results in the long term (patient/graft survival, excellent renal function). However, belatacept does not only block CD28, it also blocks CTLA-4, thereby increasing the rate of early episodes of acute cellular rejections, an observation made in pivotal studies of belatacept-based immunosuppression. One alternative strategy would be to block specifically CD28, leaving the CTLA-4 pathway functional. The most advanced anti-CD28 monoclonal antibody in the clinic is FR104. Results in nonhuman primate models make it very promising in the setting of autoimmune diseases, such as rheumatoid arthritis, and in experimental kidney transplantation. The blockade of CD40-CD40L pathway mostly relies on anti-CD40 antibodies, some of which resulting in B cell depletion. Phase II results of one of these are awaited. Finally, the ultimate aim of these costimulation blockade antibodies is to achieve efficient immunosuppression in the setting of kidney transplantation without using CNIs because of the many side effects of the latter.

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