Clinical Nephrology, Vol. 73 – No. 5/2010 (333-343)

Review

ISSN 0301-0430

Novel immunosuppressive agents in kidney transplantation

J.E. Cooper and A.C. Wiseman

University of Colorado – Denver and Health Sciences Center, Transplant Center, Aurora, CO, USA

Key words
kidney transplant – immunosuppression – clinical trials-review

Abstract. The last several decades have seen a substantial decrease in the prevalence of acute allograft rejection in kidney transplant recipients, while equivalent improvements in long-term graft function have not been realized. As a result, the primary focus of new immunosuppressive drug development has expanded to include ease of use, improved side effect profiles, and reduced nephrotoxicity in addition to the more traditional goal of improved short-term outcomes. A number of novel drugs are currently under investigation in Phase I, II, or III clinical trials primarily to replace the nephrotoxic but highly effective calcineurin inhibitors. ISA247 (voclosporine) is a cyclosporine (CsA) analog with reduced nephrotoxicity in Phase III study. AEB071 (sotrastaurin), a protein kinase C inhibitor, and CP-690550, a JAK3 inhibitor, are small molecules in Phase II studies. Everolimus is derived from the mTOR inhibitor sirolimus and is in Phase III study. Belatacept is a humanized antibody that inhibits T-cell costimulation and has shown encouraging results in multiple Phase II and III trials. Alefacept and Efaluzimab are humanized antibodies that inhibit T-cell adhesion and are in Phase I and II clinical trials. This article reviews the mechanisms of action as well as published and preliminary results of the Phase I – III clinical trials involving these novel immunosuppressive agents.

Received
May 29, 2009;
accepted in revised form
November 6, 2009

Correspondence to
A.C. Wiseman, MD
University of Colorado – Denver and Health Sciences Center,
Transplant Center,
1635 N Aurora Court,
AOP 7089, MS F749,
Aurora, CO 80045,
USA
Alexander.wiseman@ucdenver.edu

Introduction

The introduction of the calcineurin inhibitors (CNIs) cyclosporine (CsA) and tacrolimus together with the development of antiproliferative agents such as mycophenolate mofetil (MMF) and antibody induction agents, has resulted in vast improvements in acute rejection (AR) rates and short-term graft survival over the last three decades in patients receiving kidney transplants [10]. While this progress initially was predicted to lead to a corresponding improvement in long-term graft function from the late 1980's to the mid 1990's [23], a more recent analysis has shown a lack of improvement in the relative risk of graft failure for those transplanted in 1995 through 2000 despite a reduction in acute rejection rates of nearly 50% during that time [35]. One potential explanation for this lack of improvement in long-term graft survival is the nephrotoxicity imparted by CNIs over time. This hypothesis is supported by histological data obtained from protocol biopsies of kidney-pancreas recipients over 10 years that identified chronic CNI nephrotoxicity in 50% of grafts at 2 years and in 100% of grafts at 10 years post-transplant [41].

Despite the overt nephrotoxicity of CNIs, these agents remain the cornerstone of maintenance immunosuppression regimens due to their efficacy in preventing acute rejection. According to the Scientific Registry of Transplant Recipients, CNIs are used in 95% of immunosuppression protocols at the time of hospital discharge after kidney transplantation in the United States. However, as the focus of immunosuppression has shifted away from further reduction in the incidence of AR and towards preservation of long-term function, a significant number of novel immunosuppressive agents are undergoing development as a replacement for CNIs. In this article we review the mechanisms of action, preclinical, and Phase I – III completed or ongoing clinical trials of 7 novel immunosuppressive agents. While not comprehensive of all drugs currently in development [64], these agents were selected for review based upon their common features of a similar immune cell target (the T-lymphocyte) and a similar focus in drug development (CNI minimization or elimination).

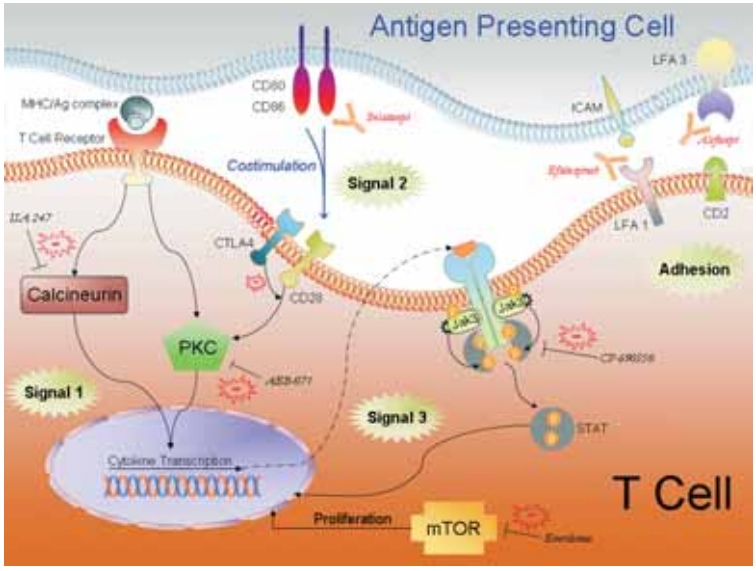

Figure 1. 3-signal model of T-cell activation. Signal 1 is initiated with the presentation of antigen by HLA molecules located on antigen presenting cells (APCs) to the T-cell receptor. Several downstream signal transduction pathways including calcineurin are activated, resulting in transcription factor activity and protein production. Signal 2 is a result of CD 80 and CD86 on APCs binding to CD28 on T-cells leading to costimulation. Signal 2 serves to enhance the Signal 1 response by further augmenting transcriptional activity and cytokine production, in part by protein kinase C activation. Signal 3 leads to cell proliferation and is a result of both cytokines binding to receptors on T-cells with subsequent activation of the JAK/STAT pathway as well as mTOR activity. T-cell adhesion to APCs involves LFA-1 on T-cells and LFA-3 on APCs. Novel immunosuppression agents interfere at various steps within the T-cell activation and adhesion pathways and are shown according to their mechanism of action.

The 3-signal model of alloimmune recognition

In this review, novel immunosuppressive agents are organized according to their mechanism of action. In order to understand the inherent differences between these agents it is practical to briefly review the signaling events that comprise the alloimmune response (Figure 1). Donor antigens are recognized and presented to T-lymphocytes by antigen-presenting cells (APC) from either donor (direct pathway) or host (indirect pathway) origin. "Signal 1" is initiated when the major histocompatibility complex/antigenic peptide molecule present on the APC engages the T-cell receptor leading to T-cell activation via the CD3 complex. This leads to induction of several signal-transduction pathways, including the calcium-calcineurin pathway [71]. Signal 2 is a costimulatory event that serves to amplify the initial activation event. One such APC/T-cell costimulatory signal that has been well described is the T-cell CD28 and CD40L interaction with APC CD80/86 and CD40, respectively [12]. Signaling events that result from Signals 1 and 2 lead to transcription factor production and subsequent expression of IL-2 and IL-15. These cytokines, as well as others, activate the mammalian target of rapamycin (mTOR) via PI3K and, along with JAK/STAT signal transduction pathways, propagate the lymphocyte cell cycle. Together with de novo nucleotide synthesis, this last step results in cell proliferation and is termed "Signal 3". Clonal expansion of lymphocytes ensues, with infiltration of the allograft resulting in the inflammatory response seen in acute graft rejection.

Signal 1 blockade: ISA247 (voclosporin)

ISA247, also termed voclosporin, is a semisynthetic structural analog of CsA that results in immunosuppression by inhibition of the calcineurin signal transduction pathway. Voclosporin is structurally similar to CsA with the exception of a modification at the amino acid-1 functional group. This has resulted in a small molecule that offers both more robust binding to calcineurin, as well as faster elimination of metabolites [2]. This novel calcineurin inhibitor is under investigation for the treatment of psoriasis as well as post-renal transplant immunosuppression. If indeed voclosporin could provide immunosuppressive efficacy with less drug and metabolite exposure, the drug may be more predictable with less intrapatient variability than CsA and tacrolimus and thus decrease the potential for nephrotoxicity.

Several in vitro experiments as well as in vivo animal studies of voclosporin have supported these pharmacologic predictions. In vitro studies of calcineurin activity in human blood show a 2.5-fold greater inhibitory effect of voclosporin compared to CsA [2]. Studies in rabbits, rats, and dogs have demonstrated less nephrotoxicity as determined by serum creatinine as well as histological changes at 30 days [2]. Additional studies in non-human pri-

mates show a greater degree of immunosuppression with voclosporin despite significantly lower blood concentrations and overall exposure when compared to CsA [56]. When comparing the survival times of renal allografts in non-human primates treated with voclosporin or CsA, statistically longer allograft survival was achieved despite lower serum drug levels in those treated with voclosporin [21].

A placebo-controlled Phase III trial studying the efficacy of voclosporin in the treatment of plaque psoriasis has illustrated the relatively mild nephrotoxic nature of this molecule. Of 336 patients assigned to either 0.2, 0.3, or 0.4 mg/kg twice daily, reductions in glomerular filtration rate (GFR) were seen in only 2% of patients at 3 months [46]. In kidney transplantation, the 6-month results of the PROMISE trial have recently been published in abstract form [20]. In this multicenter open-label study, 334 recipients of renal allografts were randomized to receive voclosporin at 0.2, 0.4, or 0.8 mg/kg twice daily or tacrolimus in addition to mycophenolate mofetil (MMF) and steroids. The incidence of biopsy-proven acute rejection (BPAR) was low in all 3 treatment groups: 11%, 8%, and 3% in the 0.2, 0.4, and 0.8 mg/kg arms, respectively, while BPAR was seen in 9% of the tacrolimus-treated group [20]. However, no differences in GFR were noted in this trial, reducing enthusiasm for this agent as a potential advance over existing CNIs. Final results with an average of 24 months follow-up are due to be published shortly, and further evaluation of efficacy and safety in a Phase III trial is planned for the latter part of this year. Given that both CsA and tacrolimus are available in generic form, trials of voclosporin must demonstrate a significant improvement in GFR or a reduction in the histological changes of CNI nephrotoxicity and/or graft fibrosis to justify widespread utilization.

Signals 1 and 2 blockade: AEB071 (sotrastaurin)

Activation of T-cell receptors (Signal 1) plus costimulatory signaling via CD28 (Signal 2) leads to protein kinase C (PKC) signaling and IL-2 production. PKC represents a diverse signaling family of at least 10 isoforms based on cofactor requirements. While the role of individual PKC isoforms in immune system signaling is under investigation, the α, β, and θ isoforms appear to have clear roles in either T- or B-cell signaling [3, 59]. Sotrastaurin is a small molecule that inhibits PKC activity, including the α, β, and θ isoforms. Similar to CNIs, this molecule achieves immunosuppression, predominantly via inhibition of PKCθ. However, in vitro studies have shown sotrastaurin effectively inhibits IL-2 production with minimal effect on typical downstream targets of calcineurin such as NFAT [17], a mechanism distinct from that of the CNIs. These findings have led to optimistic predictions that sotrastaurin could convey a comparable degree of immunosuppression without the long-term nephrotoxicity that is characteristic of CsA and tacrolimus.

Nonhuman primate studies have validated the in vitro findings with sotrastaurin blocking IL-2 and T-cell activation without effecting proliferation [69], with similar results seen in healthy human volunteers [55]. In a pilot study of patients with psoriasis, clinical severity was markedly reduced by 69% following a 2-week course of sotrastaurin. This effectiveness may be explained by the inhibitory effect of sotrastaurin on both lymphocytes (via inhibition of the isoform PKCθ) and keratinocytes (via inhibition of PKCα) [54]. When used alone at full dose or in combination with CsA at partial doses, sotrastaurin prolonged renal allograft survival in primates [4, 69]. Unfortunately, initial results from two Phase II human studies have not proven to be as promising. One trial in which patients were initially placed on tacrolimus/sotrastaurin/corticosteroids and then underwent conversion from tacrolimus to mycophenolate (MPA) at three months resulted in an increased incidence of the primary efficacy endpoint (acute rejection, graft loss, or death) in the sotrastaurin arm [8]. Similarly, in a second trial a de novo CNI-free regimen of sotrastaurin/MPA/steroids was compared to the control arm of tacrolimus/MPA/steroids, a higher acute rejection rate was noted in the sotrastaurin arm [19]. These studies were stopped prematurely and published data are not yet forthcoming. Given these initial disappointments, attention has turned to exploring sotrastaurin in combination with an mTOR inhibitor (everolimus, see later discussion) in a third Phase II trial, under the hy-

Table 1. Clinical outcomes from a Phase II study of either high or low intensity belatacept vs. CsA control . All patients received basiliximab induction with MMF and corticosteroid maintenance.

Clinical parameter	High intensity belatacept (n = 74)	Low intensity belatacept (n = 71)	CsA control (n =73)
6-month acute rejection (primary endpoint)	7%	6%	8%
GFR (cc/min, measured at 12 months)	66	62	53*
Chronic allograft nephropathy (CAN: 12-month protocol biopsy)	29%	20%	44%
Subclinical rejection (12-month protocol biopsy)	9%	20%	11%

*$p < 0.05$ belatacept arms vs. CsA arm.

pothesis that mTOR inhibition may provide greater immunosuppressive efficacy than mycophenolate in the absence of CNIs (information available at www.clinicaltrials.gov, identifier NCT00504543).

Signal 2 blockade: belatacept

Costimulation (Signal 2) is a crucial step in the activation of T-cells, serving to amplify and sustain immune responses by inducing multiple signal transduction pathways that lead to cytokine production and ultimately cell proliferation [12, 22]. T-cells undergoing Signal 1 without a Signal 2 response will become unresponsive and potentially undergo apoptosis [24]. The most extensively studied costimulatory pathway operates through interactions between the T-cell surface molecule CD28 and the B7 family of molecules, with CD80 (B7-1) and CD86 (B7-2) on the surface of APCs serving as ligands for CD28 [32, 50] (Figure 1). Cytotoxic T-lymphocyte antigen (CTLA4) is a T-cell surface molecule homologous to CD28 that also binds CD80/86 on the APC. In contrast to CD28, however, CTLA4/CD80-86 interactions send an inhibitory signal [70] and are therefore important for regulatory feedback to costimulatory signaling. This inhibitory signaling pathway has become an attractive target for novel immunosuppressant development.

Two humanized CTLA4-Ig fusion proteins have been developed to inhibit costimulatory signaling, that consist of the extracellular domain of CTLA4 fused with the Fc portion of human Ig (Figure 2). Abatacept competitively binds CD80/86 with higher affinity than CD28, but is less potent than native CTLA4 in inhibiting costimulation, due to a lower binding affinity for and a rapid dissociation from CD80/86 [30]. While this protein was unsuccessful in a primate model of renal transplant [27], it has demonstrated efficacy and is currently available for human use in rheumatoid arthritis. A second generation CTLA4-Ig fusion protein was isolated by screening variants of CTLA4-Ig and identifying a protein with 2-fold greater binding affinity for CD80 and 4-fold greater affinity for CD86 [30]. LEA29Y, or belatacept, has subsequently undergone development for use in organ transplantation.

Nonhuman primate models of kidney transplant showed intravenous belatacept as monotherapy provided superior graft survival compared to its predecessor abatacept (45 vs. 8 days, respectively) [30]. In humans, a Phase II double-blinded placebo-controlled trial to evaluate safety and tolerability of belatacept and abatacept was performed in 214 patients with rheumatoid arthritis showing effectiveness, tolerability, and excellent safety profiles [38]. These studies led to an important Phase II trial published by Vincenti et al. in 2005 [66] comparing high intensity (10 mg/kg for 11 doses up to day 169) or low intensity (10 mg/kg for 5 doses up to day 85) CNI-free belatacept regimens vs. a CsA-based control arm in 218 patients. All patients received basiliximab induction with maintenance mycophenolate mofetil (MMF) and corticosteroids, and after completing their respective dosing regimen each experimental arm received belatacept at 5 mg/kg doses every 4 – 8 weeks to day 365. All three arms had comparable safety profiles and acute rejection rates,

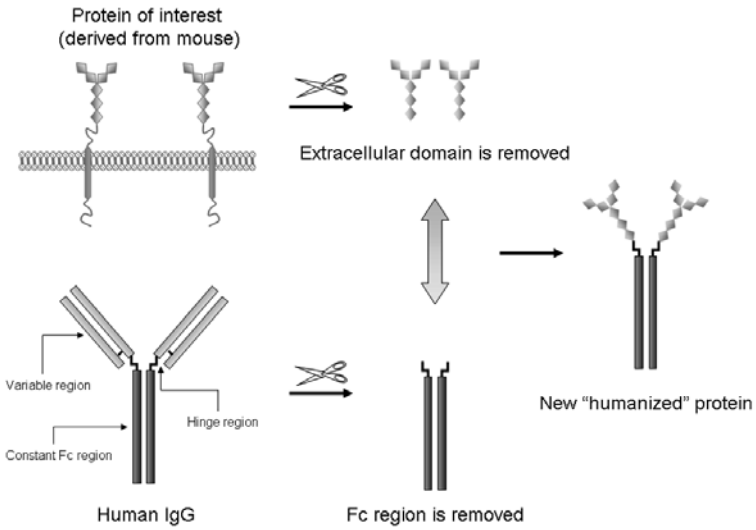

Figure 2. Production of humanized proteins. The extracellular domain of the protein of interest is fused to the Fc domain from human IgG. This results in antibodies that are more "human-like" compared to their murine monoclonal antibody predecessors, with reduced immunogenicity and improved tolerability.

while both belatacept groups showed improved glomerular filtration rates (GFR, 66 and 62 ml/ min in the high- and low-intensity belatacept groups, respectively, vs. 53 ml/min for CsA), less chronic allograft nephropathy (CAN, 29% and 20% in the high- and low-intensity belatacept groups, respectively, vs. 44% for CsA), as well as improved blood pressure, cholesterol, and A1C hemoglobin profiles at 12 months (Table 1). Despite promising findings in the primary and secondary endpoints (acute rejection, renal function and histology), one safety concern was in the incidence of post transplant lymphoproliferative disorder (PTLD). Three cases of PTLD were identified in the high-intensity belatacept arm vs. none in the low-intensity or CsA arms. Continued follow-up of 128 of the original 218 patients showed no new cases of PTLD in either belatacept arm, with one new case in the CsA arm. These data, presented thus far in abstract form only, confirmed initial findings of low acute rejection, death, and graft loss rates that are comparable between groups [40].

The Phase II success of belatacept has led to several larger, 3-year randomized, prospective Phase III trials in kidney transplantation comparing high- and low-intensity belatacept to CsA with all patients receiving basiliximab, MMF, and steroids. In recipients of standard criteria deceased donors and living donors (the BENEFIT study), preliminary 1-year results show significantly improved GFR at 12 months (high intensity, 65 ml/min, low intensity, 63 ml/min, vs. CsA, 50 ml/min) and less CAN despite an increased incidence of AR in the belatacept groups (high intensity, 22%, low intensity, 17%, vs. CsA, 7%) [67]. In recipients of extended criteria donor (ECD) kidneys (the BENEFIT-EXT study, with identical treatment arms and dosing as the BENEFIT study), 1-year results demonstrate similar graft survival and acute rejection across all three groups and improved renal function [15]. PTLD again was more common in the belatacept arms and was primarily associated with EBV-seronegative recipients of kidneys from seropositive donors and/or prior depleting antibody therapy for treatment of acute rejection.

To date, belatacept is the only agent in development that has demonstrated an advantage in renal preservation in large clinical trials compared to CNIs. In the future, the benefits of a reduction in CAN and improved GFR must be weighed against the higher risk of rejection (over 2-fold higher incidence when compared to CsA in the BENEFIT study), the possibility of an increased risk of PTLD, and finally, a route of administration (currently only parenterally available) that requires adequate intravenous access. Given these considerations, belatacept remains an agent with the potential to impact future transplant immunosuppression, provided that safety and dose administration can be clarified and the tradeoff for a higher rate of rejection can accepted or perhaps minimized with other chronic immunosuppressive agents.

Signal 3 blockade: CP-690550 and everolimus

CP-690550

As a result of costimulatory Signal 2, interleukins such as IL-2 and IL-15 are induced and act to trigger cell proliferation (Signal 3) through a signal transduction cascade that is largely initiated by cell surface cytokine receptors. These receptors signal via interaction of the common γ (cγ) subunit [1, 43] with Janus kinases (JAKs). JAKs are cytoplasmic ty-

rosine kinases that participate in the signaling of a broad range of cell surface receptors including members of the cytokine receptor family. There are four mammalian JAKs: JAK1, 2, 3, and tyrosine kinase-2. Receptor-mediated activation of JAKs results in autophosphorylation of the receptor and recruitment of signal transducers and activators of transcription (STATs) which in turn are transported to the nucleus where they regulate gene expression [13, 57]. In contrast to ubiquitous expression of other JAK subtypes, JAK3 is predominantly expressed in hematopoietic cells where it uniquely binds to cγ [37, 74], making it an attractive target for novel immunosuppression agents. The obligatory role of this pathway in immune responses is evidenced by the human severe combined immunodeficiency syndrome that results from mutations in either the cγ subunit or JAK3 itself [7, 33].

CP-690550 is a synthetic orally available inhibitor of JAK3. In vitro assays of IL-2 mediated T-cell proliferation demonstrated robust immunosuppressive potential with a 50% inhibitory concentration (IC50) of only 11 nM [11]. Despite very high potency, this molecule maintains reasonable selectivity for JAK3; an important quality as inhibition of JAK2 results in impaired hematopoiesis and anemia [47]. Initial studies in animal models were encouraging with prolongation of murine cardiac and nonhuman primate renal allograft survival [5, 6, 11] when used either alone or in combination with MMF.

A double-blinded, placebo-controlled Phase I trial of CP-690550 to assess the 28-day safety and tolerability of either low (5 mg b.i.d.), medium (15 mg b.i.d.), or high (30 mg b.i.d.) doses in stable renal transplant recipients has been published [61]. Patients received additional immunosuppression with MMF and steroids, and a CNI if in the low- or medium-dose cohort. Most adverse events were either infectious or gastrointestinal and were mild-to-moderate in severity. A significant decrease in hemoglobin levels was seen in the medium- and high-dose arms compared to control, likely secondary to crossover inhibition of JAK2. This finding was corroborated in a follow-up study of CP-690550's effect on peripheral hematologic parameters in 8 stable renal transplant patients that showed an 8% mean decrease in hemoglobin [62].

A 6-month Phase II trial of CP-690550 at 15 or 30 mg b.i.d. vs. a tacrolimus control arm in 61 patients has been completed [9]. All patients received induction with an IL-2 receptor antagonist and maintained on MMF and steroids. In the high-dose arm, an increased incidence of BK virus nephropathy and CMV infection led investigators to modify the protocol prior to study completion, implementing a planned MMF withdrawal and a more rapid steroid taper. This subsequently led to a significant increase in acute rejection in the high-dose arm (21.1%). However, the low-dose arm provided low acute rejection rates (5.3%, vs. 4.8% in the tacrolimus arm) and excellent renal function (76.9 ml/min, vs. 77.4 ml/min in the tacrolimus arm) at 6 months. This has prompted a second Phase II study investigating a lower-dose strategy of CP-690550 at 15 mg b.i.d. for either 3 or 6 months followed by 10 mg b.i.d. up to 1 year, versus CsA control with full doses of MMF and maintenance prednisone in each arm.

An additional trial is comparing even lower doses of 5 or 10 mg b.i.d. in combination with MMF/prednisone to a tacrolimus/MMF/prednisone-based control arm (information available at www.clinicaltrials.gov, identifiers NCT0083756 and NCT0010639, respectively). Thus, while CP-690550 may ultimately provide an attractive option for CNI minimization with a potent and novel mechanism of immunosuppression, the therapeutic window is under continued investigation. One potential limiting side effect of JAK3 inhibitors is anemia, induced by concomitant JAK2 inhibition of hematopoietic cells, which occurred in 30% of patients in the completed Phase II trial. Several additional molecules with high selectivity for JAK3 are under development to minimize JAK2 inhibition-related anemia [63].

Everolimus

Everolimus (Certican) is an inhibitor of cell proliferation derived from the naturally occurring immunosuppressant sirolimus (SRL). Like SRL, everolimus blocks mTOR activity, resulting in inhibition of cell proliferation by cell cycle arrest at the G1 phase. This class of immunosuppressants has received attention for their potential to allow for

decreased exposure to CNIs when used in combination. Indeed, multiple clinical trials have shown SRL to provide equal or improved efficacy in early CNI withdrawal [25, 28, 44] and late CNI conversion [39, 51] strategies, while its usefulness in CNI avoidance strategies is less clear [16, 18, 31].

Everolimus was approved for use in de novo kidney transplantation in Europe in 2005 but has yet to be approved by the FDA at the time of this manuscript in the United States due to concerns of enhanced nephrotoxicity when combined with a CNI. Initial preclinical studies showed everolimus and CsA to have synergistic immunosuppressive effects in vitro and in vivo [52] as well as beneficial effects on vascular remodeling [52] that is seen in chronic allograft nephropathy. However, a Phase III trial comparing 2 doses of everolimus (1.5 and 3 mg/day) to MMF in 588 patients also receiving steroids and CsA (initial trough goal 100 – 300 ng/ml) resulted in an increased mean creatinine and decreased GFR in the everolimus arms despite similar incidences of the primary endpoint (acute rejection, graft loss, or death) in the 3 cohorts at 3 years. This finding was apparent during interim analyses and necessitated an amendment to CsA trough goals from 100 from 300 ng/ml to 50 ng/ml [68]. Thus, everolimus appeared to provide adequate immunosuppression as evidenced by similar AR rates when compared to MMF, with concern for significant potentiation of CsA nephrotoxicity.

To date, outcomes with everolimus in the context of de novo CNI avoidance or later CNI withdrawal have not been reported. However, 2 Phase III studies were completed examining 2 fixed doses of everolimus (1.5 and 3 mg/day) in a regimen with low dose CsA, steroids, with or without basiliximab induction [60]. Both studies showed similar renal function between groups, as well as time to the primary efficacy endpoint (acute rejection, graft loss, or death at 12 months) with lower acute rejection rates in the study that included basiliximab induction. The risk of acute rejection was found to be significantly higher in those with average everolimus trough levels < 3.0 ng/ml. Frequently reported adverse effects seem to be similar to those seen with SRL and included hypercholesterolemia and lymphocele, while proteinuria was minimal. Several trials investigating the efficacy of dose-adjusted everolimus and low-dose CNI vs. standard MMF and CNI regimens are ongoing. The results of these trials will be critical to determine if the synergistic nephrotoxicity with mTOR/CNI combination can be mitigated by rigorous minimization of CNI and defined exposure of everolimus by trough level monitoring.

Additional clinical trials with everolimus are exploring other minimization strategies, including everolimus-based CNI-free protocols, everolimus-based steroid-free protocols, early (3 months) and late (12 – 96 months) conversion of CNI to everolimus, and early (3 months) or late (12 months) CNI withdrawal from everolimus-based regimens. These ongoing trials may permit a comparison of everolimus to the sirolimus data from trials of similar design [16, 48, 51]. In order for everolimus to distinguish itself as an alternative to CNIs, it should perform at least as well or better than these prior mTOR results in preventing acute rejection, with the possibility that a shorter half-life and better therapeutic monitoring may lead to reduced side effects and nephrotoxicity than sirolimus.

Anti-adhesion molecules: alefacept and efaluzimab

Alefacept

In addition to CD28/CD80-86-mediated costimulation, up-regulation of T-cell activation is also influenced by interactions between the CD2 receptor, present on NK and T-cells, and the more ubiquitously expressed cell surface glycoprotein LFA-3. Costimulation that results from LFA-3 engagement of the CD2 receptor is likely a result of both mediation of T-cell signals as well as improved cell-cell adhesion [36]. Alefacept is a humanized LFA-3/IgG_1 fusion protein that binds to CD2 receptors and has been shown to inhibit T-cell responses both in vitro and in vivo [34, 36].

Alefacept was approved by the FDA in 1993 for use in plaque psoriasis [29]. Based on its effectiveness in this T-cell-mediated disorder, alefacept has been developed as an adjunct immunosuppressant in organ transplantation. Studies of alefacept efficacy in animal models of transplantation have shown

reduced T-cell infiltration of skin grafts in mice [58], improved cardiac allograft survival in nonhuman primates [26], and in addition to SRL and donor antigen administration, prolongation of kidney allograft survival in rhesus monkeys compared to SRL alone [14]. Recently, efficacy in nonhuman primate kidney transplantation was reported when combined with costimulatory blockade and sirolimus [72]. In humans, aside from its use in psoriasis, alefacept has been effective in chronic refractory graft-versus-host disease [53]. A Phase II trial in renal transplant recipients to determine if alefacept permits CNI minimization is ongoing. In this trial, alefacept is used in combination with low-dose tacrolimus, with or without MMF, and compared to a control arm of standard tacrolimus, MMF, and steroids with basiliximab induction. Early results from this trial should become available in 2011.

Efaluzimab

Lymphocyte-associated function-1 (LFA-1) is a member of the β_2 integrin family of proteins composed of a covalently linked α- (CD11a) and β- (CD18) chain [45]. This protein, present on both T- and B-lymphocytes, interacts with intracellular adhesion molecules (ICAMs) present on APCs and endothelium to enhance cell-cell interactions [45]. LFA-1/ICAM interactions have been implicated in leukocyte binding and trafficking as well as T-cell costimulation [42]. Efaluzimab is a humanized anti-LFA-1 antibody specific for the CD11a subunit that blocks LFA-1/ICAM interactions and subsequently inhibits T-cell activation in vitro [73]. A preclinical trial of efaluzimab as a single agent in nonhuman primate cardiac allografts showed increased graft survival compared to controls [49].

In 2003 efaluzimab was approved for the treatment of psoriasis and is currently under investigation for use in a number of clinical conditions including asthma, psoriatic arthritis and rheumatoid arthritis. In the kidney transplant setting, weekly subcutaneous efaluzimab at low (0.5 mg/kg) or high (2 mg/kg) doses in 38 renal transplant recipients was published as a Phase I/II trial [65]. Other maintenance immunosuppression included either low-dose CsA, SRL, and steroids, or high-dose CsA, MMF, and steroids. The incidence of acute rejection was low in all groups (10 – 11%) indicatng adequate immunosuppression with these protocols. Unfortunately, PTLD was diagnosed in 3/10 patients in the high-dose efaluzimab/high-dose CsA group, a finding suggestive of over-immunosuppression in these individuals. If future studies are to continue (dependent upon proprietary interests of the manufacturer), lower doses of efalizumab (1 mg/kg) will likely be used in the context of a CNI-free or CNI-conversion strategy [64]. However, safety concerns have resulted in the suspension of this agent by the manufacturer for future kidney transplant trials.

Conclusions

While the addition of CNIs to immunosuppression regimens revolutionized the field of kidney transplant by drastically reducing the incidence of acute rejection, the inherent nephrotoxic effects of these agents prompts consideration of alternative immunosuppressive strategies. The complex nature of lymphocyte activation provides us with a variety of signals in which pharmacologic interference is possible, resulting in a number of clinical trials that are currently evaluating small molecules and humanized proteins directed against a broad range of activation events. Until the ultimate goal of complete allograft tolerance is reached, the study of the complexities of immune response will continue to provide targets for the development of immunosuppressive agents that are more potent and specific, and less nephrotoxic, than our current therapeutic options.

Conflict of interest

J.E. Cooper has no conflicts of interest to declare. A.C. Wiseman has received grant support and speaking honoraria from Wyeth, and consulting support from Gilead, Novartis, and Bristol Meyers-Squib.

References

[1] *Asao H, Okuyama C, Kumaki S, Ishii N, Tsuchiya S, Foster D, Sugamura K.* Cutting edge: the common

gamma-chain is an indispensable subunit of the IL-21 receptor complex. J Immunol. 2001; *167:* 1-5.

[2] *Aspeslet L, Freitag D, Trepanier D, Abel M, Naicker S, Kneteman N, Foster R, Yatscoff R.* ISA(TX)247: a novel calcineurin inhibitor. Transplant Proc. 2001; *33:* 1048-1051.

[3] *Baier G.* The PKC gene module: molecular biosystematics to resolve its T cell functions. Immunol Rev. 2003; *192:* 64-79.

[4] *Bigaud M, Riesen S, Menninger K, Barbet I, Jean C, Beerli C, Audet M, Blancher A, Heusser C, Wagner J, Morris RE.* NVP-AEB071 (AEB), a novel oral inhibitor of early T cell activation, prolongs the survival of non-human primate (NHP) kidney allografts when used as monotherapy or at non-effective doses combined with a non-effective dose of cyclosporine (CsA) (abstract #546). Am J Transplant. 2006; *6:* 250.

[5] *Borie DC, Larson MJ, Flores MG, Campbell A, Rousvoal G, Zhang S, Higgins JP, Ball DJ, Kudlacz EM, Brissette WH, Elliott EA, Reitz BA, Changelian PS.* 690,550 with mycophenolate mofetil to prevent kidney allograft rejection in nonhuman primates. Transplantation. 2005; *80:* 1756-1764.

[6] *Borie DC, Changelian PS, Larson MJ, Si MS, Paniagua R, Higgins JP, Holm B, Campbell A, Lau M, Zhang S, Flores MG, Rousvoal G, Hawkins J, Ball DA, Kudlacz EM, Brissette WH, Elliott EA, Reitz BA, Morris RE.* Immunosuppression by the JAK3 inhibitor CP-690,550 delays rejection and significantly prolongs kidney allograft survival in nonhuman primates. Transplantation. 2005; *79:* 791-801.

[7] *Buckley RH.* The multiple causes of human SCID. J Clin Invest. 2004; *114:* 1409-1411.

[8] *Budde K, Becker T, Asderakis A, Pietruck F, Grinyo JM, Rigotti P, Dantal J, Ng J, Barten MJ, Weber M.* AEB071, a Novel Protein Kinase C-Inhibitor: First Clinical Results of an AEB071 Plus Tacrolimus Regimen in Renal Transplant Recipients (abstract #391). American Transplant Congress 2009 abstracts. 2009.

[9] *Busque S, Leventhal J, Brennan DC, Steinberg S, Klintmalm G, Shah T, Mulgaonkar S, Bromberg JS, Vincenti F, Hariharan S, Slakey D, Peddi VR, Fisher RA, Lawendy N, Wang C, Chan G.* Calcineurin-inhibitor-free immunosuppression based on the JAK inhibitor CP-690,550: a pilot study in de novo kidney allograft recipients. Am J Transplant. 2009; *9:* 1936-1945.

[10] *Chan L, Gaston R, Hariharan S.* Evolution of immunosuppression and continued importance of acute rejection in renal transplantation. Am J Kidney Dis. 2001; *38:* 2-9.

[11] *Changelian PS, Flanagan ME, Ball DJ, Kent CR, Magnuson KS, Martin WH, Rizzuti BJ, Sawyer PS, Perry BD, Brissette WH, McCurdy SP, Kudlacz EM, Conklyn MJ, Elliott EA, Koslov ER, Fisher MB, Strelevitz TJ, Yoon K, Whipple DA, Sun J, Munchhof MJ, Doty JL, Casavant JM, Blumenkopf TA, Hines M, Brown MF, Lillie BM, Subramanyam C, Shang-Poa C, Milici AJ, Beckius GE, Moyer JD, Su C, Woodworth TG, Gaweco AS, Beals CR, Littman BH, Fisher DA, Smith JF, Zagouras P, Magna HA, Saltarelli MJ, Johnson KS, Nelms LF, Des Etages SG, Hayes LS, Kawabata TT, Finco-Kent D, Baker DL, Larson M, Si MS, Paniagua R, Higgins J, Holm B, Reitz B, Zhou YJ, Morris RE, O'Shea JJ, Borie DC.* Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor. Science. 2003; *302:* 875-878.

[12] *Clarkson MR, Sayegh MH.* T-cell costimulatory pathways in allograft rejection and tolerance. Transplantation. 2005; *80:* 555-563.

[13] *Darnell JE Jr, Kerr IM, Stark GR.* Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science. 1994; *264:* 1415-1421.

[14] *Dhanireddy K, Zhang X, Leopardi F, Johnson L, Kirk A.* Alefacept (LFA-3-Ig), portal venous donor specific transfusion, and sirolimus prolong renal allograft survival in non-human primates (abstract #1627). The 2006 World Transplant Congress. 2006.

[15] *Durrbach A, Medina-Pestana JD, Vanrenterghem Y, Vincenti F, Florman S, Block AJ, Garg P, Sen K, Grinyo J.* Primary Outcomes from a Randomized, Phase III Study of Belatacept vs Cyclosporine in ECD Kidney Transplants (BENEFIT-EXT Study) (abstract #27). American Transplant Congress 2009 abstracts. 2009.

[16] *Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gurkan A, Margreiter R, Hugo C, Grinyo JM, Frei U, Vanrenterghem Y, Daloze P, Halloran PF.* Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med. 2007; *357:* 2562-2575.

[17] *Evenou JP, Wagner J, Zenke G, Brinkmann V, Wagner K, Kovarik J, Welzenbach KA, Weitz-Schmidt G, Guntermann C, Towbin H, Cottens S, Kaminski S, Letschka T, Lutz-Nicoladoni C, Gruber T, Hermann-Kleiter N, Thuille N, Baier G.* The potent protein kinase C-selective inhibitor AEB071 (sotrastaurin) represents a new class of immunosuppressive agents affecting early T-cell activation. J Pharmacol Exp Ther. 2009; *330:* 792-801.

[18] *Flechner SM, Goldfarb D, Solez K, Modlin CS, Mastroianni B, Savas K, Babineau D, Kurian S, Salomon D, Novick AC, Cook DJ.* Kidney transplantation with sirolimus and mycophenolate mofetil-based immunosuppression: 5-year results of a randomized prospective trial compared to calcineurin inhibitor drugs. Transplantation. 2007; *83:* 883-892.

[19] *Friman S, Banas B, Chan L, Mulgaonkar S, Nashan B, Soergel M, Vincenti F, Wissing KM, Witte S, Woodle ES.* AEB071, a Novel Protein Kinase C-Inhibitor: Evaluation of an AEB071 Plus Mycophenolate Regimen in Renal Transplant Recipients (abstract #458). American Transplant Congress 2009 abstracts. 2009.

[20] *Gaber AO, Mulgaonkar S, Gaston R, Jevnikar A, Meier-Kriesche HU.* ISA247: A Phase IIB Multicenter, Open Label, Concentration-Controlled Trial in de novo Renal Transplantation (abstract #LB06). Am J Transplant. 2008; *8:* 336.

[21] *Gregory CR, Kyles AE, Bernsteen L, Wagner GS, Tarantal AF, Christe KL, Brignolo L, Spinner A, Griffey SM, Paniagua RT, Hubble RW, Borie DC, Morris RE.* Compared with cyclosporine, ISATX247 significantly prolongs renal-allograft survival in a nonhuman primate model. Transplantation. 2004; *78:* 681-685.

[22] *Gudmundsdottir H, Turka LA.* T cell costimulatory blockade: new therapies for transplant rejection. J Am Soc Nephrol. 1999; *10:* 1356-1365.

[23] *Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D.* Improved graft sur-

vival after renal transplantation in the United States, 1988 to 1996. N Engl J Med. 2000; *342:* 605-612.

[24] *Jenkins MK, Taylor PS, Norton SD, Urdahl KB.* CD28 delivers a costimulatory signal involved in antigen-specific IL-2 production by human T cells. J Immunol. 1991; *147:* 2461-2466.

[25] *Johnson RW, Kreis H, Oberbauer R, Brattstrom C, Claesson K, Eris J.* Sirolimus allows early cyclosporine withdrawal in renal transplantation resulting in improved renal function and lower blood pressure. Transplantation. 2001; *72:* 777-786.

[26] *Kaplon RJ, Hochman PS, Michler RE, Kwiatkowski PA, Edwards NM, Berger CL, Xu H, Meier W, Wallner BP, Chisholm P, Marboe CC.* Short course single agent therapy with an LFA-3-IgG_1 fusion protein prolongs primate cardiac allograft survival. Transplantation. 1996; *61:* 356-363.

[27] *Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, Hong X, Thomas D, Fechner JH Jr, Knechtle SJ.* CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. Proc Natl Acad Sci USA. 1997; *94:* 8789-8794.

[28] *Kreis H, Oberbauer R, Campistol JM, Mathew T, Daloze P, Schena FP, Burke JT, Brault Y, Gioud-Paquet M, Scarola JA, Neylan JF.* Long-term benefits with sirolimus-based therapy after early cyclosporine withdrawal. J Am Soc Nephrol. 2004; *15:* 809-817.

[29] *Krueger GG, Ellis CN.* Alefacept therapy produces remission for patients with chronic plaque psoriasis. Br J Dermatol. 2003; *148:* 784-788.

[30] *Larsen CP, Pearson TC, Adams AB, Tso P, Shirasugi N, Strobertm E, Anderson D, Cowan S, Price K, Naemura J, Emswiler J, Greene J, Turk LA, Bajorath J, Townsend R, Hagerty D, Linsley PS, Peach RJ.* Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. Am J Transplant. 2005; *5:* 443-453.

[31] *Larson TS, Dean PG, Stegall MD, Griffin MD, Textor SC, Schwab TR, Gloor JM, Cosio FG, Lund WJ, Kremers WK, Nyberg SL, Ishitani MB, Prieto M, Velosa JA.* Complete avoidance of calcineurin inhibitors in renal transplantation: a randomized trial comparing sirolimus and tacrolimus. Am J Transplant. 2006; *6:* 514-522.

[32] *Lenschow DJ, Walunas TL, Bluestone JA.* CD28/B7 system of T cell costimulation. Annu Rev Immunol. 1996; *14:* 233-258.

[33] *Macchi P, Villa A, Giliani S, Sacco MG, Frattini A, Porta F, Ugazio AG, Johnston JA, Candotti F, O'Shea JJ et al.* Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). Nature. 1995; *377:* 65-68.

[34] *Majeau GR, Meier W, Jimmo B, Kioussis D, Hochman PS.* Mechanism of lymphocyte function-associated molecule 3-Ig fusion proteins inhibition of T cell responses. Structure/function analysis in vitro and in human CD2 transgenic mice. J Immunol. 1994; *152:* 2753-2767.

[35] *Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B.* Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. Am J Transplant. 2004; *4:* 378-383.

[36] *Miller GT, Hochman PS, Meier W, Tizard R, Bixler SA, Rosa MD, Wallner BP.* Specific interaction of lymphocyte function-associated antigen 3 with CD2 can inhibit T cell responses. J Exp Med. 1993; *178:* 211-222.

[37] *Miyazaki T, Kawahara A, Fujii H, Nakagawa Y, Minami Y, Liu ZJ, Oishi I, Silvennoinen O, Witthuhn BA, Ihle JN et al.* Functional activation of Jak1 and Jak3 by selective association with IL-2 receptor subunits. Science. 1994; *266:* 1045-1047.

[38] *Moreland LW, Alten R, Van den Bosch F, Appelboom T, Leon M, Emery P, Cohen S, Luggen M, Shergy W, Nuamah I, Becker JC.* Costimulatory blockade in patients with rheumatoid arthritis: a pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA-4Ig and LEA29Y eighty-five days after the first infusion. Arthritis Rheum. 2002; *46:* 1470-1479.

[39] *Mulay AV, Cockfield S, Stryker R, Fergusson D, Knoll GA.* Conversion from calcineurin inhibitors to sirolimus for chronic renal allograft dysfunction: a systematic review of the evidence. Transplantation. 2006; *82:* 1153-1162.

[40] *Mulebacher F, Larsen C, Agarwal M, Vincenti F.* Long-term safety of belatacept: 6 year results of a Phase II study (abstract #621). XXII International Congress of the Transplant Society: http://tc08proceedings.cmsaustralasia.com/Abstracts.pdf#page = 218. 2008; 218.

[41] *Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD,Chapman JR.* The natural history of chronic allograft nephropathy. N Engl J Med. 2003; *349:* 2326-2333.

[42] *Nicolls MR, Gill RG.* LFA-1 (CD11a) as a therapeutic target. Am J Transplant. 2006; *6:* 27-36.

[43] *Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS, McBride OW, Leonard WJ.* Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. Cell. 1993; *73:* 147-157.

[44] *Oberbauer R, Segoloni G, Campistol JM, Kreis H, Mota A, Lawen J, Russ G, Grinyo JM, Stallone G, Hartmann A, Pinto JR, Chapman J, Burke JT, Brault Y, Neylan JF.* Early cyclosporine withdrawal from a sirolimus-based regimen results in better renal allograft survival and renal function at 48 months after transplantation. Transpl Int. 2005; *18:* 22-28.

[45] *Ostermann G, Weber KS, Zernecke A, Schroder A, Weber C.* JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes. Nat Immunol. 2002; *3:* 151-158.

[46] *Papp K, Bissonnette R, Rosoph L, Wasel N, Lynde CW, Searles G, Shear NH, Huizinga RB, Maksymowych WP.* Efficacy of ISA247 in plaque psoriasis: a randomised, multicentre, double-blind, placebo-controlled Phase III study. Lancet. 2008; *371:* 1337-1342.

[47] *Parganas E, Wang D, Stravopodis D, Topham DJ, Marine JC, Teglund S, Vanin EF, Bodner S, Colamonici OR, van Deursen JM, Grosveld G, Ihle JN.* Jak2 is essential for signaling through a variety of cytokine receptors. Cell. 1998; *93:* 385-395.

[48] *Pearson TC, Patel A, Scandling J, Shidban H, Weir M, Patel D.* Efficacy and Safety of Mycophenolate Mofetil (MMF)/Sirolimus (SRL) Maintenance Therapy after Calcineurin Inhibitor (CNI) Withdrawal in Renal Transplant Recipients: Final Results of the Spare-the-Nephron (STN) Trial (abstract #129). Am J Transplant. 2008; *8:* 213.

[49] *Poston RS, Robbins RC, Chan B, Simms P, Presta L, Jardieu P, Morris RE.* Effects of humanized mono-

clonal antibody to rhesus CD11a in rhesus monkey cardiac allograft recipients. Transplantation. 2000; *69:* 2005-2013.

[50] *Reiser H, Stadecker MJ.* Costimulatory B7 molecules in the pathogenesis of infectious and autoimmune diseases. N Engl J Med. 1996; *335:* 1369-1377.

[51] *Schena FP, Pascoe MD, Alberu J, del Carmen Rial M, Oberbauer R, Brennan DC, Campistol JM, Racusen L, Polinsky MS, Goldberg-Alberts R, Li H, Scarola J, Neylan JF.* Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and safety results from the CONVERT trial. Transplantation. 2009; *87:* 233-242.

[52] *Schuurman HJ, Cottens S, Fuchs S, Joergensen J, Meerloo T, Sedrani R, Tanner M, Zenke G, Schuler W.* SDZ RAD, a new rapamycin derivative: synergism with cyclosporine. Transplantation. 1997; *64:* 32-35.

[53] *Shapira MY, Abdul-Hai A, Resnick IB, Bitan M, Tsirigotis P, Aker M, Gesundheit B, Slavin S, Or R.* Alefacept treatment for refractory chronic extensive GVHD. Bone Marrow Transplant. 2009; *43:* 339-343.

[54] *Skvara H, Dawid M, Kleyn E, Wolff B, Meingassner JG, Knight H, Dumortier T, Kopp T, Fallahi N, Stary G, Burkhart C, Grenet O, Wagner J, Hijazi Y, Morris RE, McGeown C, Rordorf C, Griffiths CE, Stingl G, Jung T.* The PKC inhibitor AEB071 may be a therapeutic option for psoriasis. J Clin Invest. 2008; *118:* 3151-3159.

[55] *Slade A, Agyemang A, Sieberling M, Schmouder R.* NVP-AEB071: Human Pharmacodynamics in Combination with Mycophenolic Acid (abstract #396). Am J Transplant. 2006; *6:* 199.

[56] *Stalder M, Birsan T, Hubble RW, Paniagua RT, Morris RE.* In vivo evaluation of the novel calcineurin inhibitor ISATX247 in non-human primates. J Heart Lung Transplant. 2003; *22:* 1343-1352.

[57] *Stepkowski SM, Kirken RA.* Janus tyrosine kinases and signal transducers and activators of transcription regulate critical functions of T cells in allograft rejection and transplantation tolerance. Transplantation. 2006; *82:* 295-303.

[58] *Sultan P, Schechner JS, McNiff JM, Hochman PS, Hughes CC, Lorber MI, Askenase PW, Pober JS.* Blockade of CD2-LFA-3 interactions protects human skin allografts in immunodeficient mouse/human chimeras. Nat Biotechnol. 1997; *15:* 759-762.

[59] *Tan SL, Parker PJ.* Emerging and diverse roles of protein kinase C in immune cell signalling. Biochem J. 2003; *376:* 545-552.

[60] *Tedesco-Silva H Jr, Vitko S, Pascual J, Eris J, Magee JC, Whelchel J, Civati G, Campbell S, Alves-Filho G, Bourbigot B, Garcia VD, Leone J, Esmeraldo R, Rigotti P, Cambi V, Haas T.* 12-month safety and efficacy of everolimus with reduced exposure cyclosporine in de novo renal transplant recipients. Transpl Int. 2007; *20:* 27-36.

[61] *van Gurp E, Weimar W, Gaston R, Brennan D, Mendez R, Pirsch J, Swan S, Pescovitz MD, Ni G, Wang C, Krishnaswami S, Chow V, Chan G.* Phase I dose-escalation study of CP-690 550 in stable renal allograft recipients: preliminary findings of safety, tolerability, effects on lymphocyte subsets and pharmacokinetics. Am J Transplant. 2008; *8:* 1711-1718.

[62] *van Gurp EA, Schoordijk-Verschoor W, Klepper M, Korevaar SS, Chan G, Weimar W, Baan CC.* The effect of the JAK inhibitor CP-690,550 on peripheral immune parameters in stable kidney allograft patients. Transplantation. 2009; *87:* 79-86.

[63] *Velotta JB, Deuse T, Haddad M, Masuda E, Park G, Carroll D, Taylor V, Robbins RC, Schrepfer S.* A novel JAK3 inhibitor, R348, attenuates chronic airway allograft rejection. Transplantation. 2009; *87:* 653-659.

[64] *Vincenti F, Kirk AD.* What's next in the pipeline. Am J Transplant. 2008; *8:* 1972-1981.

[65] *Vincenti F, Mendez R, Pescovitz M, Rajagopalan PR, Wilkinson AH, Butt K, Laskow D, Slakey DP, Lorber MI, Garg JP, Garovoy M.* A Phase I/II randomized open-label multicenter trial of efalizumab, a humanized anti-CD11a, anti-LFA-1 in renal transplantation. Am J Transplant. 2007; *7:* 1770-1777.

[66] *Vincenti F, Larsen C, Durrbach A, Wekerle T, Nashan B, Blancho G, Lang P, Grinyo J, Halloran PF, Solez K, Hagerty D, Levy E, Zhou W, Natarajan K, Charpentier B.* Costimulation blockade with belatacept in renal transplantation. N Engl J Med. 2005; *353:* 770-781.

[67] *Vincenti F, Charpentier B, Medina-Pestana JD, Rostaing L, Vanrenterghem Y, Di Russo GB, Garg P, Lin CS, Larsen C.* Primary Outcomes from a Randomized, Phase III Study of Belatacept vs Cyclosporine in Kidney Transplant Recipients (BENEFIT Study) (abstract #4). American Transplant Congress 2009 abstracts. 2009.

[68] *Vitko S, Margreiter R, Weimar W, Dantal J, Kuypers D, Winkler M, Oyen O, Viljoen HG, Filiptsev P, Sadek S, Li Y, Cretin N, Budde K.* Three-year efficacy and safety results from a study of everolimus versus mycophenolate mofetil in de novo renal transplant patients. Am J Transplant. 2005; *5:* 2521-2530.

[69] *Wagner J, Zenke G, Brinkmann V, Pally C, Bigaud M, Burkhart C, Cottens S, Jung T, Rordorf C, Morris RE.* The first-in-class oral protein kinase C (PKC) inhibitor NVP-AEB071 (AEB) prolongs renal allograft survival in non-human primates (NHP) and suppresses lymphocyte proliferation at safe exposures in human proof of concept sutdies (abstract #57). Am J Transplant. 2006; *6:* 86.

[70] *Walunas TL, Bakker CY, Bluestone JA.* CTLA-4 ligation blocks CD28-dependent T cell activation. J Exp Med. 1996; *183:* 2541-2550.

[71] *Wange RL, Samelson LE.* Complex complexes: signaling at the TCR. Immunity. 1996; *5:* 197-205.

[72] *Weaver TA, Charafeddine AH, Agarwal A, Turner AP, Russell M, Leopardi FV, Kampen RL, Stempora L, Song M, Larsen CP, Kirk AD.* Alefacept promotes co-stimulation blockade based allograft survival in nonhuman primates. Nat Med. 2009; *15:* 746-749.

[73] *Werther WA, Gonzalez TN, O'Connor SJ, McCabe S, Chan B, Hotaling T, Champe M, Fox JA, Jardieu PM, Berman PW, Presta LG.* Humanization of an anti-lymphocyte function-associated antigen (LFA)-1 monoclonal antibody and reengineering of the humanized antibody for binding to rhesus LFA-1. J Immunol. 1996; *157:* 4986-4995.

[74] *Witthuhn BA, Silvennoinen O, Miura O, Lai KS, Cwik C, Liu ET, Ihle JN.* Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells. Nature. 1994; *370:* 153-157.