Beyond calcineurin inhibitors: emerging agents in kidney transplantation

Kassem Safa, Leonardo V. Riella, and Anil Chandraker

Purpose of review
Despite their effectiveness, calcineurin inhibitors (CNIs) represent a major obstacle in the improvement of long-term graft survival in transplantation. The identification of new agents to implement CNI-free regimens is the focus of current transplant research. The purpose of this review is to summarize the novel immunosuppressive agents, including details about their mechanisms of action, stages of development, potential benefits and challenges.

Recent findings
Targeting costimulation with belatacept is now an option for controlling the alloimmune response and has proved to be more effective in preserving long-term allograft function than CNIs despite an increased rate of acute rejection in some studies. mTOR inhibitors are also promising with their remarkable antineoplastic properties, though frequent side-effects may limit their broader use. Other agents under development include JAK inhibitors, CD40 blockade and leukocyte adhesion blockers, with unique potential benefits and side-effects in transplantation.

Summary
Novel immunosuppressive agents are now available for use in CNI-free regimens in solid organ transplantation. Timing of initiation as well as long-term efficacy and safety are questions that remain to be answered in future clinical trials.

Keywords
calcineurin inhibitors, costimulation, immunosuppressive drugs, mTOR inhibitors

INTRODUCTION

The calcineurin inhibitors (CNIs) changed the history of solid organ transplantation by significantly reducing the incidence of acute rejection and thereby improving 1-year allograft survival. Nevertheless, the impact of CNIs on long-term allograft survival has not been as impressive for a variety of reasons. Firstly, reducing acute rejection episodes does not appear to influence long-term graft survival as significantly; secondly, CNIs are nephrotoxic, and are associated with a poor metabolic and cardiovascular profile; and lastly, other factors not positively modified by the CNIs may contribute to long-term graft failure such as chronic antibody-mediated rejection (AMR), BK virus infection and nonimmunologic allograft injury [1].

Hypothetically, an ideal immunosuppressant would be a single agent, with simple dosage, acting specifically on the cells mediating rejection, and be without interactions with other medications and with minimal side-effects including toxicity. The CNIs, cyclosporin A (CsA) and tacrolimus (Tac), require constant monitoring of drug levels and have a wide array of both immune dose-related and other toxicities. CNIs' common toxicity profile is shown in Fig. 1 [2–11]. The fact that CNIs are far from ideal and have a limiting/negative impact on long-term graft survival has led to a search for CNI-free regimens in transplantation. In this article, we review new immunosuppressive agents that have emerged over the last few years and we also describe the potential options beyond CNIs for solid organ transplantation including new agents in the preclinical phases, those on the track for clinical use and those that have already received regulatory approval.

Transplantation Research Center, Brigham and Women's Hospital, Boston, Massachusetts, USA

Correspondence to Anil Chandraker, 75 Francis Street, MRB-4, Boston, MA 02115, USA. Tel: +1 617 732 7412; e-mail: achandraker@partners.org

Curr Opin Nephrol Hypertens 2013, 22:689–697
DOI:10.1097/MNH.0b013e328365b3e6

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

KEY POINTS

- Long-term kidney allograft survival is impacted by CNIs, which triggers the look for new immunosuppressive agent to use in CNIs-free regimen.
- Belatacept, a costimulation blocker, is has been shown to preserve kidney function despite an increase in acute rejection episodes.
- CD40 blockade is potentially promising and being studied in early clinical phases.
- Despite their side-effects, mTor inhibitors continue to be attractive in terms of immunosuppressive potential and antineoplastic as well as antiviral capabilities.
- Optimal dosage and combinations of newer agents such as JAK inhibitors need to be further studied.

IMMUNE RESPONSE MODEL AND THERAPEUTIC TARGETS

The three-signal model of T-cell activation is a very simplified way to summarize the complex and overlapping pathways that lead to a T-cell mediated response to alloantigens and thus the allograft. Each of these three signals involves multiple surface proteins and cytoplasmic signaling molecules that provide a potential target for immunosuppressive agents (Fig. 2). Whether cell adhesion, immunologic synapse formation, costimulation or intracellular signaling, each is a target for blockade that results in variable immunosuppressive effect and adverse-reactions profile (Table 1).

COSTIMULATION BLOCKERS

Following T-cell recognition of alloantigen (signal 1), costimulatory signals (signal 2) are required for full T-cell activation to occur; a classic example of T-cell costimulation is CD28 interaction with the B7 ligands (CD80/CD86). The 'positive' signal provided by CD28 engagement with CD80/CD86 on antigen-presenting cells (APCs) is counterbalanced by cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4); the latter is upregulated on activated T cells and engages with CD80/CD86 to dampen the T-cell response [12]. Inhibition of the CD28–CD80/86 pathway using CTLA-4-Ig fusion proteins has proved to be a more successful strategy than direct CD28-targeted blockade. The latter, although effective in nonhuman primates (NHP) and rodents models, in which a superagonistic anti-CD28 antibody has been shown to lead to an expansion of regulatory T cells and suppression of pro-inflammatory cytokine secretion, has been less successful in humans. In fact, when tested in human controls, a surprisingly severe cytokine release syndrome occurred in an early phase clinical trial, an effect found to be species specific to humans [13]. CD28 blockade continues to be an attractive approach

- Renal
  - Acute
    - o Reduction of GFR and afferent arteriolopathy [2]
    - o Delayed recovery from post-transplantation ATN and toxic tubulopathy [3]
    - o TMA [4]
  - Chronic
    - o IFTA and striped fibrosis
    - o Arterioral hyalinosis
    - o FSGS [3]
- Neurologic
  - Mild
    - o Tremor, neuralgia, neuropathy [5]
  - Severe
    - o Psychosis, hallucinations, blindness
    - o Seizures, ataxia [5]
    - o PRES [6]
- Dermatological
  - Alopecia/hair loss (mainly tacrolimus) [7]
  - Hypertrichosis/hirsutism (mainly CsA) [8]
- Metabolic
  - NODAT [9]
  - Posttransplant hyperlipidemia [10]
  - Hyperuricemia and gout [11]

FIGURE 1. Calcineurin inhibitors' common toxicity profile. GFR, glomerular filtration rate; ATN, acute tubular necrosis; TMA, thombotic microangiopathy; IFTA, interstitial fibrosis and tubular atrophy; PRES, posterior reversible encephalopathy syndrome; CsA, cyclosporin A; NODAT, new-onset diabetes after transplant.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

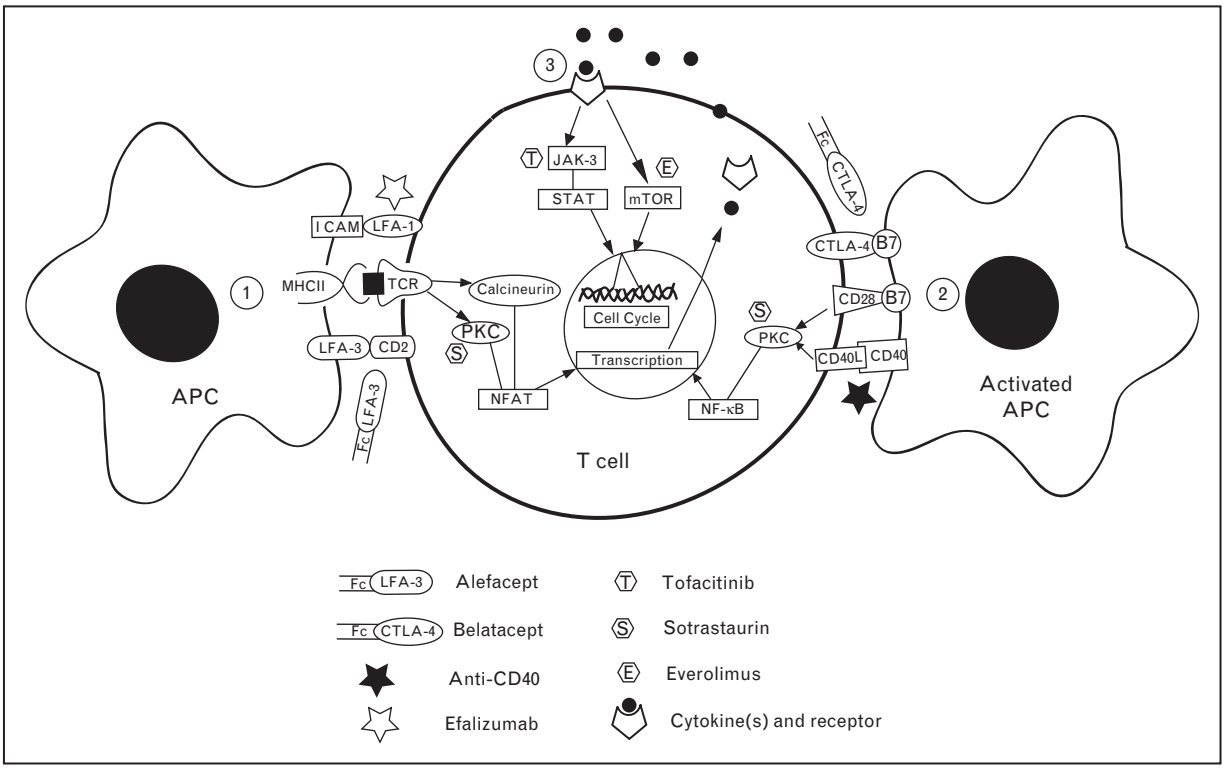

FIGURE 2. Simplified scheme of T-cell activation, proliferation and therapeutic targets. The first signal (1) consists of the immunologic synapse between MHC II on the antigen-prsenting cell (APC), and the antigen and the T-cell receptor (TCR) on the T cell; LFA-1/ICAM and CD2/LFA-3 interactions help stabilizing this synapse and may provide co-stimulatory signaling to the T cell. This engages the T cell through complex cell signaling including the calcineurin and PKC pathways and leads to upregulation of CD28 and CD40L on the T cell, which is now primed to receive co-stimulatory signals from the APC through CD40 and the B7 family. This second signal (2), leads to another cascade of intracellular messengers that reach the nucleus and lead to increased transcription of cytokines (IL-2, INFγ and so on) and their receptors, which eventually provide the final signal (3), to allow the cell to go into the S phase and subsequently proliferate.

however, with selective blocking anti-CD28 molecules being investigated in place of the super-agonistic antibodies [14▪].

Belatacept

Although an alluring pathway, clinical application of CD28:CD80/CD86 blockade has taken more than a quarter century to implement clinically after the initial discovery of the inhibitory CTLA-4 molecule. Abatacept, a CTLA-4-Ig fusion protein, was approved for the treatment of rheumatoid arthritis in 2005 [15]. Lacking the expected efficacy in NHP models of transplantation, abatacept was modified by substituting two amino acids, which gave birth to belatacept, a much more CD80/CD86 avid molecule with increased immunosuppressive potency and efficacy leading to prolonged allograft survival in NHP [16] and heralding its use in clinical trials of transplantation.

In 2005, the belatacept study group published results from a Phase II study that enrolled renal transplant patients to receive induction therapy with basiliximab and maintenance immunosuppression with mycophenolate mofetil and prednisone with randomization to receive either CsA or belatacept at two different dose intensities. Belatacept proved to be noninferior to CsA in preventing biopsy-proven acute rejection (BPAR) at 6 months. Results suggested better glomerular filtration rate (GFR) and a lower incidence of chronic allograft nephropathy (CAN) as well as a trend for better cardiovascular and metabolic profiles. However, a higher incidence of posttransplantation lymphoproliferative disorder (PTLD) was noticed in the high intensity belatacept-treated group [17]. The BENEFIT Study [18], a Phase III clinical trial followed; over the course of 3 years patients receiving a living donor or standard criteria deceased donor kidney transplant were randomized to similar arms as in the original belatacept study. Although a higher incidence of BPAR occurred with belatacept, it was associated with similar 12-month patient and graft survival, better GFR, less CAN and better

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Table 1. New immunosuppressive agents tested in calcinuerin-inhibitor-free regimens

Agent	Type	Mechanism of action	Clinical trial status	Route	Benefit(s)	Side-effects
Belatacept	Fusion protein	Costimulation blocker	FDA Approved	IV infusion	Efficacy in preventing rejection, better GFR, good metabolic and cardiovascular profiles	Increased risk of PTLD
Everolimus	Small molecule	mTor inhibitor	FDA approved in combination with CNI Ongoing trials for use in CNI sparing regimens	PO with level monitoring	Better GFR, antineoplastic and potential antiviral effects	Impaired wound healing, poor metabolic profile, edema
Sotrastaurin	Small molecule	PKC inhibitor	Phase II studies in combination with low dose CNI or everolimus; halted	PO	Better allograft survival if combined with CsA (NHP). clinically high rates of rejection	GI upset, tachycardia
Tofacitinib	Small molecule	JAK inhibitor	Phase II	PO	Better GFR, less CAN	Infectious and neoplastic
Efalizumab	Monoclonal antibody	LFA-1 Blocker	Phase II (withdrawn) Preclinical	SQ injection	Efficacy in islet cell transplantation	PML PTLD
Alefacept	Fusion protein	CD2 blocker	Preclinical on NHP	IM injection	Effector-memory T-cell depletion	AMR rhCMV reactivation
Anti CD40	Monoclonal antibody	Costimulation blocker	Preclinical Phase II	IV/SQ	Increased graft survival in preclinical models, less thrombogenic than anti-CD154	Not defined yet.

CAN, chronic allograft nephropathy; CNI, calcineurin inhibitor; GFR, glomerular filtration rate; NHP, nonhuman primates; PML, progressive multifocal leukoencephalopathy; PTLD, posttransplant lymphoproliferative disorder; rhCMV, rhesus cytomegalovirus.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

cardiovascular and metabolic profile when compared with CsA. Intriguingly, the less intensive belatacept arm was associated with less BPAR episodes than the more intensive arm. Again, a higher incidence of PTLD was particularly noted in the belatacept-treated groups and was associated with EBV-negative status prior to transplantation. Similar results were found in the BENEFIT-EXT trial, which studied the use of belatacept in transplant recipients who received kidneys from extended criteria donors [19].

In June 2011, the US Food and Drug Administration (FDA) approved belatacept (at the less intensive dose), for prophylaxis of organ rejection in adult kidney transplant recipients with a warning against its use in EBV-seronegative patients [20]. Three-year outcomes from both mentioned trials [21,22▪] showed similar patient and graft survival between the two arms with improved metabolic and cardiovascular profiles in belatacept-treated recipients. PTLD continued to be a principal safety concern, as did tuberculosis. Despite a higher incidence of acute cellular rejection in the belatacept treated groups, GFR continued to be better than in the CNI-treated patients. Better GFR was also noted at 5-year follow-up of patients from the initial belatacept Phase II trial [23]. The consistent increase in the incidence of BPAR might be related to several unforeseen consequences of CD80/CD86 blockade by belatacept. By blocking CD28 signaling, CTLA-4-Ig inhibits not only the proliferation of effector T cells, but also regulatory T cells [24▪]. Furthermore, memory T cells have been shown to be resistant to costimulation blockade [25] and CTLA4-Ig may favor Th17 (pro-inflammatory) cell differentiation [24▪]. Lastly, by blocking CD80/CD86, CTLA-4-Ig not only interrupts the positive signal through CD28 but also preferentially allows for signaling through T-cell surface CTLA-4 meant to both inhibit T-cell differentiation and enhance the susceptibility of effector T cells to suppression [26]. Further studies are still needed to confirm the reasons for higher rates of rejection with belatacept treatment.

Anti CD40

Another potential target for costimulation inhibition is the CD154:CD40 pathway, which was proven preclinically to have a major impact in allograft rejection.

Pharmacologic blockade of this pathway via anti-CD154 antibodies showed clinical efficacy in autoimmune diseases [27] and prolonged allograft survival in preclinical models; further clinical advancement was halted, however, because of thromboembolic complications attributed to the expression of CD154 on platelets [28]. This led to attempts to block the other ligand of the CD40:CD154 pair, that is CD40. Blockade of CD40 in NHP kidney transplantation, as well as in porcine islet cell xenotransplantation, has shown promising results without the concomitant thromboembolic complications [29▪,30,31▪▪]. Thus, early-phase clinical trials are being conducted in kidney transplantation. A phase IIa trial comparing efficacy and safety of ASKP1240, an anti-CD40 monoclonal antibody, as a CNI-sparing regimen in preventing kidney transplant rejection is underway (clinicaltrials.gov NCT01780844). As regulatory T-cell generation is independent from CD40:CD40L, targeting CD40 might have a potential advantage compared with CTLA-4-Ig.

SIGNALING INHIBITORS

The transduction of the diverse signals generated after antigen recognition, costimulation or cytokine release requires intracellular messengers that regulate the synthetic and proliferative machinery. Messengers such as mTor, PKC and JAK pathways are now therapeutic targets leading to immunosuppression (Fig. 1).

mTor inhibitors

Isolated before CsA, the potent antifungal agent rapamycin, also known as sirolimus, did not find its way to clinical use in transplantation until the late 1990s. Rapamycin forms a complex with the tacrolimus-binding protein (FKBP), which binds to a kinase called the mammalian target of rapamycin (mTOR). TOR is crucial in transduction of signal 3 of the T cell response and its blockade arrests the cell cycle in the G1 phase and inhibits T-cell proliferation [32,33].

Difficulties in the formulation of rapamycin led to the development of its hydroxyethyl derivative everolimus, with a similar mechanism of action but much more predictable pharmacokinetics. Clinical trials using everolimus followed, first in combination with CNIs then in CNI-sparing regimens.

In early trials, in renal transplantation, rapamycin combination with CsA resulted in fewer BPAR episodes when compared with a combination of azathioprine with CsA. This was, however, at the expense of multiple side-effects including decreased renal function, and worse cardiovascular and metabolic profiles [34]. Other similar combination regimens with CNI showed a marked efficacy in preventing rejection, but a higher incidence of aggravated nephrotoxicity attributed to the CNI [35,36]. Combining low dose everolimus with low

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

dose CsA did result in similar GFR, BPAR, graft loss and death rates as in standard dose CsA with mycophenolic acid [37]. These findings led to FDA approval of everolimus in combination with low dose CsA for low-to-moderate risk kidney transplant patients. Investigating everolimus as a base of a CNI-free regimen followed with the ZEUS study, which showed that switching CsA to everolimus at 4.5 months after transplantation was associated with a significant improvement in GFR at 12 months despite an initial increase of BPAR rates [38].

Potential advantages of sirolimus include regression of PTLD and Kaposi's sarcoma [39] as well as potential antiviral activity reported against cytomegalovirus (CMV) [40▪▪], hepatitis C virus [41] and BK virus [42]. Also, conversion from CNI to sirolimus in kidney transplant patients with a history of skin cancer was associated with longer skin cancer-free survival [43▪▪,44].

These advantages are at the expense of a number of side-effects that result in a higher discontinuation rate of mTOR inhibitors in clinical trials and practice. Side-effects include new onset diabetes, hyperlipidemia, anemia, proteinuria, oral ulcers, diarrhea, impaired wound healing, interstitial pneumonitis and edema [45].

Finally, preclinical studies in NHP of combination of sirolimus and belatacept as a CNI-free/steroid-sparing regimen in allogeneic kidney as well as islet cell transplantation has shown promising results [46,47▪▪]. Clinical studies of similar combinations are ongoing (clinicaltrials.gov NCT00565773, NCT00455013).

Janus kinase inhibitors

Following T-cell activation, a wide array of cytokines shares the Janus kinase (JAK) and the signal transducer and activator of transcription (STAT) system as their mechanism of signal transduction. By binding to their receptors, cytokines such as interleukin (IL)-2, interferon (IFN)-γ and others activate JAK, which in turn phosphorylates STAT leading to its translocation to the nucleus, DNA binding and subsequent gene transcription regulation [48].

Tofacitinib is an oral agent that inhibits JAK-3 and subsequently blunts IL-2-induced cell proliferation [49]. Recently receiving regulatory approval for use in rheumatoid arthritis, tofacitinib seems to be a potentially promising agent in the transplant setting. In a recent Phase II clinical trial in kidney transplantation, patients received basiliximab, corticosteroids, mycophenolic acid and were randomized to receive either tofacitinib at different doses or CsA. Tofacitinib proved noninferior to CsA in preventing acute rejection and was associated with better kidney function and less fibrosis at 1 year posttransplant. However, more infectious and neoplastic complications (PTLD) were observed in the tofacitinib groups [50▪▪]. Another Phase II study with pending results compared tofacitinib to tacrolimus in kidney transplantation (clinicaltrials.gov NCT00106639).

Protein kinase C inhibitors

Protein kinase C (PKC) is a family of kinases involved in multiple signal transduction pathways in different cell phenotypes; several PKC isotypes are involved in the immune response: promoting nuclear factor of activated T cells (NFAT) and nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) transactivation and subsequent IL-2 secretion; and playing crucial roles in B-cell and macrophages activation [51].

Sotrastaurin is a low-molecular weight PKC inhibitor that prevents T-cell activation independently from the calcineurin pathway [52]. Sotrastaurin proved to be effective in treating psoriasis [53] and in a preclinical heart transplantation model [54]. This has led to clinical trials to evaluate its efficacy in kidney transplantation as a platform for CNI-free regimens: two phase II studies of sotrastaturin with or without tacrolimus, were terminated early because of a higher incidence of acute rejection in the sotrastaurin alone arm [55,56]. In both studies the most common side effect was gastrointestinal upset in addition to a higher incidence of tachycardia.

Interestingly, a synergy of sotrastaurin with nonefficacious doses of either CsA or everolimus was noted, leading to investigation on combination therapy; indeed, sotrastaurin prolonged kidney allograft survival when combined with CsA in NHP. This was independent from the potential pharmacokinetic interactions between the two agents due to the common metabolism via CYP3A4 [57]. Yet, recently published results of a Phase II study comparing different doses of sotrastaurin to CsA, both in combination with everolimus and prednisone, showed higher failure rates, more gastrointestinal and cardiac adverse events, more deaths and graft loss in the sotrastaurin arms [58▪▪]. This resulted in the halting of trials studying this agent in kidney transplantation.

ADHESION AND IMMUNOLOGIC SYNAPSE INHIBITORS

Cellular adhesion has been well recognized as a crucial element in stabilizing the T-cell/APC interaction as well as the generation of the immune

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

response; indeed individuals with leukocytes adhesion deficiency (LFA-1 deficiency) develop severe and recurrent infections. Blocking these molecules constitutes yet another therapeutic option to suppress the immune system.

Lymphocyte function-associated antigen-1 antagonists

Lymphocyte function-associated antigen (LFA-1, CD11a) is an integrin family member; it interacts with intercellular adhesion molecules (ICAM), primarily ICAM-1. LFA-1 plays a critical role in the architecture of the immunological synapse, lymphocyte trafficking and transendothelial migration as well as a potential role as a costimulatory molecule [59].

Efalizumab is a recombinant humanized monoclonal antibody against LFA-1. It was initially tried successfully in psoriasis with good results. A phase II trial in kidney transplantation used efalizumab as an induction agent administered over 12 weeks with maintenance immunosuppression with prednisone, mycophenolate mofetil and CsA or sirolimus, half dose CsA and prednisone. Although BPAR at 6 months was diagnosed in only four of 38 patients enrolled, there was a high incidence of PTLD [60]. The agent was withdrawn in a phased voluntary fashion from the market in 2009 after several cases of progressive multifocal leukoencephalopathy (PML) [61]. Reports suggested that efalizumab suppresses T-cell defense against JC virus and inhibits lymphocyte migration across the blood–brain barrier, causing PML [62▪]. Before withdrawal, efalizumab showed efficacy in clinical islet cell transplantation with high rates of insulin independence [63]. Yet, efalizumab continues to be investigated preclinically, and recent data showed that combining it with costimulatory blockade prolonged murine allogeneic skin graft survival by suppressing alloreactive memory T-cells and promoting regulatory T cell retention in draining lymph nodes [25].

Lymphocyte function-associated antigen-3 antagonists

CD2 is expressed on T cells and interacts with LFA-3 on APCs and stabilizes the immunologic synapse; also the LFA-3/CD2 signaling contributes to the proliferation and activation of effector-memory and cytotoxic T cells [64]

Alefacept is a fusion protein of LFA-3 to the Fc portion of human IgG1. The LFA-3 portion of alefacept binds to CD2, which is upregulated on memory T cells. CD2 blockade leads to both a decrease in costimulatory signaling to the T cell and its apoptosis by natural killer cells binding the Fc portion of alefacept [65]. Targeting this pathway is particularly interesting as it overcomes the resistance of memory T cells to blockade of the B7/CD28 pathway.

Initial studies on NHP showed additive effect in prolonging renal allograft survival when given with CTLA4-Ig and sirolimus. This effect was attributed mainly to a selective depletion of effector-memory T cells [66]. However, a later study by the same group showed no additional benefit of alefacept when added to belatacept and sirolimus; on the contrary, addition of alefacept diminished allograft survival, decreased the number of circulating regulatory T cells, and was associated with increased rhCMV reactivation [46]. Interestingly, in the search for a model of AMR, rhesus macaques underwent mismatched kidney transplantation and were treated with anti-CD3 immunotoxin as well as tacrolimus with or without alefacept. All animals treated with alefacept developed alloantibodies and AMR [67▪▪]. The authors suggested a potential contribution of an early effector memory T-cell proliferation in promoting this observation. Yet, a recent clinical trial using alefacept in addition to tacrolimus, resulted in significant reduction of memory T cells after 3 weeks of treatment; the addition of alefacept to standard triple immunosuppression did not show a clear benefit [68▪], but its trial in other CNI-free regimens should still be considered.

CONCLUSION

After decades of use of CNIs in solid organ transplantation, alternatives to this class are being investigated in an attempt to improve the long-term graft survival. Belatacept and everolimus have reached regulatory approval and are being used in CNI-free regimens. Both have marked efficacy with good preservation of renal function over time despite the increased incidence of BPAR; however, belatacept is associated with more PTLD and infectious complications, while sirolimus/everolimus use is limited by a wide array of toxicity and a poor tolerability. It will be crucial to study the combination of lower doses of these agents to possibly merge their advantages and minimize their side effects. Costimulation blockade of the CD40–CD154 pathway is also back in the pipeline in the form of anti-CD40 agents, which in theory do not block the generation of regulatory T cells, directly inhibit B-cell activation and preserve the negative costimulatory signals. Among the small molecules, the JAK inhibitor tofacitinib has been shown to be effective in preventing rejection; yet its infectious

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

and neoplastic side effects will require further studies to define its ideal dose in combination with other immunosuppressive agents.

The major limitation of the new agents is the lack of long-term follow-up studies in transplantation. Therefore, CNIs will most likely continue to be the cornerstone of immunosuppression for the next 10 years. Their use as an initial mode of immunosuppression prior to conversion to a different agent may help minimize the short-term higher rates of acute rejection seen with more novel immunosuppressive agents. Another possibility would be the use of CNIs in lower doses in combination with other agents in order to minimize side-effects. Finally, reviewing the emerging immunosuppressive agents leaves no doubt that immunosuppression for solid organ transplantation will continue to be a multipronged approach and it will unlikely be a single agent based on the complexity of the alloimmune response.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Vincenti F. Are calcineurin inhibitors-free regimens ready for prime time? Kidney Int 2012; 82:1054–1060.
2. Fellstrom B. Cyclosporine nephrotoxicity. Transpl Proc 2004; 36 (Suppl 2): 220S–223S.
3. Liptak P, Ivanyi B. Primer: Histopathology of calcineurin-inhibitor toxicity in renal allografts. Nat Clin Prac Nephrol 2006; 2:398–404.
4. Pisoni R, Ruggenenti P, Remuzzi G. Drug-induced thrombotic microangiopathy: incidence, prevention and management. Drug Safety 2001; 24:491–501.
5. Bechstein WO. Neurotoxicity of calcineurin inhibitors: impact and clinical management. Transplant Int 2000; 13:313–326.
6. Akutsu N, Iwashita C, Maruyama M, *et al.* Two cases of calcineurin inhibitor-associated reversible posterior leukoencephalopathy syndrome in renal transplant recipients. Transplant Proc 2008; 40:2416–2418.
7. Tricot L, Lebbe C, Pillebout E, *et al.* Tacrolimus-induced alopecia in female kidney-pancreas transplant recipients. Transplant Dec 2005; 80:1546–1549.
8. Palestine AG, Nussenblatt RB, Chan CC. Side effects of systemic cyclosporine in patients not undergoing transplantation. Am J Med 1984; 77:652–656.
9. Chakkera HA, Mandarino LJ. Calcineurin inhibition and new-onset diabetes mellitus after transplantation. Transplantation 2013; 95:647–652.
10. Deleuze S, Garrigue V, Delmas S, *et al.* New onset dyslipidemia after renal transplantation: is there a difference between tacrolimus and cyclosporine? Transplant Proc 2006; 38:2311–2313.
11. Sparta G, Kemper MJ, Neuhaus TJ. Hyperuricemia and gout following pediatric renal transplantation. Pediatr Nephrol 2006; 21:1884–1888.
12. Najafian N, Sayegh MH. CTLA4-Ig: a novel immunosuppressive agent. Expert Opin Investig Drugs 2000; 9:2147–2157.
13. Schraven B, Kalinke U. CD28 superagonists: what makes the difference in humans? Immunity 2008; 28:591–595.
14. ■ Poirier N, Blancho G, Vanhove B. CD28-specific immunomodulating antibodies: what can be learned from experimental models? Am J Transplant 2012; 12:1682–1690.
This article oultines the history of targetting CD28 and the major adverse events in clinical trial. It describes the potential role of selective CD28 blockers that lack the above effects.
15. Bluestone JA, St Clair EW, Turka LA. CTLA4Ig: bridging the basic immunology with clinical application. Immunity 2006; 24:233–238.
16. Larsen CP, Pearson TC, Adams AB, *et al.* Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. Am J Transplant 2005; 5:443–453.
17. Vincenti F, Larsen C, Durrbach A, *et al.* Costimulation blockade with belatacept in renal transplantation. N Engl J Med 2005; 353:770–781.
18. Vincenti F, Charpentier B, Vanrenterghem Y, *et al.* A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). Am J Transplant 2010; 10:535–546.
19. Durrbach A, Pestana JM, Pearson T, *et al.* A phase III study of belatacept versus cyclosporine in kidney transplants from extended criteria donors (BENEFIT-EXT study). Am J Transplant 2010; 10:547–557.
20. Archdeacon P, Dixon C, Belen O, *et al.* Summary of the US FDA approval of belatacept. Am J Transplant 2012; 12:554–562.
21. Wojciechowski D, Vincenti F. Belatacept for prevention of acute rejection in adult patients who have had a kidney transplant: an update. Biologics 2012; 6:385–393.
22. ■ Pestana JO, Grinyo JM, Vanrenterghem Y, *et al.* Three-year outcomes from BENEFIT-EXT: a phase III study of belatacept versus cyclosporine in recipients of extended criteria donor kidneys. Am J Transplant 2012; 12:630–639.
This article details results from 3-year follow-up of patients receiving extended criteria deceased donor kidney transplants and treated with belatacept.
23. Vincenti F, Blancho G, Durrbach A, *et al.* Five-year safety and efficacy of belatacept in renal transplantation. J Am Soc Nephrol 2010; 21:1587–1596.
24. ■ Riella LV, Liu T, Yang J, *et al.* Deleterious effect of CTLA4-Ig on a Treg-dependent transplant model. Am J Transplant 2012; 12:846–855.
This is a in-vivo model of transplantation that highlights the importance of the B7:CD28 pathway in regulatory T-cell homeostasis. The findings in this article might explain why higher rates of acute rejection occur with belatacept.
25. Kitchens WH, Haridas D, Wagener ME, *et al.* Combined costimulatory and leukocyte functional antigen-1 blockade prevents transplant rejection mediated by heterologous immune memory alloresponses. Transplantation 2012; 93:997–1005.
26. Magee CN, Boenisch O, Najafian N. The role of costimulatory molecules in directing the functional differentiation of alloreactive T helper cells. Am J Transplant 2012; 12:2588–2600.
27. Sidiropoulos PI, Boumpas DT. Lessons learned from anti-CD40L treatment in systemic lupus erythematosus patients. Lupus 2004; 13:391–397.
28. Kawai T, Andrews D, Colvin RB, *et al.* Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. Nat Med 2000; 6:114.
29. ■ Badell IR, Thompson PW, Turner AP, *et al.* Nondepleting anti-CD40-based therapy prolongs allograft survival in nonhuman primates. Am J Transplant 2012; 12:126–135.
This is a preclinical study of CD40 targeting in nonhuman primate islet cell transplant model.
30. Thompson P, Cardona K, Russell M, *et al.* CD40-specific costimulation blockade enhances neonatal porcine islet survival in nonhuman primates. Am J Transplant 2011; 11:947–957.
31. ■■ Lowe M, Badell IR, Thompson P, *et al.* A novel monoclonal antibody to CD40 prolongs islet allograft survival. Am J Transplant 2012; 12:2079–2087.
This is another preclinical islet cell transplant model, with improved graft survival in animals treated with an anti-CD40 antibody added to basiliximab and sirolimus. Will set forth for a potential clinical use.
32. Halloran PF. Sirolimus and cyclosporin for renal transplantation. Lancet 2000; 356:179–180.
33. Sehgal SN. Sirolimus: its discovery, biological properties, and mechanism of action. Transplant Proc 2003; 35 (3 Suppl):7S–14S.
34. Kahan BD. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. The Rapamune US Study Group. Lancet 2000; 356:194–202.
35. Vitko S, Margreiter R, Weimar W, *et al.* 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.
36. Lorber MI, Mulgaonkar S, Butt KM, *et al.* Everolimus versus mycophenolate mofetil in the prevention of rejection in de novo renal transplant recipients: a 3-year randomized, multicenter, phase III study. Transplantation 2005; 80: 244–252.
37. Tedesco Silva H Jr, Cibrik D, Johnston T, *et al.* Everolimus plus reduced-exposure CsA versus mycophenolic acid plus standard-exposure CsA in renal-transplant recipients. Am J Transplant 2010; 10:1401–1413.
38. Budde K, Becker T, Arns W, *et al.* Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. Lancet 2011; 377:837–847.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

39. Boratynska M, Watorek E, Smolska D, *et al.* Anticancer effect of sirolimus in renal allograft recipients with de novo malignancies. Transplant Proc 2007; 39:2736–2739.

40. ■■ Havenith SH, Yong SL, van Donselaar-van der Pant KA, *et al.* Everolimus-treated renal transplant recipients have a more robust CMV-specific CD8+ T-cell response compared with cyclosporine- or mycophenolate-treated patients. Transplantation 2013; 95:184–191.

Findings of this report might explain the mechanism of lower CMV-related disorder in patients treated with everlolimus.

41. Kawahara T, Asthana S, Kneteman NM. m-TOR inhibitors: what role in liver transplantation? J Hepatol 2011; 55:1441–1451.

42. Liacini A, Seamone ME, Muruve DA, Tibbles LA. Anti-BK virus mechanisms of sirolimus and leflunomide alone and in combination: toward a new therapy for BK virus infection. Transplantation 2010; 90:1450–1457.

43. ■■ Euvrard S, Morelon E, Rostaing L, *et al.* Sirolimus and secondary skin-cancer prevention in kidney transplantation. N Engl J Med 2012; 367:329–339.

In this multicenter trial, switching to sirolimus from CNIs proved to be efficacious in the prevention of recurrence of skin cancer in transplant patients.

44. Campbell SB, Walker R, Tai SS, *et al.* Randomized controlled trial of sirolimus for renal transplant recipients at high risk for nonmelanoma skin cancer. Am J Transplant 2012; 12:1146–1156.

45. Stallone G, Infante B, Grandaliano G, Gesualdo L. Management of side effects of sirolimus therapy. Transplantation 2009; 87 (Suppl 8):S23–S26.

46. Lo DJ, Anderson DJ, Weaver TA, *et al.* Belatacept and sirolimus prolong nonhuman primate renal allograft survival without a requirement for memory T cell depletion. Am J Transplant 2013; 13:320–328.

47. ■■ Lowe MC, Badell IR, Turner AP, *et al.* Belatacept and sirolimus prolong nonhuman primate islet allograft survival: adverse consequences of concomitant alefacept therapy. Am J Transplant 2013; 13:312–319.

Preclinical data showing a benefit/synergy of combining mTOR inhibition with costimulation blockade.

48. O'Shea JJ, Holland SM, Staudt LM. JAKs and STATs in immunity, immunodeficiency, and cancer. N Engl J Med 2013; 368:161–170.

49. Changelian PS, Flanagan ME, Ball DJ, *et al.* Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor. Science 2003; 302:875–878.

50. ■■ Vincenti F, Tedesco Silva H, Busque S, *et al.* Randomized phase 2b trial of tofacitinib (CP-690,550) in de novo kidney transplant patients: efficacy, renal function and safety at 1 year. Am J Transplant 2012; 12:2446–2456.

This article proves clinical efficacy of JAK inhibition (as a CNI-free regimen) in preventing rejection in kidney transplant patient. Infection and malignancy rates were higher compared with CsA.

51. Yamashita K, Todo S. Sotrastaurin, a new selective protein kinase C inhibitor, on the way. Transplantation 2012; 93:146–147.

52. Kovarik JM, Neuhaus P, Cillo U, *et al.* Sotrastaurin single-dose pharmacokinetics in de novo liver transplant recipients. Transplant Int 2011; 24:276–283.

53. Skvara H, Dawid M, Kleyn E, *et al.* The PKC inhibitor AEB071 may be a therapeutic option for psoriasis. J Clin Invest 2008; 118:3151–3159.

54. Weckbecker G, Pally C, Beerli C, *et al.* Effects of the novel protein kinase C inhibitor AEB071 (sotrastaurin) on rat cardiac allograft survival using single agent treatment or combination therapy with cyclosporine, everolimus or FTY720. Transplant Int 2010; 23:543–552.

55. Budde K, Sommerer C, Becker T, *et al.* Sotrastaurin, a novel small molecule inhibiting protein kinase C: first clinical results in renal-transplant recipients. Am J Transplant 2010; 10:571–581.

56. Friman S, Arns W, Nashan B, *et al.* Sotrastaurin, a novel small molecule inhibiting protein-kinase C: randomized phase II study in renal transplant recipients. Am J Transplant 2011; 11:1444–1455.

57. Bigaud M, Wieczorek G, Beerli C, *et al.* Sotrastaurin (AEB071) alone and in combination with cyclosporine A prolongs survival times of nonhuman primate recipients of life-supporting kidney allografts. Transplantation 2012; 93:156–164.

58. ■■ Tedesco-Silva H, Kho MM, Hartmann A, *et al.* Sotrastaurin in calcineurin inhibitor-free regimen using everolimus in de novo kidney transplant recipients. Am J Transplant 2013; 13:1757–1768.

This clinical trial comparing sotrastaurin to CsA both in combination with everolimus, showed higher treatment discontinuation, deaths and graft losses with sotrastaurin. It was followed by the halting of clinical studies of this agent.

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

60. Vincenti F, Mendez R, Pescovitz M, *et al.* 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.

61. Crow JM. Therapeutics: silencing psoriasis. Nature 2012; 492:S58–59.

62. ■ Schwab N, Ulzheimer JC, Fox RJ, *et al.* Fatal PML associated with efalizumab therapy: insights into integrin alphaLbeta2 in JC virus control. Neurology 2012; 78:458–467.

This article highlights the course of fatal PML in two patients treated with LFA-1 antagonist and describes a potential mechanism for the increased susceptibility to JC virus after treatment with this agent.

63. Posselt AM, Szot GL, Frassetto LA, *et al.* Islet transplantation in type 1 diabetic patients using calcineurin inhibitor-free immunosuppressive protocols based on T-cell adhesion or costimulation blockade. Transplantation 2010; 90:1595–1601.

64. Durrbach A, Francois H, Beaudreuil S, *et al.* Advances in immunosuppression for renal transplantation. Nat Rev Nephrol 2010; 6:160–167.

65. Ellis CN, Krueger GG, Alefacept Clinical Study G. Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes. N Engl J Med 2001; 345:248–255.

66. Weaver TA, Charafeddine AH, Agarwal A, *et al.* Alefacept promotes co-stimulation blockade based allograft survival in nonhuman primates. Nat Med 2009; 15:746–749.

67. ■■ Page EK, Page AJ, Kwun J, *et al.* Enhanced de novo alloantibody and antibody-mediated injury in rhesus macaques. Am J Transplant 2012; 12:2395–2405.

In this article, the addition of alefacept to tacrolimus after depletion therapy with anti-CD3 in a nonhuman primate kidney transplant model resulted in increased alloantibody production and antibody-mediated rejection.

68. ■ Rostaing L, Charpentier B, Glyda M, *et al.* Alefacept combined with tacrolimus, mycophenolate mofetil and steroids in de novo kidney transplantation: a randomized controlled trial. Am J Transplant 2013; 13:1724–1733.

In this trial, alefacept or placebo was added to standard immunosuppression for kidney transplantation. The study did not reveal benefit in the addition of alefacept to tacrolimus.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.