CLINICAL INVESTIGATION

Immunosuppression and renal transplant rejection: review of current and emerging therapies

Clin. Invest. (2011) 1(6), 859–877

Roberto Marcén
Servicio de Nephrología, Hospital Ramón y Cajal, Universidad de Alcalá de Henares, Ctra de Colmenar Viejo km 9.1, 28034 Madrid, Spain
Tel.: +34 913 369 017
Fax: +34 913 368 000
E-mail: rmarcen.hrc@salud.madrid.org

Acute rejection associated with chronic rejection and graft loss was a common complication after renal transplantation. Introduction of the new immunosuppressive agents dramatically decreased its incidence, but the negative impact on graft survival persisted. This has been in part attributed to the fact that the new immunosuppressive agents could only control those rejection episodes, without an effect on graft survival. So, differences in acute rejection rates in clinical trials were not followed by improvements in graft survival. Moreover, recent studies suggest that antibody-mediated injury, which is not controlled by the currently used immunosuppressive agents, plays an important role in long-term graft loss. New immunosuppressive agents, such as rituximab and bortezomib, targeting humoral mechanisms of rejection, or belatacept, which preserves graft function, could improve long-term outcomes.

Keywords: alemtuzumab • belatacept • bortezomib • calcineurin inhibitors • mTOR inhibitors • mycophenolic acid • rejection • rituximab

In the last 14 years transplant immunosuppression has changed completely, with the appearance of several novel potent immunosuppressive agents, such as tacrolimus (TAC), mycophenolate mofetil (MMF), mTOR inhibitors and polyclonal and monoclonal antibodies, which have dramatically decreased the incidence of acute rejection. Acute rejection was a common complication before cyclosporine (CsA). Its incidence reached above 70% in the first months [1,2] and it accounted for between 50 and 70% of graft losses 1–3 years after grafting [1–4]. The introduction of CsA in the early 1980s reduced the risk of acute rejection to 30–50% [1–3,5], but still accounted for approximately 50% of graft losses in deceased donor graft recipients during the first 12 months in some studies [6]. Acute rejection was considered a risk factor of chronic allograft nephropathy and poor graft outcome [7–13], and consequently it was thought that reducing its incidence would improve the results of kidney transplantation. The combinations of the new immunosuppressive agents with different actions on the immune system have been successful in reducing the incidence of acute rejection to levels as low as 10–15% [14–24]. However, despite the dramatic decrease in the incidence of acute rejection, long-term results have improved little, if any, in the last years, according to registry data [25,26] and retrospective studies [27]. Nowadays, the importance of acute rejection as a predictive risk factor of poor graft outcome and its reduction as a therapeutical end point is being questioned [28]. However, the standardization of renal biopsy interpretation has permitted the identification and grading of the lesions of rejection [29] and the improvement in the technology of antibody detection has permitted a better understanding of its mechanisms [30].

The purpose of this article is to review the incidence of acute rejection according to the new immunosuppressive agents, the types of rejection and impact of graft rejection on graft function, early graft loss and long-term outcomes.

FUTURE SCIENCE part of fsg

Definition & types of acute rejection

There are three major forms of rejection; hyperacute, acute and chronic. Classic acute rejection usually develops within the first month after grafting but may develop later. The diagnosis of acute rejection was based on clinical changes such as fever, malaise, tenderness over the graft, graft enlargement and oliguria, biochemical changes such as increases in serum creatinine and decreases in glomerular filtration rate, and histological changes that constituted the gold standard of diagnosis of rejection. Since the introduction of CsA, the frequency and severity of clinical symptoms decreased and graft function deterioration was very frequently the only manifestation of rejection. In recipients with delayed graft function, core biopsy performed on the first days after grafting and repeated at regular time intervals, while lack of graft function remained, constituted the only diagnostic procedure. However, the utility of graft biopsies in predicting graft outcomes was poor due to the subjectivity in the interpretation of extension and severity of histological lesions. Consequently, the need of standardization of renal biopsy interpretation induced researchers to develop a schema, which originated in Banff, Canada in 1991. The Banff schema distinguishes and grades the lesions to diagnose acute rejection [29,31–33]. Several studies have demonstrated its utility [34–36] and it is now widely used. Chronic rejection is an entity included in the term called chronic allograft nephropathy, clinically characterized by a slow decline in graft function, generally associated with hypertension and proteinuria. As in the case of acute rejection, the Banff schema has also classified the type of histological findings characteristic of this entity [31–33].

From the etipathogenic point of view, there are two types of rejection, T cell- and antibody-mediated acute rejection and T cell- and antibody-mediated chronic rejection [32,33]. T cell-mediated rejection is the most common type of early rejection and tubulitis and vasculitis are its cardinal features [31]. The changes suggested to be caused by chronic active T cell-mediated rejection are disruption of elastica and inflammatory cells in the fibrotic intima [31,32]. Acute and chronic humoral rejection could both be mediated by alloantibodies to HLA class I and II and other antigens [37,38]. The acute rejection is characterized by C4d deposition in peritubular capillaries in the graft biopsies and the presence of circulating antibodies to donor HLA class I or II antigens [32,33,39–42]. Chronic rejection is characterized as acute rejection, by deposition of C4d in peritubular capillaries and circulating antibodies to HLA and other antigens. In addition, its diagnosis also requires at least three of the following four lesions: arterial intimal fibrosis, interstitial fibrosis/tubular atrophy, duplication of the glomerular basement membrane and lamination of peritubular capillary basement membranes [32,39–42]. The performance of protocol biopsies has allowed identification of a new pathological entity called subclinical rejection, characterized by stable graft function and tubulointerstitial infiltration. As histological lesions can progress, treatment with increased immunosuppression and high-dose corticosteroids has been recommended [43].

Immunosuppressive drugs

There are several groups of immunosuppressive agents: corticosteroids, calcineurin inhibitors (CNIs), antimetabolites and mTOR inhibitors, used in the prevention of acute rejection, and polyclonal and monoclonal antibodies, which are used both as induction therapy and treatment of acute rejection **(Box 1)**. Immunosuppressive regimens are generally composed of corticosteroids plus one CNI, CsA or TAC, and one antimetabolite, MMF

Box 1. Immunosuppressive agents used in renal transplantation.

Corticosteroids

Calcineurin inhibitors
- Cyclosporine
- Tacrolimus

Antimetabolites
- Azathioprine
- Mycophenolic acid:
  - Mycophenolate mofetil
  - Mycophenolate sodium

mTOR inhibitors
- Sirolimus
- Everolimus

Co-stimulation blockers
- Belatacept

Proteasome inhibitors
- Bortezomib

Induction therapy
- Polyclonal antibodies:
  - Immune globulin
  - Atgam
  - Thymoglobulin
- Monoclonal antibodies:
  - IL-2R antibodies: basiliximab and daclizumab
  - Anti-CD52 antibodies: alemtuzumab
  - Anti-CD20 antibodies: rituximab
  - Anti-complement protein C5 antibodies: eculizumab

Other immunosuppressive agents
- Sphingosine 1-phosphate receptor agonists: FTY720
- Protein kinase C inhibitors: AEB071
- Depleting T-effector memory cells: alefacept
- Janus kinase inhibitors: CP-690550

or enteric coated (EC)-mycophenolic acid (MPA), or alternatively, one mTOR inhibitor, sirolimus (SRL) or everolimus (EVL) plus a polyclonal or monoclonal antibody as induction therapy.

Corticosteroids

Corticosteroids have been used as maintenance immunosuppressive therapy, combined with azathioprine (AZA) and as treatment of acute rejection, since the early days of transplantation. Corticosteroids have specific and nonspecific immunosuppressive and anti-inflammatory actions. As maintenance therapy, corticosteroids are still used in combination with the new immunosuppressive protocols: Neoral®/MMF, Neoral/SRL or EVL TAC/MMF, TAC/SRL. Even today, in acute rejection episodes they constitute the first therapeutical step. Owing to their toxic effects, controversy still exits as to whether or not they should be a component of maintenance immunosuppressive regimens, but there is some concern about recommending withdrawal or avoidance [44].

In the CsA/AZA era, corticosteroid withdrawal was associated with a statistically significant risk of acute rejection [45]. However, the appearance of more potent immunosuppressive agents has led to steroid avoidance or steroid withdrawal protocols, as can be observed in US registries [46,47]. The effect of steroid avoidance has been examined in Phase II pilot studies [48], randomized trials [49–53] and retrospective single-center [54,55] or registry studies [47]. In a single-arm, prospective, Phase II study of 57 recipients treated with daclizumab for 5 weeks, 1 g twice daily of MMF and CsA microemulsion (CsA-ME), the acute rejection rate was 25%, which the authors considered to be a positive result [48]. In a randomized, multicenter study, patients received no steroids, steroids to day seven, or standard steroids with CsA-ME, EC-MPA and basiliximab, and the incidence of biopsy-proven acute rejection was significantly lower in those receiving standard steroids than in those receiving steroids to day 7, or no steroids. However, the authors believe that steroid withdrawal by the end of the first week post-transplant may offer a more favorable risk-benefit balance than complete steroid avoidance [51]. Preliminary results from a European trial (Daclizumab plus Tacrolimus plus Mycophenolate plus Steroids Withdrawal vs Standard Therapy [CARMEN] study), in which recipients receiving TAC/MMF were randomized to one single preoperative steroid dose and daclizumab or standard steroid dose, demonstrated that the incidence of rejection was similar (16.5%) in both groups [50]. However, in another European study in which TAC/MMF/steroids was compared with TAC/MMF/no steroids and TAC monotherapy/basiliximab, the incidence of acute rejection was threefold lower in the steroid control group than the other (8.2 vs 26.5 and 30.5%; $p < 0.001$) [52]. In patients with thymoglobulin induction, steroid withdrawal after day 5 was associated with low rejection rates (5%) at 6 months in the three regimens compared; CsA/MMF, high-level TAC/low-level SRL or low-level TAC/high-level SRL [49]. In the Astellas Corticosteroid Withdrawal Study, in which patients on TAC/MMF and induction therapy (IL-2R-antibody or thymoglobulin) were randomized to early corticosteroid cessation (7 days) or to long-term low-dose corticosteroid therapy, 5-year results showed higher biopsy-proven acute rejection rates, near to statistical significance, and some cardiovascular and bone disease risk benefits in the corticosteroid withdrawal group and similar patient and graft outcomes [53]. In the *post hoc* analysis, chronic allograft nephropathy incidence was higher in the withdrawal group, but no protocol biopsies were performed in the study [53]. Several researchers have investigated the effects of late corticosteroid withdrawal [56–58]. In a retrospective study perfomed in Spain, corticosteroid withdrawal was not associated with a worse graft outcome [58]. Data from the Collaborative Transplant Study demonstrated good long-term graft outcomes and no worsening of graft function in patients on CsA and steroid withdrawal after 6 months [56]. A recent meta-analysis, including nine randomized trials (seven CsA-based and two TAC-based immunosuppression) in which steroids were withdrawn between 3–6 months, demonstrated that steroid withdrawal was associated with increased rates of acute rejection in patients on CsA but not on TAC. However, graft survival at 3 years was similar in the two groups [59]. The Kidney Disease Improving Global Outcome guidelines suggest that if corticosteroids are used beyond the first week after transplantation, they have to be continued rather than withdrawn, but with low quality of evidence [60]. According to the previous studies, early corticosteroid withdrawal in patients on TAC/MMF and induction therapy seemed to have low impact on the risk of acute rejection. Late withdrawal was associated with increased risk of acute rejection, but without impact on graft and patient outcomes. Steroid-free immunosuppression or steroid withdrawal offer some benefit in terms of lower lipid levels and new onset diabetes. In our own experience, corticosteroid withdrawal in low-risk patients on treatment with Neoral/MMF, Neoral/SRL, TAC/MMF or TAC/SRL after 6 months of transplantation resulted in a low risk of rejection, and it is our policy to withdraw corticosteroids in these recipients.

Calcineurin inhibitors

Cyclosporine

Cyclosporine was the first CNI available for the prevention of acute rejection. Its immunosuppressive mechanism of action is through the formation of a complex

with its cytoplasmatic receptor protein, cyclophilin. This complex binds with calcineurin, a calcium-activated phosphatase. Its inhibition impairs the expression of cytokine genes that produce cell activation, such as IL-2, IL-4, IFN-γ and TNF-α. CsA also enhances the expression of TGF-β, which inhibits IL-2 and the generation of cytotoxic T lymphocytes. CsA in combination with corticosteroids, with or without AZA, was the base of the immunosuppression in renal transplantation for almost 20 years. Shortly after the introduction of CsA, several toxic effects attributable to the drug became apparent, including hypertension, hyperlipidemia, post-transplant diabetes mellitus, neurological disturbances such as tremor and seizures, and cosmetic complications such as hirsutism and nephrotoxicity.

The original oil-based CsA formulation had variable bioavailability and pharmacokinetics after oral administration. These characteristics could have contributed to the appearance of acute rejection episodes. A water-soluble CsA-ME formulation was designed to improve the pharmacologic activity of the drug. Trials comparing the new and old formulations showed lower acute rejection rates with CsA-ME in *de novo* renal transplant recipients [61–63] and these findings were confirmed by a meta-analysis including 23 studies [64]. As pharmacokinetic studies showed a poor correlation between predose levels and CsA exposure, a single marker of CsA exposure was sought, with CsA level at 2 h post-dose considered to be the best single predictor of AUC. However, in studies performed to assess if a better CsA monitoring resulted in improved graft outcomes, no differences in the number and severity of rejection episodes was found between C0 and C2 CsA monitoring in *de novo* renal transplant recipients [65,66].

Tacrolimus

Tacrolimus came out approximately 14 years ago. It has substituted CsA as the CNI in the immunosuppressive regimens. The agent has a similar mechanism of action but the cytoplasmatic receptor protein is the TAC-binding protein. In the first trials in which TAC was compared with CsA in combination with AZA, there was a significant reduction in the incidence of acute rejection and in the requirements of antilymphocyte treatments for corticosteroid-resistant rejection in TAC-treated recipients at 12 months [20,21]. Protocol biopsies of the patients included in the American trial comparing CsA and TAC have shown a low frequency of subclinical rejection, without differences between the two agents [67]. No differences in graft survival in the intent to treat analysis were observed at 5 years, but when crossovers from CsA to TAC were taken into account, an enhanced graft survival was observed in the TAC group [68].

The previous results were obtained with the standard CsA formulation. With the availability of CsA-ME, new trials were designed to determine whether TAC was still superior in preventing acute rejection when compared with CsA-ME. In a European trial involving 557 patients from 50 centers, biopsy-proven acute rejection was significantly lower with TAC during the first 6 months [22]; however, there were no differences in graft and patient outcomes at 2 and 3 year follow-up, and more patients were changed from CsA-ME to TAC [69,70]. A retrospective paired kidney analysis failed to show differences in acute rejection rates and graft survival between CsA-ME and TAC, but graft function was superior for TAC [71]. These findings were not confirmed by the Efficacy Limiting Toxicity Elimination-Symphony study. In this trial 1645 patients were randomized to receive either a standard-dose of CsA/MMF/corticosteroids, low-dose CsA/MMF/corticosteroids, low-dose TAC/MMF/corticosteroids or low-dose SRL/MMF/corticosteroids; the last three groups also received daclizumab induction. The incidence of biopsy-proven acute rejection in the TAC group at 12 months was half of that in the standard-dose CsA and in low-dose CsA groups, and a third of that in the low-dose SRL group. In addition, allograft survival was superior and graft function better in the low-dose TAC at 12 months [23], but these differences were reduced at 2 and 3 years, in part due to the transition from one treatment to another [72].

With regard to chronic rejection, protocol biopsies have shown that true T-cell chronic rejection was uncommon in patients on treatment with CNIs [73]. One important point to be considered is that a modest reduction of TAC exposure early after transplantation improved the histological findings in protocol biopsies, without increasing the incidence of transplant glomerulopathy, which is a form of humoral chronic rejection [74]. In addition, TAC permited withdrawal of MMF or steroids without graft risk at 6 months [75] and 3 years [76]. When toxicities of both CNI agents were analyzed, TAC had similar nephrotoxic effects as CsA. Other toxicities such as hypertension, hyperlipidemia and hirsutism were more common with CsA, while glucose intolerance, tremor and alopecia were more common with TAC. Although TAC has almost completely substituted CsA as CNI in the immunosuppressive regimens of renal transplantation, the control group in trials with new immunosuppressive agents, such as in belatacept or AEB071 trials, are CsA-based.

■ Antimetabolites

Azathioprine

Azathioprine, an analogue of the 6-mercaptopurine, interferes with the purine nucleotide synthesis that is necessary for cell proliferation. Its use has converted

the transplant from a research procedure to a routine treatment. When AZA and corticosteroids were the only immunosuppressive agents, the incidence of acute rejection rates reached 60–80% [1–3]. The agent was used combined with CsA and constituted part of the immunosuppressive regimens of the first trials comparing CsA and TAC, and for the pivotal trials evaluating the efficacy and security of MMF. Today, as will be commented later, it has been substituted by MMF, EC-MPA and mTOR inhibitors, and it is indicated in pregnant or wishing to be pregnant women, in the case of gastrointestinal intolerance to MPA or to reducing immunosuppression costs.

Mycophenolic acid

There are two formulations of MPA: MMF, the morpholinoethyl ester of MPA, and EC mycophenolate sodium (MPS). Both are converted in the liver to MPA, which is the active compound. MPA is a potent inhibitor of inosine monophosphate dehydrogenase (IMPDH), an enzyme involved in the synthesis of guanosine nucleotides, essential for DNA synthesis. IMPDH blockade results in selective suppression of T- and B-lymphocyte proliferation. Three pivotal trials, USA, European and Tricontinental [14–16], compared the efficacy and safety of MMF 2 and 3 g in combination with CsA and corticosteroids, with CsA/AZA or placebo and corticosteroids, in the prevention of acute rejection. The incidence of biopsy-proven rejection was lower and the rejection episodes less severe in the MMF groups than in the placebo/AZA groups, in all three studies. However, graft and patient survival at 3 years was similar in the three arms [77–79].

In patients on TAC/prednisone, several trials have demonstrated that the addition of MMF reduced the incidence and severity of acute rejection [80–83]. With regards to the recommended dose, 1 g/day has provided an optimal efficacy and safety [84]. Since CNIs are associated with nephrotoxicity and increased rates of cardiovascular risk factors, immunosuppressive regimens that permit dose reduction of these agents have been developed. As was commented before, the Symphony study has demonstrated that an MMF-based regimen permitted the administration of lower doses of concomitant immunosuppressive agents, with excellent results mostly in the low-dose TAC/MMF/corticosteroids [23,72].

Similar safety and efficacy have been observed with the two MPA formulations in the *de novo* [85], stable renal transplant recipients in control trials [86] and in data from the United Network for Organ Sharing/Organ Procurement and Network registry [87]. However, in a retrospective study, patients on treatment with EC-MPS experienced a lower incidence of rejection and biopsy-proven rejection, than those on MMF [88]. In addition, MPA is not a specific treatment for chronic antibody-mediated rejection, but changing CsA and AZA to TAC and MMF resulted in a decrease of anti-HLA class I and II antibodies and stabilization of graft function [89].

However, the efficacy and safety of MMF or EC-MPS compared with AZA is under debate. Data from the Scientific Registry of Transplant Recipients has demonstrated a lower incidence of acute graft rejection at 1 and 4 years [90–92] and a protective effect against declining graft function in the MMF group compared with the AZA group [91]. These results did not agree with those reported from the UK registry, in a paired kidney analysis of 238 recipients, comparing the effects of AZA or MMF combined with CNI and corticosteroids. This study demonstrated that the incidence of acute rejection was higher and graft survival at 7 years lower in the MMF patients [93]. Moreover, data from an American registry (Scientific Registry of Transplant Recipients) has demonstrated an increased rejection rate among AZA/TAC recipients versus MMF/TAC patients, but similar outcomes. However, patients were more likely to be switched to MMF after initiation on AZA (29%), as compared with switching from MMF to AZA (9%) [94]. In a controlled clinical trial (Mycophenolate Steroids Sparing study) similar rejection rates were observed in the MMF recipients as in those in AZA, both in combination with CsA-ME [95,96]. When the safety profile of MMF and AZA was compared, each agent had some advantages over the other. Gastrointestinal disorders were more common in MMF and AZA was associated with an increased risk of cancer [96,97]. In addition, due to the higher cost of MMF compared with AZA and the long-term risk–benefit profile, some authors do not justify the use of MMF [93,95]. However, despite all previous considerations, AZA is not used in most current immunosuppressive protocols. Most physicians believe that MMF and EC-MPA reduce the risk of early-acute and late rejection with less long-term deterioration of graft function than AZA. The combination of TAC/MMF or EC-MPA with or without corticosteroids is the most common immunosuppressive regimen used nowadays [46,98].

■ mTOR inhibitors

Sirolimus

Sirolimus is a macrolide antibiotic that binds to the same cytoplasm-binding as TAC (the FK binding protein). This complex engages to a specific cell cycle regulatory protein called mTOR. mTOR is a regulatory kinase in the process of cell division, its inhibition results in suppression of cytokine-driven T-cell proliferation and the progression from the G1 to S phase of the cell cycle. SRL combined with CsA or TAC results in synergistic immunosuppressive activity, due to their different mechanisms of action on the immune response.

Phase III studies showed that the use of SRL with CsA and corticosteroids reduced the incidence and severity of biopsy-proven acute rejection, when compared with AZA or placebo [18,19]. This reduction reached 40.5% for SRL 2 mg/day and 53.7% for SRL 5 mg/day [19]. In a pilot study where SRL was compared with CsA, both combined with AZA/corticosteroids, the incidence of biopsy-proven acute rejection was similar in both groups, reaching approximately 40% [99].

In TAC-based regimens, the addition of SRL to TAC/corticosteroids reduced the incidence of biopsy-proven rejection [100,101]. In trials comparing the combination of TAC/corticosteroids with SRL or MMF, there were no differences in the incidence of acute rejection and in the frequency of antilymphocyte-antibody therapy for steroid-resistant rejection between the groups, but the incidence of hyperlipidemia was lower and graft function better in the MMF group [102–104]. Other trials and retrospective studies have shown lower graft survival and poorer function in TAC/SRL than in TAC/MMF recipients at 3 years, despite similar incidence and severity of acute rejection [105,106]. Retrospective registry studies have confirmed previous findings that TAC/SRL was associated with poorer graft function and graft survival than TAC/MMF [107].

Everolimus

Everolimus is a derivative of SRL, with potent antiproliferative and immunosuppressive effects, but with more favorable pharmacokinetics. In Phase II trials, in which 3 mg EVL combined with full-dose or reduced-dose CsA-ME in combination with basiliximab and steroids, no differences were found at 3 years in the incidence of biopsy-proven rejection, biopsy-proven allograft nephropathy or graft outcomes [108]. Similar results were reported in more recent trials [109–111]. When EVL 1.5 and 3 mg/day was compared with MMF in combination with CsA-ME in *de novo* renal graft recipients, the incidence of biopsy-proven rejection was similar in the three groups [112,113], as well as the incidence of biopsy-proven chronic allograft nephropathy, but proteinuria was more frequent in the EVL groups [113]. In trials where standard EVL exposure and low CsA exposure were compared with high EVL exposure and very low CSA exposure, no differences were found in the acute rejection rates between the groups [114]. These results suggest that EVL allows reduction of CsA-ME exposure with low rejection rates and similar efficacy as MMF. In addition, EVL reduced the incidence of antibody-mediated rejection at 12 and 36 months compared with MMF [114]. When compared with EC-MPA and EVL/low-dose, CsA-ME was associated with better graft function than EC-MPA/standard-dose CsA-ME [115]. EVL could also be combined with low-dose TAC or standard-dose TAC and basiliximab. Low-exposure TAC was not associated with increasing risk of rejection [116]. It seems that EVL is superior to MMF and EC-MPA in preventing acute rejection and preserving graft function. There are no trials in which the efficacy and security of SRL and EVL have been compared.

mTOR inhibitors display several toxicities. In the immediate post-transplant period they delay the recovery of graft function, impair the healing of the surgical wound and increase the incidence of lymphoceles. In addition, they increase the levels of serum cholesterol and triglycerides, the need for lipid-lowering agents, have a proteinuric effect [113] and should be avoided in proteinuric patients [117]. Letavernier *et al*. have characterized the histopathological and immunohistochemical changes in patients developing proteinuria after SRL exposure [118]. SRL could produce podocyte dysregulation, leading to the classic lesion of *de novo* focal segmental glomerulosclerosis. mTOR can also produce thrombocytopenia and anemia. Despite the initial expectation about the important role to be played by these agents in transplantation, physicians have limited mTOR inhibitors use due to their side effects.

CNI-free immunosuppression

Immunosuppression with CNIs is associated with nephrotoxicity and increased incidence and severity of cardiovascular risk factors. The availability of new and potent immunosuppressive agents has induced researchers to design CNI-free regimens. Two main types of studies have been designed: CNI withdrawal after the first months or complete avoidance. The results of CsA withdrawal are controversial, since there are studies in which CsA withdrawal at three months was not followed by a higher risk of graft rejection in patients on SRL or EVL and corticosteroids [119–122]. In MPA-based regimens, CsA withdrawal was associated with an increased incidence of acute rejection [123,124] and graft loss, but with improvement in graft function in some studies [123]. A recent study has reported encouraging results of CsA withdrawal at 24 days in low-risk recipients [125]. Similar studies have been designed with TAC-based regimens. Early TAC elimination in low-risk recipients from a triple regimen of SRL/TAC/corticosteroids can be achieved with low incidence of biopsy-proven rejection [126]. The effects of late CNI withdrawal were assessed by the SRL Renal Conversion Trial. This trial was designed to evaluate the safety and efficacy of conversion from CNI-based, CsA or TAC, to SRL-based maintenance immunosuppression in 830 patients transplanted between 6 and 120 months before randomization, who were stratified into two groups according to their baseline-calculated glomerular filtration rate: 20–40 ml/min or more than 40 ml/min. However, the Drug Safety Monitoring

Board halted the enrollment of the 20–40 ml/min stratum when during a protocol-specified review of data, the primary safety end point of acute rejection, graft loss or death was reached by 16.7% of SRL conversion and 0% of CNI continuation patients. The incidence of biopsy-proven acute rejection was similar for SRL conversion and CNI continuation, and no significant differences in graft and patient outcomes were observed at 12 and 24 months [127]. A meta-analysis including 19 randomized control trials has shown that CNI minimization or elimination was safe, with increased risk of rejection only after elective CNI elimination [128]. The pattern of adverse effects in patients converted to SRL was consistent with the safety profile of SRL. CNI withdrawal and conversion to mTOR inhibitors should be considered in patients with CNI nephrotoxicity and in those with high risk of malignancies.

Several trials have investigated the incidence of biopsy-proven rejection and graft and recipient outcomes in CNI-free immunosuppression. Flechner *et al*. compared SRL and CsA after basiliximab induction and MMF with steroids, and no differences were found in the incidence of biopsy-proven acute rejection between the two groups [129]. In a long-term follow-up, SRL/MMF immunosuppression was associated with fewer graft losses by chronic allograft nephropathy [130]. In another randomized trial, SRL was compared with TAC and similar acute rejection rates were observed in the SRL/MMF/prednisone group as in the TAC/MMF/prednisone group [131]. However, the Symphony study showed a higher incidence of biopsy-proven acute rejection in the group on SRL/MMF than in the groups on low-dose TAC, standard or low-dose CsA [23]. Moreover, studies in which protocol biopsies were systematically taken, have demonstrated a higher incidence of subclinical rejection in patients on CNI-free regimens during the first months after grafting. Consequently, initial treatment with CNIs was recommended to minimize early immunological injury [73]. Initial CNI-free immunosuppression with MMF and SRL or EVL does not confer significant benefits with standard immunosuppression and studies in which CNIs have been substituted by the new immunosuppressive agents are ongoing.

■ Co-stimulation blockers

Belatacept is a co-stimulation blocker that binds CD80/CD86 on antigen presenting cells, preventing T-cell activation [132]. It has been used to substitute the CNI immunosuppressive agents. In a Phase II trial, belatacept combined with both MMF and corticosteroids was compared with CsA/MMF and corticosteroids. A total of 218 recipients were included in the study and were assigned to intensive belatacept, less-intensive belatacept and CsA. The incidence of biopsy-proven rejection was low, between 6 and 8% at 6 months. However, protocol biopsies at 6 months demonstrated a higher incidence of subclinical rejection in less-intensive belatacept than in intensive belatacept or CsA (20, 9 and 11%, respectively). In addition, the incidence of post-transplantation lymphoproliferative disorders was higher in the belatacept groups than in the CsA group but graft survival was similar in the three groups [24]. Data gained over 5 years, obtained from 128 recipients, demonstrated no increased incidence of acute rejection after 1 year in the intravenous belatacept group, stable graft function, low side-effect rates and a slightly better cardiovascular profile [133]. In Phase III trials, in which recipients received a kidney from a living or standard criteria deceased donor, belatacept in more-intensive and less-intensive regimens were also associated at 12 months with higher rejection rates, but with better graft function and cardiovascular/metabolic profiles, a trend towards less chronic allograft nephopathy and similar graft survival as CsA [134]. Moreover, the incidence of rejection was similar for belatacept and CsA in extended criteria donor transplants [135]. In addition, a switch from CNI therapy to belatacept after 6 months was associated with a low frequency of acute rejection and improvement in renal function [136]. The high patient persistence with intravenous belatacept, graft-function stabilization in the long term, few side effects and no need for therapeutical drug monitoring, suggest than belatacept could be a promising immunosuppressive agent. However, it should not be used in Epstein–Barr virus-seronegative recipients due to the increased risk of post-transplant lymphoproliferative diseases.

■ Proteasome inhibitor (bortezomib)

Donor-specific antibody-mediated rejection is a complication associated with poor graft outcome. Bortezomib, a selective inhibitor of the 26S proteasome with significant activity against mature plasma cells, which has been used in the treatment of plasma cell disorders, could also be effective in the treatment of humoral rejection by depleting the antibody producing plasma cell [137,138].

The first experiences with bortezomib were as therapy for antibody and cell-mediated rejection in a small series of renal transplant patients. Everly *et al*. reported the efficacy of the drug used alone or combined with rituximab, antithymocyte globulin (ATG) or plasmapheresis, in the treatment of six patients with humoral rejection with minimal toxicity [139]. Other reports with a reduced number of patients, in which bortezomib was used combined with rituximab and plasmapheresis, confirmed similar results [140]. Moreover, in some studies, bortezomib demonstrated a rapid donor-specific

antibody reduction or elimination in patients with antibody-mediated rejection [139,140]. However, in other studies one cycle of bortezomib administered as the sole desensitization therapy failed to demonstrate any effect on anti-HLA antibodies [141]. It also reduced when combined with plasmapheresis, antibody levels in the absence of clinical manifestations of allograft dysfunction [142]. In non-immunosuppressed sensitized recipìents the drug used alone had a modest effect on circulating antibodies against HLA antigens [143]. Bortezomib combined with other agents has been used for inducing tolerance with promising results [144]. It seems that bortezomib combined with plasmapheresis and/or rituzimab is useful in treating humoral rejection but when administered alone has little impact in decreasing donor-specific antibodies. The efficacy of this agent needs to be prospectively evaluated.

Induction therapy

Polyclonal antibodies

Intravenous immune globulins

Intravenous immune globulin (IVIG) preparations are made from pooled plasma from thousands of blood donors. They were developed for treating humoral immune deficiency and systemic inflammatory disorders, but they have been proven to be useful in clinical transplantation. The exact mechanisms of action are not well defined, immunoglobulin molecules modify cell-mediated immune functions and antibody production, induce and expand T-regulatory cell populations and have inhibitory effects on complement activation and injury [145]. IVIGs have been shown to be more effective in reducing panel reactive antibody levels and transplant outcomes than placebo [146]. IVIGs effectivity on desensitization improved when used in combination with rituximab with good graft and patient outcomes. Although 29% of recipients presented antibody-mediated rejection episodes, most of them were controlled with treatment [147]. IVIGs have also been used for treatment of antibody-mediated rejection alone or combined with rituximab and plasma exchange. The combination of IVIGs with plasmapheresis and rituximab improved the success rate in the treatment of antibody-mediated rejection compared with IVIG alone [148].

Antithymocyte globulin

Polyclonal antibodies, antilymphocyte globulin (ALG) and ATG have been used since the late 1960s for the treatment of steroid-resistant rejection and as induction therapy [149]. In a recent paper, Cantarovich *et al.* reported long-term results of ALG induction in patients on CsA/AZA/prednisone. The incidence of acute rejection was significantly lower in patients on ALG-induction but graft and patient outcomes were similar at 20 years [150]. Data from the US Renal Data System, in which patients receiving ATG were compared with no-induction therapy, support these findings [151].

Thymoglobulin and anti-T-lymphocyte immune globulin (Atgam®) are the two polyclonal antibody products used in the present era. Thymoglobulin is a purified immunoglobulin solution produced by the immunization of rabbits with human thymocytes. Atgam is a non-pasteurized, purified γ-globulin solution obtained by the immunization of horses with human thymocytes. When both polyclonal antibodies were compared in randomized trials, the incidence and severity of acute rejection were found to be lower in the thymoglobulin cohort than in the Atgam cohort [17]. Data at 5 and 10-year follow-ups have confirmed the superiority of thymoglobulin with respect to Atgam induction in the prevention of graft rejection and in graft survival [152,153]. Moreover, thymoglobulin was more effective than Atgam in the treatment of acute rejection [154]. There is no agreement in the time when the first dose of polyclonal antibody products must be administered or in the dose quantity. In one study, intraoperative administration of thymoglobulin reduced the incidence of rejection when compared with postoperative administration, but the differences did not reach statistical significance [155]. Others have reported similar results of 3–4 day treatment of thymoglobulin as those of a 7-day course in prospective [156] or retrospective studies [157]. The effect of thymoglobulin in decreasing the risk of acute rejection has been observed in patients treated with CsA-ME-based and TAC-based immunosupppression, with the lowest rates in TAC-treated recipients [158]. In CNI-free regimens, induction therapy with thymoglobulin in combination with SRL/MMF/corticosteroids resulted in similar incidence of acute rejection episodes as TAC/MMF/corticosteroids, but with higher risks of adverse effects and graft loss [159]. Polyclonal antibodies increase the incidence of infections, which varies with the number of courses. They also increase the risk of cancer, particulaly of lymphoma, and Epstein–Barr virus-negative recipients are at the highest risk. As commented later, thymoglobulin seems to be the elective induction therapy in high-risk recipients.

Monoclonal antibodies

Anti-IL-2 receptor antibodies

Two anti-IL-2 receptor antibodies (IL-2RA), basiliximab and daclizumab, have been used in induction therapy in renal transplantation. Basiliximab is a chimeric and daclizumab is a humanized murine antibody to CD25, the α subunit of IL-2 receptor. These agents inhibit IL-2-mediated activation and proliferation of T cells in transplant recipients. No differences have been observed in the prevention of acute rejection

between basiliximab or daclizumab in control trials [160] or retrospective studies [161]. Two meta-analyses have confirmed these results [162,163].

Basiliximab has been compared with Atgam and thymoglobulin with variable results. In some studies, there were no differences in the incidence of biopsy-proven rejection between basiliximab and Atgam [164] or basiliximab and thymoglobulin [165–167]. However, in patients at high risk for acute rejection or delayed graft function, there was a lower incidence of biopsy-proven acute rejection, and the need for antibody treatment was less frequent in the patients treated with thymoglobulin than in those on IL-2RA [168,169]. Data from the Organ Procurement and Transplantation Network registry, which includes 19,137 patients transplanted in 2001–2005 who received maintenance therapy with TAC/MMF at transplant discharge, have shown that those treated with thymoglobulin had lower rates of composite 6-month outcome in which acute rejection was included, than those treated with basiliximab or no induction therapy [170]. It seems that in low-risk recipients, no differences in biopsy-proven rejection, graft survival or graft function have been observed between IL-2RA and thymoglobulin, but thymoglobulin seems to be superior in high-risk recipients. IL-2RA induction reduces acute allograft rejection but not graft loss.

Alemtuzumab (Campath 1H)

Alemtuzumab is a humanized immunoglobulin IgG1 monoclonal antibody directed against CD52, an abundant cell surface glycoprotein expressed in circulating T and B cells and to a lesser extent in other cells: natural killer cells, monocytes and macrophages. Alemtuzumab causes cell death through complement-mediated cell lysis and antibody-mediated cellular cytotoxicity [171]. The first report of alemtuzumab came from Cambridge University, where the drug was used with low-dose CsA-ME monotherapy [172]. In a retrospective study from the same group, the incidence of acute rejection at 5 years in the alemtuzumab patients was similar to that found in a cohort control group on CsA/AZA/corticosteroids, but acute rejection episodes ocurred later [173]. Another retrospective study from Wisconsin University demonstrated no differences between patients treated with alemzutumab and those treated with other immunosuppressive regimens. However, when patients with delayed graft function were separately examined, alemzutumab significantly reduced the incidence of rejection compared with the other cohorts [174]. In a randomized control trial, Margreiter *et al.* compared the efficacy and safety of alemtuzumab and TAC monotherapy with TAC/MMF/corticosteroids, and observed that acute rejection rates and severity at 12 months were significantly lower in the alemtuzumab group [175]. In studies in which alemtuzumab was compared with thymoglobulin, both combined with TAC/MMF, alemtuzumab was associated with lower incidence of biopsy-proven rejection [176]. However, Ciancio *et al.* observed similar rejection rates at 24 months, approximately 20%, in patients treated with either of the three combinations: TAC/MMF/alemtuzumab and TAC/MMF/corticosteroids with thymoglobulin or daclizumab [177]. In patients on TAC/MMF/corticosteroid-free immunosuppression, alemtuzumab was similar in efficacy to basiliximab despite the lower TAC and MMF exposure in the alemtuzumab group [178]. In kidney transplantation from cardiac-death donors, alemtuzumab did not confer any advantage with respect to thymoglogulin or IL-2RA on graft outcomes [179]. These results agree with those from the United Network for Organ Sharing/Organ Procurement and Network registry, in which alemtuzumab induction, when compared with no induction, ATG and IL-2RAs, did not show any benefit in the prevention of acute rejection at 6 months and 1 year after transplant, due to the tendency to reduce maintenance immunosuppression [180]. In a pilot study, Flechner *et al.* reported that alemtuzumab combined with steroids, MMF/SRL/corticosteroids was associated with higher than expected acute rejection rates (38%) and with an excessive morbidity [181]. Alemtuzumab has also been used as first-line treatment of biopsy-proven rejection. In a retrospective study including 15 patients, all rejection episodes responded to treatment with different doses of alemtuzumab, but there was an excess of early infection-associated death [182]. According to previous data, alemtuzumab induction could be an effective strategy for minimization of CNIs or corticosteroid avoidance in low-risk recipients.

Rituximab

Rituximab is a chimeric monoclonal antibody directed against CD20, a transmembrane protein expressed by all mature B cells. Rituximab eliminates B cells by complement-dependent cytotoxicity, antibody-dependent cellular toxicity and stimulation of the apoptotic pathway [183]. In renal transplantation it has been used as post-transplantation lymphoproliferative disorder treatment in ABO-incompatible transplantations, as antibody-mediated rejection treatment in desensitization in HLA-sensitized patients and as induction therapy. The use of rituximab in induction therapy is controversial. Clatworthy *et al.* reported an increased incidence of rejection in patients treated with rituximab and postulate that the drug may have a rejection-provoking effect by depletion of immunoregulatory B cells [184]. While, Tydén *et al.*, in a placebo-controlled study in which all patients were treated with TAC/MMF/corticosteroids, observed a tendency towards fewer and milder rejection episodes in the rituximab (one single dose of 375 mg/m^2)

Table 1. Incidence of rejection and graft survival in selected trials.

Trial name/author (year)	Patients (n)	Immunosuppression	Acute rejection rate (%)	Period of analysis (years)	Graft function	Graft survival (%)	Ref.
USRTMMSG (1995 and 1999)	499	CsA/AZA/steroids CsA/MMF 2 g/steroids CsA/MMF 3 g/steroids + ATG all groups	53.6 35.9 (p = 0.0015) 36.7 (p = 0.0021)	3	Not available, but SCr levels were not different along the study	74.7 81.1 77.4 (NS) Intent-to-treat	[14,78]
EMMCSG (1995 and 1999)	491	CsA/PLA/steroids CsA/MMF 2 g/steroids CsA/MMF 3 g/steroids	46.4 17.0 (p < 0.001) 13.8 (p < 0.001)	3	Not available	78.0 84.8 81.2 Intent-to-treat	[15,77]
Mathew (1999)	503	CsA/AZA/steroids CsA/MMF 2 g/steroids CsA/MMF 3 g/steroids	35.5 19.7 15.9	3	1.70 ± 0.1 mg/dl 1.78 ± 0.1 mg/dl 1.56 ± 0.1 mg/dl	81.4 84.8 80.2 Intent-to-treat	[16,79]
Vincenti *et al.* (2002)	412	TAC/AZA/Pred. CsA/AZA/Pred. Induction Atgam or OKT3	30.7 46.6 (p = 0.001)	5	1.4 mg/dl 1.7 mg/dl (p = 0.0014)	64.3 61.6 (p = 0.558) Intent-to-treat	[68]
Kramer *et al.* (2008)	560	TAC/AZA/Pred. CsA-ME/AZA/Pred.	35.1 52.5 (p < 0.001)	3	67.3 ± 23.6 ml/min 64.0 ± 23.9 ml/min	88.0 86.9 Intent-to-treat	[70]
Russ *et al.* (2005)	430	SRL/CsA/steroids SRL/CsA withdrawal at 3 months/steroids	Not available but similar in the two groups	4	43.8 ml/min 58.3 ml/min (p < 0.001)	84.2 91.5 (p = 0.026)	[120]
Abramowicz *et al.* (2005)	151	CsA/MMF 2 g/steroids MMF 2 g/steroids (CsA withdrawal)	1.0 16.0 (p = 0.003)	5	61.7 ml/min 67.4 ml/min (p = 0.050)	87 81 (p = 0.322)	[123]
Ekberg *et al.* (2009)	958	CsA/MMF/steroids Low-dose CsA/MMF/steroids + daclizumab Low-dose TAC/MMF/steroids + daclizumab MMF/SRL/steroids + daclizumab	27 27 14 (p < 0.0001) 39	3	65.3 ± 26.2 ml/min 64.0 ± 23.1 ml/min 68.6 ± 23.8 ml/min (p = 0.039 vs low-dose CsA) 65.3 ± 26.2 ml/min	87 89 90 85 Intent-to-treat	[172]

Table 1. Incidence of rejection and graft survival in selected trials.

Trial name/author (year)	Patients (n)	Immunosuppression	Acute rejection rate (%)	Period of analysis (years)	Graft function	Graft survival (%)	Ref.
Vincenti *et al.* (2010)	218	Belatacept/MMF/steroids CsA/MMF/steroids	+6% late acute rejection	5	77.2 ± 22.7 ml/min 59.3 ± 15.3 ml/min		[133]
Cantarovich *et al.* (2008)	123	CsA/AZA/Pred. CsA/AZA/Pred. + ALG	75 28 (p < 0.0001)	20	128 ± 56 µmol/l 145 ± 47 µmol/l (p = 0.29)	43 47 (p = 0.50)	[150]
Hardinger *et al.* (2004)	72	CsA-ME/AZA/Pred. + thymoglobulin CsA-ME/AZA/Pred. + Atgam	8 34	5	1.9 ± 0.7 mg/dl 1.5 ± 0.7 mg/dl (p = NS)	77 54 (p = 0.046)	[153]

ALG: Antilymphocyte globulin; ATG: Antithymocyte globulin; Atgam: Anti-T-lymphocyte immune globulin; AZA: Azathioprine; Csa: Cyclosporine; EMMCSG: European Mycophenolate Mofetil Cooperative Study Group; ME: Microemulsion; MMF: Mycophenolate mofetil; NS: Nonsignificant; PLA: Placebo; Pred.: Prednisone; SCr: Serum creatine; SRL: Sirolimus; TAC: Tacrolimus; USRTMMSG: US Renal Transplant Mycophenolate Mofetil Study Group.

group compared with the controls [185]. In a retrospective study in living donor transplants treated with TAC/MMF/corticosteroids, induction with rituximab was associated with similar rates of antibody-mediated rejection but lower rates of acute T cell-mediated rejection [186]. Several noncontrolled studies with a small number of cases have shown the efficacy and safety of rituximab combined with plasma exchange and IVIG in the treatment of acute humoral rejection [187–190]. It has also been used with intravenous IVIG or plasmapheresis in the treatment of patients with chronic antibody-mediated rejection, with promising results [191]. The benefits of rituximab as an induction therapy in patients on TAC/MMF/corticosteroid were low. Combined with IVIG and plasmapheresis, rituximab was effective in desensitization and the treatment of acute and chronic antibody-mediated rejection. Long-term multicenter trials are needed to evaluate the efficacy, security and optimal dose regimen for rituximab.

Eculizumab

Eculizumab is a humanized monoclonal antibody against complement protein C5. It binds to the C5 protein, inhibiting its cleavage to C5a and C5b and preventing the formation of the membrane attack complex. It is used in the treatment of paroxismal nocturnal hemoglobinuria [192]. There are several single case reports in which eculizumab was used as rescue therapy in renal-transplant recipients with catastrophic antiphospholipid antibody syndrome [193], with hemolitic uremic syndrome [194,195] and in severe antibody-mediated rejection associated with rituximab, plasmapheresis and IVIG [196].

■ Other immunosuppressive agents

FTY720, a sphingosine receptor agonist with equivalent efficacy to MMF [197–200], is no longer used due to macular toxicity. Other agents such as AEB071, a protein kinase inhibitor [201], and alefacept, a fusion protein that depletes T effector memory cells and used in the treatment of psoriasis [202], are now in Phase II studies. The first trial with a Janus kinase inhibitor, in which the agent showed comparable efficacy and safety to TAC, has been recently published [203]. The first case of a successful kidney transplantation without immunosuppression in a patient with previous haploidentical hematopoietic stem cell transplantation has been published [204], and this opens new alternatives to transplant immunosuppression.

Impact of acute rejection on later graft outcome

The relationship between acute graft rejection rates and graft outcome is paradoxical. The new immunosuppressive agents have been very effective in decreasing the incidence of biopsy-proven acute rejection to

very low percentages, below 15% with most regimens. However, reduction of biopsy-proven rejection rates has not been systematically followed by similar improvements in graft outcomes according to retrospectve and registry studies [25–27,205,206]. Most recent trials comparing different immunosuppressive regimens showing significant differences in rejection rates have failed to show similar differences in graft survival at 3–10 years (Table 1). On the other hand, the impact on graft outcome varies according to the type of acute rejection. Broad analyses of some series in which patients on treatment with the new immunosuppressive agents were included, showed that acute rejection was associated with an increased risk for chronic rejection and graft loss in the long-term follow up [205–207]. The belatacept trials reflected these controversial issues, patients treated with belatacept had a higher incidence of acute rejection than those treated with CsA, but a better preservation of graft function [24,134,135]. Some authors believe that a less severe rejection crisis without functional effect is controlled by the new immunosuppressive therapies, but rejection with a more severe functional impact could persist [25,26,207,208]. This hypothesis is supported by the fact that antibody-mediated injury, not controlled by the available immunosuppressive agents, plays an important role in late kidney allograft failure [209,210].

Future perspective

The new immunosuppressive agents have failed to improve late graft outcomes despite the dramatic reduction in the incidence of acute rejection. Moreover, there is a growing body of evidence about the importance of humoral mechanisms in acute and chronic rejection, in which the immunosuppressive agents used in the present era have limited efficacy. The immunosuppressive agents that permit the avoidance of CNIs and improve the cardiovascular risk profile, or those with depleting effects on antibody produced cells, have to be tested in long-term controlled trials.

Acknowledgements

The author thanks Mary Harper for her assistance in preparing the English version of this article.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- The new calcineurin inhibitor (CNI) tacrolimus, associated with mycophenolic acid and with induction therapy, has decreased the incidence of acute rejection to below 15%.
- Moreover, the availability of potent immunosuppressive agents has permitted corticosteroid avoidance/minimization.
- Owing to its efficacy and safety profile, the combination of tacrolimus/mycophenolate mofetil/corticosteroids is the immunosuppressive regimen most commonly used in kidney transplantation.
- The most potent immunosuppressive agents for preventing acute rejection, CNIs, are nephrotoxic and consequently could have a negative impact on long-term graft outcome.
- mTOR inhibitors are less nephrotoxic than CNIs, but they have not replaced CNIs in *de novo* kidney transplants since they do not give additional benefits in the prevention of acute rejection and owing to their side effects.
- Belatacept does not improve the acute rejection rates when compared with CsA but does preserve graft function.
- Bortezomib combined with intravenous immune globulin and plasmapheresis could be useful in desensitization and in the treatment of refractary acute rejection.
- Poyclonal antibodies, such as tymoglobulin, are the elective induction therapy in high-risk recipients.
- Alemtuzumab induction could be an effective strategy for minimization of CNIs or corticosteroid avoidance in low-risk recipients.
- Rituximab combined with intravenous immune globulin and plasmapheresis is effective in desensitization and in the treatment of acute and chronic antibody-mediated rejection.
- Despite the low incidence of acute rejection, long-term graft survival has not improved to the same extent according to registry and single-center data.
- The lack of improvement of long-term graft outcome could be, in part, due to the fact that most of the immunosuppressive drugs only control those T cell-mediated rejection episodes without effect on humoral mechanisms of acute and chronic rejection or to the nephrotoxicity of the most potent immunosuppressive agents (CNIs).
- New immunosuppressive agents such as belatacept, which preserve graft function, rituximab and bortezomib, which have a depleting effect on antibody producing cells, could improve long-term outcomes.

Bibliography

Papers of special note have been highlighted as:

- ■ of interest
- ■■ of considerable interest

1 Ponticelli C, Minetti L, Quarto di Palo F *et al.* The Milan clinical trial with cyclosporine in cadaveric renal transplantation. A three-year follow-up. *Transplantation* 45(5), 908–913 (1988).

2 Marcen R, Pascual J, Teruel JL *et al.* Outcome of cadaveric renal transplant patients treated for 10 years with cyclosporine. Is chronic allograft nephropathy the major cause of graft loss? *Transplantation* 72(1), 57–62 (2001).

3 Najarian JS, Fryd DS, Strand M *et al.* A single institution, randomized, prospective trial of cyclosporine versus azathioprine-antilymphocyte globulin for immunosuppression in renal allograft recipients. *Ann. Surg.* 201(2), 142–157 (1984).

4 The Canadian Multicentre Transplant Study Group. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. Analysis at three years. *N. Engl. J. Med.* 314(19), 1219–1225 (1986).

5 Gulanikar AC, MacDonald AS, Sungurtekin U, Belitsky P. The incidence and impact of early rejection episodes on graft outcome in recipients of first cadaver kidney transplants. *Transplantation* 53(2), 323–328 (1992).

6 Lindholm A, Ohlman S, Albrechtsen D, Tufveson G, Persson H, Persson NH. The impact of acute rejection episodes on long-term graft function and outcome in 1374 primary renal transplants treated by 3 cyclosporine regimens. *Transplantation* 56(2), 307–315 (1993).

7 Linholm A, Albrechtsen D, Tufveson G, Karlberg I, Persson NH, Groth C-G. A randomized trial of cyclosporine and prednisolone versus cyclosporine, azathioprine, and prednisolone in primary cadaveric renal transplantation. *Transplantation* 54(4), 621–631 (1992).

8 Marcén R, Orofino L, Pascual J *et al.* Delayed graft function does not reduce the survival of renal transplant allografts. *Transplantation* 66(4), 461–466 (1998).

9 Humar A, Kerr S, Gillingham KJ, Matas AJ. Features of acute rejection that increase risk for chronic rejection. *Transplantation* 68(8), 1200–1203 (1999).

10 Matas AJ, Gilligham KJ, Payne WD, Najarian JS. The impact of an acute rejection episode on long-term renal allograft survival (t1/2). *Transplantation* 57(6), 857–859 (1994).

11 Vereerstraeten P, Abramowicz D, de Pauw L, Kinnaert P. Absence of deleterious effect on long-term kidney graft survival of rejection episodes with complete functional recovery. *Transplantation* 63(12), 1739–1743 (1997).

12 Cosio FG, Pelletier RP, Falkenhain ME *et al.* Impact of acute rejection and early allograft function on renal allograft survival. *Transplantation* 63(11), 1611–1615 (1997).

13 Ishikawa A, Flechner SM, Goldfarb DA *et al.* Quantitative assessment of the first acute rejection as a predictor of renal transplant outcome. *Transplantation* 68(9), 1318–1324 (1999).

14 Sollinger HW, for the US Renal Transplant Mycophenolate Mofetil Study Group. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. *Transplantation* 60(3), 225–232 (1995).

■■ **Evidence of the reduction of the impact on lowering acute rejection rates of mycophenolate mofetil (MMF) when compared with azathioprine.**

15 European Mycophenolate Mofetil Cooperative Study Group. Placebo-controlled study of mycophenolate mofetil combined with cyclosporin and corticosteroids for prevention of acute rejection. *Lancet* 345(8961), 1321–1325 (1995).

■■ **Confirmed the findings of the US Renal Transplant Mycophenolate Mofetil Study Group about the efficacy of MMF in the prevention of acute rejection.**

16 The Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. *Transplantation* 61(7), 1029–1037 (1996).

■■ **Third pivotal study showing the efficacy of MMF in the prevention of renal transplant rejection.**

17 Brennan DC, Flavin K, Lowell JA *et al.* A randomized, double-blinded comparison of thymoglobulin versus Atgam for induction immunosuppressive therapy in adult renal transplant recipients. *Transplantation* 67(7), 1011–1018 (1999).

18 Kahan BD, for the Rapamune US Study Group. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomized multicentre study. *Lancet* 356(9225), 194–202 (2000).

19 MacDonald AS, for the Rapamune Global Study Group. A worldwide, Phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. *Transplantation* 71(2), 271–280 (2001).

20 Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS, for the FK506 kidney transplant study group. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. *Transplantation* 63(7), 977–983 (1997).

■■ **Presents the first evidence of the superiority of tacrolimus compared with cyclosporine in the prevention of acute rejection.**

21 Mayer AD, Dmitrewski J, Squifflet J-P *et al.* Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of allograft rejection. A report of the European Tacrolimus Multicenter Renal Study Group. *Transplantation* 64(3), 436–443 (1997).

22 Margreiter R, for the European Tacrolimus versus Ciclosporin Microemulsion Renal Transplantation Study Group. Efficacy and safety of tacrolimus compared with ciclosporin microemulsion in renal transplantation: a randomized multicenter study. *Lancet* 359(9308), 741–746 (2002).

23 Ekberg H, Tedesco-Silva H, Dermibas A *et al.*, for the ELITE-Symphony Study. Reduced exposure to calcineurin inhibitors in renal transplantation. *N. Engl. J. Med.* 357(25), 2562–2575 (2007).

■■ **Presents the efficacy and safety of low-dose tacrolimus combined with MMF compared with other immunosuppressive regimens. Also provides evidence that *de novo* calcineurin inhibitor-free immunosuppression does not adequately prevent acute rejection.**

24 Vincenti F, Larsen C, Durrbach A *et al.*, for the Belatacept Study Group. Costimulation blockade with belatacept in renal transplantation. *N. Engl. J. Med.* 353(8), 770–781 (2005).

25 Meier-Kriesche H-U, 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.* 4(3), 378–383 (2004).

■ **Evidence of the lack of graft-outcome improvement with the new immunosuppressive agents, despite lower rejection rates.**

26 Meier-Kriesche H-U, Ojo AO, Hanson JA *et al.* Increased impact of acute rejection on chronic allograft failure in recent era. *Transplantation* 70(7), 1098–1100 (2000).

27 Marcén R, Fernández-Rodriguez A, Rodríguez-Mendiola N *et al.* Evolution of rejection rates and kidney graft survival. A historical analysis. *Transplant. Proc.* 41(6), 2357–2359 (2009).

28 Hariharan S, McBride MA, Cohen EP. Evolution of endpoints for renal transplant outcome. *Am. J. Transplant.* 3(8), 933–941 (2003).

29 Solez K, Axelsen RA, Benediktsson H *et al.* International standardization of criteria for the histologic diagnosis of renal allograft rejection: the Banff working classification of kidney transplant pathology. *Kidney Int.* 44(3), 411–422 (1993).

■■ **Presents for the first time a valuable classification of histologic changes in kidney grafts.**

30 Gebel H, Bray R. Sensitization and sensitivity. Defining the unsensitized patient. *Transplantation* 69(7), 1370–1374 (2000).

31 Racusen LC, Solez K, Colvin RB *et al.* The Banff working classification on renal allograft pathology. *Kidney Int.* 55(2), 713–723 (1999).

32 Solez K, Colvin RB, Racusen LC *et al.* Banff´05 meeting report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy (´CAN¨). *Am. J. Transplant.* 7(3), 518–526 (2007).

■ **Identifies the different causes of progressive loss of graft function and eliminates the confusing term chronic allograft nephropathy.**

33 Solez K, Colvin RB, Racusen LC *et al.* Banff 07 classification of renal allograft pathology: updates and future directions. *Am. J. Transplant.* 8(4), 753–760 (2008).

34 Gaber LW, Moore LW, Alloway RR *et al.* Correlation between Banff classification, acute renal rejection scores and reversal of rejection. *Kidney Int.* 49(2), 481–487 (1996).

35 Gaber LW, Schoroeder TJ, Moore LW, Shakouh-Amiri MH, Gaber AO. The correlation of Banff scoring with reversibility of first and recurrent rejection episodes. *Transplantation* 61(12), 1711–1715 (1996).

36 Colvin RB, Cohen A, Siaontz C *et al.* Evaluation of the pathologic criteria for acute allograft rejection: reproductibility, sensitivity and clinical correlation. *J. Am. Soc. Nephrol.* 8(12), 1930–1941 (1997).

37 Terasaki PI. Humoral theory of transplantation. *Am. J. Transplant.* 3(6), 668–673 (2003).

38 Scornik JC, Guerra G, Schold JD, Srinivas TR, Dragun D, Meier-Kriesche H-U. Value of post-transplant antibody tests in the evaluation of patients with renal graft dysfunction. *Am. J. Transplant.* 7(7), 1808–1814 (2007).

39 Takemoto SK, Zeevi A, Feng S *et al.* National conference to assess antibody-mediated rejection in solid organ transplantation. *Am. J. Transplant.* 4(7), 1033–1041 (2004).

40 Racusen LC, Haas M. Antibody-mediated rejection in renal allografts: lessons from pathology. *Clin. J. Am. Soc. Nephrol.* 1(3), 415–420 (2006).

41 Colvin RB. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. *J. Am. Soc. Nephrol.* 18(4), 1046–1056 (2007).

42 Gloor J, Cosio F, Lager DJ, Stegall MD. The spectrum of antibody-mediated renal allograft injury: implications for treatment. *Am. J. Transplant.* 8(7), 1367–1373 (2008).

43 Kee TY-S, Chapman JR, O´Connell PJ *et al.* Treatment of subclinical rejection diagnosed by protocol biopsy of kidney transplants. *Transplantation* 82(1), 36–42 (2006).

44 Augustine JJ, Hricik DE. Steroid sparing in kidney transplantation: changing paradigs, improving outcomes and remaining questions. *Clin. J. Am. Soc. Nephrol.* 1(5), 1080–1089 (2006).

45 Kasiske BL, Chakkera HA, Louis TA, Ma JZ. A meta-analysis of immunosuppression withdrawal trials in renal transplantation. *J. Am. Soc. Nephrol.* 11(10), 1910–1917 (2000).

46 Meier-Kriesche H-U, Li S, Gruessner RWG *et al.* Immunosuppression: evolution in practice and trends, 1994–2004. *Am. J. Transplant.* 6(5), 1111–1131 (2006).

47 Luan FL, Steffick DE, Gadegbeku C, Norman SP, Wolfe R, Ojo AO. Graft and patient survival in kidney transplant recipients selected for *de novo* steroid-free maintenance immunosuppression. *Am. J. Transplant.* 9(1), 160–168 (2009).

48 Cole E, Landsberg D, Russell D *et al.* A pilot study of steroid-free immunosuppression in the prevention of acute rejection in renal allograft recipients. *Transplantation* 72(5), 845–850 (2001).

49 Kandaswamy R, Melancon JK, Dunn T *et al.* A prospective randomized trial of steroid-free maintenance regimens in kidney transplant regimes-an interim analysis. *Am. J. Transplant.* 5(6), 1529–1536 (2005).

50 Rostaig L, Cantarovich D, Mourad G *et al.* Corticosteroid-free immunosuppression with tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. *Transplantation* 79(7), 807–814 (2005).

51 Vincenti F, Schena FP, Paraskevas S *et al.*, on behalf of the FREEDOM Study Group. A randomized, multicenter study of steroid avoidance, early steroid withdrawal or standard steroid therapy in kidney transplant recipients. *Am. J. Transplant.* 8(2), 307–316 (2008).

52 Vitko S, Klinger M, Salmela K *et al.* Two corticosteroid-free regimens-tacrolimus monotherapy after basiliximab administration and tacrolimus/mycophenolate mofetil – in comparison with a standard triple regimen in renal transplantation: results of the Atlas study. *Transplantation* 80(12), 1735–1741 (2005).

53 Woodle ES, First MR, Pirsch J, Shihab F, Gaber AO, Van Veldhuisen P, for the Astellas Corticosteroid Withdrawal Study Group. A prospective, randomized, double blind, placebo-controlled multicenter trial comparing early (7 day) corticosteroid cessation versus long-term, low-dose corticosteroid therapy. *Ann. Surg.* 248(4), 564–577 (2008).

54 Matas A, Kandaswamy R, Humar A *et al.* Long-term immunosuppression, without maintenance prednisone, after kidney transplantation. *Ann. Sur.* 240(3), 510–517 (2004).

55 Humar A, Gillingham K, Kandaswamy R, Payne W, Matas A. Steroid avoidance regimens: a comparison of outcomes with maintenance steroids versus continued steroid avoidance in recipients having an acute rejection episode. *Am. J. Transplant.* 7(8), 1948–1953 (2007)

56 Opelz G, Dohler B, Laux G, for the Collaborative Transplant Study. Long-term prospective study of steroid withdrawal in kidney and heart transplant recipients. *Am. J. Transplant.* 5(4), 720–728 (2005).

57 Rama I, Cruzado JM, Gil-Vernet S *et al.* Steroids can be safely withdrawn from cyclosporine and mycophenolate mofetil-treated renal allograft recipients: long-term results. *Transplantation* 80(2), 164–168 (2005).

58 Gonzalez–Molina M, Gentil MA, Burgos D *et al.* Effect of long-term steroid withdrawal in renal transplant recipients: a retrospective cohort study. *NDT Plus* 3(Suppl. 2), ii32–ii36 (2010).

59 Pascual J, Galeano C, Royuela A, Zamora J. A systematic review on steroid withdrawal between 3 and 6 months after kidney transplantation. *Transplantation* 90(4), 343–349 (2010).

60 KDIGO Clinical Practice Guidelines for the Care of Kidney Transplant Recipients. Kidney disease: improving global outcomes (KDIGO) transplant work group. *Am. J. Transplant.* 9(Suppl. 3), S1–S157 (2009)

61 Barone G, Bunke CM, Choc MG *et al.* Safety and tolerability of Neoral® vs Sandimmune®: 1-year data in primary renal allograft recipients. *Transplant. Proc.* 28(4), 2183–2186 (1996).

62 Keown PA, for the Canadian and International Neoral Study Groups. Use of Cyclosporine microemulsion (Neoral®) in *de novo* and stable renal transplantation: clinical impact, pharmacokinetic consequences and economic benefits. *Transplant. Proc.* 28(4), 2147–2150 (1996).

63 Pollard SG, Lear PA, Ready AR, Moore RH, Johnson RWG, on behalf of the UK Neoral Renal Study Group. Comparison of microemulsion and conventional formulations of cyclosporine A in preventing acute rejection in the novo kidney transplant recipients. *Transplantation* 68(9), 1325–1331 (1999).

64 Shah MB, Martin JE, Schroeder TJ, First MR. The evaluation of the safety and tolerability of two formulations of cyclosporine: Neoral® and sandimmune. A meta-analysis. *Transplantation* 67(11), 1411–1417 (1999).

65 Kyllonen LE, Samella KT. Early cyclosporine C0 and C2 monitoring in the novo kidney transplant patients: a prospective randomized single-center pilot study. *Transplantation* 81(7), 1010–1015 (2006).

66 Knight SR, Morris PJ. The clinical benefits of cyclosporine C2-level monitoring: a systematic review. *Transplantation* 83(12), 1525–1535 (2007).

67 Solez K, Vincenti F, Filo R. Histopathologic findings from 2-year protocol biopsies from US multicenter kidney transplant trial comparing tacrolimus versus cyclosporine. A report of the FK506 transplant study group. *Transplantation* 66(12), 1736–1740 (1998).

68 Vincenti F, Jensik CS, Filo RS, Miller J, Pirsch J. A long-term comparison of tacrolimus (FK506) and cyclosporine in kidney transplantation: evidence for improved allograft survival at five years. *Transplantation* 73(5), 775–782 (2002).

69 Krämer BK, Montagnino G, del Castillo D *et al.,* for the European Tacrolimus versus Ciclosporin Microemulsion Renal Transplantation Study Group. Efficacy and safety of tacrolimus compared with cyclosporin A microemulsion in renal transplantation 2 year follow-up results. *Nephrol. Dial. Transplant.* 20(5), 968–973 (2005).

70 Krämer BK, del Castillo D, Margreiter R *et al.,* for the European Tacrolimus versus Ciclosporin Microemulsion Renal Transplantation Study Group. Efficacy and safety of tacrolimus compared with ciclosporin A in renal transplantation: three-year observational studies. *Nephrol. Dial. Transplant.* 23(7), 2386–2392 (2008).

71 Kaplan B, Schold JD, Meier-Kriesche H-U. Long-term graft survival with Neoral® and tacrolimus:a paired kidney analysis. *J. Am. Soc. Nephrol.* 14(11), 2980–2984 (2003).

72 Ekberg H, Bernasconi C, Tedesco-Silva H *et al.* Calcineurin inhibitor minimization in the symphony study: observational results 3 years after transplantation. *Am. J. Transplant.* 9(8), 1876–1885 (2009).

73 Nankivell BJ, Borrows RJ, Fung CL-S, O´Connell PJ, Allen RDM, Chapman JR. The natural history of chronic allograft nephropathy. *N. Engl. J. Med.* 349(24), 2326–2333 (2003).

74 Cosio FG, Amer H, Grande JP, Larson TS, Stegall MD, Griffin MD. Comparison of low versus high tacrolimus levels in kidney transplantation: assessment of efficacy by protocol biopsies. *Transplantation* 83(4), 411–416 (2007).

75 Vanrenterghem Y, van Hooff JP, Squifflet J-P *et al.* Minimization of immunosuppressive therapy after renal transplantation: results of a randomized controlled trial. *Am. J. Transplant.* 5(1), 87–95 (2005).

76 Pascual J, van Hoof JP, Salmela K *et al.,* on behalf of all participating investigators. Three-year observational follow-up of a multicenter, randomized trial on tacrolimus-based therapy with withdrawal of steroids or mycophenolate mofetil after renal transplantation. *Transplantation* 82(1), 55–61 (2006).

77 European Mycophenolate Mofetil Cooperative Study Group. Mycophenolate mofetil in renal transplantation: 3-year results from the placebo-controlled trial. *Transplantation* 68(3), 391–396 (1999).

78 US Renal Transplant Mycophenolate Study Group. Mycophenolate mofetil in cadaveric renal transplantation. *Am. J. Kidney Dis.* 34(2), 296–303 (1999).

79 Mathew TH, for the Tricontinental Mycophenolate Mofetil Renal Transplant Study Group. A blinded, long-term, randomized multicenter study of mycophenolate mofetil in cadaveric renal transplantation. Results at three years. *Transplantation* 65(11), 1450–1454 (1998).

80 Shapiro R, Jordan ML, Scantlebury VP *et al.* A prospective, randomized trial of tacrolimus/prednisone versus tacrolimus/prednisone/mycophenolate mofetil in renal transplant recipients. *Transplantation* 67(3), 411–415 (1999).

81 Miller J, Mendez R, Pirsch JD, Jensik SC, for the FK506/MMF Dose-Ranging Kidney Transplant Study Group. Safety and efficacy of tacrolimus in combination with mycophenolate mofetil (MMF) in cadaveric renal transplant recipients. FK506/MMF Dose-Ranging Kidney Transplant Study Group. *Transplantation* 69(5), 875–880 (2000).

82 Johnson C, Ahsan N, Gonwa T *et al.* Randomized trial of tacrolimus (Prograf®) in combination with azathioprine or mycophenolate mofetil versus cyclosporine (Neoral®) with mycophenolate mofetil after cadaveric kidney transplantation. *Transplantation* 69(5), 834–841 (2000).

83 Gonwa T, Johnson C, Ahsan N *et al.* Randomized trial of tacrolimus + mycophenolate mofetil or azathioprine versus cyclosporine + mycophenolate mofetil after cadaveric kidney transplantation: results at three years. *Transplantation* 75 (12), 2048–2053 (2003).

84 Squifflet J-P, Backman L, Claesson K *et al.,* for the European Tacrolimus-MMF Renal Study Group. Dose optimization of mycophenolate mofetil when administered with a low dose of tacrolimus in cadaveric renal transplant recipients. *Transplantation* 72(1), 63–69 (2001).

85 Salvadori M, Holzer H, de Mattos A *et al.,* on behalf of the ERL B301 Study Groups. Enteric-coated mycophenolate sodium is therapeutically equivalent to mycophenolate mofetil in *de novo* renal transplant patients. *Am. J. Transplant.* 4(2), 231–236 (2003).

86 Budde K, Curtis J, Knoll G *et al.,* on behalf of the ERL B301 Study Groups. Enteric coated mycophenolate sodium can be safely administered in maintenance renal transplant patients: results of a 1-year study. *Am. J. Transplant.* 4(2), 237–243 (2003).

87 Irish W, Arcona S, Gifford RJ, Baillie GM, Cooper M. Enteric-coated mycophenolate sodium versus mycophenolate mofetil maintenance immunosuppression: outcomes analysis of the United Network for Organ Sharing/Organ Procurement and Transplantation Network database. *Transplantation* 90(1), 23–30 (2010).

88 Sollinger HW, Sundberg AK, Leverson G, Voss BJ, Pirsch JD. Mycophenolate mofetil versus enteric-coated mycophenolate sodium; a large, single-center comparison of dose adjustments and outcomes in kidney transplant recipients. *Transplantation* 89(4), 446–451 (2010).

89 Theruvath TP, Saidman SL, Mauiyyedi S *et al.* Control of antidonor antibody production with tacrolimus and mycophenolate mofetil in renal allograft recipients with chronic rejection. *Transplantation* 72(1), 77–82 (2001).

90 Meier-Kriesche H-U, Steffen BJ, Hochberg AM *et al.* Long-term use of mycophenolate mofetil is associated with a reduction in the incidence and risk of late rejection. *Am. J. Transplant.* 3(1), 68–73 (2003).

91 Meier-Kriesche H-U, Steffen BJ, Hochberg AM *et al.* Mycophenolate mofetil versus azathioprine therapy is associated with a significant protection against long-term renal allograft function deterioration. *Transplantation* 75(8), 1341–1346 (2003).

92 Meier-Kriesche H-U, Morris JA, Chu AH *et al.* Mycophenolate mofetil vs azathioprine in a large population of elderly renal transplant patients. *Nephrol. Dial. Transplant.* 19(11), 2864–2869 (2004).

93 Shah S, Collett D, Johnson R *et al.* Long-term graft outcome with mycophenolate mofetil and azathioprine: a paired kidney analysis. *Transplantation* 82(12), 1634–1639 (2006).

94 Schold JD, Kaplan B. AZA/tacrolimus is associated with similar outcomes as MMF/tacrolimus among renal transplant recipients. *Am. J. Transplant.* 9(9), 2067–2074 (2009).

95 Remuzzi G, Lesti M. Goti E *et al.*, for the MYSS Study Group. Mycophenolate mofetil versus azathioprine for prevention of acute rejection in renal transplantation (MYSS): a randomized trial. *Lancet* 364(9433), 503–512 (2004).

96 Remuzzi G, Cravedi P, Costantini M *et al.*, for the MYSS Study Group. Mycophenolate mofetil versus azathioprine for prevention of chronic allograft dysfunction in renal transplantation: the MYSS follow-up randomized, controlled clinical trial. *J. Am. Soc. Nephrol.* 18(6), 1973–1985 (2007).

97 Kauffman HM, Cherik WS, McBride MA, Cheng Y, Hanto DW. Post-transplant *de novo* malignancies in renal transplant recipients: the past and present. *Transpl. Int.* 19(8), 607–620 (2006).

98 Morales JM, Marcén R, Andrés A *et al.* Renal transplantation in the modern immunosuppressive era in Spain: four-year results from a multicenter database focus on post-transplant cardiovascular disease. *Kidney Int.* 74(Suppl. 111), S94–S99 (2008).

99 Groth CG, Bäckman L, Morales JM *et al.*, for the Sirolimus European Renal Transplant Study Group. Sirolimus (rapamycin)-based therapy in human renal transplantation. Similar efficacy and different toxicity compared with cyclosporine. *Transplantation* 67(7), 1036–1042 (1999).

100 Van Hooff JP, Squifflet J-P, Wlodarczyk Z, Vanrenterghem Y, Paczek L. A prospective randomized multicenter study of tacrolimus in combination with sirolimus in renal transplant recipients. *Transplantation* 75 (12), 1934–1939 (2003).

101 Vitko SW, Wlodarczyk Z, Kyllönen L *et al.*, on behalf of the TERRA Study Group. Tacrolimus combined with two different dosages of sirolimus in kidney transplantation: results of a multicenter study. *Am. J. Transplant.* 6(3), 531–538 (2006).

102 Gonwa T, Mendez R, Yang HC *et al.*, for the Prograf Study Group. Randomized trial of tacrolimus in combination with sirolimus or mycophenolate mofetil in kidney transplantation: results at 6 months. *Transplantation* 75(8), 1213–1220 (2003).

103 Mendez R, Gonwa T, Yang HC, Weinstein S, Jensik S, Steinberg S, for the Prograf Study Group. A prospective, randomized trial of tacrolimus in combination with sirolimus or mycophenolate mofetil in kidney transplantation: results at 1 year. *Transplantation* 80(3), 303–309 (2005).

104 Ciancio G, Burke GW, Gaynor JJ *et al.* A randomized long-term trial of tacrolimus and sirolimus versus tacrolimus and mycophenolate mofetil versus cyclosporine (Neoral®) and sirolimus in renal transplantation. I. Drug interactions and rejection at 1 year. *Transplantation* 77(2), 244–258 (2004).

105 Gallon L, Perico N, Dimitrov BD *et al.* Long-term renal allograft function on a tacrolimus-based, pred-free maintenance immunosuppression comparing sirolimus vs MMF. *Am. J. Transplant.* 6(7), 1617–1623 (2006).

106 Gralla J, Wiseman AC. Tacrolimus/sirolimus versus tacrolimus/mycophenolate in kidney transplantation: improved 3-year graft and patient survival in recent era. *Transplantation* 87(11), 1712–1719 (2009).

107 Meier-Kriesche H-U, Schold JD, Srinivas TR, Howard RJ, Fujita S, Kaplan B. Sirolimus in combination with tacrolimus is associated with worse renal allograft survival compared with mycophenolate mofetil combined with tacrolimus. *Am. J. Transplant.* 5(9), 2273–2280 (2005).

108 Nashan B, Curtis J, Ponticelli C , Mourad G, Jaffe J, Haas T, on behalf of the 156 Study Group. Everolimus and reduced-exposure cyclosporine in *de novo* renal-transplant recipients: a three-year Phase II, randomized, multicenter, open-label study. *Transplantation* 78(9), 1332–1340 (2004).

109 Vitko S, Tedesco H, Eris J *et al.* Everolimus with optimized cyclosporine dosing in renal transplant recipients: 6-month safety and efficacy results of two randomized studies. *Am. J. Transplant.* 4(4), 625–635 (2004).

110 Tedesco-Silva H, Vitko S, Pascual J *et al.*, on behalf of the 2306 and 2307 Study Groups. 12-month safety and efficacy of everolimus with reduced exposure cyclosporine in the novo renal transplant recipients. *Transplant. Int.* 20(1), 27–36 (2007).

111 Tedesco-Silva H, Kim YS, Lackova E *et al.* Everolimus with reduced-dose cyclosporine as a strategy for optimizing long-term renal function: results from a randomized study in 833 *de novo* renal-transplant recipients. *Transplant. Int.* 22(Suppl. 1), 186 (2009).

112 Vitko S, Margreiter R, Weimar W *et al.*, for the RAD B201 Study Group. Three-year efficacy and safety results from a study of everolimus versus mycophenolate mofetil in the novo renal transplant recipients. *Am. J. Transplant.* 5(10), 2521–2530 (2005).

113 Lorber MI, Mulgaonkar S, Butt KMH *et al.* Everolimus versus mycophenolate mofetil in the prevention of rejection in the novo renal transplant recipients: a 3-year randomized, multicenter, Phase III study. *Transplantation* 80(2), 244–252 (2005).

114 Salvadori M, Scolani MP, Bertoni E *et al.* Everolimus with very low-exposure cyclosporine A in *de novo* kidney transplantation: a multicenter, randomized, controlled trial. *Transplantation* 88(10), 1194–1202 (2009).

115 Bertoni E, Larti A, Farsetti S *et al.* Cyclosporine (CyA) very low dose with everolimus (E) high dose is associated with better outcomes in renal transplant patients with respect to standard treatment with EC-MPS (M). *Transplant. Int.* 22(Suppl. 1), 91 (2009).

116 Chan L, Greenstein S, Hardy MA *et al.*, for the CRADUS09 Study Group. Multicenter, randomized study of the use of everolimus with tacrolimus after renal transplantation demonstrated its effectiveness. *Transplantation* 85(6), 821–826 (2008).

117 Ruiz JC, Campistol JM, Sanchez-Fructuoso A *et al.* Increase of proteinuria after conversion from calcineurin inhibitor to sirolimus-based treatment in kidney transplant patients with chronic allograft dysfunction. *Nephrol. Dial. Transplant.* 21(11), 3252–3257 (2006).

118 Letavernier E, Bruneval P, Mandet C *et al.* High sirolimus levels may induce focal segmental glomerulosclerosis *de novo*. *Clin. J. Am. Soc. Nephrol.* 2(2), 326–333 (2007).

■■ **First evidence of the sirolimus-induced lesion in kidney allografts.**

119 Oberbauer R, Kreis H, Johnson RWG *et al.*, for the Rapamune Maintenance Regimen Study Group. Long-term improvement in renal function with sirolimus after early cyclosporine withdrawal in renal transplant recipients: 2-year results of the Rapamune Maintenance Regimen Study. *Transplantation* 76(2), 364–370 (2003).

120 Russ G, Segoloni G, Oberbauer R *et al.*, for the Rapamune Maintenance Regimen Study Group. Superior outcomes in renal transplantation after early cyclosporine withdrawal and sirolimus maintenance therapy, regardless of baseline function. *Transplantation* 80(9), 1204–1211 (2005).

121 Lebranchu Y, Thierry A, Toupance O *et al.* Efficacy on renal function of early conversion from cyclosporine to sirolimus 3 months after renal transplantation: concept study. *Am. J. Transplant.* 9(5), 1115–1123 (2009).

122 Bemelman FJ, de Maar EF, Press RR *et al.* Minimization of maintenance immunosuppression early after renal transplantation: an interim analysis. *Transplantation* 88(3), 421–428 (2009).

123 Abramowicz D, Rial MC, Vitko S *et al.*, on behalf of the Cyclosporine Withdrawal Study Group. Cyclosporine withdrawal from a mycophenolate mofetil-containing immunosuppressive regimen: results of a five-year, prospective, randomized study. *J. Am. Soc. Nephrol.* 16(7), 2234–2240 (2005).

124 Ekberg H, Grinyó J, Nashan B, on behalf of the CAESAR study. Cyclosporine sparing with mycophenolate mofetil, daclizumab and corticosteroids in renal allograft recipients: the CAESAR study. *Am. J. Transplant.* 7(3), 560–570 (2007).

125 Guba M, Pratschke J, Hugo C *et al.*, for the SMART-study group. Renal function, efficacy, and safety of sirolimus and mycophenolate mofetil after short-term calcineurin inhibitor-based quadruple therapy in the novo renal transplant patients: one year analysis of a randomized multicenter trial. *Transplantation* 90(2), 175–183 (2010).

126 Grinyó JM, Campistol JM, Paul J *et al.* Pilot randomized study of early tacrolimus withdrawal from a regimen with sirolimus plus tacrolimus in kidney transplantation. *Am. J. Transplant.* 4(8), 1308–1314 (2004).

127 Schena FP, Pascoe MD, Albery J *et al.*, for the sirolimus CONVERT trial study group. Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-months efficacy and safety results from the CONVERT trial. *Transplantation* 87(2), 233–242 (2009).

128 Moore J, Middleton L, Cockwell P *et al.* Calcineurin inhibitor sparing with mycophenolate in kidney transplantation: a systematic review and meta-analysis. *Transplantation* 87(4), 591–605 (2009).

129 Flechner SM, Kurian SM, Solez K *et al.* *De novo* kidney transplantation without use of calcineurin inhibitors preserves renal structure and function at two years. *Am. J. Transplant.* 4(11), 1776–1785 (2004).

130 Flechner SM, Goldfarb D, Solez K *et al.* Kidney transplantation with sirolimus and mycophenolate mofetil-based immunosuppression: 5-year results of a randomized prospective trial compared with calcineurin inhibitor drug. *Transplantation* 83(7), 883–889 (2007).

131 Larson TS, Dean PG, Stegall MD *et al.* Complete avoidance of calcineurin inhibitors in renal transplantation: a randomized trial comparing sirolimus and tacrolimus. *Am. J. Transplant.* 6(3), 514–522 (2006).

132 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.* 5(3), 443–453 (2005).

133 Vincenti F, Blancho G, Durrbach A *et al.* Five-year safety and efficacy of belatacept in renal transplantation. *J. Am. Soc. Nephrol.* 21(9), 1–10 (2010).

■ **Demonstrates the possibility of immunosuppression without calcineurin inhibitor with similar results.**

134 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.* 10(3), 535–546 (2010).

135 Durrbach A, Pestana JM, Pearson T *et al.* A Phase III study of balatacept versus cyclosporine in kidney transplants from extended criteria donor (BENEFIT-EXT study). *Am. J. Transplant.* 10(3), 547–557 (2010).

136 Rostaing L, Massari P, Duro Garcia V *et al.* Switching from calcineurin inhibitor-based regimens to a belatacept-based regimen in renal transplant recipients: a randomized Phase II study. *Clin. J. Am. Soc. Nephrol.* 6, 430–439 (2011).

137 Everly JJ, Walsh RC, Alloway RR, Woodle ES. Proteasome inhibition for antibody-mediated rejection. *Curr. Opin. Organ Transplant.* 14(6), 662–665 (2009).

138 Perry DK, Burns JM, Pollinger HS *et al.* Proteasome inhibition causes apoptosis of normal human plasma cells preventing alloantibody production. *Am. J. Transplant.* 9(1), 201–209 (2009).

139 Everly MJ, Everly JJ, Susskind B *et al.* Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. *Transplantation* 86 (12), 1754–1761 (2008).

■ **Provides, for the first time, evidence that proteasome inhibition also inhibits cellular and humoral rejection.**

140 Walsh RC, Everly JJ, Brailey P *et al.* Proteasome inhibitor-based primary therapy for antibody-mediated renal allograft rejection. *Transplantation* 89(3), 277–284 (2010).

141 Sberro-Soussan R, Zuber J, Suberbielle-Boissel C *et al.* Bortezomib as the sole post-renal transplantation desensitization agent does not decrease donor-specific anti-HLA antibodies. *Am. J. Transplant.* 89(3), 681–686 (2010).

142 Trivedi HL, Terasaki PI, Feroz A *et al.* Abrogation of anti-HLA antibodies via proteasome inhibition. *Transplantation* 87(10), 1555–1561 (2009).

143 Wahrmann M, Haidinger M Körmöczi GF *et al.* Effect of the proteasome inhibitor bortezomib on humoral immunity in two presensitized renal transplant candidates. *Transplantation* 89(11), 1385–1390 (2010).

144 Triveri HL, Terasaki PI, Feroz A *et al.* Clonal deletion with bortezomib followed by low or no maintenance immunosuppression in renal allograft recipients. *Transplantation* 90(2), 221–222 (2010).

145 Jordan SC, Toyoda M, Vo AA. Intravenous immunoglobulin a natural regulator of immunity and inflammation. *Transplantation* 88(1), 1–6 (2009).

146 Jordan SC, Tyan D, Stablein D *et al.* Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial. *J. Am. Soc. Nephrol.* 15(12), 3256–3262 (2004).

147 Vo AA, Peng A, Toyoda M *et al.* Use of intravenous immune globulin and rituximab for desensitization of highly HLA-sensitized patients awaiting kidney transplantation. *Transplantation* 89(9), 1095–1102 (2010).

148 Lefaucheur C, Nochy D, Andrade J *et al.* Comparison of combination plasmapheresis/IVIg/anti-CD20 versus high dose IVIg theatment of antibody-mediated rejection. *Am. J. Transplant.* 9(5), 1099–1107 (2009).

149 Gaber AO, Monaco AP, Russell JA, Lebranchu Y, Mohty M. Rabbit antithymoglobulin (Thymoglobulin): 25 year and new frontiers in solid organ transplantation and haematology. *Drugs* 70(6), 691–732 (2010).

150 Cantarovich M, Durrbach A, Hiesse C, Ladouceur M, Benoit G, Charpentier B. 20-year follow-up results of a randomized controlled trial comparing antilymphocyte globulin induction to no induction in renal transplant recipients. *Transplantation* 86(12), 1732–1737 (2008).

151 Gill JS, Johnston O, Rose CL *et al.* Are there any benefits to using depleting antibodies in low risk kidney transplant recipients? *Am. J. Transplant.* 8(Suppl. 2), 215 (2008).

152 Hardinger KL, Schnitzler MA, Miller B *et al.* Five-year follow up of thymoglobulin versus Atgam induction in adult renal transplantation. *Transplantation* 78(7), 136–141 (2004).

153 Hardinger KL, Rhee S, Buchanan P *et al.* A prospective, randomized, double-blinded comparison of thymoglobulin versus Atgam for induction immunosuppressive therapy: 10 year results. *Transplantation* 86(7), 947–952 (2008).

154 Gaber AO, First MR, Tesi R J *et al.* Results of the double-blind, randomized, multicenter, Phase III clinical trial of thymoglobulin versus Atgam in the treatment of acute rejection episodes after renal transplantation. *Transplantation* 66(1), 29–37 (1998).

155 Goggins WC, Pascual MA, Powelson JA *et al.* A prospective, randomized, clinical trial of intraoperative versus postoperative thymoglobulin in adult cadaveric renal transplant recipients. *Transplantation* 76(5), 798–802 (2003).

156 Agha IA, Rueda J, Alvarez A *et al.* Short course induction immunosuppression with thymoglobulin for renal transplant recipients. *Transplantation* 73(3), 473–475 (2002).

157 Klem P, Cooper JE, Weiss AS *et al.* Reduced dose rabbit anti-thymocyte globulin induction for prevention of acute rejection in high-risk kidney transplant recipients. *Transplantation* 88(7), 891–896 (2009).

158 Charpentier B, Rostaing L, Berthoux F *et al.* A three-arm study comparing immediate tacrolimus therapy with antithymocyte globulin induction therapy followed by tacrolimus or cyclosporine A in adult renal transplant recipients. *Transplantation* 75(6), 844–851 (2003).

159 Glotz D, Charpentier B, Abramovicz D *et al.* Thymoglobulin induction and sirolimus versus tacrolimus in kidney transplant recipients receiving mycophenolate mofetil and steroids. *Transplantation* 89(12), 1511–1517 (2010).

160 Kandus A, Arnol M, Omahen K *et al.* Basiliximab versus daclizumab combined with triple immunosuppression in deceased donor renal transplantation: a prospective, randomized study. *Transplantation* 89(8), 1022–1027 (2010).

161 Pham K, Kraft K, Thielke J *et al.* Limited-dose daclizumab versus basiliximab: a comparison of cost and efficacy in preventing acute rejection. *Transplant. Proc.* 37(2), 899–902 (2005).

162 Adu D, Cockwell P, Ives NJ, Shaw J, Weatley K. Interleukin-2 receptor monoclonalantibodies in renal transplantation: meta-analysis of randomized trials. *BMJ* 326(7393), 789–794 (2003).

163 Webster AC, Playford EG, Higgins G, Chapman JR, Craig JC. Interleukin 2 receptor antagonists for renal transplant recipients: a meta-analysis of randomized trials. *Transplantation* 77(2), 166–176 (2004).

164 Sollinger H, Kaplan B, Pescovitz MD *et al.* Basiliximab versus antithymocyte globulin for prevention of acute renal allograft rejection. *Transplantation* 72(12), 1915–1919 (2001).

165 Lebranchu Y, Bridoux F, Buchler M *et al.* Immunoprophylaxis with basiliximab compared with antithymocyte globulin in renal transplant patients receiving MMF-containing triple therapy. *Am. J. Transplant.* 2(1), 48–56 (2002).

166 Mourad G, Rostaing L, Legendre C, Garrigue V, Thervet E, Durand D. Sequential protocols using basiliximab versus antithymocyte globulins in renal-transplant patients receiving mycophenolate mofetil and steroids. *Transplantation* 78(4), 584–590 (2004).

167 Kyllönen LE, Eklund BH, Pesonem EJ, Salmela KT. Single bolus antithymocyte globulin versus basiliximab induction in kidney transplantation with cyclosporine triple immunosuppression: efficacy and safety. *Transplantation* 84(1), 75–82 (2007).

168 Brennan DC, Daller JA, Lake KB, Cibrik D, del Castillo D. Rabbit antithymocyte globulin versus basiliximab in renal transplantation. *N. Engl. J. Med.* 355(19), 1967–1977 (2006).

169 Noël C, Abramowicz D, Durand D *et al.* Daclizumab versus antithymocyte globulin in high-immunological-risk renal transplant recipients. *J. Am. Soc. Nephrol.* 20(6), 1385–1392 (2009).

170 Willoughby LW, Schnitzler MA, Brennan DC *et al.* Early outcomes of thymoglobulin and basiliximab induction in kidney transplantation: application of statistical approaches to reduce bias in observational comparisons. *Transplantation* 87(10), 1520–1529 (2009).

171 Weaver TA, Kirk A. Alemtuzumab. *Transplantation* 84(12), 1545–1547 (2007).

172 Calne R, Friend P, Moffatt S *et al.* Prope tolerance, perioperative campath 1H, and low-dose cyclosporin monotherapy in renal allograft recipients. *Lancet* 351(9117), 1701–1702. (1998).

173 Watson CJE, Bradley JA, Friend PJ *et al.* Alemtuzumab (CAMPATH 1H) induction therapy in cadaveric kidney transplantation-efficacy. *Am. J. Transplant.* 5(6), 1347–1353 (2005).

174 Knechtle SJ, Fernandez LA, Pirsch JD *et al.* Campath-1H in renal transplantation : the University of Wisconsin experience. *Surgery* 136(4), 754–760 (2004).

175 Margreiter R, Klempnauer J, Neuhaus P, Muehlbacher F, Boesmueller C, Calne RY. Alemtuzumab (Campath-1H) and tacrolimus monotherapy after renal transplantation: results of a prospective randomized trial. *Am. J. Transplant.* 8(7), 1480–1485 (2008).

176 Farney AC, Doares W, Rogers J *et al.* A randomized trial of alemtuzumab versus antithymocyte globulin induction in renal and pancreas transplantation. *Transplantation* 88(6), 810–819 (2009).

177 Ciancio G, Burke GW, Gaynor JJ. A randomized trial of thymoglobulin vs alemtuzumab (with lower dose maintenance immunosuppression) vs daclizumab in renal transplantation at 24 months of follow-up. *Clin. Transplant.* 22(2), 200–211 (2008).

178 Kaufman DB, Leventhal JR, Axelrod D, Gallon LG, Parker MA, Stuart FP. Alemtuzumab induction and prednisone-free maintenance immunotherapy in kidney transplantation: comparison with basiliximab induction-long-term results. *Am. J. Transplant.* 5(2), 2539–2548 (2005).

179 Schadde E, D´Alessandro AM, Knechtle SJ *et al.* Alemtuzumab induction and triple maintenance immunotherapy in kidney transplantation from donors after cardiac death. *Transpl. Int.* 21(7), 625–636 (2008).

180 Huang E, Cho YW, Hayashi R, Bunnapradist S. Alemtuzumab induction in deceased donor kidney transplantation. *Transplantation* 84(7), 821–828 (2007).

181 Flechner SM, Friend PJ, Brockmann J *et al.* Alemtuzumab induction and sirolimus plus mycophenolate mofetil maintenance for CNI and steroid-free kidney transplant immunosuppression. *Am. J. Transplant.* 5(12), 3009–3014 (2005)

182 Clatworthy MR, Friend PJ, Calne RY *et al.* Alemtuzumab (CAMPATH-1H) for the treatment of acute rejection in kidney transplant recipients: long-term follow-up. *Transplantation* 87(7), 1092–1095 (2009).

183 Pescovitz MD. Rituximab, an anti-CD20 monoclonal antibody: history and mechanism of action. *Am. J. Transplant.* 6(5), 859–866 (2006).

184 Clatworthy MR, Watson CJ, Plotnek G *et al.* B-cell-depleting induction therapy and acute cellular rejection. *N. Engl. J. Med.* 360(25), 2683–2685 (2009).

185 Tydén G, Genberg H, Tollemar J *et al.* A randomized, double blind, placebo-controlled, study of single-dose rituximab as induction in renal transplantation. *Transplantation* 87(9), 1325–1329 (2009).

186 Takagi T, Ishida H, Shirakawa H, Shimizu T, Tanabe K. Evaluation of low-dose rituximab induction therapy in living related kidney transplantation. *Transplantation* 89(12), 1466–1470 (2010).

187 Mulley WR, Hudson FJ, Tait BD *et al.* A single low-fixed dose of rituximab to salvage renal transplants from refractory antibody-mediated rejection. *Transplantation* 87(2), 286–289 (2009).

■ **Presents data showing that low-dose rituximab with immune globulin preparations and plasmapheresis controls refractory acute humoral rejection.**

188 Faguer S, Kamar N, Guilbeaud-Frugier C *et al.* Rituximab therapy for acute humoral rejection after kidney transplantation. *Transplantation* 83(9), 1277 (2007).

189 Lefaucheur C, Nochy D, Andrade J *et al.* Comparison of combination plasmapheresis/IVIg/antiCD20 versus high-dose IVIg in the treatment of antibody-mediated rejection. *Am. J. Transplant.* 9(5), 1099–1107 (2009).

190 Kaposztas Z, Pdder H Mauiyyedi S *et al.* Impact of rituximab therapy for treatment of acute humoral rejection. *Clin. Transplant.* 23(1), 63–73 (2009)

191 Fehr T, Rüsi B, Fischer A, Hopfer H, Wüthrich RP, Gaspert A. Rituximab and intravenous immunoglobulin treatment of chronic antibody-mediated kidney allograft rejection. *Transplantation* 87(12), 1837–1841 (2009).

192 Hillmen P, Young NS, Schubert J *et al.* The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N. Engl. J. Med.* 355(12), 1233–1243 (2006).

193 Lonze BE, Singer AL, Montgomery RA. Eculizumab and renal transplantation in a patient with CAPS. *N. Engl. J. Med.* 362(18), 1744–1745 (2010).

194 Nurnberger J, Witzke O, Opazo Saez A, Vester U, Baba HA, Kribben A. Eculizumab for atypical hemolytic-uremic syndrome. *N. Engl. J. Med.* 360(5), 542–544 (2009).

195 Larrea CF, Cofan F, Oppenheimer F, Campistol JM, Escolar G, Lozano M. Efficacy of eculizumab in the treatment of recurrent atypical hemolitic-uremic syndrome after renal transplantation. *Transplantation* 89(7), 903–904 (2010).

196 Locke JE, Magro CM, Singer AL *et al.* The use of antibody to complement protein C5 for salvage treatment of severe antibody-mediated rejection. *Am. J. Transplant.* 9(1), 231–236 (2009).

197 Mulgaonkar S, Tedesco H, Oppenheimer F *et al.,* for the FTYA121 study group. FTY720/cyclosporine regimens in *de novo* renal transplantation: a 1-year dose-finding study. *Am. J. Transplant.* 6(8), 1848–1857 (2006).

198 Tedesco-Silva H, Mourad G, Kahan BD *et al.* FTY720, a novel immunomodulator: efficacy and safety results from the first Phase 2A study in the novo renal transplantation. *Transplantation* 79(11), 1553–1560 (2005).

199 Tedesco-Silva H, Szakaly P, Shoker A *et al.,* for the FTY720 2218 clinical study group. FTY720 versus mycophenolate mofetil in the novo renal transplantation: six months results of a double-blind study. *Transplantation* 84(7), 885–892 (2007).

200 Salvadori M, Budde K, Charpentier B *et al.,* for the FTY720 0124 Study Group. FTY720 versus MMF with cyclosporine in the novo renal transplantation: a 1-year, randomized controlled trial in Europe and Australasia. *Am. J. Transplant.* 6(12), 2912–2921 (2006).

201 Matz M, Weber U, Mashreghi M-F *et al.* Effects of the new immunosuppressive agents AEB071 on human immune cells. *Nephrol. Dial. Transplant.* 25(7), 2159–2167 (2010).

202 Weaver TA, Charafeddine AH, Agarwal A *et al.* Alefacept promotes costimulation blockade based allograft survival in primates. *Nat. Med.* 15(7), 746–749 (2009).

203 Busque S, Leventhal J, Brennan DC *et al.* Calcineurin-inhibitor-free immunosuppression based on the JAK inhibitor CP-690,550: a pilot study in *de novo* kidney allograft recipients. *Am. J. Transplant.* 9(8), 1936–1945 (2009).

204 Fangman J, Kathrin Al-Ali H, Sack U *et al.* Kidney transplant from the same donor without maintenance immunosuppression after previous hematoietic stem cell transplant. *Am. J. Transplant.* 11(1), 156–162 (2011).

205 McDonald S, Russ G, Campbell S, Chadban S. Kidney transplant rejection in Australia and New Zealand: relationship between rejection and graft outcome. *Am. J. Transplant.* 7(5), 1201–1208 (2007).

206 Opelz G, Döhler B, for the Collaborative Transplant Study Report. Influence of time of rejection on long-term graft survival in renal transplantation. *Transplantation* 85(5), 661–666 (2008).

207 Pallardó LM, Sancho A, Capdevila L, Franco A. Acute rejection and late renal transplant failure: risk factors and prognosis. *Nephrol. Dial. Transplant.* 19(Suppl. 3), iii38–iii42 (2004).

208 Moreso F, Alonso A, Gentil MA *et al.* Improvement in late renal allograft survival between 1990 and 2002 in Spain: results from a multicentre case control study. *Transplant. Int.* 23(9), 907–913 (2010).

209 El-Zoughby ZM, Stegall MD, Lager DJ *et al.* Identifying specific causes of kidney allograft loss. *Am. J. Transplant.* 9(3), 527–535 (2009).

210 Gaston RS, Cecka JM, Kasiske BL *et al.* Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. *Transplantation* 90(1), 68–74 (2010).