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.
**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 and to predict graft function. Core biopsy has been recommended as the gold standard of diagnosing acute rejection, by deposition of C4d in 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.**

<table>
<thead>
<tr>
<th><strong>Corticosteroids</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calcineurin inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td><em>■</em> Cyclosporine</td>
<td></td>
</tr>
<tr>
<td><em>■</em> Tacrolimus</td>
<td></td>
</tr>
<tr>
<td><strong>Antimetabolites</strong></td>
<td></td>
</tr>
<tr>
<td><em>■</em> Azathioprine</td>
<td></td>
</tr>
<tr>
<td><em>■</em> Mycophenolic acid:</td>
<td></td>
</tr>
<tr>
<td>- Mycophenolate mofetil</td>
<td></td>
</tr>
<tr>
<td>- Mycophenolate sodium</td>
<td></td>
</tr>
<tr>
<td><strong>mTOR inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td><em>■</em> Sirolimus</td>
<td></td>
</tr>
<tr>
<td><em>■</em> Everolimus</td>
<td></td>
</tr>
<tr>
<td><strong>Co-stimulation blockers</strong></td>
<td></td>
</tr>
<tr>
<td><em>■</em> Belatacept</td>
<td></td>
</tr>
<tr>
<td><strong>Proteasome inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td><em>■</em> Bortezomib</td>
<td></td>
</tr>
<tr>
<td><strong>Induction therapy</strong></td>
<td></td>
</tr>
<tr>
<td><em>■</em> Polyclonal antibodies:</td>
<td></td>
</tr>
<tr>
<td>- Immune globulin</td>
<td></td>
</tr>
<tr>
<td>- Atgam</td>
<td></td>
</tr>
<tr>
<td>- Thymoglobulin</td>
<td></td>
</tr>
<tr>
<td><em>■</em> Monoclonal antibodies:</td>
<td></td>
</tr>
<tr>
<td>- IL-2R antibodies: basiliximab and daclizumab</td>
<td></td>
</tr>
<tr>
<td>- Anti-CD52 antibodies: alemtuzumab</td>
<td></td>
</tr>
<tr>
<td>- Anti-CD20 antibodies: rituximab</td>
<td></td>
</tr>
<tr>
<td>- Anti-complement protein C5 antibodies: eculizumab</td>
<td></td>
</tr>
<tr>
<td><strong>Other immunosuppressive agents</strong></td>
<td></td>
</tr>
<tr>
<td><em>■</em> Sphingosine 1-phosphate receptor agonists: FTY720</td>
<td></td>
</tr>
<tr>
<td><em>■</em> Protein kinase C inhibitors: AEB071</td>
<td></td>
</tr>
<tr>
<td><em>■</em> Depleting T-effector memory cells: alefacept</td>
<td></td>
</tr>
<tr>
<td><em>■</em> Janus kinase inhibitors: CP-690550</td>
<td></td>
</tr>
</tbody>
</table>
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 exists 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 micro-emulsion (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 performed 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 permitted 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 compar-
ing 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 mor-
pholinoethyl 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 inhibi-
tor of inosine monophosphate dehydrogenase (IMPDH),
an enzyme involved in the synthesis of guanosine nucle-
etides, 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 corti-
costeroids, with CsA/AZA or placebo and corticoste-
roids, 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 pla-
ceso/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 asso-
ciated with nephrotoxicity and increased rates of cardio-
vascular risk factors, immunosuppressive regimens that
permit dose reduction of these agents have been devel-
oped. As was commented before, the Symphony study has
demonstrated that an MMF-based regimen permit-
ted 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 demon-
strated 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 anal-
ysis of 238 recipients, comparing the effects of AZA or
MMF combined with CNI and corticosteroids. This
study demonstrated that the incidence of acute rejec-
tion 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 com-
mon 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–ben-
etit 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 proto-
cols. 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 syn-
ergistic immunosuppressive activity, due to their dif-
ferent 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 anti-proliferative 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 lymphocites. 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 [125]. 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 nephropathy 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 therapeutic 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 recipients 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 rituximab 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 effectiveness 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 immunosuppression, 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, particularly 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 occurred later [173]. Another retrospective study from Wisconsin University demonstrated no differences between patients treated with alemtuzumab and those treated with other immunosuppressive regimens. However, when patients with delayed graft function were separately examined, alemtuzumab 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 thymoglobulin 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 on 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 transplantsations, 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/m²)
Table 1. Incidence of rejection and graft survival in selected trials.

<table>
<thead>
<tr>
<th>Trial name/author (year)</th>
<th>Patients (n)</th>
<th>Immunosuppression</th>
<th>Acute rejection rate (%)</th>
<th>Period of analysis (years)</th>
<th>Graft function</th>
<th>Graft survival (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>USRTMMSG (1995 and 1999)</td>
<td>499</td>
<td>CsA/AZA/steroids</td>
<td>53.6</td>
<td>3</td>
<td>Not available, but Scr levels were not different along the study</td>
<td>74.7</td>
<td>[14,78]</td>
</tr>
<tr>
<td>EMMCSG (1995 and 1999)</td>
<td>491</td>
<td>CsA/PLA/steroids</td>
<td>46.4</td>
<td>3</td>
<td>Not available</td>
<td>78.0</td>
<td>[15,77]</td>
</tr>
<tr>
<td>Mathew (1999)</td>
<td>503</td>
<td>CsA/AZA/steroids</td>
<td>35.5</td>
<td>3</td>
<td>1.70 ± 0.1 mg/dl</td>
<td>81.4</td>
<td>[16,79]</td>
</tr>
<tr>
<td>Vincenti et al. (2002)</td>
<td>412</td>
<td>TAC/AZA/Pred.</td>
<td>30.7</td>
<td>5</td>
<td>1.4 mg/dl</td>
<td>64.3</td>
<td>[68]</td>
</tr>
<tr>
<td>Kramer et al. (2008)</td>
<td>560</td>
<td>TAC/AZA/Pred.</td>
<td>35.1</td>
<td>3</td>
<td>67.3 ± 23.6 ml/min</td>
<td>88.0</td>
<td>[70]</td>
</tr>
<tr>
<td>Russ et al. (2005)</td>
<td>430</td>
<td>SRL/CsA/steroids</td>
<td>Not available but similar in the two groups</td>
<td>4</td>
<td>43.8 ml/min</td>
<td>84.2</td>
<td>[120]</td>
</tr>
<tr>
<td>Abramowicz et al. (2005)</td>
<td>151</td>
<td>CsA/MMF 2 g/steroids</td>
<td>1.0</td>
<td>5</td>
<td>61.7 ml/min</td>
<td>87</td>
<td>[123]</td>
</tr>
<tr>
<td>Ekberg et al. (2009)</td>
<td>958</td>
<td>CsA/MMF/steroids</td>
<td>27</td>
<td>3</td>
<td>65.3 ± 26.2 ml/min</td>
<td>87</td>
<td>[172]</td>
</tr>
</tbody>
</table>
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 paroxysmal 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 hemolytic 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
Review: Clinical Trial Outcomes

Marcén

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 retrospective 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 refractory acute rejection.
- Polyclonal 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.


16 The Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. The efficacy and safety of low-dose mycophenolate mofetil (MMF) when compared with azathioprine.


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


Review: Clinical Trial Outcomes

Marcén


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


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


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)


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).


Review: Clinical Trial Outcomes

Marcén


115 Bertoni E, Larrt 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).

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


Review: Clinical Trial Outcomes

Marcén


Immunosuppression & renal transplant rejection


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


