IMMUNOSUPPRESSIVE STRATEGIES IN SOLID ORGAN TRANSPLANTATION

Sonia Lin, Pharm.D., BCPS

Reviewed by Judy Cheng, Pharm.D., FCCP, BCPS With Added Qualifications in Cardiology; and Nicole M. Sifontis, Pharm.D., BCPS

Learning Objectives

1. Justify the rationale for using multiple drug regimens in recipients of solid organ transplants.
2. Analyze the limitations of established immunosuppressive strategies.
3. Evaluate the relative benefits and risks of withdrawal and avoidance immunosuppressive strategies.
4. Design and implement immunosuppressive strategies aimed at decreasing toxicity and improving long-term patient outcomes.
5. Design and implement effective immunosuppressive strategies for immunological high-risk transplant recipients.

Introduction

The field of solid organ transplantation has made great progress over the past decade. Many new agents have joined the armamentarium of immunosuppressive drugs for use in various combinations of immunosuppression regimens. Also, a few drugs with novel mechanisms of action are currently being evaluated in early clinical trials. The use of these new immunosuppressive drugs has significantly decreased the rates of acute rejection (AR) over the past 10 years. In kidney transplantation, AR rates were high in the 1960s with allograft survival at 1 year being 50%. In the cyclosporine (CSA) era, AR rates decreased significantly with a corresponding increase in allograft survival rates to more than 80%. The use of more potent immunosuppressive therapy over the past 10 years has further decreased AR rates and improved allograft survival rates in kidney transplant recipients to about 15% and 90%–95%, respectively, at 1 year post-transplantation.

Despite the significant improvement in lower AR rates and increased 1-year allograft survival rates, there has not been a proportionate corresponding improvement in overall 5–10-year long-term allograft survival. Death-censored allograft survival, which takes into consideration patients who died with a funtioning graft, has also significantly decreased. Although AR is a strong predictor of poor graft survival, the relative importance of other confounding variables may be changing over time. First, there has been a widening gap between the number of patients on the transplant waiting list and the number of available organs for transplantation. Strategies that have been implemented in an effort to expand the donor pool to minimize this gap include using organs obtained from expanded criteria donors (ECDs) and organs obtained from expanded donors experiencing cardiac death. These strategies may lead to an increased risk of delayed allograft function, which is an independent risk factor for poor allograft survival. Second, a trend toward transplantation in the elderly due to improved patient survival rates in the general population, as well as the transplant population, can also result in an increased risk of delayed allograft function. Finally, adverse effects of intensive immunosuppression may be more apparent due to improved patient survival. Over-immunosuppression can lead to an increased incidence of complications such as chronic allograft nephropathy (CAN) and polyoma virus nephropathy, as well as increased incidence of cardiovascular risk factors, such as hypertension, lipid abnormalities, and glucose intolerance. These variables can have a detrimental effect on long-term allograft function.

Thus, current and future trends in immunosuppression for solid organ transplantation are focused on strategies to minimize immunosuppressive therapy without compromising their efficacy. Long-term complications such as infection and malignancy are still a concern. The two main drug classes that are targeted for drug minimization are corticosteroids and calcineurin inhibitors (CNIs). Regimens incorporating new immunosuppressive drugs that facilitate minimization of agents in these two drug classes in kidney transplantation are the main focus of this chapter; however,

Abbreviations in this Chapter

AR	Acute rejection
AUC	Area under the curve
AZA	Azathioprine
CAN	Chronic allograft nephropathy
CNI	Calcineurin inhibitor
CSA	Cyclosporine
CYP	Cytochrome P450
ECD	Expanded criteria donor
EC-MPS	Enteric-coated mycophenolate sodium
GI	Gastrointestinal
HLA	Human leukocyte antigen
IL	Interleukin
IVIG	Intravenous immunoglobulin
MHC	Major histocompatibility complex
MMF	Mycophenolate mofetil
MPA	Mycophenolic acid
PRA	Panel reactive antibodies

reference is made to use of drugs for immunosuppressive drug therapy for other solid organ transplant types in other solid organ transplant types. Significant progress has been made in the area of tolerance induction, which is promising for alleviating the need for lifelong immunosuppressive therapy altogether. Defining effective therapy to prevent or delay the onset of end-organ failure is also a focus of ongoing research.

With the increasing complexity of immunosuppressive drug regimens, there is a growing need for the presence of a clinical pharmacist as an integral member of a multidisciplinary transplant team. The pharmacist's knowledge focuses on the safety and efficacy of drug use through application of pharmacokinetic and pharmacodynamic principles to specific drugs. This application of knowledge allows pharmacists to more effectively design and implement therapeutic plans for complex drug regimens, as well as proactively identify and manage potential adverse drug reactions and drug interactions. Collaboration with physicians and transplant coordinators with regard to therapeutic drug monitoring and drug counseling brings pharmacists one step closer to individualizing immunosuppressive regimens that improve patient compliance and ultimately, long-term graft and patient survival.

Overview of the Principles of Immunosuppression

The immune response is described in a simplified three-signal model. Initiation of the response is triggered by T-cell receptor recognition of a foreign antigen (Class II antigen) presented by the major histocompatibility complex (MHC) on antigen-presenting cells such as B lymphocytes, dendritic cells, and macrophages. This interaction is referred to as "Signal 1". Co-stimulatory molecules, CD80 and CD86, which are present on the surface of antigen-presenting cells must also interact with the co-stimulatory receptor, CD28, on the T-cell surface before full T-cell activation occurs. This co-stimulatory interaction is referred to as "Signal 2." The combination of Signal 1 and Signal 2 leads to the activation of three-signal transduction pathways, one of which is the calcium-calcineurin pathway, triggering production of cytokines such as interleukin (IL)-2 by activated T cells. These molecules bind to CD25, which are IL-2 receptors on the surface of the activated T cells, to trigger the mammalian target of rapamycin to induce T-cell proliferation and further cytokine production. This step is referred to as "Signal 3." The absence of Signal 2 leads to T-cell unresponsiveness and a halt in the rest of the immune cascade.

Immunosuppressive regimens generally consist of triple-drug maintenance therapy that includes drugs that act at different levels of the immune cascade. Lymphocyte-depleting induction agents act at Signal 1 by destroying circulating T cells and B cells, inhibiting the first step of the immune response. Calcineurin inhibitors and IL-2 receptor antagonists act at Signal 1 and Signal 3, respectively, by inhibiting cytokine production and IL-2-mediated activation of the mammalian target of rapamycin. Antiproliferative drugs such as azathioprine (AZA) and mycophenolate mofetil (MMF), which inhibit purine synthesis, in conjunction with mammalian target of rapamycin inhibitors such as sirolimus and everolimus, act downstream from Signal 3 to ultimately inhibit T-cell proliferation. New immunosuppressive drugs that are being evaluated target other parts of the immune cascade. For example, belatacept acts at Signal 2 by binding to CD80 and CD86 to inhibit binding to the costimulating receptor, CD28 (Figure 1-1).

Phases of Immunosuppression
Induction Drugs

Biologic induction drugs are used for initial immunosuppression in 70%–80% of solid organ transplants that are performed currently, with the exception of liver transplants in which only 20% of procedures use induction therapy. Induction therapy is used for the prevention of AR post-transplant and can be either polyclonal or monoclonal. The antilymphocyte preparations such as murine-derived CD3 monoclonal antibody, muromonab (OKT3), and antithymocyte globulin are also indicated for treatment of AR or refractory, steroid-resistant rejection post-transplant. Over the past decade, there has been a dramatic shift from the use of OKT3 and equine-derived polyclonal antithymocyte antibody (Atgam) to better-tolerated drugs such as rabbit-derived antithymocyte antibody (Thymoglobulin) and anti-IL-2 monoclonal antibodies (basiliximab and daclizumab). More recently, an increasing number of transplant centers are starting to use alemtuzumab (Campath-1H), an anti-CD52 monoclonal antibody, for induction therapy.

Antibody therapy is used in immunosuppressive regimens to delay the introduction of maintenance treatment with CNI drugs and to treat patients whose immune systems are sensitized. Antibody therapy is being incorporated into

Halloran PF. Immunosuppressive drugs for kidney transplantation. N Engl J Med 2004;351:2715–29.
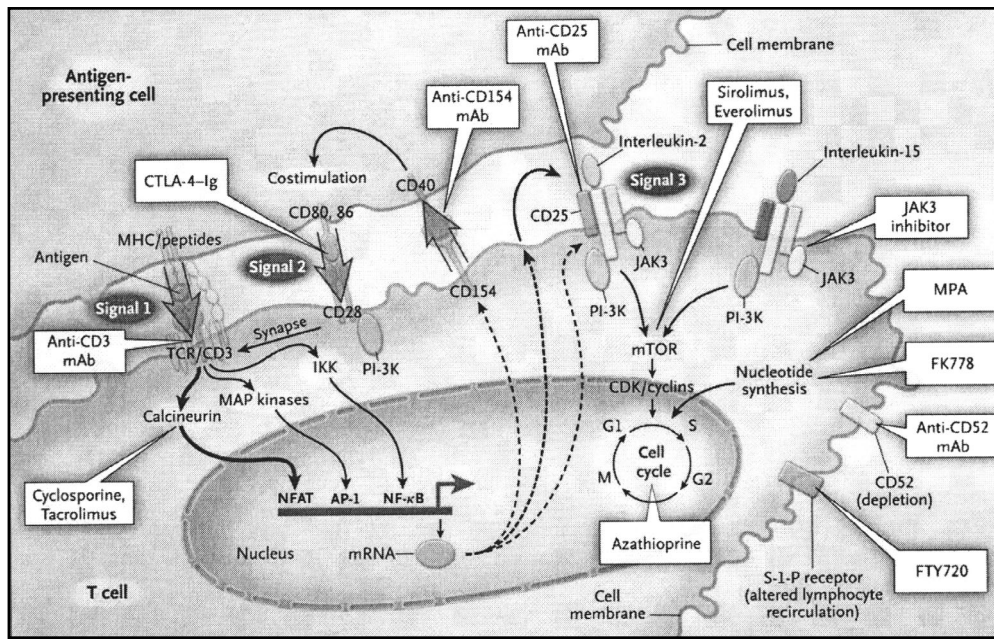
Figure 1-1. The immune cascade and the mechanisms of action of immunosuppressive therapy.
AP-1 = activator protein 1; CDK = cyclin-dependent kinase; CTLA-4-Ig = cytotoxic T-lymphocyte-associated antigen 4 immune globulin; IKK = inhibitor of nuclear factor-B kinase; JAK3 = janus kinase 3; mAb = monoclonal antibody; MAP = mitogen-activated protein; MHC = major histocompatibility complex; MPA = mycophenolic acid; mTOR = Mammalian target of rapamycin; NFAT = nuclear factor of activated T-cells; NF-kB = nuclear factor Kappa-B; PI-3K = phosphoinositide-3-kinase; S-1-P = sphingosine-1-phosphate; TCR = T-cell receptor.
Adapted with permission from the Massachusetts Medical Society and Halloran PF. Drug therapy: immunosuppressive drugs of kidney transplantation. N Engl J Med 2004;351:2715–29.

immunosuppression minimization protocols that foster CNI and steroid withdrawal or avoidance. The role of antibody therapy in immunologic conditioning and tolerance induction is also currently being evaluated.

Maintenance Immunosuppression

Standard maintenance immunosuppressive regimens consist of a three-drug combination to simultaneously target the immune response at various levels. Calcineurin inhibitors are used in most transplant recipients on discharge from the hospital after the transplant procedure. The trend has been decreased CSA use with a corresponding rise in tacrolimus use. Cyclosporine and tacrolimus are typically used in daily maintenance doses of 3–5 mg/kg and 0.15–0.3 mg/kg, respectively. Therapeutic drug monitoring incorporates target trough levels of 150–250 ng/mL and 5–10 ng/mL for CSA and tacrolimus, respectively, after the first 3 months post-kidney transplantation. Antiproliferative drugs are also a common part of triple-drug therapy and a more noticeable shift has occurred from the use of AZA to

MMF. Routine therapeutic drug monitoring is not required with antiproliferative therapy, although its clinical value in MMF therapy is being evaluated. Finally, corticosteroids continue to be used as part of a triple-drug immunosuppressive regimen; however, long-term use has declined almost 10% between 1993 and 2002, indicating the trend toward adopting corticosteroid withdrawal and avoidance protocols. This trend spans across all types of solid organ transplants.

Treatment of Rejection

The incidence of AR in recipients of a kidney transplant has declined steadily from 38% to 15% over the past decade. A similar trend has also been observed post-liver transplantation. Treatment of acute cellular rejections in kidney transplantation is based on the Banff 97 Classification criteria, which is a grading system that can also be applied to acute humoral rejections and CAN. Classification is somewhat subjective and is based on the severity of injury to the nephronic structures. The mainstay

Helderman JH, Bennett WM, Cibrik DM, Kaufman DB, Klein A, Takemoto SK. Immunosuppression: practice and trends. Am J Transplant 2003;3(suppl 4):41–52.

2004 Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients: Transplant Data 1994–2003. Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation, Rockville, MD; United Network for Organ Sharing, Richmond, VA; University Renal Research and Education Association, Ann Arbor, MI, 2004:IV-1–14.

van Gelder T, Shaw LM. The rationale for and limitations of therapeutic drug monitoring for mycophenolate mofetil in transplantation. Transplantation 2005;80:S244–S53.

Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. Kidney Int 1999;55:713–23.

Racusen LC, Colvin RB, Solez K, et al. Antibody-mediated rejection criteria – an addition to the Banff 97 classification of renal allograft rejection. Am J Transplant 2003;3:708–14.

of treatment for AR remains high-dose intravenous corticosteroids for mild to moderate episodes and antibody therapy for severe episodes or those that are unresponsive to corticosteroid therapy. Intravenous immunoglobulin (IVIG) has been used for humoral rejections, and sirolimus may play a role as rescue therapy in the treatment of corticosteroids and anti-lymphocytic resistant rejections.

Traditional Immunosuppressive Regimens

Calcineurin Inhibitor-Based Immunosuppressive Regimens

Calcineurin inhibitors have been an integral part of immunosuppressive therapy for kidney transplantation since the 1980s. They are also the backbone of maintenance immunosuppressive regimens post-liver and post-heart transplant. Cyclosporine and tacrolimus, two CNIs used in clinical practice, inhibit the production of T-cell growth factors like IL-2 by binding with intracellular proteins called immunophilins, to inhibit the calcium/calmodulin-activated phosphatase, calcineurin. This CNI blocks transcriptional activation of the early T-cell specific genes and ultimately results in inhibition of T-cell proliferation. Both CNIs are effective in reducing the frequency of AR after a kidney transplant when used in combination with the newer immunosuppressive drugs. Their adverse effect profiles are similar, but slight nuances exist. Knowledge of these nuances will allow clinicians to tailor individual immunosuppressive therapy based on patient-specific variables.

Cyclosporine-Based Regimens

Cyclosporine is a lipophilic cyclic polypeptide CNI that was originally manufactured in an oil-based formulation (Sandimmune) in the early 1980s. Its use was hindered by poor and erratic absorption, despite its relative success in preventing AR in recipients of solid organ transplant. The availability of a microemulsion formulation of CSA (Neoral) in the mid 1990s has significantly improved bioavailability and minimized the variability in pharmacokinetic characteristics seen with the original oil-based formulation. Hence, it is now easier to maintain CSA drug concentrations in the narrow therapeutic range to ensure effective immunosuppression while minimizing adverse effects. Reliable concentrations have been demonstrated in kidney transplants, as well as liver and heart/lung transplants, leading to lower dose requirements to obtain the same level of efficacy. Whole blood CSA levels that are measured 2 hours after dose administration correlated best with an abbreviated area under the curve (AUC) (taken from 0 to 4 hours after administration of CSA), reflecting overall drug exposure. However, this is often clinically not feasible due to the need for specific timing of blood samples with respect to dose administration. Thus, trough CSA levels are more commonly used.

Acute rejection rates and overall patient and allograft survival were comparable up to 2 years post-transplant between the two CSA formulations in *de novo* kidney recipients and in recipients of a second kidney transplant. A trend toward lower AR rates and a lower requirement of monoclonal antibody treatment for AR was observed in patients receiving CSA microemulsion.

The dose-limiting effect of CSA is paradoxically nephrotoxicity, which occurs as a result of direct vasoconstriction on the kidney vasculature. Cyclosporine-induced nephrotoxicity can present as a reversible decline in glomerular filtration rate in up to 35% of patients and can progress to irreversible dysfunction in up to 15%. The latter is significantly associated with CAN in kidney transplant recipients, as well as nonrenal transplant recipients, and can limit long-term allograft survival. Other notable adverse effects with CSA include mild to moderate hypertension, neurotoxicity, which can range in symptoms from mild headache and tremor to seizures and coma, hepatic dysfunction, hyperlipidemia, glucose intolerance, and cosmetic effects such as hirsutism and gingival hyperplasia.

Tacrolimus-Based Regimens

Tacrolimus is a macrolide CNI that differs from CSA in that it binds to a specific immunophilin-binding protein called FK-binding protein 12 to inhibit T-cell activation through calcineurin inhibition, whereas CSA complexes with a different immunophilin called cyclophilin to exert similar pharmacological effects. Tacrolimus is also 10–100 times more potent than CSA in its immunosuppressant activities and it may play a role in corticosteroid reduction protocols. Therapeutic drug monitoring of tacrolimus can be performed using whole blood trough concentrations, which correlates very well with overall drug exposure, allowing optimal individual dose titration for efficacy and toxicity.

Tacrolimus has been an effective immunosuppressant when used in triple-drug therapy regimens containing AZA or MMF in combination with corticosteroids, with or without the addition of an antilymphocyte antibody for induction therapy. It has also been effective as rescue therapy for AR in patients treated with CSA-based immunosuppressive regimens. The adverse effect profile is similar to that of CSA. Differences in the incidence of specific adverse effects are discussed below.

Cyclosporine-Based Versus Tacrolimus-Based Regimens
Efficacy

In the AZA-era, tacrolimus-based therapy demonstrated a significant advantage over CSA-based therapy using the conventional oil-based formulation of CSA. Biopsy-proven AR rates after 1 year were 30.7% and 46.4%, respectively, and more patients receiving CSA were switched to tacrolimus due to refractory rejections. Allograft survival rates were similar between tacrolimus and CSA at 3 years (81.9% and 77.8%, respectively) and 5 years (64.3% and

Dunn CJ, Wagstaff AJ, Perry CM, Plosker GL, Goa KL. Cyclosporin: An updated review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral) in organ transplantation. Drugs 2001;61:1957–2016.

61.6%, respectively) post-transplantation. Patient survival rates were also comparable. Unfortunately, it is difficult to make definitive conclusions regarding comparative efficacy from these results due to significant changes that were made to the immunosuppressive regimens with the availability of the microemulsion formulation of CSA and the new antiproliferative drug, MMF. However, similar results were seen at 6 months when tacrolimus was compared directly with CSA microemulsion in regimens containing AZA and prednisone (AR 20% vs. 37%; p<0.001; patient survival 99.3% vs. 98.5%; graft survival 94.8% vs. 91.9%).

In the current era of immunosuppression, the use of MMF closed the gap in efficacy between tacrolimus and CSA microemulsion. Acute rejection rates of 15% and 20%, respectively, were similar after 1 year; however, patients treated with tacrolimus were less likely to require antilymphocytic therapy for corticosteroid-resistant rejection (4% vs. 11%; p=0.05). No significant differences in patient and allograft survival were seen after 2 years. African-American patients or patients who had delayed graft function seemed to have better outcomes with tacrolimus treatment. Tacrolimus may also be over CSA preferred in ABO-incompatible kidney transplantation and patients who have immune systems that are highly sensitized, based on excellent allograft outcomes in uncontrolled studies.

Tolerability

There are slight differences in the adverse effect profiles between tacrolimus and CSA. In particular, tacrolimus-induced nephrotoxicity is likely dose-related and thus amenable to dose reductions. Significantly lower serum creatinine concentrations were reported after 5 years of therapy in patients receiving tacrolimus compared with patients receiving CSA microemulsion. Tacrolimus is also associated with a better cardiovascular adverse effect profile, demonstrated by significantly lower systemic blood pressures and serum total cholesterol and low-density lipoprotein concentrations. Both CNIs are associated with an increased risk of developing post-transplant diabetes mellitus, defined as requiring insulin therapy, which can present in up to 20% of treated patients. The mechanism of CNI-induced post-transplant diabetes mellitus differs depending on the drug administered. Cyclosporine causes a reduction in pancreatic β-cell volume through inhibition of DNA and RNA synthesis, whereas tacrolimus causes morphological damage to β-cells and impaired insulin synthesis and secretion. Patient-specific variables that increase the risk of post-transplant diabetes mellitus include increased recipient age, African-American ethnicity, presence of pretransplant glucose intolerance, obesity, hepatitis C, and concomitant use of high-dose corticosteroids. The diabetogenic effect of tacrolimus is dose-dependent; thus, risk can be minimized by adjusting tacrolimus doses to maintain lower trough concentrations, as well as using lower prednisone doses. Finally, tacrolimus is consistently associated with a higher incidence of tremor compared with CSA (35%–54% vs. 12%–34%), whereas cosmetic adverse effects such as hirsutism, gingivitis, and gingival hyperplasia are seen more frequently in patients treated with CSA. No differences were seen in the incidence of opportunistic infections or malignancy between the two drugs.

Withdrawal and Avoidance Immunosuppressive Strategies

Rationale for Immunosuppressive Withdrawal and Avoidance Strategies

Historically, the main goal of immunosuppressive therapy was the prevention of AR episodes that can have a significant negative impact on allograft survival. In the current immunosuppressive era, the effectiveness of current drug combinations is such that AR rates are so low that establishing superiority in AR rates of new immunosuppressive drugs is an impossible challenge given the sample size required to achieve statistical significance. The current concern of most transplant clinicians is managing late allograft loss. The leading causes of late kidney allograft loss in recipients of a kidney transplant are CAN and death with a functioning graft. Although one would expect that infections and malignancy are the most common causes of death, death from cardiovascular disease occurs more frequently, accounting for 30.1% of deaths in primary kidney transplant patients. Calcineurin inhibitors and corticosteroids are the most widely used drugs in current immunosuppressive regimens and are the most likely drugs to increase risk of cardiovascular death as a result of their hypertensive, lipemic, diabetogenic, and/or nephrotoxic effects. Thus, the focus of immunosuppression minimization is on the withdrawal or avoidance of either or both of these two drug classes. The emergence of infections of organisms such as polyoma virus, which principally resides in the kidney, can be the culprit in 3%–5% of graft losses. Infection with this virus ultimately has an impact on cardiovascular risk because kidney dysfunction has recently been shown to be an independent risk factor for mortality in kidney transplantation. Although malignancies are not the most frequent cause of death, their impact should not be overlooked. Malignancies can occur in up to 40% of patients 20 years after transplantation, resulting in 10% of deaths. The most common malignancies include skin cancer and post-transplant lymphoproliferative disorders, such as non-Hodgkin's lymphoma. An increased risk of cervical, breast, and colorectal cancer has also been observed in the transplant population. Newer immunosuppressive drugs, such as sirolimus, may have anticancer effects and may be

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 2003;75:2048–53.

Fellstrom B, Jardine AG, Soveri I, et al. Renal dysfunction is a strong and independent risk factor for mortality and cardiovascular complications in renal transplantation. Am J Transplant 2005;5:1986–91.

Table 1-1. Comparison of Pharmacokinetic Parameters Between Calcineurin Inhibitors and Sirolimus

Drug	F (%)	Vd (L/kg)	Protein Binding (%)	Excretion	T ½ (hours)	Oral CL (L/kg/hour)
Cyclosporine	10–89	4.5	80	Biliary	8.4	0.3–0.4
Tacrolimus	25	1	73	Biliary	12	0.06
Sirolimus	15	12	92	Biliary	62	0.21

CL = clearance; F = bioavailability; T½ = half-life; Vd = volume of distribution.

preferable in patients who have a predisposition for developing malignancy.

Strategies that reduce the risk of long-term immunosuppressive adverse effects without compromising efficacy are the trend for future immunosuppressive regimens. Due to the complexity of the immune system and the wide intra- and inter-patient variability in immunologic response, fine-tuning of immunosuppressive drug doses is difficult. ImmuKnow is a relatively new assay that assesses lymphocytic activity by measuring the amount of adenosine-triphosphate synthesized in CD4 cells during the early response to stimulation as a reflection of cell-mediated immunity. There is no clear correlation between adenosine triphosphate levels and clinical outcomes, thus, no specific guidelines are provided on how to modify immunosuppressive therapy based on the results of this assay. However, it can provide clinicians with a sense of a patient's overall immune status. The adenosine triphosphate concentrations generally range between 226 ng/mL and 524 ng/mL in stable transplant recipients who have moderate immune response. Patients with adenosine triphosphate concentrations less than 225 ng/mL or greater than 525 ng/mL may be at risk for complications secondary to over- or under-immunosuppression, respectively. Development of future assays that give more precise measurements of immune status would be invaluable to better guide clinicians in tailoring immunosuppressive regimens to optimize efficacy and minimize toxicity.

Calcineurin Inhibitor Withdrawal and Avoidance Strategies

A major limitation of continued CNI use as part of a standard maintenance immunosuppression regimen is its contribution to the increased incidence of CAN. Chronic allograft nephropathy is currently the leading cause of kidney allograft failure. It is also the most common cause of kidney dysfunction in kidney organ transplantation. Kidney biopsies from patients with CAN are characterized by tubular atrophy and interstitial fibrosis that is widespread through the kidney and is graded on a CAN scale of I–III based on severity of the fibrosis. Calcineurin-inhibitor toxicity, along with chronic rejection, is one of the most significant causes of CAN. The prevalence of CAN at 2 years post-kidney transplantation was reported to be 72.3% and 62.0% in CSA- and tacrolimus-treated patients, respectively. Other contributing factors include AR, cytomegalovirus infection, hypertension, proteinuria, hyperlipidemia, and more recently, polyoma virus. Several

strategies have been developed to safely limit or avoid the use of CNIs: CNI withdrawal, CNI avoidance, or CNI conversion to sirolimus. These strategies incorporate newer immunosuppressive drugs that are not known to have harmful kidney effects, such as sirolimus and MMF.

Sirolimus

Sirolimus is a macrocyclic lactone that suppresses the immune cascade by binding to intracellular immunophilins, known as FK-binding proteins, which in turn bind to the mammalian target of rapamycin to cause inhibition of mammalian target of rapamycin-mediated signal-transduction pathways leading to cell cycle arrest of T lymphocytes and B lymphocytes in the mid-to-late G1 phase, even after cell stimulation. Sirolimus also blocks IL-2-induced proliferation of T cells (see Figure 1-1) and B cells.

Pharmacokinetics

Sirolimus exhibits large intra- and inter-patient variability with respect to its pharmacokinetic parameters. It has highly variable oral absorption and a narrow therapeutic index (see Table 1-1 for a comparison of pharmacokinetic parameters). Therapeutic drug monitoring is done by obtaining trough sirolimus concentrations, which correlate well with AUC at steady-state. A minimum sirolimus trough concentration of 5 ng/mL is the clinical threshold for preventing AR with an upper limit of around 15 ng/mL as the threshold for adverse effects from sirolimus therapy. Lower sirolimus concentrations (5–10 ng/mL) are recommended in patients receiving concomitant CNI therapy. Concentrations should be monitored no more frequently than on a weekly basis, given the drug's long half-life.

Adverse Effects

Dyslipidemias occur commonly in patients receiving sirolimus therapy and is dose-dependent. Hypercholesterolemia with total cholesterol concentrations greater than 240 mg/dL and hypertriglyceridemia with serum triglyceride concentrations greater than 200 mg/dL develops in 60%–80% and 70%–79%, respectively, of transplant recipients in the first 2 years after transplantation. These rates of hypercholesterolemia and hypertriglyceridemia are compared with pre-transplantation rates of 11% and 32%, respectively. Persistent dyslipidemia with sirolimus therapy can put the transplant patient at increased cardiovascular risk. Doses of 2 mg and 5 mg are

Guba M, Graeb C, Jauch KW, Geissler EK. Pro- and anti-cancer effects of immunosuppressive agents used in organ transplantation. Transplantation 2004;77:1777–82.

associated with 0.7 and 1.2 additional cardiovascular deaths per 1000 patients per year, respectively. The usual therapeutic strategies for treatment of hyperlipidemia, such as diet, lifestyle modification, and lipid-lowering therapy, should be implemented.

Dose-dependent thrombocytopenia defined as a platelet count less than 150×10^3 cells/mm^3 is also frequently seen in up to 50% of patients receiving sirolimus therapy and usually occurs in conjunction with leukopenia defined as white blood cell count less than 5000 cells/mm^3. Platelet counts usually reach a nadir within the first 4 weeks of therapy and spontaneously resolve with time.

Gastrointestinal (GI) tract adverse effects include diarrhea, liver function abnormalities, ileus, and internal hemorrhoids. Diarrhea can occur in 16%–38% of patients and typically occurs when higher blood concentrations are maintained. This adverse effect will subside with time. Liver function abnormalities are more persistent and can be seen in 7%–16.3% of patients.

Treatment with sirolimus can lead to an increased frequency of postoperative lymphoceles or perinephric fluid collections compared with that seen with CNI therapy (38.1% vs. 17.6%). Also, although impaired wound healing is relatively uncommon, it can pose as a clinical dilemma particularly in the setting of obesity, diabetes, infection, rejection, and increased age. For example, temporary discontinuation of sirolimus therapy may be warranted in an obese patient with obesity and diabetes who is admitted for persistent drainage from his or her surgical site 1 month post-cholecystectomy. However, the risk of AR should always be weighed against the benefits of continued treatment when contemplating temporary or permanent withdrawal of immunosuppressive therapy.

Interstitial pneumonitis is a rare but potentially fatal dose-dependent pulmonary complication that can occur within the first year of sirolimus therapy. Patients commonly present with dyspnea on exertion and a dry cough. Other symptoms include fatigue, fever, and hemoptysis. The pneumonitis is characterized by bilateral alveolo-interstitial pulmonary infiltrates on chest radiograph and computed tomography scans. Treatment consists of withdrawal or dose reduction of sirolimus; clinical symptoms and radiologic tests should improve within 3 weeks with complete resolution within 3 months. This adverse effect has been described in patients with kidney transplants, as well as liver, heart, lung, and islet cell transplants.

Although sirolimus carries a relatively low potential for nephrotoxic effects compared with other immunosuppressive drugs, it has been shown to delay the time to graft function or the established delayed graft function, which is defined as requiring dialysis within the first week post-transplantation. Tubular toxicity resulting in hypokalemia and hypophosphatemia has also been reported in 8%–27% of treated patients.

Sirolimus may have an advantage over other currently available immunosuppressive drugs. The incidence of malignancies appears to be lower, particularly with respect to skin cancer.

Role in Immunosuppressive Therapy

Sirolimus was initially used for prophylaxis of rejection after kidney transplantation in combination with CSA and corticosteroids. This combination, which included daily sirolimus doses of 2 mg or 5 mg, improved AR rates at 6 months and 1 year compared with the standard regimen of CSA, AZA, and corticosteroids. However, allograft function measured by creatinine clearance at 1 year was significantly lower in patients receiving sirolimus. This decreased function was due to the pharmacokinetic interaction, whereby sirolimus and CSA each increased the blood concentration of the other, potentiating the nephrotoxic effects of CSA and necessitating the use of lower CSA doses. Although, adding sirolimus to regimens of low-dose tacrolimus and corticosteroids also led to significantly lower creatinine clearance than regimens containing low-dose tacrolimus and MMF, comparable 1-year outcomes in terms of AR rate, graft function, as well as patient and graft survival, were observed.

The role of sirolimus in immunosuppressive therapy has evolved over the past several years. Currently, sirolimus is used in dual- or triple-drug therapy regimens to allow safe minimization of CNI exposure to improve kidney allograft function in patients with a low to moderate immunological risk profile.

In one approach, patients with a low-to-moderate immunological risk profile, such as Caucasians receiving their first kidney transplant, may be initiated on a maintenance immunosuppressive regimen consisting of sirolimus, standard doses of CNI and prednisone. No initial induction therapy is required. Two to 3 months post-transplantation, CNI withdrawal may be attempted with continuation of dual-therapy with sirolimus and prednisone in patients with adequate and stable kidney function (serum creatinine concentrations less than 4.5 mg/dL) who have not had any significant AR episodes in the preceding month. Sirolimus therapy should be optimized by targeting trough blood concentrations of up to 30 ng/mL. There may be an increased risk for AR upon withdrawal of CNI; however this has been shown to have no significant effect on overall allograft loss or patient survival. This risk of AR may also be offset by a significant improvement in graft function, measured by serum creatinine concentrations and creatinine clearance, and a significant reduction in the risk of hypertension (see Table 1-2). These positive effects on renal function have been documented by histologic changes in CAN on biopsy. Withdrawal of CNI has not been shown to have any impact on total cholesterol and triglyceride levels or the incidence of post-transplant diabetes mellitus.

In a second approach, patients being treated with CNI-based immunosuppression who have been kidney transplant recipients for at least 2–4 months may be offered the option to switch their CNI to sirolimus. Patients who have suboptimal allograft function as characterized by kidney

Kasiske B, Cosio FG, Beto J, et al; National Kidney Foundation. Clinical practice guidelines for managing dyslipidemias in kidney transplant patients: a report from the Managing Dyslipidemias in Chronic Kidney Disease Work Group of the National Kidney Foundation Kidney Disease Outcomes Quality Initiative. Am J Transplant 2004;4(suppl 7):13–53.

Table 1-2. Relative Risk of Major Outcomes for CNI Withdrawal Compared with Continued CNI Therapy

Outcomes	Relative Risk	p-value	Difference in Absolute Risk	p-value
AR at 1 year (after CNI withdrawal)	2.36	p=0.003	6%	p=0.002
Overall risk of AR	1.59	p=0.001	8%	p=0.0006
Graft loss	0.87	p=0.66	0%	p=0.32
Patient survival	0.88	p=0.76	——	——
Renal function				
SCr	——	——	- 0.19 mg/dL	p<0.0001
CrCl	——	——	+ 7.49 mL/minute	p=0.00001
Hypertension	0.56	p=0.0006	——	——

AR = acute rejection; CNI = calcineurin inhibitor; CrCl = creatinine clearance; SCr = serum creatinine.
Mulay AV, Hussain N, Fergusson D, Knoll GA. Calcineurin inhibitor withdrawal from sirolimus-based therapy in kidney transplantation: A systematic review of randomized trials. Am J Transplant 2005;5:1748–56.

biopsy or slowly declining kidney function are also eligible to be switched to sirolimus therapy. The CNI can be slowly tapered over 2 weeks or can be stopped abruptly. If the CNI is stopped abruptly, a loading dose of sirolimus should be given to quickly obtain trough concentrations in the target therapeutic range of 5–15 ng/mL. Sirolimus trough concentrations should be monitored weekly until a stable concentration is achieved. Dose changes should not be made more frequently than every week or every other week.

The role of sirolimus in CNI-sparing or avoidance regimens for other solid organ transplant types is yet to be defined. In recipients of liver transplants, the potential benefits of sirolimus were offset by increased wound complications and hepatic artery thrombosis. In recipients of heart transplants with coronary allograft vasculopathy, those who received sirolimus had less cardiovascular events than those continued on their immunosuppression regimen; however, the long-term effects of hyperlipidemia secondary to sirolimus therapy is unknown.

Mycophenolate Mofetil

Mycophenolate mofetil was first introduced into clinical practice in 1995. It is an antiproliferative immunosuppressive drug that selectively inhibits the rate-limiting enzyme, inosine monophosphate dehydrogenase, required for *de novo* purine synthesis of guanosine nucleotide which is essential for the proliferation of T and B lymphocytes. Inhibition of inosine monophosphate dehydrogenase is noncompetitive and reversible.

Pharmacokinetics

Mycophenolate mofetil is a morpholinoethyl ester of its active metabolite, mycophenolic acid (MPA). It is rapidly hydrolyzed to its active form after oral administration. Mycophenolic acid is primarily converted to an inactive metabolite, MPA glucuronide in the GI tract and liver, which is then excreted in the urine. Other metabolites include acyl glucuronide, which has demonstrated in vitro activity, and two other active metabolites. Mycophenolic acid also undergoes enterohepatic recirculation; this process incorporates transport of MPA-glucuronide into the bile, conversion back to MPA by gut bacteria, and reabsorption of MPA into the circulation. Alterations in the process of enterohepatic recirculation can lead to changes in the overall exposure of MPA. Concomitant administration of MMF with CSA or metronidazole can lead to decreased enterohepatic recycling of MPA and MPA-glucuronide. Cyclosporine inhibits biliary excretion of MPA-glucuronide from hepatocytes into the GI tract and metronidazole kills the anaerobic bacteria required for conversion of MPA-glucuronide to MPA in the GI tract. These two mechanisms lead to reductions in the AUC of MPA and subsequent reduction in overall MPA exposure.

Alterations in the pharmacokinetics of MPA can also occur in patients who have kidney dysfunction. Mycophenolic acid is highly bound to serum albumin (97%–98%). Reduced urinary excretion can lead to significant accumulation of uremic toxins and MPA glucuronide, both of which compete with MPA for albumin-binding sites leading to elevations in the free fraction of MPA. Clinically, this may result in dose-dependent adverse effects.

Adverse Effects

The most common adverse effects reported with MMF therapy are GI and hematological in nature. Gastrointestinal tract complaints are commonly diarrhea, nausea, vomiting, and abdominal pain. Anemia and leukopenia with severe neutropenia (absolute neutrophil count less than 500 cells/μL) are the major hematological abnormalities. Temporary dose reduction or discontinuation of MMF may be implemented depending on the severity of the adverse event. Anemia and neutropenia may be treated with recombinant human erythropoietin and a granulocyte-colony stimulating factor, respectively. Higher doses of MMF (e.g., 3 g/day) were associated with a higher risk for cytomegalovirus infection in all three of the pivotal trials; however, these trials preceded routine prophylaxis against cytomegalovirus disease. If adverse effects are severe enough to warrant discontinuation of MMF, an alternate drug should be substituted in its place or an increase in one of the patient's other immunosuppressant drug doses should be made to maintain overall net immunosuppression.

Mulay AV, Hussain N, Fergusson D, Knoll GA. Calcineurin inhibitor withdrawal from sirolimus-based therapy in kidney transplantation: a systematic review of randomized trials. Am J Transplant 2005;5:1748–56.

Fung J, Kelly D, Kadry Z, Patel-Tom K, Eghtesad B. Immunosuppression in liver transplantation—beyond calcineurin inhibitors. Liver Transpl 2005;11:267–80.

Lindenfeld J, Miller GG, Shakar SF, et al. Drug therapy in the heart transplant recipient. Part II: immunosuppressive drugs. Circulation 2004;110:3858–65.

Role in Immunosuppressive Therapy

Initially, MMF was used at 2–3 g/day in immunosuppressive regimens containing CSA and corticosteroids, with or without the use of antithymocyte induction therapy. Its efficacy in preventing AR at 6 months when used in this manner was demonstrated in three pivotal trials. It was also demonstrated that MMF was more effective at preventing rejection when compared with AZA, suggesting that it was a more potent antiproliferative agent. The pooled results of these trials at 3 years confirmed the long-term efficacy of MMF in graft survival; odds ratio for graft failure of 0.73 when MMF is given at a dose of 2 g/day. The efficacy of MMF in combination with tacrolimus- and sirolimus-based regimens has also been established. Acute rejection rates and patient and graft survival were similar to that of CSA-based regimens. However, significantly better graft outcomes in patients with delayed graft function and improved kidney function was seen with tacrolimus, MMF, and prednisone triple therapy and the sirolimus, MMF, and prednisone combination, respectively than was seen with the CSA-based regimens. Current immunosuppressive regimens incorporate MMF as a mainstay of standard triple-drug therapy.

Although MMF is successful in preventing AR, its clinical use is being further explored. With the increasing incidence of CAN, the role of MMF in CNI-sparing regimens has been a focus. Improvement in long-term outcomes of CNI withdrawal with the use of sirolimus prompted evaluating the feasibility of transplantation with CNI avoidance altogether. However, initial attempts with a combination of high-dose sirolimus with initial target concentrations of 30 ng/mL, AZA, and prednisone resulted in AR rates of more than 40%. In addition, numerous sirolimus-related adverse effects were reported and premature discontinuation of treatment occurred in more than 50% of patients. The use of MMF, instead of AZA, in combination with sirolimus and basiliximab induction therapy resulted in an improvement in AR rates of 6.4% compared with using AZA as part of the regimen. It also allowed use of lower target sirolimus concentrations to minimize the incidence of adverse effects. When this new combination was compared with an identical regimen substituting CSA for MMF, similar rates of AR, patient survival, and graft survival were reported. However, the sirolimus and MMF regimen resulted in significantly better kidney function at 1 year than the sirolimus and CSA regimen. Serum creatinine concentrations were 1.3 mg/dL and 1.8 mg/dL and glomerular filtration rates were 81 mL/minute and 61 mL/minute for the MMF and CSA groups, respectively. This difference translated into a greater proportion of patients having normal kidney biopsies after 2 years (67% vs. 21%, respectively). Mycophenolate mofetil has also inhibited sirolimus-induced pro-fibrotic effects in kidney allografts; thus, the combination could constitute a potentially protective mechanism against development of CAN.

Another focus in the expanding role of MMF is the preservation of long-term kidney allograft function in patients with declining kidney function. The use of MMF in this manner has been best characterized by the "Creeping Creatinine" Study. In this study, recipients of a kidney transplant who were maintained on a CSA-based dual- or triple-drug immunosuppressive regimen and had deteriorating kidney function were eligible for a change in drug therapy to a combination of MMF and corticosteroid therapy, with CSA withdrawal over a 6-week period to minimize the risk of AR. Patients had a serum creatinine concentration between 1.1 mg/dL and 4.5 mg/dL and a calculated creatinine clearance of more than 20 mL/minute. Stabilization or an improvement in kidney function, characterized by a flat or positive slope of the creatinine-versus-time plot, was observed in almost 60% of patients after 6 months with an increased mean creatinine clearance at both 6 and 12 months. No ARs were observed. Significant improvements were also seen in serum cholesterol concentrations after CSA withdrawal. A transient decrease in hemoglobin concentrations was observed in patients during concomitant initiation of MMF and withdrawal of CSA. This was likely due to the pharmacokinetic interaction between CSA and MMF that was previously discussed, whereby CSA inhibits the biliary excretion of MPA-glucuronide. Withdrawal of CSA leads to an increase in the enterohepatic recirculation of MPA-glucuronide, which results in higher MPA plasma concentrations and consequently more suppression of erythropoiesis. An increase in the incidence of GI tract effects can also be expected. This interaction underscores and supports the need for therapeutic drug monitoring of MMF to ensure appropriate use with respect to drug dosing. This monitoring may also help to minimize the toxicities associated with MMF, particularly the GI adverse effects. Pharmacists need to be cognizant of this and other drug interactions that can occur as a result of ongoing modification of immunosuppressive therapy. The study results present an alternative immunosuppressive strategy in patients experiencing CNI-induced nephrotoxicity; however, general applicability of these results may not be practical due to the limited description of baseline patient characteristics. Variables that categorize patients as high-risk recipients, such as ethnicity and those with a high level of donor-specific antibodies, were not presented. It may be inferred that the majority of patients were likely Caucasian, and thus low-risk recipients because the participating centers were in Europe and the United Kingdom.

Mycophenolate mofetil is not as widely used in other solid organ transplants compared with kidney transplants. Large variations in the pharmacokinetics of MMF are seen in recipients of liver transplants that are directly related to the degree of liver dysfunction. Liver dysfunction impairs protein synthesis and MPA glucuronidation, which results in an increased free fraction of MPA and prolonged MPA half-life, respectively, leading to a high incidence of GI and hematological effects. However, MMF plays a small role as a kidney-sparing drug in patients with CNI-induced nephrotoxicity. In recipients of heart transplants, MMF has been significantly more effective than AZA in preventing

Dudley C, Pohanka E, Riad H, et al. Mycophenolate mofetil substitution for cyclosporine A in renal transplant recipients with chronic progressive allograft dysfunction: the "Creeping Creatinine" Study. Transplantation 2005;79:466–75.

Table 1-3. Comparison of Pharmacokinetic Parameters Between EC-MPS and MMF Under Twice-Daily Dosing

Pharmacokinetic parameter	EC-MPS	MMF
Absolute bioavailability	72%	94%
C_{max} (mcg/mL)	19.2	20.2
Time to C_{max} (hours)	2.3	0.9 (p<0.01)
AUC (mcg•hour/mL)	56.0	55.7
Half-life (hours)	8–16	13–17

AUC = area under the curve; C_{max} = maximum concentration; EC-MPS = enteric-coated mycophenolate sodium; MMF = mycophenolate mofetil.

rejection and improving mortality rates. Despite this, the use of MMF is not widespread in heart transplantation and is reserved for high-risk patients and those experiencing kidney dysfunction.

Enteric-Coated Mycophenolate Sodium

In an attempt to minimize GI toxicity associated with MMF, enteric-coated mycophenolate sodium (EC-MPS), also known as Myfortic, was introduced by Novartis. This delayed-release formulation was designed to release the active drug (MPA) in the neutral pH of the small intestine. At equivalent doses (EC-MPS 720 mg and MMF 1000 mg contain equimolar amounts of MPA), pharmacokinetic parameters are similar between EC-MPS and MMF with the exception of the significantly prolonged time to reach maximal plasma MPA concentrations for EC-MPS. Absolute bioavailability of MPA appears to be greater with MMF (see Table 1-3).

This new formulation is similar in efficacy to MMF when used in patients with *de novo* kidney transplants, as well as patients who had previously been receiving stable treatment with MMF. Therapeutic outcome measures of biopsy-proven AR, biopsy-proven chronic rejection, and treatment failure after 1 year of treatment are presented in Table 1-4. The most common adverse effects of EC-MPS were GI in nature, and the rate of occurrence was similar to patients receiving MMF.

Concomitant administration of EC-MPS with antacids and bile acid sequestrants can decrease overall MPA exposure. The available dosage forms are 180-mg and 360-mg tablets. Two clinical trials are currently being conducted to evaluate the efficacy and tolerability of EC-MPS. The Myfortic Prospective Multicenter Study is a large, prospective, open-label, multicenter study that evaluates the use of EC-MPS in combination with CSA and corticosteroids as maintenance immunosuppression in *de novo* and stable kidney transplant recipients. The effect of EC-MPS on GI tract symptoms and quality of life will be assessed in an open-label, multicenter study of short duration called the Patient Reported Outcome on GI Symptoms.

Belatacept

Belatacept is a new investigational immunosuppressive drug that selectively binds to costimulatory ligands (CD80 and CD86) on the surface of antigen-presenting cells, blocking their interaction with surface co-stimulatory receptors (CD28) located on T cells, leading to inhibition of T-cell activation (Signal 2).

In a Phase II study, belatacept was shown to be non-inferior to CSA in preventing AR at 6 months in recipients of kidney transplants when used in combination with basiliximab induction therapy, MMF, and corticosteroids. Kidney function and systolic blood pressure measurements were significantly better in the belatacept group, and lipid concentrations were similar despite the requirement for less lipid-lowering drugs compared with patients receiving CSA. There was also no difference in the incidence of infection or malignancy between the groups. Belatacept may play a future role as an immunosuppressant that aids in the preservation of glomerular filtration rate and decreases the incidence of CAN. However, the use of belatacept may be limited in that it requires patients to attend clinic for monthly intravenous administration.

Corticosteroid Withdrawal and Avoidance Strategies

Corticosteroids remain a key component of most immunosuppressive protocols. However, the benefits derived from a corticosteroid-based regimen are offset by the numerous long-term complications, such as increased susceptibility to infection, impaired wound healing, increased cardiovascular risk factors (glucose intolerance, hyperlipidemia, and hypertension), cataracts, and osteoporosis. All of these complications contribute to increased long-term morbidity and mortality after transplantation. Withdrawing steroids may minimize these complications; however, the benefits of withdrawal must be weighed against the risk of precipitating rejection.

A few factors should be considered when designing a corticosteroid withdrawal or avoidance protocol. One factor is determining the target population. Patients who are at high risk for corticosteroid-related adverse events would

Table 1-4. Efficacy and Safety Outcome Measures of EC-MPS Compared with MMF

Outcomes (%)	*De novo* Kidney Transplant Recipients		Maintenance Kidney Transplant Patients	
	EC-MPS	MMF	EC-MPS	MMF
Acute rejection	2.8	6.2	1.3	3.1
Chronic rejection	22.5	24.3	3.8	4.9
Treatment failures	26.3	28.1	2.5	6.1
GI complaints	81	80	60	61

EC-MPS = enteric-coated mycophenolate sodium delayed-release; GI = gastrointestinal; MMF = mycophenolate mofetil.

Vincenti F, Larsen C, Durrbach A, et al. Costimulation blockade with belatacept in renal transplantation. N Engl J Med 2005;353:770–81.

likely derive the most clinical benefit from corticosteroid minimization. These patients include women who are postmenopausal, patients with a prior history of malignancy, and possibly those with significant cardiovascular risk factors such as diabetes. Pediatric patients may also benefit from decreased corticosteroid exposure in terms of growth and body morphology. However, patients who are immunologically at high risk for graft rejection or failure, such as those of African-American descent, may not be ideal candidates for corticosteroid minimization. The use of potent induction drugs in this population may overcome this risk and is discussed later in this chapter. Another major factor to be considered is the timing of corticosteroid withdrawal. Late steroid withdrawal allows patients to develop some degree of steroid dependence, theoretically leading to an increased risk of AR on withdrawal compared with limiting exposure to a few days post-transplant.

Preliminary efforts of late corticosteroid withdrawal (after 3–6 months post-transplantation) in patients on triple-drug therapy with CSA, MMF, and prednisone resulted in a significant increase in ARs compared with those patients who were continued on prednisone therapy. Although no significant differences were observed between the two groups in overall patient and graft survival at 1 year, the effect of steroid withdrawal on graft longevity is unknown. Steroid withdrawal decreased actuarial 5-year graft survival rates from 85% in patients who remained on prednisone to 73% in the withdrawal group. Protocols incorporating MMF may be of added benefit given the drug interaction that exists between MMF and corticosteroids. Corticosteroids induce the activity of uridine diphosphate glucuronosyltransferase, the specific enzyme responsible for MPA metabolism; thus, discontinuation of corticosteroids can result in return to normal enzyme activity and lead to a relative increase in overall MPA exposure. This mechanism likely compensates for the decrease in overall net immunosuppression with the interruption of corticosteroid treatment, minimizing the overall risk of AR. Definite improvements in blood pressure, serum lipids, and bone density were observed; however, rejection rates with MMF-containing corticosteroid withdrawal regimens were still significantly higher than those patients who were maintained on corticosteroids.

The availability of newer immunosuppressive drugs, particularly induction drugs, has prompted evaluation of a different approach to achieve successful corticosteroid withdrawal. Corticosteroid avoidance or early discontinuation within the first week after transplantation may be beneficial in that there is minimal opportunity for development of corticosteroid dependence. Induction therapy may facilitate these two strategies by overcoming the excess risk of AR during the critical period immediately post-transplant. Muromonab-CD3 (OKT-3), an older monoclonal antibody, is a murine-derived agent that has been used as induction therapy, as well as treatment of steroid-resistant rejection. This agent results in rapid depletion of T cells by binding to the CD3 complex on the T-cell surface. Its use has fallen out of favor as it has been associated with a cytokine release syndrome that manifests as fever, chills, headache, GI tract symptoms, and, more significantly, pulmonary edema and acute respiratory distress. In addition, use of OKT-3 can induce production of anti-murine antibodies, precluding repeated use. Modern agents such as monoclonal antibodies directed at IL-2 receptors and lymphocyte-depleting drugs have been evaluated as alternative induction drugs for the purpose of corticosteroid withdrawal.

Interleukin-2 Receptor Antagonists

Interleukin-2 receptor antagonists are monoclonal antibodies that have a high-binding affinity and specificity for the CD25 antigen on the IL-2 receptors located on activated T cells. Administration of these drugs leads to saturation of the IL-2 receptors, resulting in inhibition of T-cell proliferation. Basiliximab (chimeric) and daclizumab (humanized) differ slightly in the composition of their antibody sequences and their half-lives (7–14 days vs. 20 days, respectively). Both drugs are administered intravenously and have an adverse effect profile similar to that of placebo.

Basiliximab and daclizumab are both effective in facilitating minimization of corticosteroid exposure. The majority of data are focused on the early withdrawal of corticosteroids. Successful corticosteroid withdrawal within the first 3–7 days after transplant has been shown with the use of two doses of basiliximab 20 mg in combination with CSA and MMF. After 1 year, AR and patient/graft survival rates were 16%–20% and 95%–100%, respectively, with more than 70% of patients remaining on regimens that were prednisone-free. Daclizumab has also been an effective induction agent for corticosteroid withdrawal after one intravenous dose of methylprednisolone 500 mg in combination with tacrolimus and MMF. Daclizumab was administered as a two-dose regimen of 1 mg/kg intraoperatively and on day 14 after transplantation. Outcomes were relatively similar to those reported with basiliximab. Efficacy of these agents in preventing ischemic reperfusion injury and delayed graft function is limited.

Lymphocyte-Depleting Drugs
Antithymocyte Globulin

Antithymocyte globulin (Thymoglobulin) is a rabbit-derived polyclonal antibody that has potent T-cell depleting activity. Induction therapy with antithymocyte globulin can be used with a maintenance regimen of CSA and MMF to allow for early withdrawal of corticosteroids. Patients with delayed graft function may need a prolonged course of antithymocyte globulin for up to 10 days to delay CNI introduction, promoting good graft recovery by minimizing the risk of additive CNI nephrotoxicity. Antithymocyte globulin doses of 1.25–1.5 mg/kg, typically infused over 6 hours, are administered for 5 days with CSA at target trough concentrations of 150–200 ng/mL in the first

Ahsan N, Hricik D, Matas A, et al. Prednisone withdrawal in kidney transplants recipients on cyclosporine & MMF—a prospective randomized study. Steroid Withdrawal Study Group. Transplantation 1999;68:1865–74.

Vincenti F. Interleukin-2 receptor antagonists and aggressive steroid minimization strategies in kidney transplant patients. Transplant Int 2004;17:395–401.

Table 1-5. Outcomes of Long-Term Prednisone-Free Immunosuppression with Antithymocyte Induction Therapy

Outcomes	1 year	5 years
Rejection rates		
Acute	11%	15%
Chronic	2%	13%
Mean serum creatinine (mg/dL)	1.6	1.7
Patient survival	97%	91%
Graft survival		
Overall	95%	84%
Death-censored	98%	92%

3 months and MMF 1 g 2 times/day. A 6-day corticosteroid regimen is started with an intravenous dose of methylprednisolone 500 mg infused intraoperatively and oral doses are tapered over the next 5 days. Corticosteroid doses should be administered 30 minutes before administration of antithymocyte globulin, in conjunction with diphenhydramine and acetaminophen to prevent the cytokine release syndrome. This syndrome is caused by T-cell activation and can cause symptoms such as fever, chills, dyspnea, and chest pain. The major outcomes of antithymocyte globulin therapy at 1 year and 5 years are summarized in Table 1-5. More than 80% of patients remained on prednisone-free immunosuppressive regimens with significantly lower rates of corticosteroid-related adverse effects, such as cytomegalovirus infection, post-transplant diabetes mellitus, avascular necrosis, osteoporosis, cataracts, and non-post-transplant lymphoproliferative disease malignancy. A maintenance regimen of tacrolimus and sirolimus can also be used with antithymocyte globulin with similar efficacy.

Alemtuzumab

Alemtuzumab, marketed in the United States as Campath, is a recombinant humanized CD52-specific monoclonal antibody that has labeled approval for use in the treatment of β-cell chronic lymphocytic leukemia. Human CD52 is a cell-surface antigen that is densely expressed on T cells and B cells, eosinophils, and some populations of monocytes, macrophages, and dendritic cells. Alemtuzumab reacts with this antigen to produce profound depletion of T-cells from peripheral blood for several months. It also has less marked effects on B cells and monocytes.

The use of alemtuzumab in organ transplantation has been focused on corticosteroid-sparing protocols. It has successfully been used as a two-dose regimen of 20 mg in conjunction with CSA monotherapy, achieving target CSA trough concentrations of 75–125 ng/mL. No corticosteroids were used, with the exception of 250–500 mg of intravenous methylprednisolone administered 30 minutes before each dose of alemtuzumab to minimize the first-dose cytokine-release syndrome. Alemtuzumab has also been used in a two-dose regimen of 0.3 mg/kg or as a single-dose regimen of 30 mg in combination with low-dose tacrolimus (target trough concentrations of 5–8 ng/mL) and MMF with seemingly better results than the two-dose combination regimen with CSA monotherapy. Biopsy-confirmed AR rates at 1 year, which tended to occur in the later post-transplant period at 5–6 months, were reported to range from 9% to 15% with excellent patient and graft survival rates at 1 year and 3 years. Acute rejection rates after 5 years of follow-up were 30%, with patient and death-censored graft survival rates of almost 90% and 80%, respectively, with more than 90% of patients remaining prednisone-free. There appears to be no compromise in kidney function or increased risk of cytomegalovirus infection, polyoma virus infection, post-transplant lymphoproliferative disease, or malignancy with alemtuzumab therapy despite its potent immunosuppressive activity. Preliminary results with alemtuzumab induction therapy to allow corticosteroid-free immunosuppression are promising; however more widespread use awaits results from larger prospective studies. Its concomitant role in the induction of tolerance is also being evaluated and is discussed later in this chapter.

Immunosuppressive Strategies in Special Populations

High Immunological Risk Transplant Recipients
African-American Population

Successful kidney transplantation in the African-American population is challenging for a variety of reasons. As potential recipients of kidney transplants, African Americans are disproportionately at a disadvantaged position. This population is inherently at increased risk for developing kidney failure requiring dialysis and although kidney transplantation is the treatment of choice for kidney failure, the rate of transplantation in African-American patients is significantly lower than their Caucasian cohorts as a result of limited organ availability and, more importantly, the combination of environmental and genetic factors. The relative importance of these factors remains unclear.

Socioeconomic Factors

African Americans compose almost 40% of patients with kidney failure who are treated by dialysis, but they only receive about 25% and 15% of the cadaveric kidneys and kidneys from living donors, respectively. The racial disparities begin early and continue at every step in the transplantation process. First, African-American patients are less likely to be identified as candidates for kidney transplantation before and after the start of dialysis compared with their Caucasian cohorts. After evaluation for transplant, they are also less likely to identify a potential living donor; when and if one is identified, that donor is less likely to be a suitable candidate due to underlying hypertension or glucose intolerance. Due to fewer living donors available, African-American recipients are more dependent on the cadaveric waiting list, which consists of overwhelmingly white donors. Inherent differences in ABO blood types between African-American and Caucasian patients (a greater proportion of African Americans have blood type B compared with a greater proportion of Caucasians with blood type A) lead to an increased

incidence of sensitization in African-American recipients to any given donor and almost a 2-fold longer waiting time on the list for a cadaveric kidney. These barriers are compounded by impaired access to immunosuppressive drugs and adherence to required drugs once access has been obtained. Pharmacists can play a key role in minimizing this barrier through management of drug assistance programs and drug counseling sessions during routine patient visits to the clinic.

Genetic Factors

The African-American population demonstrates differences in their immunologic response to the transplanted allograft compared with their Caucasian counterparts. In addition to the racial disparity between cadaveric donors and recipients leading to decreased human leukocyte antigen (HLA) matching, African Americans express greater polymorphism in MHC than Caucasians, resulting in less correlation between functional immune response and HLA matching. African Americans also exhibit an increased expression of co-stimulatory molecules resulting in a stronger immunologic response to foreign antigens (Signal 2). Previous exposure to red blood cell transfusions also increases the risk for sensitization before transplantation. The deleterious impact of hypertension on kidney function, despite good blood pressure control with drugs, may predispose African-American patients to decreased allograft survival. The deleterious effect of high blood pressure despite good control may be explained by the expression of larger amounts of transforming growth factor-β in African-American patients, which can promote kidney fibrosis.

Finally, immunosuppressive drugs display different pharmacokinetics in African Americans that puts them at risk for reduced overall allograft survival once they have received an organ transplant. In general, pharmacokinetic differences are seen mainly with CNIs, CSA and tacrolimus. Bioavailability, maximum drug concentrations, and overall drug exposure as measured by AUC were all reduced and drug clearance was increased for both drugs in African Americans compared with Caucasians receiving similar drug doses. These alterations appear to be specific to the female gender for CSA, but have no gender specificities that apply to tacrolimus. Cyclosporine and tacrolimus are both metabolized by the cytochrome P450 (CYP) 3A subfamily enzyme system and are substrates for p-glycoprotein, which is a product of the multidrug-resistant gene MDR1, a human drug transporter gene. Polymorphic differences in the gene expression of CYP3A and p-glycoprotein are primarily responsible for the pharmacokinetic differences of CNIs in African-American patients. The increased polymorphic expression of CYP3A4 and CYP3A5 activity, as well as p-glycoprotein activity, by African Americans results in the requirement for higher doses of CSA and tacrolimus to achieve similar average steady-state concentrations.

Sirolimus also shares the same metabolic pathway as CNIs; however, ethnicity has little effect on the pharmacokinetic parameters of sirolimus due to large intrapatient and interpatient variability. African Americans tend to have more favorable clinical outcomes with higher sirolimus doses. No variations in the pharmacokinetics of the antiproliferative drugs, MMF and AZA, have been observed in African Americans to date.

Immunosuppressive Strategies

African Americans seem to benefit from more aggressive immunosuppression to achieve similar patient and allograft outcomes as Caucasian transplant patients. Minimization of immunosuppression should still be attempted in this population as they are predisposed to diabetes, hypertension, and poorer allograft outcomes based on their ethnic background. Historically, African Americans were more likely to have AR with discontinuation of corticosteroid therapy. However, induction therapy with antithymocyte globulin and newer maintenance immunosuppressive drugs may make withdrawal of corticosteroids after 1 week post-transplant more feasible, leading to a beneficial effect on cardiovascular risk factors.

No prospective studies that compare the efficacy of monoclonal antibodies with that of antithymocyte globulin are available. Studies that are available were not specifically conducted in African-American patients, but did have African-American participants. In general, monoclonal antibodies seem to be more effective than polyclonal antibodies in preventing rejection in patients who are at low immunologic risk, but the converse seems true for patients who are at high immunologic risk. However, allograft outcomes and kidney function in African Americans treated with basiliximab or antithymocyte globulin were similar based on a retrospective analysis.

Mycophenolate mofetil was also effective when used in the African-American population and demonstrated a dose-dependent effect in lowering AR rates when doses were increased from 2 g/day to 3 g/day. This effect was not observed in Caucasians. The use of MMF in combination with basiliximab and CSA allows safe withdrawal of corticosteroid in the early post-transplant period at days 3–7 with no compromise in the rate of AR and patient or allograft survival.

In summary, similar rates of patient and allograft survival at 1 year can be achieved in African-American recipients compared with their Caucasian counterparts with the newer immunosuppressive drugs that are available for use today. However, these success rates diverge beyond the early post-transplant period.

Highly Sensitized Transplant Recipients

Kidney transplantation as the preferred treatment for patients with kidney failure treated by dialysis but is a limited resource due to organ availability. Patients who are

Dirks NL, Huth B, Yates CR, Meibohm B. Pharmacokinetics of immunosuppressants: a perspective on ethnic differences. Int J Clin Pharmacol Ther 2004;42:701–18.

Gredericks S, Holt DW. Pharmacogenomics of immunosuppressive drug metabolism. Curr Opin Nephrol Hypertens 2003;12:607–13.

Haririan A, Morawski K, Sillix DH, et al. Induction therapy with basiliximab versus antithymocyte globulin in African-American kidney transplant recipients. Transplantation 2005;79:716–21.

highly sensitized with panel reactive antibodies (PRAs) of greater than 20% have an increased immunological risk for rejection. High levels of preformed anti-HLA antibodies occur as a result of exposure to non-self HLA antigens. The most common risk factors for having high PRA levels are prior failed transplants, multiple pregnancies, and prior multiple red blood cell transfusions. About 30% of patients who are awaiting kidney transplantation are highly sensitized, with almost 50% having PRA levels of greater than 80%. As the PRA level increases, the likelihood of finding an immunologically compatible kidney becomes more difficult, because patients are more likely to have alloantibodies to a greater number of potential donors resulting in a T-cell and/or B-cell positive crossmatch which precludes successful transplantation. This sensitization leads to patients waiting on the transplant list for a prolonged time period or expiring before ever receiving a transplant.

Preventive strategies to minimize the incidence of sensitization are limited. Little can be done with regard to high PRA levels due to prior transplants or multiple pregnancies; however, the use of recombinant human erythropoietin should be encouraged for the treatment of anemia to prevent the need for red blood cell transfusions. Also, the availability of various desensitization treatment protocols designed to decrease PRA levels allow transplantation in situations that were previously considered immunologic contraindications, including transplantation across the ABO-incompatible blood groups. Therapeutic treatment with IVIG has been evaluated as an approach to decreasing anti-HLA antibody reactivity. Intravenous immunoglobulin is a potent immunomodulatory drug that is derived from pooled human plasma. The immunomodulatory properties of IVIG include inhibition of T-cell activation and proliferation, neutralization of antibody production and reduction in IL-2 production. In patients with persistent PRA levels greater than or equal to 50%, treatment with monthly infusions of high-dose IVIG 2 g/kg (Gamimune N 10%) for 4 months with an additional infusion at 12 months and 24 months significantly increased the rate of transplantation and shortened the time to transplantation with allograft survival of 80% at 2 years. The most common adverse effects related to IVIG therapy include infusion-related reactions and headache after dose administration.

Treatment with IVIG is not being used routinely in highly sensitized patients despite promising results. Intravenous immunoglobulin does not entirely eliminate sensitization as PRA levels will rebound back to baseline levels after 6 months. However, IVIG has a more prolonged effect on anti-HLA donor-specific antibodies, which may not be adequately represented by the measured PRA levels. In addition, the use of IVIG is expensive, with the cost of the first four doses reaching almost $20,000. An alternate desensitization strategy uses IVIG in combination with plasmapheresis, allowing IVIG to be given at lower doses of 100 mg/kg. Plasmapheresis is an extracorporeal immunoadsorption technique that removes circulating donor-specific alloantibodies in patients who are T-cell and/or B-cell crossmatch positive against their potential living donor. Therapy is continued on an alternate-day basis

until donor-specific antibody titers in the recipient are negative. The feasibility of using lower doses of IVIG with antibody induction drugs, such as daclizumab and antithymocyte globulin, to minimize drug cost is also being evaluated.

Retransplantation

Kidney allograft failure is one of the most common causes for kidney failure requiring treatment by dialysis in patients in the United States, falling fourth after diabetes mellitus, hypertension, and glomerulonephritis. Patients who are awaiting retransplantation are at higher immunologic risk than transplant-naïve patients because they have been previously exposed to alloantigens, resulting in higher PRAs. However, the benefits of decreased mortality from transplantation disappear after allograft failure, and mortality rates increase back up to and may exceed that of patients who are transplant-naïve on dialysis. Thus, retransplantation would be preferred, particularly in patients with diabetes. Historically, the success rate of retransplantation has been low compared with the outcome of the first allograft. More recently, these rates have improved with the availability of pretransplant screening, such as flow cytometry crossmatching and HLA matching, and improved post-transplant management with more potent immunosuppressive drugs. Induction antibody therapy in conjunction with CSA-based triple-drug maintenance immunosuppressive regimens resulted in patient and allograft survival rates at 1 year, 5 years, and 10 years post-retransplantation similar to patients who undergo transplantation for the first time, despite high PRA levels (PRAs greater than or equal to 25%) in 70% of the patients who were prior organ recipients. The majority of the patients received AZA as the antiproliferative drug. Factors that significantly affected survival of the second graft were the degree of HLA-DR (one of the three important MHC genes) mismatching and the number of ARs. Multiple organ transplants increase the risk for infections and malignancy, which can also adversely affect long-term allograft success. However, overall outcome with retransplantation is significantly better than with lifetime dialysis.

The ethical issues associated with retransplantation are an ongoing discussion. The decision to retransplant a patient who lost his or her first graft due to drug noncompliance or reasons that were beyond the patient's control, such as thrombosis or hyperacute rejection, needs to be weighed against the ethics of restricting a suitable patient to lifelong dialysis with the current success rates of retransplantation. In addition, consideration must be given to the allocation of limited organs to a patient who is awaiting his or her first transplant in contrast to a retransplanted patient who will likely have a longer waiting time due to sensitization from his or her first allograft. In general, a patient who has a primary biopsy-proven, nonfunctioning allograft will typically return to the waiting list without any loss of accumulated waiting time, pending approval from the United Network for Organ Sharing. Those who experience allograft failure after a period of good allograft function return to the waiting list and begin accruing waiting time from time zero. Finally, the decision to re-list a patient who

Table 1-6. Criteria for Expanded Donors

Donor Condition	Donor Age Categories		
	10–49	50–59	≥60
CVA + HTN + SCr > 1.5		X	X
CVA + HTN		X	X
CVA + SCr > 1.5		X	X
HTN + SCr > 1.5		X	X
CVA			X
HTN			X
SCr > 1.5			X
None of the above			X

CVA = cerebrovascular accident was cause of death; HTN = history of hypertension at any time; SCr = serum creatinine; X = Expanded Criteria Donor.

http://www.unos.org/policiesandbylaws/policies/docs/policy_70.doc.

has allograft failure due to documented drug noncompliance is often at the discretion of the transplant team.

Recipients of Organs from Marginal or Extended Criteria Donor

One strategy that has been implemented by United Network for Organ Sharing to address the growing gap between the list of patients waiting for a transplant and the number of available organs is through the use of ECDs. The criteria for ECDs are provided in Table 1-6. These donors may have risk factors that lead to worsened allograft outcomes; however, it could provide the only possibility of life in patients who have an urgent clinical need for an organ or have no other choice but to wait on the transplant list because of prior sensitization. Based on data from the Scientific Registry of Transplant Recipients, the use of ECDs has resulted in a 14.3% increase in the number of recovered organs and a corresponding 7.7% increase in the number of transplants performed. Patient, allograft, and death-censored graft survival in patients who receive an organ from an ECD are, although slightly lower, similar to recipients of non-ECD organs with the current standard immunosuppressive regimen of antithymocyte globulin induction therapy and maintenance immunosuppressive therapy with tacrolimus, MMF, and prednisone. Patients who are over age 40 or of African-American or Asian ethnicity and on the transplant waiting list for more than 1350 days may derive an increased survival benefit from using ECD kidneys. However, caution should be applied to directing ECD kidneys to patients who are being retransplanted as outcomes are significantly worse than if they received a standard criteria donor kidney.

Recipients of Organs from ABO-Incompatible Donors

Compatibility in ABO-blood group between recipient and donor has historically been a prerequisite for kidney transplantation, due to the significant risk of hyperacute rejection from circulating preformed specific antibodies against donor blood type antigens, in a recipient with an incompatible blood group. This risk posed a significant disadvantage to patients who had blood types that were less common (blood group B or O compared with blood group A) as it further exacerbated the growing gap between the number of patients waiting for a kidney and the number of organs available. Creative strategies are being explored to minimize this gap. Among the Caucasian population with blood type A, about 20% express the subtype A2, which is known to be much less antigenic than the A1 subtype. This lesser antigenicity potentially allows successful transplantation of kidneys from A2 donors to blood type B or O recipients with little risk of hyperacute rejection. However, if the recipient has anti-A2 antibody titers of greater than 1:16, plasmapheresis can be used effectively as preconditioning monotherapy or in combination with IVIG to facilitate transplantation from a potential donor who has an incompatible ABO blood group. Transplantation from non-A2 donors (type A1 and B) has also demonstrated feasibility if recipients had a splenectomy and underwent plasmapheresis with or without IVIG therapy. Alternatively, rituximab, an anti-CD20 monoclonal antibody, in a single dose of 375 mg/m^2 can be administered in place of a splenectomy before transplantation. Maintenance immunosuppression post-transplantation generally consisted of a triple-drug combination with a CNI, antiproliferative drug, and corticosteroids. With the exception of patients with baseline anti-blood group antibody titers of greater than 1:16, graft survival rates in ABO-incompatible transplants appear to be similar to those who are ABO-compatible. These strategies provide additional alternatives to increasing the donor organ supply to patients who otherwise would be waiting for significantly prolonged periods on the cadaveric-donor list.

Future Immunosuppressive Strategies

Tolerance Induction Immunosuppressive Strategies

Since the introduction of allotransplantation in the 1950s, significant strides have been made in the prevention of AR episodes for prolonging long-term allograft survival. However, a threshold appears to have been reached at which the benefits of transplantation has equilibrated with the risks associated with immunosuppressive drugs that are required for maintaining good allograft function. Thus, the focus of immunosuppression has shifted from the management of short-term outcomes to the prevention of long-term complications. Strategies to induce immunological tolerance are of utmost interest for many reasons, all of which ultimately lead to eliminating the need for long-term immunosuppressive drug therapy.

True immunological tolerance has been difficult to attain due to the complex nature of the immune system. Major barriers to achieving tolerance include the pre-existing donor-specific antibodies and immunity that is induced by

Fehr T, Sykes M. Tolerance induction in clinical transplantation. Transpl Immunol 2004;13:117–30.

infectious agents. Early attempts at inducing tolerance involved using total lymphoid irradiation to destroy the lymphocytes and antibodies that mediate allograft rejection. This approach, when used in combination with antithymocyte globulin and low-dose prednisone was not met with an acceptable success rate to offset the infectious and malignant complications induced by total lymphoid irradiation. More recent tolerogenic strategies apply the use of polyclonal and monoclonal antibodies to induce profound T-cell depletion. In particular, the use of alemtuzumab, an anti-CD52 specific antibody, has shown promising results. Recipients of kidney transplants received a single dose of intravenous methylprednisolone 500 mg before one dose of alemtuzumab 20 mg, followed by a second dose of alemtuzumab 20 mg within 24 hours after the transplant procedure. The concomitant use of low-dose immunosuppression with only CSA to achieve target trough concentrations of 75–125 ng/mL resulted in 62%–94% of patients having normal kidney function almost 1 year after transplantation. However, this was not considered true tolerance but was eventually coined "prope tolerance" or "almost tolerance" by the authors. This phenomenon has also been demonstrated in a further attempt at achieving true tolerance with the use of 3–4 doses of alemtuzumab 0.3 mg/kg preceded by intravenous corticosteroids with each dose. Rejection episodes occurred within the first 2 weeks in all patients despite lymphopenia persisting for more than 6 months. All AR episodes were responsive to therapy with high-dose corticosteroids and sirolimus, with one patient requiring additional antibody therapy. However, all patients maintained good allograft function free of additional rejection episodes and had an average creatinine clearance of 74 mL/minute after 12 months with low-dose sirolimus therapy. It is clear that complete donor-specific tolerance without the need for lifetime immunosuppression has not yet been achieved. Current research efforts are being focused on the role of antigen-presenting cells in the induction of rejection, given the occurrence of AR in the presence of profound lymphocyte depletion.

Conclusion

The impact of the long-term complications of immunosuppressive therapy on cardiovascular and renal outcomes is a growing dilemma in the field of transplantation. The relative shortage of available organs for the number of individuals awaiting transplantation is a persistent issue, and strategies to increase the donor pool, such as the use of marginal or ECDs, may be contributing to the lack of improvement in graft survival the potential improvement in graft survival.

Pharmacists have the potential to play an integral role in drug management for recipients of transplants given the growing number of drugs that exist and their potential for adverse effects and drug interactions. Pharmacist knowledge of the pharmacokinetics and pharmacodynamics of drug therapy is a crucial contribution to the long-term well-being of these patients.

Annotated Bibliography

1. Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. Am J Transplant 2004;4:378–83.

   Acute rejection rates have always had a strong inverse correlation to overall allograft survival. However, the authors of this analysis of kidney-only transplant recipients in the Scientific Registry of Transplant Recipients (SRTR) demonstrated that although the rate of acute rejection (AR) significantly declined after 1996, the risk of overall allograft loss did not proportionately decline and death-censored allograft loss increased. When allograft survival was analyzed based on the return of allograft function after the episode, it was shown to be similar in patients who had no AR and those who had AR with a return to baseline allograft function. In contrast, patients who did not return to within 5% of baseline allograft function had a 3-fold increased risk for death-censored allograft loss at 6 years. These results do not diminish the role of preventing AR in overall allograft survival, but encourages the identification of other evolving factors that may also be contributory.

2. Kauffman HM, Cherikh WS, Cheng Y, Hanto DW, Kahan BD. Maintenance immunosuppression with target-of-rapamycin inhibitors is associated with a reduced incidence of *de novo* malignancies. Transplantation 2005;80(7):883–9.

   Malignancy is known to be a complication of long-term immunosuppressive therapy. Antitumor properties have been demonstrated with sirolimus in the murine model; this is the first study to report the clinical impact of the antitumor properties of sirolimus. In this database analysis of 33,249 transplant recipients of primary deceased donor kidneys, patients received either calcineurin inhibitor (CNI) alone, mammalian target of rapamycin inhibitor alone, or CNI together with mammalian target of rapamycin inhibitor. All three regimens were used in combination with or without an antiproliferative drug. After 2.6 years of follow-up, treatment with mammalian target of rapamycin inhibitors significantly reduced the risk of developing any *de novo* malignancy by 60%. Variables that were associated with an increased risk included male gender, increased age, white race, and a history of previous malignancy. There is already a noticeable shift toward the clinical use of sirolimus in alleviating the incidence of chronic allograft nephropathy (CAN), and these data provide further evidence of the additional benefits of sirolimus-based immunosuppression.

3. Watson CJE, Firth J, Williams PF, et al. A randomized controlled trial of late conversion from CNI-based to sirolimus-based immunosuppression following renal transplantation. Am J Transplant 2005;5:2496–2503.

   The immunosuppressive efficacy of sirolimus was initially evaluated in combination regimens with early cyclosporine withdrawal, which resulted in improved allograft function and survival. This is the first controlled study evaluating the renal benefits of converting patients from CNI-based therapy to sirolimus-based therapy up to 8 years post-transplant. Forty patients with impaired allograft function receiving CNI-based immunosuppression were randomized to either continued CNI or to sirolimus-based therapy. Baseline glomerular filtration rate (GFR) values were 36.1 mL/minute and 37.8 mL/minute for the CNI and sirolimus groups, respectively. Patients switched to sirolimus therapy had a

significant improvement in mean GFR of +8.5 mL/minute greater than baseline. There was also a significant difference of 12.9 mL/minute in the GFR between the two groups at 12 months. Although further studies are required to determine if late conversion to sirolimus has an independent effect on reversing kidney dysfunction, this study supports sirolimus as an alternative immunosuppressive strategy to prevent progression of kidney dysfunction in patients receiving CNI-based therapy.

4. Miller LW. Cardiovascular toxicities of immunosuppressive agents. Am J Transplant 2002;2:807–18.

This excellent and comprehensive review highlights the importance of cardiovascular risk in the long-term management of recipients of solid organ transplants. The article addresses the prevalence and incidence of hypertension, dyslipidemia, diabetes, and coronary artery disease, and the relative contribution of CNIs, mammalian target of rapamycin inhibitors, and antiproliferative drugs on these cardiovascular risk factors. The proposed mechanisms of how each drug can lead to these adverse effects are presented, along with strategies to minimize, monitor, and manage a patient presenting with these complications. However, the reader should refer to additional literature on managing post-transplant diabetes mellitus as these topics were omitted (incidence and mechanism was presented). Regardless, this article is a definite must for clinicians who have specific interest in the cardiovascular aspects of solid organ transplantation.

5. Kirk AD, Hale DA, Mannon RB, et al. Results from a human renal allograft tolerance trial evaluating the humanized CD52-specific monoclonal antibody alemtuzumab (Campath-1H). Transplantation 2003;76(1):120–9.

Progressive success of organ transplantation is limited by the long-term complications of immunosuppression. Achieving immunologic tolerance is the key to eliminating the need for immunosuppression. As a follow-up to the recently coined phenomenon "prope tolerance," the authors attempted to induce true tolerance in 7 patients with 3–4 doses of alemtuzumab 0.3 mg/kg, each preceded by intravenous corticosteroids. Unfortunately, rejection episodes occurred early in all patients despite profound lymphopenia. All but one episode were responsive to high-dose corticosteroid therapy and sirolimus. All patients maintained good allograft function and remained free of repeat rejection after 12 months on low-dose sirolimus monotherapy. From these results, the authors seemingly failed to meet their objective. This study indicated that lymphocyte depletion should not be the main focus of tolerance induction and that monocytes play an important role in allograft rejection.

SELF-ASSESSMENT QUESTIONS

Questions 1–3 pertain to the following case.
P.G. is a 43-year-old Caucasian man with chronic kidney disease secondary to long-standing type 1 diabetes mellitus but is not yet on dialysis. His diabetes is being managed with an insulin pump and his recent hemoglobin A1C concentration is 9.1% before transplantation. His other medical history includes hypertension and anemia for which he is receiving erythropoietin therapy. He is admitted to the hospital today to receive a living donor kidney from his high school best friend.

1. Which one of the following is the most appropriate choice for induction immunosuppressive therapy?

   A. Muromonab.
   B. Basiliximab.
   C. Antithymocyte globulin.
   D. Alemtuzumab.

2. His serum creatinine immediately post-transplant decreased from 5.2 mg/dL to a nadir of 1.1 mg/dL. His white blood cell count was 12.9 x 10^3 cells/mm^3 with an absolute lymphocyte count of 500/mm^3. His hemoglobin and hematocrit levels were 10 g/dl and 29.8%, respectively, and platelet count was 267 x 10^3 cells/mm^3. Which one of the following is the most appropriate maintenance immunosuppressive regimen for P.G.?

   A. Cyclosporine, mycophenolate mofetil, and prednisone taper over 3 months to 10 mg/day.
   B. Cyclosporine, azathioprine, and rapid prednisone withdrawal in 7 days.
   C. Tacrolimus, mycophenolate mofetil, and rapid prednisone withdrawal in 7 days.
   D. Sirolimus, mycophenolate mofetil, and prednisone taper over 3 months to 10 mg/day.

Eight years later, P.G.'s creatinine clearance has slowly

declined to 20 mL/minute and discussion is initiated regarding the need for eventual hemodialysis. He currently does not exhibit any symptoms of kidney failure. P.G. is wondering whether he should approach another one of his old high school friends for a kidney donation. His panel reactive antibody (PRA) levels are 25%.

3. Which one of the following is the best strategy for managing P.G.'s chronic kidney disease in terms of improving his overall morbidity and mortality?

   A. Get re-listed on the transplant waiting list and wait for the next available cadaveric kidney.
   B. Begin approaching friends and family members about the gift of organ donation to identify a donor before the need for dialysis.
   C. Prepare to initiate dialysis when needed and start approaching friends and family members about the gift of organ donation.
   D. Prepare to initiate life-long dialysis when needed because his risk for rejection after retransplantation is high.

4. A kidney becomes available from a 65-year-old donor with a history of hypertension and death due to intracranial hemorrhage and a serum creatinine of 2.0 mg/dL at the time of tissue typing. Which one of the following is the most appropriate initial maintenance immunosuppressive regimen for P.G. at this time?

   A. Cyclosporine, sirolimus, and prednisone.
   B. Cyclosporine, azathioprine, and prednisone.
   C. Tacrolimus, mycophenolate mofetil, and prednisone.
   D. Tacrolimus, sirolimus, and prednisone.

Questions 5 and 6 pertain to the following case.
T.C. is a 63-year-old Caucasian man who has kidney failure secondary to immunoglobulin (Ig) A nephropathy. He

received a living donor kidney transplant 6 months ago, for which he received induction therapy and triple-drug maintenance therapy with tacrolimus, mycophenolate mofetil, and prednisone. His baseline serum creatinine is 1.3 mg/dL. Over the past 3 months, his serum creatinine concentrations have been slowing creeping up from baseline levels to 1.5 mg/dL, 1.7 mg/dL, and 2.3 mg/dL. The tacrolimus trough concentration from this visit is 31 ng/mL.

5. Which one of the following is the most appropriate intervention that should be implemented in T.C.'s pharmacotherapeutic regimen at this time?

    A. Hold tacrolimus and obtain a kidney biopsy to confirm acute rejection.
    B. Switch tacrolimus to sirolimus.
    C. Reduce tacrolimus dose and obtain another tacrolimus concentration in 24–48 hours.
    D. No changes to immunosuppressive drug regimen and obtain another tacrolimus concentration.

One year after transplantation and after multiple modifications in immunosuppressive therapy for managing adverse effects, T.C. is now receiving the same triple-drug regimen that he received immediately post-transplant. His new baseline serum creatinine is 1.9 mg/dL, tacrolimus trough concentration is 14 ng/mL and a protocol-driven kidney biopsy result shows signs that are indicative of chronic allograft nephropathy.

6. Which one of the following would be the most appropriate immunosuppressive strategy at this time?

    A. Switch tacrolimus to sirolimus.
    B. Continue tacrolimus with target trough concentrations of 5–10 ng/mL.
    C. Continue tacrolimus at lower doses and add sirolimus.
    D. Treat with high-dose pulse corticosteroids.

7. Assuming equal cost between immunosuppressive drugs within the same drug class, which one of the following regimens would you expect to be the most cost-effective in terms of improving overall allograft survival and long-term renal function in a hypertensive patient requiring dialysis in the first week post-transplant?

    A. Cyclosporine, azathioprine, and prednisone.
    B. Cyclosporine, mycophenolate mofetil, and prednisone.
    C. Tacrolimus, azathioprine, and prednisone.
    D. Tacrolimus, mycophenolate mofetil, and prednisone.

8. J.B. is a 41-year-old Caucasian man who received a cadaveric kidney transplant 3 years ago. He has been complaining of abdominal discomfort for the last couple of days with 2–3 bouts of nausea and vomiting. His serum creatinine is 1.8 mg/dL, which is slightly increased from his baseline serum creatinine of 1.4 mg/dL. His cyclosporine trough concentration was

245 ng/mL. His cardiac enzymes were negative. His immunosuppressive drugs include cyclosporine 125 mg 2 times/day, mycophenolate mofetil 1 g 2 times/day, and prednisone 5 mg/day. Which one of the following strategies would be the most helpful in decreasing J.B.'s gastrointestinal tract complaints?

    A. Switch patient to mycophenolate sodium delayed release.
    B. Decrease dose of mycophenolate mofetil.
    C. Decrease dose of cyclosporine.
    D. Increase dose of cyclosporine.

Questions 9–12 pertain to the following case.

T.P. is a 57-year-old African-American man with diabetes mellitus and hypertension who received a living donor kidney from his sister. His immunosuppressive therapy consists of cyclosporine 50 mg 2 times/day, mycophenolate mofetil 1 g 2 times/day, and prednisone 7.5 mg once daily; cyclosporine trough concentrations are maintained between 150 ng/dL and 170 ng/dL. His serum creatinine has been between 1.4 mg/dL and 1.5 mg/dL. His blood pressure is 158/95 mm Hg and fasting lipid profile is as follows: total cholesterol 350 mg/dL and low-density lipoprotein 135 mg/dL. An attempt was made to decrease T.P.'s prednisone dose further, but he now presents with an acute rejection episode requiring high-dose corticosteroids. His serum creatinine is now 1.9 mg/dL.

9. Which one of the following factors is most likely to put T.P. at increased risk for acute rejection episodes?

    A. Age.
    B. African American.
    C. Diabetes mellitus.
    D. Living donor kidney transplant from his sister.

10. Which one of the following would be the most appropriate adjustment of T.P.'s immunosuppressive regimen after this rejection episode based on his lipid profile?

    A. Increase his cyclosporine dose and keep the mycophenolate mofetil dose the same.
    B. Maintain the cyclosporine dose and increase mycophenolate mofetil dose.
    C. Switch cyclosporine to tacrolimus and keep the mycophenolate mofetil dose the same.
    D. Decrease the cyclosporine dose and increase the prednisone dose.

Three months after T.P.'s acute rejection episode, his serum creatinine on routine laboratory tests is 2.5 mg/dL. His immunosuppressive drug concentrations are within the target range and noncompliance with his drugs is not an issue. ImmunKnow (assay for lymphocyte activity) results showed 103 ATP ng/mL and a urinalysis showed the presence of decoy cells indicative of polyoma (BK) virus. The presence of active polyoma infection was confirmed with a plasma viral polymerase chain reaction assay = 400,000 DNA copies/mL.

11. Which one of the following is the most likely factor that would have predicted his risk for developing polyoma infection?

    A. History of diabetes.
    B. Overall degree of immunosuppression.
    C. Incomplete recovery of serum creatinine to baseline levels.
    D. T.P.'s age, gender, and ethnicity.

Several months later, T.P.'s plasma BK virus load decreased to nondetectable levels, and a repeat ImmunKnow showed 230 ATP ng/mL and serum creatinine was 2.1 mg/dL. It is decided that it would be beneficial for T.P. to switch his immunosuppressive therapy to sirolimus, mycophenolate mofetil, and prednisone at this time.

12. Which one of the following recommendations would be most appropriate to facilitate the switch in T.P.'s immunosuppressive regimen?

    A. Discontinue calcineurin inhibitor today and initiate maintenance doses of sirolimus tomorrow.
    B. Administer sirolimus as a 2 times/day regimen.
    C. Monitor sirolimus levels daily during initial titration until target levels are obtained.
    D. Monitor sirolimus levels once weekly after initiation of therapy and dosage changes.

13. M.F. is a 42-year-old Hispanic man with diabetes who received a pancreas transplant 5 years after his kidney transplant from his brother. His post-pancreas transplant course was complicated by wound dehiscence, abscess, and pancreatic fistula requiring removal of the pancreatic allograft. Cellulitis subsequently developed around the surgical site. He presents to the transplant clinic for follow-up with no major complaints, and his wound appears to be granulating slowly. His serum creatinine has increased to 2.3 mg/dL and tacrolimus trough concentration is 10 ng/mL. His immunosuppressive drugs include tacrolimus 5 mg 2 times/day and mycophenolate mofetil 500 mg 2 times/day, and he is completing a course of antibiotic drugs. Which one of the following would be the most optimal immunosuppressive strategy for M.F. at this time?

    A. Make no changes at this time but closely monitor.
    B. Discontinue tacrolimus and give a loading dose of sirolimus.
    C. Switch tacrolimus to cyclosporine.
    D. Lower his tacrolimus dose and increase mycophenolate mofetil.

14. B.B. is a 34-year-old man with diabetes who has a kidney transplant 1.5 years ago. His current immunosuppression consists of sirolimus 2 mg once daily and mycophenolate mofetil 1.5 g 2 times/day. He is no longer receiving any prophylactic drugs for infection except cotrimoxazole for recurrent urinary tract infections. He has remained corticosteroid-free. His fasting glucose concentrations are well-controlled, his serum creatinine is 1.8 mg/dL and sirolimus trough blood concentration is 7 ng/mL. Which one of the following statements is currently most applicable to B.B. with regard to his risk of experiencing adverse effects from immunosuppressive therapy?

    A. Mycophenolate mofetil increases B.B.'s risk for invasive cytomegalovirus infection.
    B. Mycophenolate mofetil increases B.B.'s risk for cardiovascular disease.
    C. Sirolimus increases B.B.'s risk for *Pneumocystis jiroveci* pneumonia.
    D. Sirolimus increases B.B.'s risk for gastrointestinal tract adverse effects.

Questions 15 and 16 pertain to the following case.

K.A. is a 39-year-old Caucasian woman with history of systemic erythematosus who just received a living related donor kidney transplant from her 30-year-old sister. Her father has diabetes mellitus and her mother is relatively healthy except for removal of a benign breast mass 2 years ago. K.A. inherited her mother's fair skin and red hair; she had a suspicious mole on her back removed 5 years ago.

15. Which one of the following would be the most appropriate immunosuppressive regimen for K.A. based on her risk factors?

    A. Cyclosporine, mycophenolate mofetil, and corticosteroids.
    B. Tacrolimus, mycophenolate mofetil, and corticosteroids.
    C. Sirolimus, mycophenolate mofetil, and corticosteroids.
    D. Tacrolimus, sirolimus, and corticosteroids.

16. Which one of the following is the most important preventive measure that you should counsel K.A. about with regard to minimizing her potential for long-term complications associated with immunosuppressive therapy?

    A. Self-administered breast examinations.
    B. Routine application of high sun-protection factor.
    C. Yearly Papanicolaou smear.
    D. Daily weight-bearing exercise.

Questions 17 and 18 pertain to the following case.

Five months after a kidney transplant, A.C. is admitted to the hospital with a fever for the past week and laboratory values reveal pancytopenia. She complains of mild shortness of breath on exertion and fatigue. Her serum creatinine is 2.1 mg/dL, white blood cell count is 2.8×10^3 cells/mm^3, hemoglobin is 7.2 g/dL, and platelet count is 103×10^3 cells/mm^3. All other laboratory values and diagnostic work-up are negative. Microbiologic cultures are all negative. Her immunosuppressive regimen consists of sirolimus and prednisone. Her sirolimus trough concentration is 11 ng/mL and her prednisone dose is currently being tapered.

17. Which one of the following statements is most applicable to A.C.'s pancytopenia?

    A. Thrombocytopenia is an immune-mediated adverse effect of sirolimus requiring discontinuation of therapy.
    B. Treatment with filgrastim is required due to the persistent nature of leukopenia associated with sirolimus therapy.
    C. Anemia is a dose-dependent adverse effect of sirolimus.
    D. The risk of thrombotic microangiopathy is increased.

Three weeks later, the abnormalities in A.C.'s laboratory values resolve, but she complains of worsening shortness of breath, persistent fever and fatigue, and the presence of a dry cough with blood-tinged phlegm. Her chest radiograph shows significant bilateral lower alveolo-interstitial pulmonary infiltrates. Sputum cultures have not grown any organisms thus far. Her immunosuppressive regimen consists of sirolimus, mycophenolate mofetil, and prednisone.

18. Which one of the following is the most appropriate thing to do regarding A.C.'s immunosuppressive therapy to manage her current symptoms?

    A. Continue current regimen with sirolimus and prednisone.
    B. Decrease sirolimus dose.
    C. Discontinue sirolimus therapy.
    D. Discontinue sirolimus therapy and give high-dose steroids to alleviate respiratory symptoms.

19. P.R. is a 55-year-old Asian woman who received her second cadaveric kidney transplant 7 months ago. She is 5'2" and weighs 102 pounds. Her first kidney allograft was lost early due to thrombosis, but she currently enjoys good function from her second allograft. She received induction therapy with basiliximab, and her maintenance immunosuppressive regimen consists of tacrolimus 5 mg 2 times/day, mycophenolate mofetil 1 g 2 times/day, and prednisone 10 mg/day. Her trough tacrolimus concentration was 21.5 ng/mL and her previous weekly levels were 19.4 ng/mL and 22.3 ng/mL. Her fasting glucose concentrations have been in the range of 180 mg/dL to 210 mg/dL. Which one of the following is the most appropriate change to P.R.'s immunosuppressive drugs?

    A. Decrease dose of tacrolimus to 4 mg 2 times/day.
    B. Decrease dose of mycophenolate mofetil to 750 mg 2 times/day.
    C. Decrease prednisone dose to 5 mg once daily.
    D. Switch tacrolimus to cyclosporine.

20. K.C. is a 17-year-old girl with kidney failure treated with dialysis due to reflux nephropathy. She will receive a living donor kidney transplant from her twin sister. However, her sister is concerned about the potential cosmetic side effects such as "steroid face, facial hair growth, hair loss, and gum overgrowth" that can occur from some of the immunosuppressive drugs. K.C. is going off to college next year and her sister is worried that K.C. will have difficulty "fitting in." She has heard of cases where no immunosuppression is required. Which one of the following statements is most applicable to K.C.?

    A. Due to the low success rates of tolerance induction, the high incidence of infectious and malignant complications outweighs the potential benefits.
    B. "Prope" tolerance (almost complete tolerance) can be achieved with the use of potent lymphocyte-depleting agents.
    C. Prope tolerance requires long-term immunosuppressive therapy; thus, K.C. is still at risk for cosmetic side effects.
    D. Tolerance induction does not apply to K.C. because she is receiving a kidney from her identical twin.

Questions 21 and 22 pertain to the following case.
G.G. is a 52-year-old Caucasian woman with kidney failure due to hypertension. She received her first kidney transplant from a cadaver donor about 6 years ago. Her history is significant for two episodes of acute rejection during the first 6 months post-transplant. Both AR episodes were treated with pulse corticosteroid therapy with return of her serum creatinine to baseline after each episode. Her serum creatinine has gradually increased over the past few years. Her immunosuppressive regimen consists of tacrolimus, sirolimus, and prednisone.

21. Which one of the following strategies will help preserve G.G.'s long-term kidney allograft function?

    A. No changes to her immunosuppressive regimen are needed.
    B. Reduce target tacrolimus 12-hour trough concentration by 25%–50%.
    C. Convert tacrolimus to cyclosporine-based regimen.
    D. Convert tacrolimus to mycophenolate mofetil-based regimen.

G.G. subsequently develops biopsy-proven chronic allograft nephropathy and needs re-transplantation. She now presents for her second kidney transplant from a 55-year-old cadaver donor who died of cerebrovascular accident and a serum creatinine of 1.7 mg/dL. Due to her previous transplant, her panel reactive antibody at the time of transplant is 45%, but the T- and B-cell crossmatch is negative.

22. Which one of the following will be the most appropriate initial immunosuppressive regimen?

    A. Basiliximab, tacrolimus, mycophenolate mofetil, and prednisone.
    B. Antithymocyte globulin, tacrolimus, mycophenolate mofetil, and prednisone.
    C. Basiliximab, sirolimus, mycophenolate mofetil, and prednisone.
    D. Antithymocyte globulin, sirolimus, mycophenolate mofetil, and prednisone.

23. A 56-year-old Caucasian man with end-stage liver disease secondary to hepatitis C and alcohol use requires a liver transplantation. Complications related to his liver failure included hepatic encephalopathy, ascites, esophageal varices, and kidney dysfunction. Other medical history includes type 2 diabetes mellitus and osteopenia. His transplant surgery was uneventful. Which one of the following is the best immunosuppressive strategy for this patient?

A. Tacrolimus, mycophenolate mofetil, and prednisone.

B. Tacrolimus, mycophenolate mofetil, and prednisone withdrawal.

C. Reduced dose of tacrolimus, mycophenolate mofetil, and prednisone (CNI sparing).

D. Sirolimus, mycophenolate mofetil, and prednisone (CNI free).

24. L.K. is a 48-year-old woman who received a liver transplant 3 years ago for hepatitis C cirrhosis. She was lost to follow-up for 2 years. After a 1-week vacation in Florida, she returns to the clinic with an elevated serum creatinine of 2.1 mg/dL from a baseline value of 1.5 mg/dL. Her current regimen consists of tacrolimus monotherapy (12-hour trough concentration is 17 ng/mL). What would be the most appropriate management strategy for her immunosuppressive regimen at this time?

A. Discontinue tacrolimus and initiate mycophenolate mofetil.

B. Reduce tacrolimus target concentration by 50% and initiate mycophenolate mofetil.

C. Discontinue tacrolimus and initiate sirolimus.

D. Discontinue tacrolimus and initiate sirolimus and mycophenolate mofetil.

25. P.J. is a 35-year-old Caucasian woman with kidney failure secondary to focal segmental glomerulosclerosis. Her medical history includes hypertension. P.J. was diagnosed with kidney failure about 1 year ago and at the time of presentation, her serum creatinine was 5.0 mg/dL and she was not yet on dialysis. Standard pre-transplant evaluation confirmed that she was a good candidate for kidney transplantation. Her immunologic workup revealed that she had a 100% panel reactive antibody due to two previous pregnancies and a red blood cell transfusion. Her husband volunteered as a potential donor. He was ABO-compatible and had four HLA-antigen mismatches with his wife. Although standard B-cell crossmatch was negative, the standard T-cell crossmatch was positive. However, flow cytometry crossmatch was positive for both T cells and B cells. Which one of the following is the best treatment option for P.J.?

A. Place P.J. on the cadaveric renal transplant waiting list.

B. Proceed with the transplant using an immunosuppressive regimen of antithymocyte globulin, tacrolimus, mycophenolate mofetil, and corticosteroids.

C. Initiate plasmapheresis and rituximab, repeat crossmatch, and proceed with transplant if crossmatch is negative.

D. Initiate high-dose intravenous Ig therapy, repeat crossmatch, and proceed with the transplant if crossmatch is negative.