# Immunosuppression in Pediatric SOT

By Barrett Crowther, Pharm.D., FAST, BCPS

Reviewed by David M. Newland, Pharm.D., M.S., BCPS; Mary Soliman, Pharm.D., BCPPS; and Yvonne Zorn, Pharm.D., BCPPS

## LEARNING OBJECTIVES

1. Evaluate risk factors for developing rejection in pediatric recipients of a solid organ transplant (SOT).
2. Devise an appropriate induction plan for a pediatric SOT recipient considering specific organ type, immunologic risk, underlying diseases, and planned maintenance immunosuppressive regimen.
3. Design a maintenance immunosuppressive regimen plan in pediatric SOT for suitability on the basis of individual recipient characteristics.
4. Assess how current strategies used to treat SOT rejection affect cellular- and antibody-mediated immune responses.

### ABBREVIATIONS IN THIS CHAPTER

| | |
|---|---|
| aHUS | Atypical hemolytic uremic syndrome |
| AMR | Antibody-mediated rejection |
| ATG | Antithymocyte globulin |
| CMV | Cytomegalovirus |
| CNI | Calcineurin inhibitor |
| dd-cfDNA | Donor-derived cell-free DNA |
| DSA | Donor-specific antibody |
| eATG | Equine antithymocyte globulin |
| EBV | Epstein-Barr virus |
| IVIG | Intravenous immunoglobulin |
| mTOR | Mammalian target of rapamycin |
| NK | Natural killer |
| PRA | Panel of reactive antibody |
| PTLD | Posttransplant lymphoproliferative disorder |
| rATG | Rabbit antithymocyte globulin |
| SOT | Solid organ transplantation |

*Table of other common abbreviations.*

## INTRODUCTION

### History of Pediatric Solid Organ Transplantation

The first successful human solid organ transplant (SOT) in the United States was in 1954. Dr. Joseph Murray performed this kidney transplant between two identical adult twins. Pediatric SOT procedures followed in the 1960s. Figure 1 outlines the timeline for the first successful pediatric abdominal and thoracic SOT procedures.

Pediatric kidney and liver transplants represent about 70% of all pediatric SOT procedures (Table 1). Although the overall annual SOT procedures in the United States steadily increased by over 20% during 2007–2017, the number of annual pediatric SOT procedures essentially remained unchanged over the past decade. The growth overall in SOT procedures has been driven by improved immunosuppressive regimen management, advances in surgical techniques, progress with testing methods, and improved disease state management before SOT.

### Allograft and Patient Survival

As benchmark metrics for U.S. transplant programs, 1-year allograft and patient survival has steadily improved in pediatric SOT for the past 20 years. However, despite short-term improvements, long-term survival of the allograft continues to be a challenge among all organ types. Factors contributing to long-term survival include, but are not limited to, rejection episodes, infectious causes, medication adverse effects, comorbid conditions, and whether the transplant was from a deceased or living donor. Living donation is common for both liver and kidney transplants, and there are case reports of unique situations in which living donor lung, intestine, and heart transplants were performed (Date 2017; Khaghani 2004). Patient age and weight also significantly contribute to the success of the transplant because of the technical difficulty of the surgical procedure in children younger

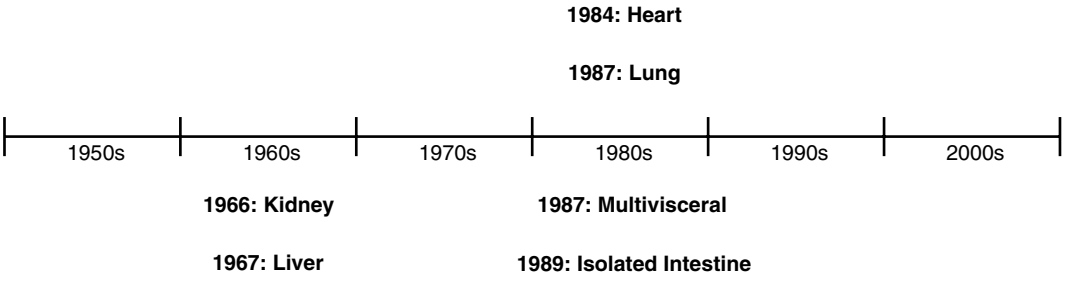


**Figure 1.** Timeline for first successful transplantation of pediatric organs.

Adapted from: Scientific Registry of Transplant Recipients [homepage on the Internet].

**Table 1.** Pediatric Organ Transplants Performed in the United States in 2017 (< 18 yr of age)

| | Overall[a] | Kidney | Liver | Heart | Lung | Intestine |
|---|---|---|---|---|---|---|
| < 1 yr | 249 | 0 | 133 | 111 | 3 | 1 |
| 1–5 yr | 575 | 183 | 249 | 107 | 4 | 21 |
| 6–10 yr | 292 | 131 | 83 | 48 | 5 | 15 |
| 11–17 yr | 783 | 432 | 134 | 165 | 32 | 10 |
| **All pediatric transplants** | **1899** | **746** | **599** | **431** | **44** | **47** |

[a]Includes kidney/pancreas, heart/lung, and pancreas.

Adapted from: DHHS. Organ Procurement and Transplantation. Transplants in the U.S. by Recipient Age.

than 1 year, ability to tailor immunosuppression on the basis of the maturation of the immune system, medication administration challenges in younger patients and patients with absorption issues, and nonadherence in the pediatric population (Hebert 2017). Figure 2 shows pediatric kidney allograft survival according to the Scientific Registry of Transplant Recipients report.

## BASELINE KNOWLEDGE STATEMENTS

Readers of this chapter are presumed to be familiar with the following:

- Interpreting basic laboratory tests
- Understanding basic immunology concepts
- Recognizing the mechanism of action and common adverse reactions with solid organ transplant maintenance immunosuppressive agents

*Table of common pediatric laboratory reference values.*

## ADDITIONAL READINGS

The following free resources are available for readers wishing additional background information on this topic.

- Wiseman AC. Immunosuppressive medications. Clin J Am Soc Nephrol 2016;11:332-43.

### Overview of Rejection

Rejection is an inflammatory response by the immune system recognizing the transplanted organ as foreign, which can lead to allograft dysfunction and loss, if untreated. One of the primary immune mechanisms for organ rejection involves naive and memory T cells and has been described using a three-signal model of alloimmune response. Signal 1 occurs when antigen-presenting cells (e.g., macrophages, B cells, dendritic cells) display antigen to $CD4^+$ T cells through the major histocompatibility complex, communicating by the T-cell receptor, causing T-cell activation. Because of this recognition response, signal 1 ultimately increases the production of interleukin (IL)-2, a cytokine responsible for T-cell activation and proliferation. Concurrent with signal 1, signal 2 occurs to verify the immune response is warranted. The antigen-presenting cell simultaneously binds to the T cell through CD80 and CD86 interaction with CD28 on the T cell, regulating clonal expansion and differentiation. This action is required for T-cell proliferation and is also called costimulation.

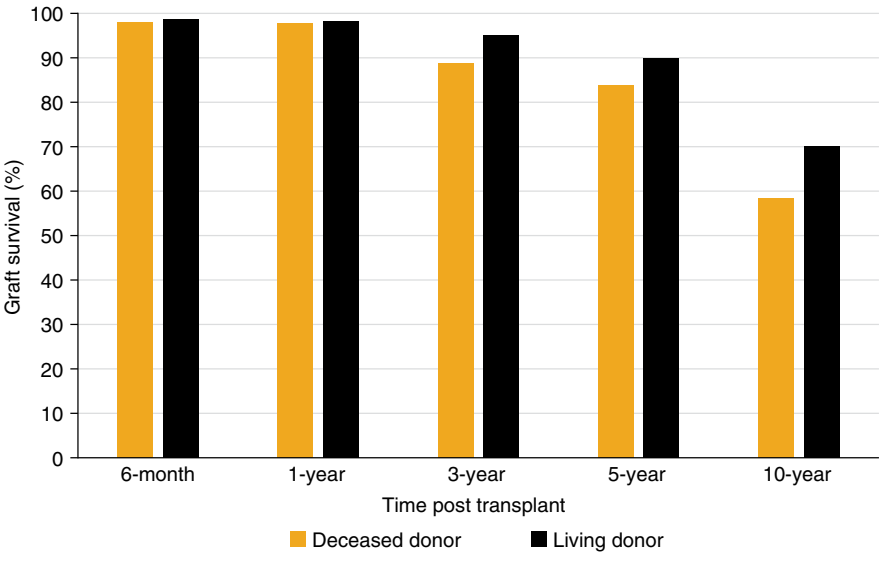


**Figure 2.** Pediatric kidney transplant graft survival rates in the United States (2017).

Adapted from: Scientific Registry of Transplant Recipients. SRTR/OPTN Annual Data Report. 2017.

Contrary to this response, signal 2 is down-regulated when CD80 and CD86 bind with cytotoxic T-lymphocyte antigen-4 (CD152), resulting in T-cell anergy or apoptosis. Signal 3 is responsible for stimulating the mammalian target of rapamycin (mTOR) when IL-2 binds to CD25, leading to the stimulation of the cell cycle. T-cell production also requires synthesis of purine nucleotides, through inosine monophosphate dehydrogenase, and pyrimidine nucleotides. Signal 3 ultimately results in protein synthesis and messenger RNA translation leading to T-cell activation and proliferation. $CD4^+$ T cells orchestrate effector responses of several other cell types, including B cells, $CD8^+$ cytotoxic T cells, $T_{DTH}$ cells, and memory T cells. A diagrammatic representation of this process can be found at in a 2004 article (Halloran 2004).

Solid organ transplant rejection is subdivided on the basis of T- or B-cell involvement in cellular and humoral rejection, respectively. Humoral rejection is also called antibody-mediated rejection (AMR) with involvement of B cells, antibodies, complement, platelets, and coagulation factors. Cellular rejection involves several implicated cells and factors, including $CD4^+$ and $CD8^+$ T cells, antigen-presenting cells, natural killer (NK) cells, adhesion molecules, and various cytokines. Mixed rejection involves components of both AMR and cellular rejection (Box 1).

### Box 1. Components Related to Humoral Rejection and Cellular Rejection

**Humoral Rejection**
- B cells
- Antibodies
- Coagulation
- Complement
- Platelets

**Cellular Rejection**
- $CD4^+$ T cells
- $CD8^+$ T cells
- Adhesion molecules
- Antigen-presenting cells
- Cytokines
- NK cells

### *Classification of Rejection*

Rejection is temporally classified on the basis of onset. Hyperacute rejection occurs within the first 24 hours of transplantation, but often within minutes of reperfusion at the time of transplantation. Hyperacute rejection primarily presents as AMR involving preexisting antibodies because of prior sensitizing events to shared donor antigens (HLAs and blood group antigens). These sensitizing events can include prior SOT, blood transfusions, and pregnancy. Hyperacute rejection is extremely harmful, resulting in interstitial hemorrhage within the transplanted organ and necrosis of the vascular endothelium, often leading to rapid loss of the transplanted organ. Hyperacute rejection is now very rare with pretransplant blood typing and advanced HLA screening techniques.

Acute rejection often occurs within the first 90 days of transplantation but can occur at any period posttransplantation. Acute rejection is a rapid and concentrated inflammatory response directed toward the transplanted organ mediated by a recipient's immune mediators interacting with donor antigens. Acute rejection presents more commonly as cellular rejection. Active AMR occurs when de novo antibodies form against donor antigens or when suppressed pretransplant donor-specific antibodies (DSAs) reemerge. Acute rejection may be prevented by an appropriate induction regimen and adherence to a tailored maintenance regimen. Ultimately, acute rejection results in allograft injury and may lead to chronic rejection.

Chronic rejection is a gradual immune and inflammatory insult to the allograft that leads to fibrosis and loss of allograft function over time. Because of this presentation, chronic rejection is a primary cause of late allograft loss.

### *Risk Factors for Rejection*

Risk factors for rejection can be categorized on the basis of organ type, immunologic factors, donor-related factors, and recipient-related factors.

For pediatric SOT, according to recent Scientific Registry of Transplant Recipients data, at 1 year posttransplantation, intestinal transplant recipients have the highest incidence of rejection, followed by liver, heart, and kidney, respectively (SRTR 2019). Degree of rejection can greatly differ among these organ types. Liver transplant recipients are less susceptible to developing HLA-related AMR. Antibody-mediated rejection because of anti-HLA antibodies in the liver transplant recipient remains a controversial topic.

Patients with preformed antibodies, either blood group antibodies or HLA antibodies, are at a significantly higher risk of rejection. The concept of ABO-compatibility for SOT matching is similar to that for blood donation, where blood type O is the universal donor and blood type AB is the universal recipient. For most SOT procedures, transplantation is contraindicated if the recipient and donor have incompatible blood groups, given the high risk of hyperacute rejection because of preformed anti-A and anti-B antibodies. Of note, successful ABO-incompatible transplants for kidney, liver, intestine, heart, and lung transplants have been performed using strategies involving plasmapheresis, intravenous immunoglobulin (IVIG), rituximab, and/or splenectomy (Urschel 2016; Warner 2006). In addition, children younger than 24 months may have more success with ABO-incompatible liver or heart transplantation, primarily because of immature immune systems leading to low isohemagglutinin (anti-A/anti-B) titers.

Potential SOT recipients are typed for HLA, which is encoded on chromosome 6 in humans. Children receive one set of antigens from each of their parents. This HLA typing is used to assess the suitability of the donor/recipient match. According to the United Network for Organ Sharing, potential SOT recipient pairs of HLA-A, HLA-B, and HLA-DR are evaluated. As, such a recipient can have up to six HLA mismatches. For example, a recipient and donor who share only HLA-DR52 but no other HLA matches would be considered a five of six HLA mismatch (2 HLA-A, 2 HLA-B, 1 HLA-DR). In pediatric kidney transplantation, the presence of at least one HLA-DR mismatch has been associated with an increased risk of rejection.

Preformed anti-HLA antibodies are typically assessed before SOT. A panel of reactive antibody (PRA) is calculated to determine the percentage of the population with which the recipient's anti-HLA antibodies would react on a scale of 0%–100%. In this manner, for many organs, PRA helps determine the difficulty of obtaining an appropriate immunologic donor. Patients with a PRA greater than 0% are considered sensitized and at increased risk of rejection for heart, lung, kidney, and intestine transplantation.

To further assess anti-HLA antibody presence, a crossmatch is performed. Traditionally, a crossmatch occurred when a patient's serum was assessed in the presence of a specific donor's lymphocytes to help determine whether preformed DSAs existed. According to donor HLA typing and assessment of a recipient's anti-HLA antibody profile, a virtual crossmatch may also be conducted. If a patient has moderate to strong preformed donor-specific anti-HLA antibodies, the virtual crossmatch is typically positive. A virtual crossmatch is a much quicker way to evaluate a donor/recipient compatibility than a physical traditional crossmatch. A positive crossmatch is typically a contraindication for kidney, intestine, lung, or heart transplantation because of the high likelihood of rejection, unless the immunosuppressive regimen is significantly modified. Typically, this involves removal of the preformed antibodies and strategies to prevent rebound production of said antibodies (e.g., plasmapheresis, IVIG, rituximab, and/or eculizumab). Furthermore, immunosuppressive induction and maintenance regimens can influence immunologic risk factors for rejection.

Several donor-related factors are associated with an increased risk of acute rejection. Cold ischemic time, the time from the clamping of the donor aorta until the anastomosis of the organ to the recipient vasculature, has been associated with increased risk of delayed graft function (in kidney transplant recipients), acute rejection, and primary nonfunction (Wu 2015). In kidney transplantation, cold ischemic times greater than 24 hours have a greater risk of acute rejection episodes than deceased donor kidney transplants with a cold ischemic time of less than 12 hours (Postalcioglu 2018; KDIGO 2009). With advances in ex vivo organ perfusion before organ implantation, the impact of cold ischemic time appears to be somewhat modifiable (O'Callaghan 2013). In addition, living donor recipients tend to have an extended survival compared with deceased donor recipients (see Figure 2).

Recipient-related factors associated with an increased risk of acute rejection in kidney transplantation include African American ethnicity and delayed graft function. Even in the setting of modern immunosuppression, delayed graft function has been associated with an increased risk of acute rejection. In pediatric liver transplant recipients, patients 0–5 months of age at the time of transplantation had a lower risk of rejection than the other age groups, indicating the influence of an immature immune system reducing the risk of rejection (Shepherd 2008). For all organ types, one of the largest concerns in the pediatric population is nonadherence leading to acute rejection and potential allograft loss (Dobbels 2010). Lung, liver, kidney, and heart transplant recipients 12–17 years of age have a significantly lower long-term allograft survival rate than do recipients younger than 12 years (Dharnidharka 2015).

### Overview of Immunosuppression for SOT

The goal of immunosuppression is to maintain low rates of acute rejection to improve long-term survival of the allograft and patient while minimizing the effects of over-immunosuppression, including serious infections, malignancies, and other problematic adverse effects from the immunosuppressive medication regimen. Especially problematic in the SOT population is the development of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections. Epstein-Barr virus disease can lead to posttransplant lymphoproliferative disorder (PTLD), necessitating reduced immunosuppression. Achieving this balance is challenging, especially in the pediatric recipient. The ultimate goal of transplant immunosuppression is to induce immunologic tolerance, where maintenance immunosuppression can be discontinued, though this is rare and depends on several unique factors. Liver transplant recipients have had the most success in investigational studies achieving tolerance, and even more so in pediatric recipients transplanted in infancy, partly because the density of memory T cells increases with age (Kamran Hejazi Kenari 2014; Li 2004). Traditionally, immunosuppression is divided into three main categories: induction, maintenance therapy, and rejection treatment. Each will be covered in detail in the following.

#### *Induction*

Induction is potent immunosuppression administered surrounding the transplant event. Typically, induction agents are administered intravenously, though there are exceptions for subcutaneous administration.

Induction therapy is used to decrease the incidence of acute rejection, decrease the rate of delayed allograft function, or induce tolerance or near tolerance, where the recipient develops decreased immune responsiveness to donor antigen. Induction agent selection depends on several factors, including the specific planned immunosuppressive maintenance regimen (e.g., steroid withdrawal, calcineurin inhibitor [CNI] minimization), presence of preformed anti-HLA or blood group antibodies at the time of transplantation, risk of posttransplant infection, anticipated delayed allograft function, and recipient disease states.

Advantages of using induction agents include decreasing the risk of early rejection and potentially permitting simpler maintenance regimens, which may decrease the risks of medication-specific adverse effects as well as patient-incurred costs from the maintenance regimen, and may decrease medication burden. Disadvantages of induction agents include costs incurred at the time of the transplant event and adverse effects associated with the specific agents.

#### *Maintenance*

The goal of maintenance immunosuppression is to prevent acute rejection while minimizing the toxicities associated with the immunosuppressive agents. Maintenance immunosuppression includes recurrently scheduled immunosuppressive agents typically administered orally or enterally. More recently, intravenous maintenance immunosuppression has gained interest, more so in the adult transplant population.

Although attempts have been made to devise one, no single test can accurately measure the overall state of immunosuppression for a transplant recipient. Evidence does not generally support the accuracy of the immune cell function assay, which measures cell-mediated immune responses, to comprehensively predict infection or rejection risk (Ling 2012). As a result, determining the minimal level of immunosuppression required for individual SOT recipients remains a challenge. Recurrent infections and evidence of viral replication, including EBV and CMV nucleic acid testing, may provide information on over-immunosuppression in an individual.

#### *Rejection Treatment*

Rejection treatment is meant to treat the individual acute rejection episodes. The strategy used to treat the rejection episode depends on the severity of rejection, classification of rejection (e.g., AMR, cellular rejection, or mixed rejection), recurrence, type of organ transplanted, and patient-specific-factors.

## INDUCTION IMMUNOSUPPRESSION

### Factors Influencing Induction Selection

Several factors affect the decision regarding induction agent selection, including immunologic risk, underlying disease states of the transplant recipient, the planned maintenance immunosuppressive regimen, and the organ(s) transplanted (which will be covered in the individual organ-specific chapters) (Gabardi 2011).

#### *Immunologic Risk*

Patients at higher immunologic risk often require more potent induction immunosuppression with a lymphocyte-depleting agent, ensuring a balance with infectious risk. This is especially true with highly immunogenic organ transplants such as kidney transplants, where preformed antibodies at the time of transplantation necessitate induction with a lymphocyte-depleting agent. Over 90% of pediatric kidney transplant recipients now receive induction with either a lymphocyte-depleting agent or a non–lymphocyte-depleting agent such as basiliximab (Hart 2019). Conversely, 56.6% of pediatric liver transplant recipients only receive corticosteroids as induction at the time of transplantation because the transplanted liver tends to be less immunogenic than other transplanted organs (Kim 2019).

Both the International Society for Heart and Lung Transplantation (ISHLT) guidelines for the care of heart transplant recipients (2010) and the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (2009) recommend antithymocyte globulin (ATG) induction for heart and kidney recipients, respectively, who are at high risk of acute rejection after transplantation (Costanzo 2010). A recent analysis of the

ISHLT database showed that use of ATG was associated with lower rates of acute rejection during the initial transplant hospital stay and lower rates of allograft loss at 1 year compared with basiliximab. Induction with ATG was also associated with higher infection rates before hospital discharge (Butts 2018). Of note, lung transplant guidelines currently only recommend ATG induction for patients at high risk of rejection (Faro 2007). Although the American Association for the Study of Liver Diseases makes no specific recommendations regarding immunosuppression, it notes that rejection rates are lower with tacrolimus-based immunosuppression and/or induction with a non–lymphocyte-depleting antibody such as basiliximab.

#### *Underlying Disease States*

Options are limited for induction modification to help prevent underlying disease recurrence after SOT. This is a subject of exploration, especially regarding recurrent glomerulonephritis after kidney transplantation, which is one of the most common causes of allograft failure in pediatric kidney transplant recipients transplanted for a glomerulonephritis. One retrospective analysis, including 116 renal transplant recipients, showed a significant risk reduction in the recurrence of primary immunoglobulin (Ig)A nephropathy with ATG induction compared with no induction or IL-2 receptor antagonist induction (Berthoux 2008).

#### *Delayed Initiation or Avoidance of CNIs*

Calcineurin inhibitors remain the backbone of immunosuppressive regimens for most pediatric SOT regimens within the United States. One of the primary concerns of full-dose CNIs is nephrotoxicity. When excluded from the maintenance regimen, CNIs are typically replaced by another maintenance agent, often an mTOR inhibitor. This maintenance replacement is often completed without respect to the induction regimen that was used. Of note, mTOR inhibitors have poor tolerability with high discontinuation rates because of adverse effects. A recent conversion study from a tacrolimus-mycophenolate-corticosteroid–based regimen to an everolimus-reduced-dose-tacrolimus regimen in pediatric kidney transplant recipients at 4–6 weeks posttransplantation had a discontinuation rate of 34.6% in the everolimus group compared with 13% in the tacrolimus-mycophenolate group, primarily because of mTOR inhibitor adverse effects (Tonshoff 2018).

Induction regimen modifications have been used to delay initiation of CNIs in nonrenal transplant recipients with renal impairment surrounding the transplant event. Data to support this practice in pediatric transplantation are limited. A retrospective review analyzed 12 pediatric liver transplant recipients who received basiliximab induction (12 mg/m$^2$ on the day of transplant and postoperative day 4) to delay CNI initiation by a mean of 3 days after transplantation compared with patients who received the standard protocol with only corticosteroids for induction and who did not have renal dysfunction. Patients with basiliximab induction and delayed initiation of tacrolimus had renal function and rates of acute rejection at 1 year posttransplantation similar to those who received the standard protocol (50% vs. 43%, p=0.62, respectively) (Mouzaki 2013). In addition, the ISHLT guidelines for the care of heart transplant recipients (2010) suggest the use of rATG induction in pediatric heart transplant recipients if CNI therapy is planned to be delayed or avoided because of renal toxicity concerns (Costanzo 2010).

#### *Steroid Avoidance or Withdrawal*

Because of the significant metabolic and endocrine adverse effects associated with corticosteroids, avoidance or withdrawal is desirable to modify these negative potential outcomes. Specific to pediatric SOT, steroid avoidance and withdrawal has been investigated to help optimize linear growth after transplantation. Growth failure has been associated not only with poor self-esteem but also with morbidity in patients with end-stage renal disease. To achieve successful corticosteroid avoidance or early corticosteroid withdrawal in pediatric SOT, studies have used either lymphocyte-depleting induction or IL-2 receptor antagonist induction. A recent meta-analysis reviewed pediatric SOT withdrawal and avoidance randomized control trials and observational studies. Fourteen studies involving 1146 kidney or liver transplant recipients were included, though no studies involving intestine, heart, lung, or pancreas transplant recipients met the criteria. The kidney transplant studies showed an improvement in height standard deviation scores, whereas the liver transplant studies (n=2) did not. Height improvement was most likely if the patients were prepubertal. Of importance, in the kidney transplant studies, corticosteroid avoidance and withdrawal was not associated with increased acute rejection, allograft failure, or decreased renal function (Tsampalieros 2017).

### Induction Choice

#### *Agents Available*

#### Corticosteroids

Corticosteroids have anti-inflammatory properties through reducing prostaglandin and cytokine synthesis, inhibiting histamine and bradykinin release, and decreasing capillary permeability. Corticosteroids, typically intravenous methylprednisolone, are administered as a large dose immediately pre- and posttransplantation (e.g., methylprednisolone 10 mg/kg on postoperative day 0 and 5 mg/kg on postoperative day 1), followed by an intravenous/oral/enteral corticosteroid taper. Secondary to several adverse effects, some transplant programs rapidly remove corticosteroids from patients' maintenance immunosuppressive regimens. Adverse effects include growth impairment, posttransplant diabetes mellitus, hypertension, growth suppression, bone metabolism disruption, delayed wound healing, peptic ulcer, acne, insomnia, emotional fluctuation, edema, and Cushing syndrome.

## Non-Lymphocyte-Depleting Agents

### *Basiliximab*

Basiliximab is a chimeric monoclonal antibody that binds to the IL-2 receptor (CD25) to prevent T-cell activation and proliferation, occupying the IL-2 receptor for around 5–8 weeks. Basiliximab clearance may be more rapid in certain situations, such as excess ascites fluid drainage post-liver transplantation (Kovarik 2002). Basiliximab is the only non–lymphocyte-depleting antibody approved for SOT induction in the United States. Basiliximab is not used to treat rejection. Basiliximab is administered as a 20- to 30-minute intravenous infusion administered within 2 hours of transplantation and repeated on postoperative day 4. The FDA-approved dosing for patients who weigh less than 35 kg is 10 mg per dose and, for patients who weigh 35 kg or more, 20 mg per dose. Because hypersensitivity reactions are extremely rare and adverse effects were not significantly greater than with placebo, premedication for basiliximab infusions is not necessary. A 2017 registry analysis of pediatric kidney transplant recipients in Australia and New Zealand (n=658) showed a significantly lower rate of acute rejection in the first 6 months after transplantation in patients who received induction with an IL-2 receptor antagonist compared with patients who did not receive antibody induction (11.7% vs. 22.8%, respectively; p<0.001). Most of these patients received initial maintenance immunosuppression with a CNI, mycophenolate, and prednisolone. When adjusted for a propensity score analysis, IL-2 receptor antagonist induction resulted in significantly lower early acute rejection rates (OR 0.64; 95% CI, 0.44–0.93) (Mincham 2017).

## Lymphocyte-Depleting Agents

### *Antithymocyte Globulin*

Rabbit antithymocyte globulin is a polyclonal antibody produced by immunizing rabbits against human lymphocytes. The antilymphocyte antibodies are extracted and pooled to produce rATG. Of importance, equine antithymocyte globulin (eATG) is also commercially available, though about 1/10th as potent as rATG. Equine ATG is not commonly used in pediatric SOT because induction with rATG in adult kidney transplantation had superior long-term 10-year composite outcomes of freedom from death, acute rejection, or graft loss compared with eATG (48% vs. 29%, respectively; p=0.011). This end point was primarily driven by freedom from acute rejection (p=0.004) (Hardinger 2008). Rabbit ATG binds T-cell surface antigens (CD3, CD4, CD8, CD28, CD2, CD5, CD45, CD154), B-cell surface antigens (CD20), and NK cell antigens (CD16, CD56), resulting in depletion of circulating T lymphocytes and modulation of T-lymphocyte activity. Although the half-life of rATG is only 2–3 days, the biologic effects on lymphocyte depletion can last more than 9–12 months.

Rabbit ATG is approved for both induction and acute cellular rejection in kidney transplantation, though it is often reserved for recurrent, steroid-resistant, or moderate to severe acute cellular rejection. Rabbit ATG is used off-label for nonrenal SOT. For induction, rATG is typically administered starting before the anastomosis of the transplanted organ, given that postoperative administration has been associated with increased rates of ischemia-reperfusion injury and delayed graft function in adult kidney transplant recipients. Optimal rATG cumulative target dosing for induction remains controversial, and monitoring of CD3 counts has been used to assess the need for additional doses on an individual patient-specific basis. According to package insert recommendations, dosing adjustments are warranted on the basis of WBC and Plt. It is recommended to decrease the dose by 50% when the WBC is 2–3 × $10^3$ cells/$mm^3$, hold the dose if the WBC is less than 2 × $10^3$ cells/$mm^3$, decrease the dose by 50% when the Plt is 50,000–70,000/$mm^3$, and hold the dose if the Plt is less than 50,000/$mm^3$.

Rabbit ATG may be administered by central or peripheral intravenous access, though central intravenous access is preferred because of the risk of phlebitis and thrombosis associated with peripheral administration. Because of these phlebitis concerns, heparin and hydrocortisone are added to the intravenous rATG preparation for pediatric patients at select transplant centers. Typically, rATG is administered over 6 hours for the first dose and may be administered over 4 hours, if tolerated, for additional doses. Premedication with corticosteroids (commonly methylprednisolone), diphenhydramine, and/or acetaminophen is often recommended to reduce the risk of severe infusion-related reactions, which present as a cytokine release syndrome (e.g., fever, chills, dyspnea, nausea/diarrhea, headache, pain, pulmonary edema). Rabbit ATG may increase the risks of chronic leukopenia and thrombocytopenia, infection, and malignancy.

### *Alemtuzumab*

Alemtuzumab (Campath) is a humanized monoclonal antibody that binds the CD52 found on activated T cells, B cells, granulocytes, NK cells, and macrophages, resulting in cell lysis. Although alemtuzumab's half-life is 11 hours to 6 days, its biologic effects on lymphocyte depletion exceed 1 year. Alemtuzumab is used off-label as an induction agent, primarily with single-arm reports in pediatric kidney transplantation and case reports in pediatric heart transplantation. In very rare cases, alemtuzumab has been reported to treat moderate to severe rejection. Alemtuzumab is administered by central or peripheral intravenous line over 2 hours or as a subcutaneous injection. Premedication with corticosteroids, acetaminophen, and/or diphenhydramine is recommended to reduce the risk of cytokine release syndrome. In addition, alemtuzumab can increase the risk of neutropenia, thrombocytopenia, and anemia as well as the risk of infections and malignancies. Adult dosing is 30 mg at the time of transplantation, but 0.5 mg/kg as a one-time dose with a maximum

dose of 30 mg has been suggested for pediatric patients. Of note, alemtuzumab was withdrawn from the U.S. market in 2012 so that it could gain approval for multiple sclerosis. In November 2014, alemtuzumab was FDA approved as a 12-mg dose for relapsing forms of multiple sclerosis. As of April 2019, alemtuzumab is available for B-cell chronic lymphocytic leukemia and several unlabeled indications, including induction for SOT, as part of a limited distribution program (Campath Distribution Program).

In one of the few comparison trials, outcomes with single-dose alemtuzumab induction in highly sensitized pediatric renal transplant recipients who underwent desensitization with IVIG and rituximab (n=15) were compared with those of pediatric kidney transplant patients who were nonsensitized receiving IL-2 receptor antagonist induction (n=35). Graft survival at 3 years posttransplantation was similar between the alemtuzumab-sensitized group and the nonsensitized group (83.9% vs. 91.3%, respectively; p=0.56). Although the 1-year acute cellular rejection rate was higher in the alemtuzumab-sensitized group (46.7% vs. 11.4%, p=0.001), the 1-year AMR rates were similar (13.3% vs. 11.4%, p=0.34). No differences in bacterial or viral infections were present. No patients in the alemtuzumab group developed PTLD, though one patient in the IL-2 receptor antagonist group did develop this disorder. Despite a much higher overall risk of poor outcomes, the alemtuzumab-sensitized group had similar outcomes, except for acute cellular rejection, than the nonsensitized group (Kim 2017).

## MAINTENANCE IMMUNOSUPPRESSION

At least 90% of pediatric kidney, heart, liver, lung, and intestine transplant recipients are discharged from their initial transplant event on maintenance tacrolimus. Over 90% of pediatric kidney, lung, and heart transplant recipients are discharged from their transplant event on a mycophenolic acid formulation, whereas this is less common in pediatric liver and intestinal transplant recipients. Maintenance corticosteroids have become less common for kidney, heart, and intestinal transplant recipients, with only 60%–70% of these patients being discharged from transplantation on a corticosteroid. Rates of maintenance corticosteroids are currently greater than 75% for pediatric lung and liver recipients.

### Maintenance Regimens

#### *Corticosteroids*

Corticosteroids are typically tapered over a set interval if the transplant recipient does not undergo a rapid steroid withdrawal or avoidance protocol. However, there is no standard steroid taper, and the taper depends on the organ transplanted, the induction agent used, the planned maintenance immunosuppressive regimen, and transplant center–specific guidelines.

#### *Calcineurin Inhibitors*

Two different CNIs are currently routinely used in SOT maintenance immunosuppression: tacrolimus and cyclosporine.

### Mechanism of Action

Both cyclosporine and tacrolimus prevent the transcription of IL-2, ultimately impeding T-cell activation and replication by inhibiting the action of calcineurin, preventing dephosphorylation and translocation of nuclear factor of activated T cells.

Cyclosporine accomplishes this by forming a complex with cyclophilin, calcium, calmodulin, and calcineurin to prevent the action of calcineurin. Tacrolimus accomplishes this by forming a complex with tacrolimus-binding protein (FKBP12), calcium and calmodulin to prevent the action of calcineurin.

### Dosing

#### *Cyclosporine*

The initial oral/enteral dosing range of cyclosporine is typically 8–18 mg/kg/day. Although cyclosporine is often dosed twice daily, thrice-daily dosing may be required in a pediatric transplant recipient because of accelerated drug clearance. Maintenance dosing may be determined on the basis of the cyclosporine trough concentration (C0) or the 2-hour post-dose concentration (C2). The conversion from oral to intravenous cyclosporine is 3:1, depending on the daily dose, because it can be administered as a continuous infusion or as divided intravenous doses.

#### *Tacrolimus*

The initial oral/enteral dosing range of tacrolimus is typically 0.05–0.2 mg/kg/day. Tacrolimus immediate-release capsules and the compounded oral suspension (either 0.5- or 1-mg/mL) are often dosed twice daily but may require thrice-daily dosing because of accelerated drug clearance in pediatric recipients. Of note, compounded suspension tends to have reduced absorption compared with immediate-release capsules (Reding 2002). Tacrolimus granules for oral suspension in 0.2- and 1-mg packets recently became commercially available. When converting from immediate-release capsules to granules, the total daily dose should remain the same. Pharmacokinetics of tacrolimus can be influenced by both age and *CYP3A5* genotyping. *CYP3A5*1/1* and *CYP3A5*1/3* expressers rapidly metabolize tacrolimus. In a pilot study of 53 pediatric transplant recipients, patients with initial tacrolimus dosing tailored to age and *CYP3A5* genotype attained therapeutic tacrolimus troughs about 1.5 days faster after transplantation (p=0.049) and had less nontherapeutic tacrolimus trough concentrations (p=0.002) than individuals who did not have tailored dosing (Min 2018). In addition, tacrolimus rate and extent of absorption decrease when administered with food (especially food with high fat content) with oral/enteral formulations. Consistency with tacrolimus

administration and food is imperative. Conversion from oral to intravenous tacrolimus is 3:1 to 4:1, depending on the daily dose, because it must be administered as a continuous infusion, typically through a dedicated line. In addition, intravenous tacrolimus has been implicated in episodes of anaphylaxis because of polyoxyethylated castor oil in the intravenous formulation. Intravenous tacrolimus is often reserved for when other routes of administration are not feasible. Tacrolimus immediate-release capsules may also be opened, placing the powder contents underneath the tongue for 10–15 minutes for sublingual administration. The liquid formulation of tacrolimus should not be used for sublingual administration. Of note, a sublingual dose may double tacrolimus exposure compared with oral/enteral administration; thus, close therapeutic drug monitoring is necessary, and a dose reduction may be warranted.

## Dosage Form Specifics

### *Modified vs. Nonmodified Cyclosporine*

Cyclosporine is available as nonmodified liquid-filled oral capsules and as an oral solution (Sandimmune), which is the original oil-based formulation that requires bile salts to emulsify the oil formulation for gut absorption. This creates high inter- and intrapatient drug exposure variability. To address this erratic drug exposure, a modified cyclosporine capsule and oral solution (Neoral, Gengraf) were manufactured as a microemulsion to reduce dependence on bile salts for absorption. Although both are currently commercially available, the modified and nonmodified formulations of cyclosporine are not interchangeable, and consistency is imperative because cyclosporine is a narrow therapeutic index drug. The pharmacokinetics of the two different formulations reinforce the inconsistencies between the two formulations, given that the half-life of the nonmodified formulation is 10–27 hours in adults and that of the modified formulation is 5–18 hours in adults. In pediatric kidney transplant recipients, the half-life of the nonmodified formulation was 7.3 hours (6.1–16.6 hours) (Burckart 1986).

### *Extended- vs. Immediate Release Tacrolimus*

Tacrolimus is available as an immediate-release capsule that has a half-life of about 8–16 hours in pediatric patients (Wallemacq 2001). More recently, extended-release formulations of tacrolimus have become available. Brand Astagraf XL is an extended-release, once-daily tacrolimus capsule that is FDA approved for pediatric and adult kidney transplant recipients. Astagraf XL's half-life is about 35–41 hours in adults. In a pharmacokinetic analysis of 33 pediatric liver, kidney, and heart transplant recipients younger than 16 years, the linear relationship of minimum concentration at 24 hours and AUC at 24 hours showed a strong positive correlation for both immediate- and extended-release tacrolimus, validating trough concentration monitoring for both formulations in the pediatric transplant population (Vondrak 2018). LCP-tacrolimus (Envarsus XR) is the newest available formulation of an extended-release, once-daily tacrolimus tablet formulation with enhanced bioavailability and lower maximum concentrations than immediate-release tacrolimus. According to the ASTCOFF and ASERTAA trials, the daily dose of LCP-tacrolimus is not daily dose equivalent to immediate-release tacrolimus. For non–African American adult kidney transplant recipients, patients require about a 30% daily dose reduction when converting from immediate-release tacrolimus to LCP-tacrolimus, whereas African American adult kidney transplant recipients require a 20% dose reduction (Trofe-Clark 2018; Tremblay 2017). The STRATO trial showed improved tacrolimus-induced hand tremors and patient-reported quality of life scores in patients converted from immediate-release tacrolimus to LCP-tacrolimus (Langone 2015). LCP-tacrolimus is not approved for pediatric use, and its safety and efficacy have not been established in the pediatric population.

## Adverse Effects

One of the most serious, yet common adverse effects with CNIs is acute nephrotoxicity, which tends to occur at similar rates with tacrolimus and cyclosporine and is dose related. At increased doses, CNIs cause vasoconstriction of afferent arterioles and reduced glomerular filtration rate (GFR), resulting in hyperkalemia, hypomagnesemia, hypertension, and renal dysfunction. In addition, there have been documented cases of acute kidney injury because of thrombotic microangiopathy/hemolytic uremic syndrome caused by CNIs, which is not dose related. In addition, CNIs can cause irreversible fibrotic changes to all components of the kidney, leading to chronic nephrotoxicity.

Calcineurin inhibitors can lead to neurotoxicity adverse effects, which are more prevalent with tacrolimus than with cyclosporine. Minor adverse effects including tremor, headache, and insomnia are most common, but rare neurotoxic adverse effects can include seizures, expressive aphasia, delirium, coma, and posterior reversible encephalopathy syndrome.

Calcineurin inhibitor–induced posttransplant diabetes mellitus tends to be more common with tacrolimus and is believed to be the result of calcineurin-inhibiting gene transcription, increasing insulin resistance, resulting in increased glucose intolerance. Cardiovascular complications associated with CNI therapy tend to be more common with cyclosporine, including hypertension and hyperlipidemia.

Calcineurin inhibitors can also cause nausea, diarrhea, and vomiting. Despite decreased GI transit time, infectious diarrhea often results in significant increases in blood concentrations of CNIs because of decreased activity of the intestinal CYP3A and the intestinal lumen efflux pump ability of P-glycoprotein in the setting of intestinal inflammation (Maezono 2005).

Especially problematic in the pediatric population, CNIs have cosmetic adverse effects. Tacrolimus can lead to alopecia, and cyclosporine can cause hirsutism and gingival hyperplasia.

### Drug Interactions

Calcineurin inhibitors are CYP3A4 substrates and moderate CYP3A4 inhibitors; thus, major CYP3A4 inhibitors increase CNI concentrations, and CYP3A4 inducers decrease CNI concentrations. Drugs that are common CYP3A4 inhibitors include diltiazem, verapamil, nicardipine, erythromycin, clarithromycin, protease inhibitors, and azole antifungals. Azole antifungals include fluconazole (often used to "boost" CNI concentrations), ketoconazole, itraconazole, posaconazole, and voriconazole. According to Lexi-Comp, when initiating voriconazole concomitantly with CNIs, the cyclosporine dose should be reduced by 50% on initiation and the tacrolimus dose reduced by 66% on initiation. Foods that inhibit CYP3A4 include grapefruit juice and products that contain grapefruit juice. Major CYP3A4 inducers include anticonvulsants, such as carbamazepine, phenobarbital, fosphenytoin, phenytoin, and rifamycins, such as rifabutin and rifampin, nafcillin, griseofulvin, and St. John's wort.

Administration of nephrotoxins with CNIs can result in additive nephrotoxicity. Nonsteroidal anti-inflammatory drugs are often avoided in patients taking CNIs, though low-dose aspirin may be used for antiplatelet activity to prevent thrombotic complications after pediatric SOT. Additional nephrotoxins such as aminoglycosides, colistin, and amphotericin B should also be avoided, if possible.

Cyclosporine has several documented interactions with various statins because of inhibition of CYP3A4 and organic anion-transporting polypeptide (OATP). Of importance, tacrolimus has not been demonstrated to inhibit OATP (Lemahieu 2005). Cyclosporine increases the AUC of lovastatin by 5- to 20-fold, simvastatin by 8-fold, and pitavastatin by 5-fold. Patients receiving CNIs, especially cyclosporine, should be monitored for signs of muscle toxicity if initiated on a statin, and conservative dosing should be used (Wiggins 2016).

### Monitoring

Because both cyclosporine and tacrolimus are narrow therapeutic index drugs, routine therapeutic drug monitoring is warranted. With cyclosporine, although trough monitoring of whole blood is poorly correlated with AUC, it tends to be most widely used because of ease of obtaining concentrations in the outpatient setting. The trough target is about 150–400 ng/mL, but the specific target is greatly depends on type of transplant, transplant center protocols, time from transplant, and other patient-specific factors. Concentration 2 hours after dose administration has shown the most positive correlation with AUC.

Tacrolimus trough concentrations correlate well with AUC. The trough target is about 5–15 ng/mL, but the specific target greatly depends on the type of transplant, transplant center protocols, time from transplantation, and other patient-specific factors.

## *Antiproliferative Agents*

### Mechanism of Action

Antiproliferative agents interfere with DNA synthesis, ultimately leading to decreased synthesis of T cells and B cells.

Azathioprine, a guanosine analog that antagonizes the metabolism of purines, is converted in the liver to 6-mercaptopurine, the active metabolite. 6-Mercaptopurine is converted to inactive metabolites by xanthine oxidase and thiopurine methyltransferase (TPMT). Because patients with intermediate, low, or absent TPMT activity are at an increased risk of bone marrow suppression, TPMT deficiency testing is recommended before azathioprine initiation to determine whether preemptive dose reduction is warranted.

Mycophenolate derivatives are noncompetitive inhibitors of inosine monophosphate dehydrogenase. The active metabolite, mycophenolic acid, inhibits the de novo synthesis pathway of guanosine nucleotides without DNA incorporation. Of note, both T cells and B cells depend on the de novo synthesis pathway for proliferation, and neither has salvage pathways, unlike other cell types.

Mycophenolate mofetil is the inactive 2,4-morpholinoethyl prodrug ester of mycophenolic acid that is cleaved to mycophenolic acid before absorption in the small intestine. Mycophenolate sodium is the sodium salt formulation of mycophenolic acid. Mycophenolate mofetil is available as a commercially available suspension, whereas mycophenolate sodium is not.

### Dosing

Table 2 contains dosing comparisons.

### Adverse Effects

See Table 2 for an adverse effect comparison. Of importance, mycophenolic acid has been associated with first-trimester pregnancy loss and congenital malformations, including, but not limited to, cleft lip/palate, external ear malformation, and anomalies of the kidneys. Because of this risk, the FDA has mandated a Risk Evaluation and Mitigation Strategies (REMS) program. The REMS components include a medication guide to be provided to the patient, health care provider education, and a voluntary centralized pregnancy registry for female exposures to mycophenolic acid during pregnancy. For females with reproductive potential who are sexually active while taking mycophenolic acid, the following combination strategies for birth control are recommended: hormonal contraception plus one barrier method or two barrier methods. Other single acceptable strategies include placement of an intrauterine device, tubal sterilization, or vasectomy of the female patient's monogamous partner. For females of childbearing potential, pregnancy testing is recommended immediately before

**Table 2.** Comparison of Antiproliferative Agents

| | MMF (CellCept) | MPS (Myfortic) | Azathioprine (Imuran) |
|---|---|---|---|
| Pediatric dosing | In patients taking cyclosporine: 1200 mg/m$^2$/day IV/PO divided twice daily (max dose of 1000 mg twice daily)<br>In patients taking tacrolimus or a CNI-free regimen: 900 mg/m$^2$/day IV/PO divided twice daily (maximum dose of 1000 mg twice daily)<br>OR<br>20–50 mg/kg/day divided twice daily<br>Of note, some transplant programs divide every 8 hr or every 6 hr | BSA < 1.19 m$^2$: MPS not recommended<br>BSA 1.19–1.58 m$^2$: 540 mg PO twice daily<br>BSA > 1.58 m$^2$: 720 mg PO twice daily<br>Of note, some transplant programs divide every 8 hr or every 6 hr | 1–2 mg/kg/day for SOT indications |
| Conversion to MPA[a] | 0.739:1 | 1:1 | N/A |
| Generic availability | Yes | Yes | Yes |
| Administration | IV – Administer over at least 2 hr<br>Oral – Do not crush; avoid inhalation | Oral – Do not crush, chew, or cut tablets | PO, IV |
| Adverse effects | Diarrhea, anemia, leukopenia, and vomiting | Diarrhea, anemia, leukopenia, and vomiting | Most common: Leukopenia, anemia, and thrombocytopenia<br>Less common: Alopecia, pancreatitis, and hepatotoxicity |
| Drug interactions | Antibiotics: Decrease MPA exposure through interference of normal gut flora<br>Cholestyramine, probenecid, antacids: Decrease MPA exposure by up to 40%<br>Cyclosporine: Decreases MPA exposure by up to 40% | Antibiotics: Decrease MPA exposure through interference of normal gut flora<br>Cholestyramine, probenecid, antacids: Decrease MPA exposure by up to 40%<br>Cyclosporine: Decreases MPA exposure by up to 40% | Allopurinol inhibits xanthine oxidase; thus, the azathioprine dose is suggested to be decreased by 75% if no other alternatives<br>Febuxostat should be avoided |
| Monitoring | Usefulness of drug concentrations (trough or abbreviated AUC) is not well defined in the pediatric transplant literature because MPA is not technically defined as a narrow therapeutic index drug | Usefulness of drug concentrations (trough or abbreviated AUC) is not well defined in the pediatric transplant literature because MPA is not technically defined as a narrow therapeutic index drug | Consider initial testing for TPMT deficiency |

[a](1 g of MMF) × 0.739 = 739 mg of MPA vs. 720 mg of mycophenolate sodium = 720 mg of MPA.

BSA = body surface area; CNI = calcineurin inhibitor; IV = intravenous(ly); MMF = mycophenolate mofetil; MPA = mycophenolic acid; MPS = mycophenolate sodium; N/A = not applicable; PO = oral(ly); SOT = solid organ transplantation.

therapy, 8–10 days after therapy initiation, and at routine clinic follow-up visits as necessary or indicated.

### Drug Interactions

See Table 2 for interaction comparisons.

### Monitoring

See Table 2 for monitoring comparisons.

#### *mTOR Inhibitors*

Two oral mTOR inhibitors, sirolimus and everolimus, are used for maintenance immunosuppressive therapy in SOT, though neither agent is commonly used de novo. Because of the nephrotoxicity associated with CNIs, the inherent antitumor properties of mTOR inhibitors, the potential benefit in slowing chronic liver transplant rejection progression with mTOR

inhibitors, and the prevention of chronic rejection in cardiac transplant with mTOR inhibitors, mTOR inhibitor use has been explored (Arora 2015). The mTOR inhibitors are added or they replace CNIs or antiproliferative agents at time intervals after the initial transplant event.

### Mechanism of Action

Both sirolimus and everolimus bind to FKBP12, forming a complex that prevents stimulation of mTOR by IL-2. The inhibition results in blockade of the cell cycle progressing from G1 to S phase and prevention of T-cell proliferation and differentiation. Of note, everolimus has a higher bioavailability and shorter half-life than sirolimus.

### Dosing

Because of its long half-life and large volume of distribution, it is recommended to start sirolimus with a loading dose of 3 mg/m$^2$ on day 1 (with a maximum initial loading dose of 6 mg), followed by a maintenance dose of 1 mg/m$^2$ once daily thereafter (with a maximum initial maintenance dose of around 2 mg, considering drug interactions and other factors). Because of the proven shorter half-life of sirolimus in younger patients than in adults, some pediatric patients may require twice-daily dosing (Schubert 2004). Follow-up dosing is based on trough concentrations because sirolimus is a narrow therapeutic index drug. The commercially available liquid formulation of sirolimus has specific instructions for administration, which includes mixing the sirolimus dose in a cup with at least 60 mL of water or orange juice and drinking it immediately. The cup should then be refilled with another 120 mL of water or orange juice, mixed, and drunk immediately. Especially in younger pediatric transplant patients, this administration volume may be a limitation.

Data for pediatric transplant dosing of everolimus are limited, though 0.8 mg/m$^2$ twice daily in combination with cyclosporine and 2 mg/m$^2$ twice daily in combination with tacrolimus has been suggested (Tonshoff 2018; Ganschow 2017; Hoyer 2003).

### Adverse Effects

The adverse effects of everolimus and sirolimus are similar, including leukopenia, anemia, thrombocytopenia, hyperlipidemia, significant proteinuria, oral ulcers, and delayed wound healing. Because of significant wound healing issues, mTOR inhibitors are typically avoided in the immediate posttransplant phase, and appropriate immunosuppressive conversion should occur before major elective surgery. In addition, when initiated in de novo recipients, sirolimus has been associated with hepatic artery thrombosis in liver transplant recipients. Because of increased serious infections and mortality in the first 3 months of transplantation, use in heart transplant is advised against in a boxed warning.

### Drug Interactions

Both everolimus and sirolimus are CYP3A4 substrates and thus have the same CYP3A4 inhibitor and inducer interactions as CNIs.

### Monitoring

Because of the narrow therapeutic index drug categorization for sirolimus and everolimus, therapeutic drug monitoring should routinely be performed. Trough concentrations (Cmin) strongly correlate with AUC exposure for both agents. In combination with minimized CNIs and corticosteroids, the suggested Cmin for sirolimus is around 5–12 ng/mL and, in regimens without CNIs, around 12–24 ng/mL in the first year posttransplantation. Depending on organ type and duration from transplantation, in combination with minimized CNIs and corticosteroids, the suggested Cmin for everolimus is around 3–8 ng/mL and, in regimens without CNIs, around 6–10 ng/mL in the first year posttransplantation.

### Role in Pediatric SOT

In addition to the aforementioned potential benefits of mTOR inhibitors, mTOR inhibitors have been associated with lower rates of CMV disease in the SOT population. When comparing 59 pediatric kidney transplant recipients who received everolimus and low-dose cyclosporine with 242 recipients who received standard-dose CNIs, patients who received everolimus had an 82% lower risk of CMV replication. In addition, in patients at highest risk of CMV (n=88), there was a significantly lower risk of CMV disease in those who received everolimus and low-dose cyclosporine than in those who received standard-dose cyclosporine (0% vs. 14.3%, p=0.046) (Hocker 2016).

Despite the potential benefits of mTOR inhibitors, tolerability remains poor, with drug discontinuation rates as the result of adverse effects often exceeding 30%–40% (Tonshoff 2018; Ganschow 2017).

## *Selective T-Cell Costimulation Blocker*

### Belatacept

#### *Mechanism of Action*

Belatacept, the newest addition to the maintenance immunosuppressive armamentarium, is a human fusion protein combining extracellular portion of CTLA-4 with constant-region fragment (Fc) of human IgG. Belatacept binds to the antigen-presenting cell through CD80 and CD86 interaction, inhibiting signal 2 and down-regulating T-cell clonal expansion and differentiation.

#### *Dosing*

Belatacept is approved for maintenance therapy in kidney transplant recipients 18 years and older in combination with mycophenolate mofetil and corticosteroids with basiliximab induction because belatacept is intended to replace CNIs in the maintenance immunosuppressive regimen.

Recommended dosing for de novo adult kidney transplant recipients is 10 mg/kg intravenously before implantation; 10 mg/kg on postoperative day 4; 10 mg/kg at the end of weeks 2, 4, 8, and 12 after transplantation; then 5 mg/kg every 4 weeks as a maintenance. Conversion from a CNI-based regimen to a belatacept-based regimen in adult kidney transplant recipients has been described by several groups showing improvement in renal function post-conversion (Grinyo 2017; Schulte 2017). Because of the increased risk of PTLD, belatacept is contraindicated in patients who are EBV seronegative or if EBV serostatus is unknown.

#### *Efficacy/Adverse Effect Profile*

The Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial (BENEFIT) showed that a belatacept-based maintenance immunosuppressive regimen in adult renal transplant recipients provides effective immunosuppression with improved long-term preservation of renal function, possible improved cardiovascular/metabolic risk profile, and less toxicity than a cyclosporine-based regimen. Concerns with belatacept arose when patients receiving belatacept had a higher incidence of PTLD and acute rejection. Although a REMS was originally a component of belatacept prescribing, as of May 2017, it is no longer required for belatacept. Other adverse effects include increased risk of infections, including tuberculosis, necessitating screening for tuberculosis before initiation; and infusion-related reactions.

#### *Monitoring Values*

While receiving belatacept, patients should be monitored for signs of adverse effects. In addition, the belatacept dose should be adjusted to the nearest 12.5 mg if a patient's body weight changes by more than 10% from the actual body weight at the time of transplantation; thus, monthly weights should be obtained.

#### *Role in Pediatric SOT*

Because of the high rates of PTLD in the BENEFIT study in EBV seronegative patients, belatacept is not currently approved for pediatric patients because of concern for development of PTLD (Vincenti 2010). Ongoing studies are cautiously reviewing its use in the pediatric population and determining optimal pediatric dosing/pharmacokinetics, including a recently completed phase I trial. The CD86 saturation with a 7.5-mg/kg dose of belatacept in adolescent kidney transplant recipients was determined to be similar to the CD86 saturation with a 5-mg/kg dose of belatacept in adult kidney transplant recipients (Moudgil 2018). This phase I trial provides necessary information to permit further investigation regarding the use of belatacept in the adolescent population, a traditionally highly nonadherent population that may benefit from monthly infusion monitoring.

## ACUTE CELLULAR REJECTION

### Pathophysiology

Acute cellular rejection involves rapid-onset allorecognition, migration of effector cells, and destruction of the transplanted allograft tissue, typically within the first 90 days of transplantation. This process involves $CD4^+$ and $CD8^+$ T cells, antigen-presenting cells, NK cells, adhesion molecules, and cytokines.

### Diagnosis

In general, acute rejection may present with fever, pain, edema, inflammation surrounding the transplant organ site, or evidence of decreased organ function. Rejection diagnosis is typically confirmed by biopsy of the transplanted organ or by preemptive treatment with evidence of improvement in transplant organ function. Performing protocol biopsies at set time intervals posttransplantation is a common practice for several organ transplant types to help diagnose subclinical rejection episodes, though biopsies carry the risk of bleeding and other major complications, including renal biopsies, endomyocardial biopsies, and transjugular liver biopsies.

Gaining significant interest in SOT is the use of specific plasma or urine biomarkers to detect allograft injury to predict acute rejection and assess the need for a transplant biopsy. Cell-free DNA is released in response to cell injury, including necrosis or apoptosis. Proof-of-concept studies exist for all SOT types in the adult population, analyzing the presence of donor-derived cell-free DNA (dd-cfDNA) from recipient-derived cell-free DNA to predict allograft injury. A recent systematic review of 47 studies (18 kidney, 7 liver, 11 heart, 1 kidney-pancreas, 5 lung, and 5 multiorgan) confirmed the validity of using dd-cfDNA to determine allograft injury for all organ types. Baseline values of dd-cfDNA are typically achieved within 2 weeks after transplantation, depending on organ type, and detection of elevated dd-cfDNA occurs before clinical signs. The predictive ability of dd-cfDNA is best for severe acute cellular rejection and AMR. The dd-cfDNA tends to have a higher negative than positive predictive value, but one of the main limitations currently is establishing individual thresholds for positive results for different organ groups and for the pediatric SOT population. In addition, the standard frequency of testing for routine monitoring has not yet been determined (Knight 2019).

### Treatment

Treatment of acute cellular rejection greatly depends on the transplanted organ, clinical manifestations, and severity of rejection. For example, recommendations to treat acute cellular rejection for pediatric liver transplantation include use of bolus corticosteroids, increased CNI exposure, or addition of an mTOR inhibitor or mycophenolic acid for patients not already maintained on either of these classes of immunosuppressants (Kelly 2013). Recommendations for treating acute

cellular rejection in pediatric kidney transplantation include initially treating with corticosteroids and using ATG for steroid-resistant rejection or recurrent acute cellular rejection (KDIGO 2009). Treatment of isolated acute cellular rejection typically involves the use of pulse corticosteroids, use of ATG, or optimization of the maintenance immunosuppressive regimen, depending on transplant organ type.

## ANTIBODY-MEDIATED REJECTION

### Pathophysiology

Antibody-mediated rejection is primarily mediated by B cells, which further maturate into memory B cells and plasma cells, which produce DSAs. Donor-specific antibodies then bind to either HLA or non-HLA on the donor transplant endothelial cells, activating and recruiting complement, NK cells, neutrophils, and macrophages, resulting in tissue damage. This endothelial cell damage may also cause platelet aggregation and thrombotic microangiopathy.

### Diagnosis

Diagnostic criteria for active AMR vary between transplant organ types. Four common diagnostic criteria exist between organ groups: evidence of endothelial cell injury, evidence of antibody involvement (including HLA and non-HLA antibodies [e.g., angiotensin II type 1 receptor antibody and endothelin-1 type A receptor antibody]), evidence of complement activation, and evidence of innate immune cells infiltrating the transplanted organ (Banasik 2014).

### Treatment

In the pediatric kidney transplant population, nonadherence to maintenance immunosuppression has been associated with the development of de novo DSAs, reinforcing the importance of adherence (Pizzo 2016). Ultimately, prevention of AMR is the best management because AMR treatment presents challenges, and therapies have limited data analyses supporting efficacy, even in the adult SOT population. Similar to acute cellular rejection, specific treatment regimens for active AMR depend on organ type. Common treatment approaches include one or more of the following therapies.

#### *Plasmapheresis*

Although antibody removal, most commonly using plasmapheresis, is considered part of standard of care for treating active AMR, data analyses to support this practice are limited. The most evidence supporting antibody removal for AMR treatment is in the kidney transplant population. In a systematic review and meta-analysis of studies investigating AMR treatment modalities in adult and pediatric kidney transplant recipients, 14,380 citations were evaluated, but only 21 studies met the inclusion criteria of controlled studies including patients with histologic evidence of AMR. Of the five randomized controlled trials evaluating antibody removal, there was no significant advantage in allograft survival with antibody removal (HR 0.76; 95% CI, 0.35–1.63). When analyzing the three randomized controlled trials with a longer follow-up of 2–5 years, antibody removal significantly improved allograft survival (HR 0.46; 95% CI, 0.26–0.82). Of the four studies specifically evaluating plasmapheresis or plasmapheresis with IVIG, three showed short-term improvement in kidney allograft function (Wan 2018).

#### *Intravenous Immunoglobulin*

Intravenous immunoglobulin is also considered standard of care for active AMR for many organ types, though like plasmapheresis, the evidence of benefit is limited by small, heterogeneous studies. In addition, plasmapheresis and IVIG are often components of desensitization regimens.

Intravenous immunoglobulin is derived from pooled donated plasma from human donors. Composition of IVIG includes 97%–98% IgG monomers with variable amounts of IgA, depending on the specific formulation. Specific pathogen IgG titer variance exists between specific formulations and even in between product-specific batches. Important properties to consider when selecting individual products for SOT recipients include IgA content, osmolality, and stabilizer. Patients who are IgA deficient are at increased risk of anaphylactic reactions, potentially because of preformed anti-IgA antibodies, depending on the IgA content of the IVIG preparation, though thresholds have not been established. Products that are hyperosmolar may increase the risk of thrombotic events. Sucrose-containing products have been associated with nephrotoxicity.

Other systemic adverse effects of IVIG include headache, fevers, rigors, fatigue, vomiting, and dizziness.

#### *Rituximab*

Rituximab is a chimeric monoclonal antibody that targets the CD20 antigen and results in B-cell lysis. CD20 is positioned on pre-B and mature B cells, but not on active plasma cells. In SOT specifically, rituximab is used as part of desensitization regimens, acting as an upstream approach to decrease preformed antibodies that result in a positive crossmatch, and to treat AMR. For the treatment of pediatric active AMR, rituximab is typically given as a 375-mg/m$^2$ intravenous infusion, with the total number of weekly doses varying. Rituximab should be premedicated with at least diphenhydramine and acetaminophen. Adverse effects include infusion reactions such as flu-like symptoms, fever, chills, headache, myalgias, pancytopenia, and increased risk of infection. Although underpowered, the RITUX ERAH trial showed that, in 38 adult kidney transplant patients with AMR, there was no difference in allograft failure and lack of renal function improvement at 1-year follow-up in patients who received rituximab or placebo at day 5 of AMR treatment in combination with plasmapheresis, IVIG, and corticosteroids. This analysis was limited by its short follow-up, sample size, and ability of study

## Patient Care Scenario

**A 16-year-old female adolescent with a medical history significant for C3 glomerulopathy presented several days ago for a renal biopsy as a workup for rejection, given a recent increase in SCr and self-admitted maintenance immunosuppressive medication nonadherence. Today, the final biopsy report suggests active AMR (positive C4d staining and many lymphocytes in the peritubular capillaries). The biopsy revealed no evidence of acute cellular rejection. In addition, the Luminex assay results reveal strong HLA Class II DSA (DQ8). The patient has not been treated for rejection in the past. Her maintenance immunosuppressive medications include prednisone 5 mg by mouth daily, mycophenolate sodium 360 mg by mouth twice daily, and tacrolimus 8 mg by mouth twice daily. Which regiment would be best to treat this active AMR rejection episode?**

**A. Basiliximab**
**B. Plasmapheresis plus IVIG**
**C. Intravenous methylprednisolone**
**D. Carfilzomib**

### ANSWER

**Only a few small, randomized controlled trials have evaluated treatment strategies for active AMR in kidney transplantation. The 2009 KDIGO guidelines recommend one or a combination of the following to treat acute AMR with or without corticosteroids: (1) plasmapheresis, (2) IVIG, (3) rituximab, and (4) lymphocyte-depleting agent (e.g., ATG). Plasmapheresis with or without IVIG to treat AMR has been associated with improved renal function and improved long-term survival of the kidney allograft, making Answer B correct. Basiliximab is used only as an induction agent and should not be used to treat active AMR. Carfilzomib has been used to treat multidrug-resistant acute AMR in lung transplant recipients, but has not been investigated in the kidney transplant population because of the nephrotoxicity associated with this agent. Intravenous methylprednisolone may be used in a regimen to treat acute AMR but should not be used as a single agent.**


1. Wan S, Ying T, Wyburn K, et al. The treatment of antibody-mediated rejection in kidney transplantation: An updated systematic review and meta-analysis. Transplantation 2018;102:557-68.
2. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant 2009;9(suppl 3):S1-S155.
3. Ensor C, Yousem S, Marrari M, et al. Proteasome inhibitor carfilzomib-based therapy for antibody-mediated rejection of the pulmonary allograft: use and short-term findings. Am J Transplant 2017;17:1380-8.


investigators to administer supplemental doses of rituximab in both groups (Sautenet 2016).

### *Proteasome Inhibitors*

The most commonly used proteasome inhibitor used to treat active AMR is bortezomib, though use of carfilzomib has been reported in adult lung transplant recipients (Ensor 2017). Carfilzomib has been associated with significant nephrotoxicity.

Bortezomib is a dipeptidyl boronic acid 26S proteasome inhibitor that disrupts cell homeostasis and results in plasma cell apoptosis. In addition to its anti-plasma cell properties, bortezomib may suppress T-cell function and inhibit IL-6 activity, leading to cell death at various stages in B-cell maturation. Bortezomib has gained interest to treat active AMR because it specifically targets the mature plasma cell, which is responsible for antibody production. Of note, bortezomib is typically reserved for AMR treatment when other strategies have failed to successfully decrease DSA titers and is often used in combination with other AMR treatment strategies. Although pediatric data analyses are extremely limited, bortezomib 1.3 mg/m$^2$/dose intravenously or subcutaneously every 72 hours × 4 doses has been used in pediatric kidney and heart transplant recipients (Roberti 2015; Morrow 2012). Potential adverse drug reactions with bortezomib include peripheral neuropathy, thrombocytopenia, and pulmonary toxicity.

### *Eculizumab*

Eculizumab is a humanized monoclonal antibody that binds complement protein C5, preventing the cleavage into C5a and C5b by C5 convertase. This activity prevents the formation of the terminal complex C5b-9 (membrane attack complex), an effector protein responsible for cell lysis. Eculizumab is FDA approved for atypical hemolytic uremic syndrome (aHUS), paroxysmal nocturnal hemoglobinuria, and myasthenia gravis. Eculizumab has been reported as a component of transplant regimens for pretransplant desensitization and as a component to treat active AMR.

Pediatric dosing of eculizumab for aHUS is outlined in Table 3. Of note, the dosing for treatment and the treatment duration for active AMR and desensitization are not well established. In adults, eculizumab may be administered as an intravenous infusion over 35 minutes, but eculizumab should be administered over 1–4 hours in pediatric patients. After plasmapheresis, fresh frozen plasma infusion, or plasma exchange, supplemental doses of eculizumab to maintain terminal complement blockade are required to maintain complement blockade. Removal of eculizumab by hemodialysis or peritoneal dialysis is unlikely because of the molecular size

**Table 3.** Eculizumab Dosing for aHUS

| Weight (kg) | Induction Dose | Maintenance Dose |
|---|---|---|
| 5 to < 10 | 300 mg weekly × 1 | 300 mg at week 2; then 300 mg every 3 wk |
| 10 to < 20 | 600 mg weekly × 1 | 300 mg at week 2; then 300 mg every 2 wk |
| 20 to < 30 | 600 mg weekly × 2 | 600 mg at week 3; then 600 mg every 2 wk |
| 30 to < 40 | 600 mg weekly × 2 | 900 mg at week 3; then 900 mg every 2 wk |
| ≥ 40 | 900 mg weekly × 4 | 1200 mg at week 5; then 1200 mg every 2 wk |

aHUS = atypical hemolytic uremic syndrome.

of eculizumab; thus, supplemental doses are not required. If possible, patients should receive the quadrivalent meningococcal conjugate vaccine at least 2 weeks before treatment with eculizumab because patients are at a 1000- to 2000-fold increased risk of meningococcal disease while receiving eculizumab. Patients 10 years and older should also receive the serogroup B meningococcal vaccine (CDC 2015). Prophylaxis with an antibacterial agent that provides coverage against encapsulated bacteria (e.g., *Neisseria meningitidis*) should be used for patients who are receiving vaccine at the time of eculizumab initiation for a duration of 2 weeks up to a lifetime. Of note, meningococcal disease has been documented in patients receiving eculizumab despite receiving a meningococcal vaccine; thus, the CDC states that antimicrobial prophylaxis can be considered for the duration of eculizumab therapy in addition to appropriate meningococcal vaccination (McNamara 2017).

Reported adverse effects of eculizumab include GI effects, increased infectious risk (primarily because of encapsulated bacteria), hypertension, headache, and infusion reactions. In addition, eculizumab may rarely cause hepatotoxicity (Hayes 2015). Because of the risk of meningococcal infections, eculizumab requires providers to enroll in a REMS program and is available only through a restricted distribution program. Patients should be educated regarding meningococcal infection risk, provided with a medication guide before each infusion, and be appropriately vaccinated with the meningococcal vaccine.

Although there are no published trials with eculizumab use for AMR prevention or treatment, another C5 inhibitor, ravulizimab-cwvz, received FDA approval in late December 2018 for the treatment of paroxysmal nocturnal hemoglobinuria in adults, with less frequent dosing than eculizumab (FDA 2018).

## CHRONIC REJECTION/CHRONIC ALLOGRAFT INJURY

### Pathophysiology

With all organ types, chronic rejection is progressive immune and inflammatory injury to the allograft that may ultimately result in allograft dysfunction and allograft loss. Chronic rejection injury is facilitated by both immune mediators (e.g., acute rejection episodes, presence of DSAs) and non-immune mediators (CMV disease, ischemia-reperfusion injury).

### Risk Factors

Acute rejection, multiple acute rejection events, and late acute rejection significantly increase the risk of chronic rejection. In pediatric liver transplantation, chronic rejection is now rare in the era of tacrolimus-based immunosuppression. However, certain risk factors such as deceased donor transplant recipients, African American recipients, patients with autoimmune-related liver disease, patients with PTLD, patients with CMV disease, and patients with multiple acute rejection episodes have an increased risk of chronic rejection (Gupta 2001).

### Prevention

For most organ types, chronic rejection is often irreversible; thus, the focus has been on prevention. Prevention of chronic rejection centers on prevention of modifiable risk factors, including avoidance of acute rejection through optimization of immunosuppression and adherence to maintenance regimens. Non-immunosuppressive therapies have been investigated to prevent chronic rejection. Although statins have decreased the rates of chronic rejection (i.e., cardiac allograft vasculopathy) in adult heart transplant recipients, these findings were not substantiated in a large registry retrospective review of children and adolescent heart transplant recipients (Greenway 2016). In the adult lung transplant population, survival without chronic rejection (i.e., chronic lung allograft dysfunction) was significantly higher in a randomized controlled trial (n=83) in patients receiving prophylactic azithromycin compared with placebo (p=0.024) (Ruttens 2016). However, because of the small volume of pediatric lung transplants on an annual basis, these findings would be difficult to investigate in the pediatric population.

## CONCLUSION

Substantial growth and development have occurred in pediatric transplant and pediatric transplant immunosuppression since the first procedures were performed. Because of the nature of transplantation, limited large-sample randomized controlled trials exist in SOT; hence, many of the current SOT practices are based on the adult population in case-controlled trials without randomization, cohort studies, case-control–analytic studies, several time-series, uncontrolled experiments, or expert opinion. Much

## Practice Points

**Immunosuppressive treatment of the SOT recipient involves a fine balance of preventing cellular- and antibody-mediated rejection and avoiding complications associated with therapies, including infections and malignancy.**

- **Despite improvements regarding short-term outcomes in pediatric SOT, long-term survival continues to be an area for improvement.**
- **The risk of rejection after transplantation is influenced by several factors, including organ type, immunologic factors, donor-related factors, and recipient-related factors.**
- **Selection of either a lymphocyte-depleting or a non–lymphocyte-depleting antibody for SOT induction depends on immunologic risk, underlying disease states of the transplant recipient, planned maintenance immunosuppressive regimen, and the organ(s) transplanted as a balance to prevent either over- or under-immunosuppression.**
- **Although mTOR inhibitors – and potentially belatacept, depending on future investigation in adolescent recipients – offer alternatives to calcineurin inhibitors for maintenance immunosuppression, toxicities and acute rejection rates have limited their more widespread use.**
- **Treatment strategies for acute rejection have been well established, but continued development regarding less invasive testing techniques, including testing dd-cfDNA, may offer a means to treat allograft injury earlier, potentially reducing long-term implications of the injury.**
- **AMR treatment continues to have limited options, though newer agents are being investigated on the basis of mechanism of action.**
- **Prevention of chronic rejection surrounds preventing acute rejection episodes, though azithromycin has shown benefit in preventing chronic lung allograft dysfunction in lung transplant recipients, and mTOR inhibitors have shown benefit in preventing vasculopathy.**

of the current practice for pediatric SOT immunosuppressive management is based on extrapolation from the adult population. Further research in the pediatric population is required to optimize immunosuppression in this unique and heterogeneous group.

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# Self-Assessment Questions

**Questions 1 and 2 pertain to the following case.**

K.C., a 5-year-old African American girl, has a medical history significant for a deceased donor renal transplant about 6 months ago for end-stage renal disease because of a small left solitary kidney at birth. She presented 2 days ago to the inpatient acute care unit for a renal biopsy as a workup for recurrent rejection, given a recent increase in SCr. K.C. also has a history of vesicoureteral reflux and hypertension related to transplantation. At the time of transplantation, she received induction with rabbit antithymocyte globulin (rATG), had a calculated PRA (cPRA) of 0%, had a 0 of 6 HLA-antigen mismatch, and was ABO blood group compatible, and the transplanted kidney had a cold ischemic time of 9 hours. She was treated 1 month ago with methylprednisolone 10 mg/kg/day intravenously for 3 days for mild acute cellular rejection. Today, the final biopsy report suggests acute cellular rejection without acute antibody-mediated rejection (AMR) (negative C4d staining). The Luminex assay revealed no evidence of donor-specific antibodies (DSAs). K.C.'s maintenance immunosuppressive medications include prednisolone 3 mg by mouth every Monday, Wednesday, Friday, and Saturday; mycophenolate mofetil 300 mg by mouth twice daily; and tacrolimus 2 mg by mouth twice daily.

1. Which one of the following was K.C.'s greatest risk factor for development of rejection?

   A. cPRA at transplantation
   B. Cold ischemic time
   C. Ethnicity
   D. Induction agent

2. Which one of the following is best to recommend for K.C.'s acute cellular rejection episode?

   A. Rituximab
   B. Intravenous rATG
   C. Plasmapheresis plus intravenous immunoglobulin (IVIG)
   D. Eculizumab

3. A 17-year-old female adolescent received a bilateral lung transplant for end-stage lung disease secondary to cystic fibrosis 8 months ago. She was readmitted to the hospital medical ICU 5 days ago for acute-on-chronic respiratory failure requiring endotracheal intubation and mechanical ventilation. The patient also has a history of gastroesophageal reflux disease, pancreatic insufficiency, and chronic kidney disease as the result of a significant history of aminoglycoside therapy. Her postoperative course was complicated by *Aspergillus flavus* and *Pseudomonas aeruginosa* pneumonia. She was noted to have alveolar infiltrates of concern for rejection and underwent a transbronchial lung biopsy yesterday, which revealed acute AMR (acute lung injury with neutrophil infiltration of the alveolar septae with capillaritis and C4d deposition in the alveolar capillaries). Furthermore, the Luminex assay revealed strong de novo DSAs to HLA-B44. Her current immunosuppressive regimen includes tacrolimus 3 mg by mouth twice daily, mycophenolate sodium 720 mg by mouth twice daily, and prednisone 10 mg by mouth once daily. On discussion, it is decided to treat the acute AMR episode with five plasmapheresis sessions, followed by immune globulin 100 mg/kg after the first four sessions and 2 g/kg after the final plasmapheresis session. When considering which immune globulin preparation to select for therapy, which one of the following stabilizing agents used in immune globulin preparations would be most likely to cause a problem for this patient?

   A. Maltose
   B. Proline
   C. Glucose
   D. Sucrose

4. A 14-year-old male adolescent with end-stage renal disease secondary to atypical hemolytic uremic syndrome (aHUS) caused by complement factor H mutation was admitted today for a deceased donor kidney transplant. Because the patient had been maintained on hemodialysis for 6 months, the pediatric nephrologist decides to initiate eculizumab at the time of transplant surgery. Which one of the following would be most important before initiating chronic eculizumab therapy in this patient?

   A. The facility administering eculizumab must enroll in the eculizumab REMS program.
   B. The pediatric nephrologist should be informed that supplemental doses of eculizumab are not required after plasmapheresis or plasma exchange if required after transplantation.
   C. The pediatric nephrologist should be informed that supplemental doses of eculizumab are recommended after hemodialysis if required after transplantation.
   D. The patient should initially receive prophylaxis with amoxicillin because meningococcal vaccines should ideally be administered at least 2 weeks before treatment with eculizumab.

5. A 15-year-old male adolescent received a deceased donor kidney transplant 1 month ago for end-stage renal disease caused by Alport syndrome. Today, he presents to the pediatric kidney transplant clinic. The patient received rATG induction, and his maintenance

immunosuppressive regimen includes tacrolimus 3 mg by mouth twice daily, mycophenolate mofetil, and prednisone. His 12-hour tacrolimus trough concentration this morning was 15.7 ng/mL, which was obtained appropriately. The patient's tacrolimus trough concentrations were 9.4 ng/mL and 9.1 ng/mL 13 and 4 days ago, respectively. The patient has had no changes in his tacrolimus dose for 3 weeks, and his target trough concentration goal is 8–10 ng/mL. Which one of the following most likely contributed to this patient's supratherapeutic tacrolimus trough concentration?

A. Presence of emesis 60 minutes after morning medication administration (including tacrolimus) 7 days ago and not taking medications again until the evening dosing time
B. Initiation of rosuvastatin 5 mg by mouth daily 2 weeks ago for hyperlipidemia
C. Initiation of nystatin swish and swallow four times daily for new-onset thrush 4 days ago
D. Initiation of a new diet limiting dietary fat and now taking medications on an empty stomach beginning 6 days ago

6. A 14-year-old male adolescent (height 61 inches, weight 65 kg) with end-stage renal disease caused by prune belly syndrome who is postoperative day 2 from a deceased donor kidney transplant is seen after a kidney transplant. He received two doses of rATG 100 mg intravenously for transplant induction and will receive a third dose of rATG today. If the patient's WBC is $2.5 \times 10^3$ cells/mm$^3$ and Plt is 25,000/mm$^3$, which one of the following adjustments to the rATG dose is best to recommend?

A. Make no change; administer rATG 100 mg intravenously.
B. Administer rATG 50 mg intravenously.
C. Hold the rATG dose.
D. Administer rATG 25 mg intravenously.

7. Three months ago, an 18-year-old man received a bilateral lung transplant for end-stage lung disease caused by pulmonary arterial hypertension. Today he is being seen in the lung transplant clinic. The patient's current immunosuppressive regimen includes tacrolimus 1.5 mg by mouth twice daily, mycophenolate sodium (Myfortic) 720 mg by mouth twice daily, and prednisone 15 mg by mouth once daily. He has been taking a stable dose of tacrolimus for the past month, and his tacrolimus trough concentration this morning is at goal. Which one of the following pairs of adverse effects is most important to monitor for in this patient?

A. GI toxicity and pancytopenia
B. Delayed wound healing and hyperlipidemia
C. Pancytopenia and nephrotoxicity
D. Hyperglycemia and neurotoxicity

8. A 13-year-old female adolescent with end-stage liver disease caused by acute liver failure of unknown etiology is to receive a living donor liver transplant today. Which one of the following is best to recommend immediately after transplantation to preserve this patient's liver function?

A. Immediate-release tacrolimus and prednisone
B. Sirolimus, immediate-release tacrolimus, and prednisone
C. Belatacept, mycophenolate mofetil, and prednisone
D. LCP-tacrolimus and prednisone

9. For which one of the following patients would sirolimus most likely be beneficial?

A. A 17-year old lung transplant recipient for cystic fibrosis who received a deceased donor lung transplant 1 day ago to decrease the risk of acute rejection
B. A 15-year-old kidney transplant recipient who is 1 month after a deceased donor kidney transplant with rATG for IgA nephropathy who would like to convert from tacrolimus to lower the risk of acute rejection
C. A 2-year-old liver transplant recipient for biliary atresia who received a deceased donor liver transplant 3 days ago who would like to decrease the risk of acute rejection
D. A 16-year-old kidney transplant recipient who is 2 months after a kidney transplant with rATG who received an organ from a cytomegalovirus (CMV) IgG positive donor and, at the time of transplantation, was CMV IgG negative, who cannot afford valganciclovir and would like to reduce the risk of CMV

10. The following patients are scheduled for living donor solid organ transplantation (SOT) with rATG induction. Which one of the following individuals is most likely to have height improvement benefits from rapid steroid withdrawal over a 3-day period?

A. A 17-year old female liver transplant candidate who is receiving a transplant for Wilson disease
B. A 16-year-old female kidney transplant candidate who receiving a transplant for IgA nephropathy
C. A 5-year old male kidney transplant candidate who is being transplanted for kidney dysplasia
D. A 13-year-old male liver transplant candidate who is being transplanted for fulminant hepatic failure of unknown etiology

**Questions 11 and 12 pertain to the following case.**

A.D., an 8-year-old Hispanic boy, is admitted to the hospital for a deceased donor heart transplant for severe dilated cardiomyopathy. On admission, it is noted that A.D. has an acute kidney injury, and his estimated GFR is 20 mL/minute/1.73 m$^2$.

A.D. has not had any recent infections and has no history of malignancy, and his Epstein-Barr virus IgG is positive.

11. Three months after transplantation with a maintenance regimen of tacrolimus (goal trough concentration of 10–12 ng/mL), mycophenolate mofetil, and prednisolone, A.D.'s estimated GFR remains 30 mL/minute/1.73 $m^2$. The decision is made to convert the patient to everolimus (goal trough concentration of 3–8 ng/mL), low-dose tacrolimus (goal trough concentration of 3–5 ng/mL), and prednisolone. Which one of the following is of most concern regarding initiation of everolimus therapy in A.D.?

    A. Increased risk of developing cardiac allograft vasculopathy
    B. Increased risk of developing proteinuria
    C. Increased risk of developing posttransplant diabetes mellitus
    D. Increased risk of developing posttransplant hypertension

12. Two months after conversion to an everolimus-, tacrolimus-, and prednisolone-based maintenance regimen, A.D. is admitted to the hospital with hypotension, general malaise, and an oral temperature on admission of 103.1°F. Rapid PCR-based blood culture testing identifies that A.D. has candidemia with *Candida albicans*. Without adjusting his maintenance immunosuppressive regimen, which one of the following is best to recommend for A.D.?

    A. Amphotericin B liposome
    B. Voriconazole
    C. Fluconazole
    D. Micafungin

13. Assuming no other confounding variables, which one of the following kidney transplant recipients has the highest risk of rejection after transplantation?

    A. A 6-year-old African American male living donor kidney transplant recipient with focal segmental glomerulosclerosis who received rATG induction
    B. A 15-year-old female deceased donor kidney transplant recipient with polycystic kidney disease with strong preformed class II anti-HLA-DR DSAs and a positive crossmatch who received rATG induction
    C. A 16-year-old male deceased donor kidney transplant recipient with polycystic kidney disease with donor cold ischemic time of 20 hours with ex vivo machine perfusion who received rATG induction
    D. A 14-year-old male living donor kidney transplant recipient with IgA nephropathy with a cPRA of 0% and a negative virtual crossmatch to his biological brother who is donating with rATG induction

14. A 15-year-old female adolescent kidney transplant recipient was transplanted 6 months ago for systemic lupus erythematous. Today, in the clinic, she is noted to have an increased SCr from a baseline of 0.5 mg/dL to 1.5 mg/dL. The patient is taking a maintenance immunosuppressive regimen of tacrolimus, mycophenolate sodium, and prednisone. She states she rarely misses taking her medications. It is decided that she needs a transplant kidney biopsy to rule out rejection, but the patient and her mother refuse. Which one of the following blood tests would provide the most accurate information regarding whether this patient has active rejection without proceeding with a transplant kidney biopsy?

    A. Immune cell function assay
    B. Tacrolimus trough concentration from the clinic this morning
    C. Donor-derived cell-free DNA (dd-cfDNA)
    D. Cystatin C

15. Assuming no other confounding variables, which one of the following SOT recipients has the highest risk of immediate rejection after transplantation?

    A. A 11-month-old boy, deceased donor kidney transplant recipient because of reflux nephropathy with a cPRA of 0%, negative virtual crossmatch and with rabbit antithymocyte globulin induction.
    B. A 6-year-old girl, deceased donor intestinal transplant recipient because of short bowel syndrome with multiple parenteral nutrition–related catheter infections with strong anti-HLA DSAs (HLA-A and HLA-DR) toward the donor with basiliximab induction.
    C. A 2-year-old girl, living donor kidney transplant recipient because of reflux nephropathy with a negative virtual crossmatch toward her mother, who is donating with basiliximab induction.
    D. A 14-year-old male adolescent, deceased donor lung transplant recipient because of cystic fibrosis with a cPRA of 0% with basiliximab induction.