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INVITED REVIEW / DAVETLİ DERLEME

# Review of Immunosuppressive Treatment in Lung Transplantation

## Akciğer Transplantasyonunda Immunosupresif Tedavinin Gözden Geçirilmesi

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Abstract

Lung transplantation is a well-established therapeutic option for selected patients with end-stage lung disease that has failed to respond to standard medical and surgical therapy. Immunosuppression is paramount to the management of these patients, the goal of which is to maintain long-term graft survival and keep the balance between infection risk and rejection of the new organ. Immunosuppression for lung transplantation can be considered under three clinical contexts: induction therapy, maintenance therapy, and anti-rejection therapy. This review provides a comprehensive update of the current status of immune-suppressive treatments in lung transplantation. Careful selection of immunosuppressive agents, inpatient management of acute complications and rejection episodes, management of maintenance immunosuppression, and meticulous long-term follow-up with monitoring for adverse drug events and drug-drug interactions are all essential measures to ensure the best patient outcomes in lung transplantation.

**KEY WORDS:** Lung, transplant, immunosuppression, review

Özet

Akciğer transplantasyonu, standart medikal ve cerrahi tedaviye başarısız yanıt veren son dönem akciğer hastalığı olan seçilmiş hastalar için iyi bir tedavi seçeneğidir. İmmunosupresyon bu hastaların tedavisinde, uzun dönem greft sağkalımını sağlama ve enfeksiyon riski ve yeni organın rejeksiyonu arasındaki dengeyi koruma hedefi için çok önemlidir. Akciğer transplantasyonu için immunosupresyon üç klinik bağlamda düşünülebilir; indüksiyon tedavisi, idame tedavisi ve anti-rejeksiyon tedavisi. Bu derleme akciğer transplantasyonunda immunosupresif tedavinin mevcut durumu hakkında kapsamlı bir güncelleme sağlamaktadır. Akciğer transplantasyonunda en iyi hasta sonuçlarını sağlamak için şunların tümü esas önlemlerdir; immunosupresif ajanların dikkatli seçimi, akut komplikasyonlar ve rejeksiyon ataklarının yatırılarak tedavisi, idame immunosupresyonun yönetimi, advers ilaç olayları ve ilaç-ilaç etkileşimleri yönünden izleme ile birlikte uzun dönem titiz takip.

**ANAHTAR SÖZCÜKLER:** Akciğer, transplantasyon, immunosupresyon, derleme

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### INTRODUCTION

Lung transplantation offers a realistic treatment option for improved survival and quality of life for selected patients with end-stage lung disease of multiple etiologies. Advances in surgical techniques and the development of effective immunosuppression in the last few decades have led to substantially improved outcomes and an exponential growth in the number of lung transplant procedures performed worldwide [1]. This increase in clinical activity has led to significant progress in our understanding of factors that may limit short-and long-term lung transplantation survival. A focus on initiatives to standardize lung transplant referral and allocation criteria to ensure fair and appropriate use of donor organs and to develop strategies that may increase donor lung availability has led to improved 1-year and overall survival rates, as reflected in the recently published International Society for Heart and Lung Transplantation (ISHLT) paper [2]. Optimal immunosuppression is paramount to the management of transplant recipients to ensure long-term graft survival by maintaining a balance between infection and rejection. This review provides a comprehensive update of the current status of lung transplantation, with a particular focus on the importance and relevance of immunosuppressive therapy.

### Historical Background

The first human lung transplant was performed in 1963 by Dr. James Hardy at the University of Mississippi-a single lung transplant in a patient with severe emphysema and left lung carcinoma [3]. The procedure was successful, but the patient died of renal failure on post-operative day 18. Further attempts to improve surgical techniques and survival times highlighted the importance of the role of immunosuppressive therapy in graft survival [4-6]. The introduction of cyclosporine in 1978 and further refinement of technique and organ preservation led to the first successful human heart-lung transplant in 1981 in a female adult with idiopathic pulmonary arterial hypertension, followed closely by the first successful isolated human lung transplant in 1983 in a patient with pulmonary fibrosis [7,8]. En bloc double-lung transplantation was first performed successfully in 1988 [9]. However, this technique was prone to complications, particularly in relation to

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**Table 1. Summary of induction immunosuppressive medications and suggested doses**

| Medication | Dose | Mechanism of action | Therapeutic drug monitoring |
|---|---|---|---|
| Anti-thymocyte globulin<br>Thymoglobulin (rabbit-derived)<br>ATGAM (horse-derived) | One vial/10 kg for 3-7 days<br>No longer used | Prevents T cell proliferation | Monitor full blood, specifically WCC for leukopenia and platelets for thrombocytopenia, and renal function. Dose changes prompted by changes in CD3 levels |
| OKT3 | 5-10 mg/day over 7-14 days | Prevents T cell activation | No routine monitoring |
| Daclizumab | 1 mg/kg/day and fortnightly for a total of five doses | Inhibits T cell proliferation and differentiation | No routine monitoring |
| Basiliximab | 20 mg day 1 and 4 post-transplant | Inhibits T cell proliferation and differentiation | No routine monitoring |
| Alemtuzumab | 30 mg one-off dose infused over 2 hours | Causes leukocyte depletion | Monitor full blood, specifically WCC for pancytopenia.<br>Infection prophylaxis important |

tracheal dehiscence. As a result, sequential bilateral lung transplantation, where the airway anastomosis is at the level of the main-stem bronchi rather than at the level of the trachea, remains the most common surgical procedure used for bilateral lung transplantation today [10]. More recent techniques include split-lung, size-reduction measures and, cadaveric bilateral lobar transplants, which have helped to improve the options of achieving lung transplantation in smaller recipients [11-13].

According to the 2013 ISHLT registry, approximately 46,069 lung transplant procedures were performed worldwide from January 1994 through to June 2012 [2]. The 2011 data show a record 3640 transplants performed, representing the highest reported number of any year to date. There has been a persistent increase in the number of bilateral lung transplants performed since the mid-1990s, with a relatively static rate of annual single-lung transplants [2]. The ISHLT registry reports a median survival of lung transplant recipients of 5.6 years, with an adjusted median survival of 7.9 years, in recipients who survived to 1 year post-transplant. Survival rates at 3 months, 1 year, 3 years, 5 years, and 10 years in the 2002-2012 era were 88%, 79%, 64%, 53%, and 31%, respectively, compared with previous 3-month and 1-year survivals of 81% and 70% in the 1996-2002 era, demonstrating a survival increase of 7% and 9%, respectively [2].

The most common indications for lung transplantation, in decreasing order, are: chronic obstructive pulmonary disease (COPD) with and without alpha-1-antitrypsin deficiency (A1ATD) (39.3%), interstitial lung disease (23.7%), cystic fibrosis (CF) (16.6%), pulmonary fibrosis (3.7%), idiopathic pulmonary arterial hypertension (3.1%), and non-CF bronchiectasis (2.7%). Referral criteria are often institution-, program-, and disease-specific but must adhere to the ISHLT guidelines outlining absolute and relative contraindications, outlined in Table 1. Due to the significant risks involved, the timing of transplantation is very important, in that the lung transplant candidate must be considered to be at or fast approaching a stage where this high-risk procedure is a necessity yet strong enough, physically and emotionally, to survive the complex surgery and adhere to the demanding post-transplant immunosuppressive medical regimen.

**Immunosuppression Therapy**

Optimal immunosuppression is perhaps the most important part of ensuring allograft lung function and graft survival after transplantation by targeting multiple immune pathways to decrease both acute and chronic rejection [14]. There are 3 stages of immunosuppression in lung transplantation; induction, maintenance, and treatment of rejection.

**Induction Immunosuppression**

Induction immunosuppressive agents are used to deplete the recipient immune system in the immediate post-transplant period, decreasing early interaction between the recipient immune cells and donor allograft antigens to prevent acute rejection [1]. Induction therapy consists of a brief regimen of T cell-depleting therapy with different protocols used by individual transplant centers, including the use of agents, such as polyclonal anti-T cell preparations (anti-thymocyte globulin) or monoclonal antibodies aimed at lymphocyte surface molecules, including CD3 (OKT3), IL-2R/CD25 (basiliximab, daclizumab) or CD52 (alemtuzumab). In brief, OKT3 prevents T cell activation, daclizumab and basiliximab inhibit T cell proliferation and differentiation, and alemtuzumab causes leukocyte depletion. A summary of induction immunosuppressant medications and doses is presented in Table 1.

**a) Anti-thymocyte globulin**

Anti-thymocyte globulin (ATG) is a polyclonal antibody product derived from the serum of horses or rabbits inoculated with human lymphocytes, after which the animal immunoglobulin (Ig) G antibodies are removed from the serum via plasmapheresis and purified. These antibodies are potent killers of human T lymphocytes and can thus induce prolonged lymphopenia. The antibodies are non-specific, in that they bind multiple sites on the T cell, causing apoptosis. Thymoglobulin is the rabbit-derived product and is administered more frequently than ATGAM, the horse-derived product, due to its higher potency and longer half-life (30 days versus 5.7 days) [14]. Comparator trials have demonstrated a 40% relative risk of death, graft loss, and rejection at 5 years post-transplant with thymoglobulin versus ATGAM and a higher event-free survival and improved quality-of-life years, without increased post-transplant lymphoproliferative disease

(PTLD) or infection rates compared to ATGAM at 10 years [15]. The first dose of thymoglobulin is usually given to the patient post-operatively; generally, a daily dose of one vial per 10 kg body weight is given in the immediate post-transplant period for 3-7 days. High-dose corticosteroids, such as methylprednisolone 125 mg eight hourly over 24 hours post-transplant, are often given in conjunction to minimize potential infusion-related reactions. Anti-thymoglobulin antibody serum sickness is a rare side effect manifested by non-specific symptoms of jaw pain, myalgia, fever, and flu-like illness, which can be seen up to several weeks after the thymoglobulin is given [16]. Additional doses may be given to target a peripheral blood CD3 count of less than 0.05 cells/μL. Monitoring of platelets and renal function is also recommended.

**Muromonab-CD3**

Muromonab-CD3 (OKT3) is a murine monoclonal antibody directed against the epsilon chain of the T cell receptor-CD3 complex, resulting in prevention of T cell activation and depletion of circulating T cells with with relative sparing of T regulatory cells [17]. A cytokine release syndrome can occur with fevers, chills, headaches, and myalgias that, in its most severe form, can lead to circulatory collapse. As for other induction agents, OKT-3 may also be associated with a higher rate of infection. Other less frequent adverse effects include seizures, aseptic meningitis, and renal insufficiency [18].

**b) Daclizumab and basiliximab**

Daclizumab and basiliximab are chimeric humanized murine monoclonal antibodies targeting the α-subunit, or tac subunit, of the IL-2 receptor (CD25) [19,20]. By binding this cell surface receptor, these antibodies inhibit T cell proliferation and differentiation. Although the two antibodies have the same mechanism of action, they have markedly different half-lives and duration of IL-2 receptor saturation, owing to different proportions of the human and murine components. Basiliximab is 25% murine, with a half-life of approximately 13 days and a 30-day average saturation of the IL-2 receptor [19]. Daclizumab is only 10% murine and therefore has a longer half-life of 20 to 40 days and an effective IL-2 saturation of 120 days [20]. Basiliximab is currently approved for dosing at 20 mg on the first and fourth days after transplant, while daclizumab is dosed at 1 mg/kg within the first day after transplant and then every 2 weeks for a total of five doses.

**Alemtuzumab**

Alemtuzumab is a humanized rat monoclonal antibody directed against CD52, an antigen found on T lymphocytes, B lymphocytes, monocytes, macrophages, natural killer (NK) cells, and eosinophils. By binding to CD52, alemtuzumab causes a depletion of leukocytes by multiple pathways, including complement-mediated cytolysis, antibody-mediated cellular toxicity, and apoptosis induction [21].

The half-life of the medication is approximately 12 days; however, different inflammatory cells have differential rates of recovery after alemtuzumab therapy, with monocyte recovery at 3 months, B cells at 12 months, and 50% T cell recovery at 36 months [14]. These long-lasting effects on immune cells can cause delayed neutropenia. In addition, alemtuzumab has been shown to result in inhomogeneous depletion of T cells, with relative sparing of T regulatory cells and memory cells. Due to the prolonged immunosuppressive effects of alemtuzumab, it is recommended that patients induced with this agent receive prolonged prophylaxis against opportunistic viral (e.g. cytomegalovirus) and fungal infections for 6 months [21]. Table 2 outlines recommended infection prophylaxis post-lung transplant.

Alemtuzumab is a recognized alternative to traditional anti-thymocyte-depleting antibodies for induction immunosuppression and has been associated with lower rates of early allograft rejection. Alemtuzumab or alternative cytolytic therapy can be used early post-transplant for patients with calcineurin inhibitor-related side effects (e.g., severe renal dysfunction) to reduce exposure to calcineurin inhibitors and thereby preserve renal function [18].

**Maintenance Immunosuppression**

Maintenance immunosuppressive therapy is based on a triple regimen composed of a calcineurin inhibitor (cyclosporine or tacrolimus), an anti-metabolite cell proliferation inhibitor (azathioprine or mycophenolate mofetil [MMF]), and corticosteroids.

Mammalian target of rapamycin (mTOR) inhibitors—for example, sirolimus and everolimus—are an alternative to replace the calcineurin inhibitor or anti-metabolite during the course of follow-up. In brief, cyclosporine and tacrolimus inhibit calcineurin, thereby decreasing IL-2 production and reducing T cell activation/proliferation; azathioprine and MMF are anti-metabolites, which deplete lymphocytes; sirolimus and everolimus are mTOR inhibitors, which arrest T cell growth; and corticosteroids suppress prostaglandin synthesis, reduce histamine/bradykinin release, decrease vascular permeability, and down-regulate cytokines. A summary of maintenance immunosuppressant medications and doses is presented in Table 3.

**a) Calcineurin inhibitors**

Calcineurin inhibitors, such as tacrolimus and cyclosporine, work to prevent IL-2-mediated CD4+ T cell activation [14]. Cyclosporine binds to cyclophilin, which prevents calcineurin de-phosphorylation of nuclear factor of activated T cells (NFAT), preventing translocation to the nucleus, which is the site of transcriptional activity in the production of inflammatory proteins. This prevents activation and proliferation of CD4+ T cells through the IL-2 pathway [22]. In addition, cyclosporine has also been shown to inhibit FOXP3 expression and potentially diminish regulatory T cell suppressor function in animal models as well as in renal transplant patients [23]. Cyclosporine is initiated early post-transplant initially at a dose of 1 mg/kg body weight, with transition to oral medication (oral dose approximately 3 times intravenous dose achieving therapeutic levels) once gastro-intestinal absorption is established, and serum drug levels are monitored throughout, with dose adjustments based upon the target trough concentration, which should be maintained between 250 and 350 ng/mL in the early post-transplant period, with later trough levels determined by frequency of rejection episodes, renal function, or infectious complications.

**Table 2. Suggested prophylactic treatment post-transplant**

| Potential pathogen | Medication | Dose | Treatment time frame | Additional information and monitoring |
|---|---|---|---|---|
| *Cytomegalovirus (CMV)* | Valganciclovir | 900 mg oral once daily | 3 months prophylaxis for donor-positive and recipient-negative patients plus 3 months CMV PCR surveillance. For donor-and recipient-positive patients, 3 months CMV PCR testing is commenced at 1 month post-transplant. | Must be adjusted in renal dysfunction |
| Pneumocystis *jiroveci (carinii) (PCP)* | Trimethoprim-sulfamethoxazole | One double-strength tablet 800 mg/160 mg BD oral three times weekly Alternatively cotrimoxazole 480 mg/d | Lifelong | May use dapsone, pentamidine, atovaquone or azithromycin 250 mg thrice weekly if allergic. Components of this prophylactic regimen are also effective at preventing *Nocardia* infection and toxoplasmosis. Potential side effects include rash, renal insufficiency, hyperkalemia, and, bone marrow suppressions |
| Candida and *Aspergillus spp* | Itraconazole<br>Inhaled amphotericin B | <br>10 mg nebulized twice daily | First 3 months after transplant<br>First 10 days after transplant | May use posaconazole or voriconazole depending on transplant center Need to monitor for drug-drug interaction with use of calcinuerin inhibitors |
| Oral thrush *(Candida spp)* | Clotrimazole troche Nystatin solution | 10 mg oral three times daily | First 6-12 months after transplantation; may be used lifelong if necessary | |

**Table 3. Summary of maintenance immunosuppressive medications and suggested doses**

| Medication | Dose | Therapeutic drug monitoring |
|---|---|---|
| Cyclosporine | 100-250 mg twice daily | Serum trough 250-350 ng/mL |
| Tacrolimus | 2-5 mg twice daily | Serum trough 5-15 ng/mL |
| Sirolimus | 5 mg daily | Serum trough 10-20 mcg/mL |
| Everolimus | 750 mcg twice daily | Serum trough 3-12 mcg/mL |
| Mycophenolate mofetil | 1000-1500 mg twice daily | No routine monitoring |
| Azathioprine | 1-3 mg/kg/day | Monitor full blood count and liver enzymes |
| Prednisolone | 5-15 mg daily | No routine monitoring |

Tacrolimus binds to immunophilin, an FK-binding protein that also inhibits calcineurin, thereby preventing activation and translocation of NFAT, with the ultimate effect being decreased IL-2 production and resultant decreased IL-2-mediated proliferation of T cells [24]. The common initial dosing for tacrolimus (half-life 12-22 hours) is 2-5 mg twice daily, but doses are individualized based upon the trough concentration, which should be maintained at 10 to 15 ng/mL early post-transplant, depending on the time from transplant and concomitant immunosuppressant medications. Tacrolimus is generally administered orally, but sublingual or intravenous tacrolimus can be considered, if intestinal absorption is not an option.

Both cyclosporine and tacrolimus are metabolized via the hepatic cytochrome P450 system in the liver; therefore, any alteration of this system, either by medications or hepatic dysfunction, will result in variable trough levels. Specifically, any medication that decreases cytochrome P450 activity can potentially lead to increased drug levels and increased toxicity, while co-administration of medications that increase P450 activity can lead to decreased drug blood levels and potentially ineffective immunosuppression. Both medications are associated with nephrotoxicity that may range from mild renal dysfunction to end-stage renal disease requiring hemodialysis [25]. This nephrotoxicity is often dose-dependent but may also be idiosyncratic and may be reversible if

the offending medication is stopped early. The side effect profiles of cyclosporine and tacrolimus are very similar, consisting of hypertension, dyslipidemia, electrolyte disturbances, hirsutism, gingival hyperplasia, and, rarely, hemolytic uremic syndrome. Tacrolimus also increases the risk of post-transplant diabetes mellitus.

In most centers, the use of tacrolimus has overtaken the use of cyclosporine, as studies have shown tacrolimus to decrease antibody production to a greater extent with better tolerability. However, the ultimate decision is often based on center experience, the patient's concomitant diseases, and associated risk factors.

**b) Anti-metabolites**

Mycophenolate mofetil (MMF) inhibits de novo purine synthesis, therefore blocking proliferation of T and B lymphocytes. Other potential mechanisms of immunosuppression include inducing apoptosis of activated T cells, decreasing expression of adhesion molecules, resulting in decreased recruitment of inflammatory cells, and decreasing inducible nitric oxide production and the resultant tissue damage. MMF has been shown to have no effect on T regulatory cell survival or suppressor function [26].

Mycophenolate mofetil may be administered either orally or intravenously. There are two mycophenolate products: Cellcept, which is an acid salt and an immediate release product, and Myfortic, which is enteric-coated to reduce the dose-limiting gastrointestinal adverse effects of the medication. Clinical trials in heart and renal transplantation have found the enteric formulation to be comparable in terms of safety and efficacy to the original formulation of MMF [14]. Co-administration of antacids, cholestyramine, and iron should be avoided, as they can decrease bioavailability. In addition, cyclosporine has been shown to decrease active drug levels by interfering with entero-hepatic recirculation. Diarrhea and gastrointestinal upset are the most notable side effects, although leukopenia and bone marrow suppression have also been observed [26].

Azathioprine is an older antimetabolite, which is converted into 6-mercaptopurine via hepatic enzymes. Its mechanism of action is to halt DNA replication and induce apoptosis in CD28 cells, causing T cell destruction. Azathioprine is both an oral and intravenous medication requiring once-daily dosing, with a consistent oral bioavailability of approximately 40% [27]. Drug levels are not routinely monitored, although accumulation of the metabolite 6-thioguanine may occur in renal disease, leading to accumulation of toxic compounds. The main toxicity associated with azathioprine is dose-dependent myelosuppression, potentially resulting in thrombocytopenia, leukopenia, and macrocytic anemia. In addition, hepatotoxicity and malignancy have been reported. Prior to initiation of azathioprine, it is recommended to test for thiopurine methyltransferase (TPMT) activity. TPMT metabolizes and subsequently inactivates azathioprine and its metabolites. Approximately 11% of the population has low TPMT levels, and 1 in 300 people has very low to inactive TPMT, resulting in increased toxicity of azathioprine with conventional treatment doses [27].

Like most immunosuppressive agents, azathioprine has multiple drug interactions. Most notable among these is allopurinol, an inhibitor of xanthine oxidase. Treatment of gout or hyperuricemia with allopurinol leads to decreased metabolism of 6-mercaptopurine and severely elevated circulating levels of azathioprine, resulting in potentially profound pancytopenia. If co-administration can not be avoided, the azathioprine dose should be reduced with close hematological monitoring.

**c) mTOR inhibitors**

Mammalian target of rapamycin (mTOR) inhibitors, such as sirolimus and everolimus, are being increasingly used in lieu of tacrolimus. They bind to FK-binding proteins downstream of IL-2 and cause inhibition of the target of rapamycin, therefore blunting IL-2-mediated T cell proliferation [28]. They are not routinely used immediately post-operatively due to their anti-proliferative effects on fibroblasts and effects on wound healing [29]. Instead, patients can be transitioned from tacrolimus to sirolimus or everolimus, once healing of the bronchial anastomosis is ensured.

Sirolimus has once-daily administration, is less nephrotoxic than calcineurin therapy, and has reduced metabolic side effects, such as hypertension or hyperlipidemia. Everolimus is a derivative of sirolimus with increased bioavailability. Both medications require therapeutic drug monitoring, with a target trough level of 10 to 20 mcg/mL for sirolimus and 3 to 12 mcg/mL for everolimus. Often, lower therapeutic levels are targeted when these drugs are prescribed in conjunction with low-dose tacrolimus or cyclosporine [14]. Of note is the pharmacokinetic interaction between sirolimus and cyclosporine. When given in combination, sirolimus can potentiate calcineurin inhibitors, thereby inducing nephrotoxicity by increasing levels of cyclosporine and potentiating mechanisms of nephropathy. Other side effects of sirolimus include dyslipidemia, hypertension, myelosuppression, skin fragility syndrome, and thrombotic microangiopathy. Multiple different pulmonary pathologies have also been associated with sirolimus, ranging from interstitial pneumonitis to organizing pneumonia, lymphocytic alveolitis, alveolar hemorrhage, and pulmonary vasculitis, when used in the lung transplant population [30].

Everolimus is a rapamycin derivative that is synthesized to have increased bioavailability compared with sirolimus. It can be administered either once or twice daily and has a shorter half-life, with more rapid onset steady state than its parent compound. This medication shares a mechanism of action with sirolimus as well as drug interactions and toxicities, aside from the combined cyclosporine-medicated renal toxicity.

**d) Corticosteroids**

Glucocorticoids have historically been the backbone of maintenance immunosuppression and are utilized for the prevention and treatment of acute rejection due to their anti-inflammatory and immunosuppressive activity. Early post-transplantation, prednisolone (1 mg/kg/day) is generally given after the initial 24 hours of induction of high-dose methylprednisolone (125 mg eight hourly for three doses), subsequently reducing in a tapering fashion to a maintenance dose of 5-10 mg daily over the ensuing months. The side effects of glucocorticoids include hyperglycemia, hyper-

tension, peptic ulcer disease, osteoporosis, cataracts, and cushingoid side effects, to name a few. Due to the significant adverse effect profile associated with prolonged use, many transplant clinicians continue to seek steroid-sparing regimens where possible.

**Treatment of Rejection**

**a) Acute cellular rejection**

Acute rejection occurs in up to 36% of lung transplant recipients within the first year [1]. Although sometimes patients may be asymptomatic, typical presentation may include a low-grade fever, leukocytosis, cough, dyspnea, pulmonary infiltrates on the chest radiograph, and/or a decline in oxygenation. A suspected diagnosis of acute vascular rejection is confirmed by trans-bronchial lung biopsy with visualization of peri-vascular lymphocytic infiltrates. In general, the majority of lung transplant centers treats uncomplicated acute rejection in the first 3 months post-transplant with a short course of intravenous corticosteroids (10 mg/kg IV methylprednisolone for 3 days followed by gradual steroid taper from 1 mg/kg/day prednisolone, tapering by 0.2 mg/kg/week to maintenance levels). Occasionally, corticosteroid-resistant cellular rejection may be noted, prompting the use of anti-thymocyte globulin (ATG), given to achieve T cell depletion for 3 to 5 days.

**b) Antibody-mediated rejection**

As with cellular rejection, antibody-mediated rejection may be noted in parallel, confirmed by the triad of donor-specific antibodies, lung allograft dysfunction, and histological confirmation of antibody-mediated lung injury, confirmed by CD4 complement deposition in lung parenchymal tissue. In general, initial treatment of cellular rejection is important. A severe episode of antibody-mediated rejection is optimally treated with antibody removal by five to seven episodes of plasmapheresis, followed by intravenous immunoglobulin therapy, and the use of rituximab (anti-CD20 monoclonal antibody) to suppress de novo antibody production [1]. MMF can be used as a B cell-targeted therapy.

**c) Chronic lung allograft dysfunction**

Despite the many therapeutic options of acute or refractory acute rejection episodes described, a proportion of patients develop chronic lung allograft dysfunction (CLAD), manifesting clinically as lung function decline with progressive small airflow obstruction, airway neutrophilia, and HRCT-confirmed features of mosaicism identified on expiratory lung images and bronchial wall thickening [31]. CLAD is an overarching term that embraces all forms of chronic lung dysfunction post-transplant, including bronchiolitis obliterans syndrome (BOS), azithromycin-responding allograft dysfunction (ARAD), and restrictive allograft dysfunction (RAD). CLAD is an unfortunate reality of lung transplantation, with approximately 50% of lung allograft recipients being affected at 5 years post-transplant.

Bronchiolitis obliterans syndrome (BOS) is defined as a persistent obstructive FEV1 decline (≥20%) in two measurements taken at least 3 weeks apart [31]. The BOS classification system is shown in Table 4. BOS is the clinical manifestation of an inflammatory bronchiolitis associated with fibrotic remodeling of the small and medium-sized airways and is characterized by progressive loss of allograft function, with development of airflow obstruction. Until recently, the development of BOS was associated with an irreversible and relentless decline in lung function, which either eventually stabilized at a very low level or, in many patients, progressed to end-stage respiratory failure, accounting for the commonest cause of death after the first post-transplant year. BOS has historically been attributed to the effects of ongoing alloimmune injury, as both the frequency and severity of acute rejection episodes have been associated with increased risk. These observations have led to the paradigm that BOS is chronic rejection of the transplanted lung, and consequently, intensification of immunosuppression was used as an attempted therapy in many affected recipients. These approaches offered, at best, a slowing in the progression of the condition in some but also contributed to infective complications that undoubtedly added to the overall mortality risk from BOS. Over the last decade, a number of clinical trials of more intensive immunosuppressive regimes from the time of transplant or after onset of BOS have failed to show an impact on the incidence of BOS or regain lost function. More recently, however, it has been appreciated that non-alloimmune insults to the lung allograft, such as the lung injury of primary graft dysfunction, viral and bacterial infections, and aspiration injury, also increase the risk of developing BOS. This suggests that cross-talk between innate immune responses and alloimmunity may play a key role and highlights the importance of inflammation in driving the process.

**Table 4.** Bronchiolitis obliterans syndrome (BOS) classification

| BOS Stage | Classification |
|---|---|
| 0 | $FEV_1$ >90% of baseline & $FEF_{25-75}$% >75% of baseline |
| 0-p (potential BOS) | $FEV_1$ 81-90% of baseline &/or $FEF_{25-75}$% ≤75% of baseline |
| 1 | $FEV_1$ 66-80% of baseline |
| 2 | $FEV_1$ 51-65% of baseline |
| 3 | $FEV_1$ ≤50% of baseline |

Azithromycin-responding allograft dysfunction (ARAD) or azithromycin-responsive BOS is defined in patients with an FEV1 increase of ≥10% after a 2-3-month treatment trial of thrice-weekly 250 mg azithromycin [31]. The first randomized, double-blind, placebo-controlled study investigating the role of azithromycin given as prophylaxis to lung transplant recipients to prevent the development of BOS in 2011 showed that over the 2-yr follow-up period, those who received azithromycin had a significantly lower incidence of BOS: 12.5% compared to 44.2% in those who received placebo. The primary outcome measure of BOS-free survival was significantly better in patients on azithromycin, but there was no significant difference in overall survival between the two treatment arms [32]. Results of further long-term trials of azithromycin in BOS are greatly anticipated.

Restrictive Allograft Dysfunction (RAD) denotes pulmonary restriction on lung function testing, in association with radio-

logical features of ground glass shadowing and upper zone pleural thickening. It is defined as a persistent decline in vital capacity (VC) and total lung capacity (TLC) that is accompanied by a decline in $FEV_1$ of >20% [31]. Similarly to BOS, these findings must be demonstrated on two measurements taken at least 3 weeks apart. The importance of recognizing this specific type of CLAD is suggested by the significantly worse survival of patients with RAS compared to recipients with obstructive BOS.

Specific therapies of benefit for CLAD, therefore, include the use of low-dose alternate-day azithromycin, anti-reflux therapy and total lymphoid irradiation (TLI) [32-34]. Gastroesophageal reflux is common post-lung transplant with recent studies suggesting that aspiration, characterised by the presence of pepsin and bile acids in bronchoalveolar lavage may be present as early as 1 month post-transplant, supporting the need for early assessment of reflux, which may inform fundoplication [33]. Early fundoplication has been associated with greater freedom from BOS and improved survival. TLI has been shown to significantly reduce the rate of decline in graft function associated with BOS. It is well tolerated and associated with few serious complications and is therefore an appropriate immunosuppressive approach in the treatment of progressive BOS [34]. Prompt treatment of Pseudomonal or fungal infection is important, as previous studies have shown that de novo colonization of the lung allograft by Pseudomonas is strongly associated with the subsequent development of BOS [35]. The use of potent anti-inflammatory therapy with high-dose prednisolone or cytolytic therapy has not been shown to be of benefit. Lung re-transplantation continues to be controversial, but survival rates have improved in patients with BOS over the past decade and thus should be considered as a treatment option in this patient population.

## DISCUSSION

The risk of allograft rejection is highest in the early period post-transplantation and generally decreases with time. Thus, most regimens employ the highest intensity of immunosuppression immediately after surgery and decrease the intensity of therapy over the first year, eventually tailoring immunosuppression intensity levels to preserve allograft function with the lowest maintenance levels of immunosuppression compatible with preventing graft rejection. Using low doses of several drugs with non-overlapping toxicities is preferable to higher, and more toxic, doses of fewer drugs whenever feasible. Combination regimens also help to block the many components of the complex immunological cascade that leads to allograft rejection. It is becoming increasingly important to avoid over-immunosuppression, which can lead to undesirable adverse effects, such as susceptibility to infection and malignancy.

Although induction therapy has proven to decrease the incidence and severity of acute and chronic rejection in other solid organ transplantations, the beneficial effects of induction therapy on acute rejection and BOS in lung transplantation have not been consistently demonstrated in clinical trials. The recent ISHLT registry data suggests a trend towards a small but statistically significant improvement in survival with the use of induction therapy when excluding deaths in the 2-week perioperative period between the years 1994-2011 and 2000-2011 [2]. Per ISHLT registry data, approximately 16% of transplant recipients were given induction therapy at the time of transplantation over the last decade. Of these, the majority was given an IL-2 receptor antagonist (68.9%); 20.4% received therapy with polyclonal anti-lymphocyte or anti-thymocyte globulin (although the proportion of patients receiving this therapy appears to be decreasing in the last 5 years in contrast to the increase in the other two agents), and the remaining 10.7% received induction with alemtuzumab [2].

Historically, maintenance immunosuppression consisted of cyclosporine, azathioprine, and low-dose prednisolone. Currently, multiple combinations of the previously discussed medications are given. Per the recent ISHLT registry data, tacrolimus is reported to be used more commonly compared with cyclosporine at 1, 5, and 10 years after transplantation. Similarly, MMF was prescribed more commonly than azathioprine, perhaps reflecting changes in practice during recent years [2]. The most common combination therapy at 5 years of follow-up consisted of tacrolimus and MMF (38%), followed by tacrolimus and azathioprine (20%), cyclosporine and MMF (8%), and cyclosporine and azathioprine (6%). Sirolimus and everolimus use remains relatively low, with less than 20% of lung transplant recipients receiving the drugs at either 1 or 5 years after transplantation; 8% of patients received maintenance immunosuppression with tacrolimus monotherapy [2]. There is no current consensus as to the optimal combination of immunosuppressive therapy in lung transplantation. Institutions evaluate donor and recipient patient risk factors for rejection and develop specific protocols to guide clinicians on which induction agent to use. Immunosuppression regimens must be tailored to the individual patient and may require modification over time.

Lung transplantation is a complex treatment that is reserved for patients with end-stage lung disease. Patients receive lung transplants for a heterogeneous group of pulmonary diseases, resulting in different patient phenotypes and individual pharmacogenomics. Given the lack of consensus as to the optimal therapeutic regimen, individual transplant centers often follow different protocols with respect to initial immunosuppression, indications to transition medications, and hierarchical ordering of medications. The goal of immunosuppression is to block T cell activation and proliferation, in turn preventing de novo antibody generation and transplanted organ dysfunction. Immunosuppression must be balanced with the risk of infection, including nosocomial infections and opportunistic pathogens. Careful selection of immunosuppressive agents, inpatient management of acute complications and rejection episodes, management of maintenance immunosuppression, and meticulous long-term follow-up with monitoring for adverse drug reactions and drug-drug interactions are all essential measures to ensure the best patient outcomes in this patient population.

### Future Research

Immunosuppression in lung transplantation remains a difficult issue, with rejection continuing to plague patient outcomes. Future multicenter trials assessing current immune suppressive therapies as well as continued research into stem cells and alloreactivity of the transplanted organ may identify new molecular targets for innovative therapies and new

pharmaceuticals that may improve survival and quality of life for lung transplant patients worldwide.

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