Derleme|Review

Immunosuppression in Lung Transplantation

Akciğer Transplantasyonunda İmmünsüpresyon

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ÖZET

Tüm organ nakillerinin başarısı için immünsüpresyon önemli bir basamaktır. İmmünsüpresif ajanların geliştirilmesi, değerlendirilmesi ve kullanımı transplante organa göre farklılık göstermektedir. Akciğer nakli sonrasında uygulanan immünsüpresif tedaviler konusunda kanıta dayalı yaklaşım, mevcut randomize kontrollü çalışmaların azlığı nedeniyle sınırlıdır. İmmünsüpresif tedavi konusunda yapılan araştırmalar az sayıda hasta içermekte ve önemli soruları yanıtsız bırakmaktadır. Bu makale akciğer nakli sonrasında uygulanan immünsüpresif tedavi üzerine genel bir bakış sunmaktadır. Makale randomize kontrollü çalışmaları ve alanında önde gelen yayınları bünyesinde barındırmaktadır. Akciğer nakli hayat kurtarıcı bir operasyon olmakla beraber akciğer nakli ile yaşam zorlayıcı olabilmektedir. Karşılaşılan güçlüklerin aşılması ise halen çözülememiş araştırmaya açık büyük bir alan olarak karşımıza çıkmaktadır.

Anahtar Kelimeler: *Akciğer nakli, immünsüpresyon, farmakoloji.*

SUMMARY

Immunosuppression is critical for the success of any organ transplant. The way that immunosuppressant medications are developed, evaluated, and applied varies from organ to organ. Making evidence-based decisions on immunosuppressive therapies in lung transplantation is a challenge due to the limited number of randomized clinical trials completed to date, the key questions that remain unanswered, and relatively small patient volumes. This review provides an overview of immunosuppressive medications that are used in lung transplantation. Wherever possible, the discussion is limited to randomized controlled trials and the most robust literature in the field. Lung transplantation is a life-saving procedure, yet living with a lung transplant can be a challenge. Learning how to best meet those challenges is a large and unfinished research agenda.

Keywords: *Lung transplant, immunosuppression, pharmacology.*

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Introduction

Immunosuppression is critical for the success of any organ transplant, and the field of lung transplantation offers a particular challenge. Survival after a lung transplant is inferior when compared with recipients of other organs due to higher risks for primary graft dysfunction, infection, acute rejection, and chronic allograft dysfunction[1].

Making evidence-based decisions on immunosuppressive therapies in lung transplantation is a challenge due to the limited number of randomized clinical trials completed to date, the key questions that remain unanswered, and relatively small patient volumes. This review provides an overview of immunosuppressive medications that are used in lung transplantation. Wherever possible, the discussion is limited to randomized controlled trials and the most robust literature in the field.

Basic Principles of Immunosuppression

Immunosuppressive protocols for lung transplant recipients will vary from center to center, and from patient to patient. Empiric or planned variance will stem from the concept of giving more aggressive regimens to patients considered more likely to reject, and less aggressive regimens to patients considered more vulnerable to the complications of over-immunosuppression. Regimen choices may reflect regional risks such as endemic infections, or cohort risks such as highly sensitized transplant candidates. For-cause or unplanned variance will stem from each individual patient's experience on the originally planned regimen. Patients with early or aggressive rejection will likely require an upgrade to the intensity of their immunosuppressive regimen whereas patients who struggle with opportunistic infections or post-transplant malignancies will likely require a downgrade.

Immunosuppressive regimens can be divided into two phases. The induction phase refers to a brief period starting from the time of transplantation and often extending several days when patients receive intravenous antibody therapies that offer an immediate immunosuppressed state. The goal of induction therapy is to provide intense immunosuppression when the risk of rejection is highest. The maintenance phase refers to the long-term regimen that a patient continues for the rest of his or her life. The intensity of a maintenance regimen often declines over time, but the presence of some immunosuppression is permanent. Key features of medications used in maintenance regimens are summarized in (Table 1).

Three general principles form the basis of induction and maintenance immunosuppressive choices[2]. The first is that immune reactivity and the tendency towards graft rejection is highest early (i.e. within the first 3-6 months post-transplant), and decreases with time. The second is that ideal regimens employ several drugs with non-overlapping toxicities. The third is that over-immunosuppression should be avoided, as it leads to unwanted complications like infection and malignancy.

Induction Immunosuppression

Non T-cell depleting antibodies: Non T-cell depleting antibody therapy refers to the interleukin-2 (IL-2) receptor antagonists basiliximab and daclizumab. The IL-2 receptor is expressed on lymphocytes. IL-2 receptor antagonists compete with IL-2 for receptor binding. The drug's ability to block IL-2 from binding to the receptor prevents the receptor from signaling, thereby preventing T cell proliferation and B cell activation.

Daclizumab is traditionally dosed as 1 mg/kg IV every two weeks for five doses whereas basiliximab is traditionally dosed as 20 mg IV on post-operative days zero and four. Both are considered benign in terms of their adverse event profile. Currently, only basiliximab remains available. Daclizumab was withdrawn from the market in 2009.

In lung transplantation, one randomized controlled trial has compared an IL-2 receptor antagonist with placebo[3] and three randomized controlled trials have compared them with T-cell depleting therapies[3-6].

Conte, et al compared daclizumab with placebo[3,4]. Twenty-five patients were randomized; 15 to daclizumab and 10 to placebo. Treatment with daclizumab 1 mg/kg IV for five doses on post-operative days 0, 7, 21, 35, and 49 did not significantly impact acute rejection rates, infection rates, or mortality.

Mullen, et al randomized 50 lung recipients to daclizumab or antithymocyte globulin (ATG)[5]. Patients randomized to daclizumab received 2 mg/kg IV within four hours post-operatively, then 1 mg/kg IV four days later. Patients randomized to ATG received 10 mg/kg infused continuously over the first 5-8 days post-operatively, stopping once the calcineurin inhibitor levels reached their target. Once ATG therapy ended, methylprednisolone 2 mg/kg IV every 12 hours for three doses was given. Maintenance immunosuppression included a calcineurin inhibitor (either tacrolimus or cyclosporine), mycophenolate

Table 1. Key features of maintenance immunosuppressive medications.

	Dosing strategy	Possible routes of administration	Able to administer through a feeding tube?	Adjust dose for renal impairment?	Adjust dose for hepatic impairment?	Major side effects
Calcineurin inhibitors						
Tacrolimus (Prograf®)	To achieve target trough concentration	Oral, Sublingual, Intravenous	Yes, use packets for suspension or a compounded solution	Drug is not renally cleared but is nephrotoxic; consider reducing target serum concentration in moderate/severe renal impairment	Drug undergoes extensive hepatic metabolism; consider lower doses in severe hepatic impairment	Nephrotoxicity, electrolyte disturbances (↓ Mag, ↓ Phos, ↑ K), neurotoxicity (tremor, headache), hyperglycemia, hypertension, hyperlipidemia, hair loss
Tacrolimus (Astagraf XL®)	To achieve target trough concentration	Oral	No			
Tacrolimus (Envarsus XR®)	To achieve target trough concentration	Oral	No			
Cyclosporine nonmodified (Sandimmune®)	n/a; use the modified formulation	n/a; use the modified formulation	n/a; use the modified formulation	Drug is not renally cleared but is nephrotoxic; consider reducing target serum concentration in moderate/severe renal impairment	Drug undergoes extensive hepatic metabolism; consider lower doses in severe hepatic impairment	Nephrotoxicity, electrolyte disturbances (↓ Mag, ↓ Phos, ↑ K), hypertension, hyperlipidemia, neurotoxicity (tremor, headache), hyperglycemia, hair growth
Cyclosporine modified (Neoral®)	To achieve target trough or target C2 concentration	Oral	Yes, use the oral solution			
Antimetabolites						
Mycophenolate mofetil (Cellcept®)	Standard dosing, or to achieve target AUC	Oral, Intravenous	Yes, use the oral solution	Renal dysfunction may lead to decreased excretion	No	Leukopenia, GI intolerance
Mycophenolate mofetil (Myfortic®)	Standard dosing	Oral	No	Renal dysfunction may lead to decreased excretion	No	Leukopenia, GI intolerance
Azathioprine (Imuran®)	Standard weight-based dosing	Oral	Yes, crush the tablets	Renal dysfunction may lead to delayed clearance; drug is cleared by hemodialysis	No but monitor for drug-induced cholestasis	Leukopenia, anemia, hepatotoxicity

Table 1. Key features of maintenance immunosuppressive medications (Continuation of Table 1).

Leflunomide (Arava®)	Standard dosing, or to achieve target trough concentration	Oral	Yes, crush the tablets	Use with caution; avoid in severe impairment	Avoid in moderate or severe impairment	Hair loss, diarrhea, nausea, hepatotoxicity
Antiproliferatives						
Sirolimus (Rapamune®)	To achieve target trough concentration	Oral	Yes, use the oral solution	No, but consider discontinuing therapy if acute renal impairment occurs when used in combination with cyclosporine	Yes, reduce to varying amounts per degree of hepatic impairment	Delayed wound healing, oral ulcers, proteinuria, hyperlipidemia, thrombocytopenia
Everolimus (Zortress®)	To achieve target trough concentration	Oral	No	No	Yes, reduce to varying amounts per degree of hepatic impairment	Delayed wound healing, oral ulcers, proteinuria, hyperlipidemia, thrombocytopenia
Costimulation blockers						
Belatacept (Nulojix®)	Standard weight-based dosing	Intravenous	n/a	No	No	Edema, diarrhea; Do not use in EBV seronegative patients due to high incidence of PTLD in that population

mofetil, and prednisone. This study was powered to detect a 33% reduction in infection rate. No significant difference was noted with regard to rejection. The number of patients experiencing an infection was not significantly different, but the daclizumab group had a significantly higher number of total infections, severe infections, and cytomegalovirus infections per patient. One-year survival was 96% in the daclizumab and 88% in the ATG group.

The remaining two trials are published in abstract but not manuscript form. Senn, et al randomized 24 lung recipients to basiliximab or ATG.6 Patients randomized to basiliximab received 20 mg IV on post-operative days one and four. Patients randomized to ATG received 3 mg/kg IV over post-operative days 0-6. Maintenance immunosuppression included cyclosporine, mycophenolate mofetil, and prednisone. Conte, et al compared daclizumab with ATG in 26 lung recipients[3,4]. Patients on daclizumab received 1 mg/kg IV for five doses on post-operative days 0, 7, 21, 35, and 49 whereas patients on ATG received 1.5 mg/kg IV on post-operative day zero then repeated daily as needed through post-operative day five in order to maintain the CD3 lymphocyte count < 5% of the total lymphocyte count. Maintenance immunosuppression included cyclosporine, mycophenolate mofetil and prednisone. No difference was found between the therapies with regard to acute rejection, bronchiolitis obliterans syndrome (BOS), or mortality.

T-cell depleting antibodies: T-cell depleting antibody therapy refers to ATG, alemtuzumab, and OKT3. The ATGs are polyclonal antibodies whereas alemtuzumab and OKT3 are monoclonal antibodies. Although their pharmacology and mechanisms are unique, the end result of these therapies is similar. All are strong immunosuppressant agents that result in T-cell depletion persisting for months after dosing.

The dosing of these therapies is varied in lung transplantation. Infusion related reactions are likely, and all of these therapies should be pre-medicated. Acetaminophen and an antihistamine are the recommended pre-medications for alemtuzumab whereas acetaminophen, an antihistamine, and corticosteroids are the recommended pre-medications for ATG therapies. OKT3 was withdrawn from the market in 2010.

Antithymocyte globulins (ATG) have been compared with placebo in three randomized controlled trials[7-9]. They have been compared with alemtuzumab in one randomized controlled trial[10].

Chaparro[7] (n= 60), Palmer[8] (n= 44), and Snell[9] (n= 223) randomly assigned lung transplant recipients to ATG or placebo. The drug products and doses were different in each study: antilymphocyte globulin during the first 7 post-operative days for Chaparro, Duke rabbit ATG at a dose of 1.5 mg/kg IV daily for three days for Palmer, and ATG-Fresenius at a single dose of 5 mg/kg or 9 mg/kg IV post-operatively for Snell. These are all different from the commercially available thymoglobulin, and it is unknown if the different products would offer different clinical outcomes. The Snell trial was revised at the interim analysis due to failure of the 5 mg/kg treatment arm, coupled with an inability to enroll enough subjects to power the endpoint between the 9 mg/kg and placebo arms. Their primary endpoint of efficacy failure (i.e. death, graft loss, acute rejection, or loss to follow-up) at one year occurred in 48%, 40%, and 37% of subjects in the 5 mg/kg, 9 mg/kg and placebo groups, respectively. The Palmer trial reported promising one year outcomes with acute rejection graded 2A or higher occurring in 23% of Duke rabbit ATG vs. 55% of the control arm (p= 0.03) with no significant difference in BOS, infection, malignancy, or survival. An eight-year follow-up on this study cohort has also been published.10 It shows that the early rejection advantage is lost at approximately six years post-transplant. By eight years post-transplant, the difference between the Duke rabbit ATG and control groups was 60% vs. 87% (p= 0.11) for BOS, similar for infectious complications, 41% vs. 14% (p= 0.09) for malignant complications, and 36% vs. 23% (p= 0.48) for survival. Overall, these trials demonstrate that ATG offers lower early acute rejection outcomes without a clear corresponding long-term benefit on BOS or mortality.

Jaksch, et al randomly assigned 60 lung transplant recipients to thymoglobulin or alemtuzumab[11]. Patients assigned to thymoglobulin received 2 mg/kg IV daily on days 0-4 post-transplant combined with tacrolimus, mycophenolate 1.5 g twice daily and corticosteroids at standard doses. Patients assigned to alemtuzumab received 30 mg IV once combined with tacrolimus at reduced target troughs, mycophenolate mofetil 750 mg twice daily, and corticosteroids at reduced doses. Acute rejection episodes with a severity of A2 or worse occurred in 17% of the thymoglobulin- and 0% of the alemtuzumab-treated patients (p= 0.019). However, BOS occurred in 3% of thymoglobulin-treated patients and 13% alemtuzumab-treated patients (p= 0.19). One- and Two-year survival was

96% and 96% in the thymoglobulin group, and 93% and 90% in the alemtuzumab group, respectively (p= 0.1). Thus, although alemtuzumab offered excellent acute rejection rates, it did not improve the more clinically relevant BOS or survival rates.

Taken together, all of these studies fail to illustrate a clear benefit of induction therapy in lung transplantation[4]. Despite that, a majority of centers elect to give induction immunosuppression[12]. The convincing evidence may be a retrospective cohort study of 3970 lung recipients from the ISHLT registry, which showed a survival benefit to giving induction[13]. At four years post-transplant, survival rates were 57%, 60%, and 64% for patients who received no induction, ATG, or IL-2 receptor antagonists, respectively (p= 0.007). The benefit persisted in the multivariate analysis for IL-2 receptor antagonists in all lung recipients and for ATG in bilateral recipients only.

Maintenance Immunosuppression

Calcineurin inhibitors: Calcineurin inhibitors refer to any of five distinct medications including two cyclosporine formulations and three tacrolimus formulations. The two cyclosporine formulations are cyclosporine nonmodified (Sandimmune®) and cyclosporine modified (Neoral®). The difference is that the modified formulation contains a microemulsion that results in better and more consistent absorption[14]. The three tacrolimus formulations include tacrolimus capsules (Prograf®), an extended release tacrolimus capsule (Astagraf XL®), and an extended release tacrolimus tablet (Envarsus XR®). Each of these formulations has a distinct pharmacokinetic profile and none is directly interchangeable with another[15].

The immunosuppressive benefit of calcineurin inhibitors results primarily from reduced IL-2 production by T cells. Intracellularly, each drug binds to its target: cyclophilin for cyclosporine and FK-binding protein for tacrolimus. The drug-target complex inhibits the phosphatase activity of calcineurin. With calcineurin unable to dephosphorylate nuclear factor of activated T cells (NF-AT), NF-AT is unable to move into the cell nucleus and initiate gene transcription for IL-2 formation. Without IL-2 production, an activated cell is incapable of mounting a substantial immune response.

Both cyclosporine products and tacrolimus are given twice a day at twelve hour intervals. The two extended release tacrolimus products are given once a day in the morning. All calcineurin inhibitor doses should be adjusted as needed to achieve target serum concentrations. Although trough concentrations are used most frequently, two-hour post-dose concentrations may be used for cyclosporine only[16]. Drug interactions, particularly those involving the cytochrome p-450 3A4 enzymes, are prevalent and clinically significant (Table 2). All calcineurin inhibitors are associated with substantial risk for adverse effects, making careful therapeutic drug monitoring essential. Adverse effects include nephrotoxicity, neurotoxicity (e.g. intention tremor, headache, seizure), hyperglycemia, hypertension, hyperlipidemia, and electrolyte disturbances (e.g. hyperkalemia, hypomagnesemia, and hypophosphatemia). Comparatively, tacrolimus is more likely to cause hyperglycemia and neurotoxicity whereas cyclosporine is more

Table 2. Common cytochrome P450 3A4 drug interactions[60].

Drugs that induce CYP 3A4 (i.e. reduce immunosuppressant exposure)	Drugs that inhibit CYP 3A4 (i.e. increase immunosuppressant exposure)
Barbiturates	Amiodarone
Carbamazepine	Azole antifungals:
Glucocorticoids	Fluconazole (at higher doses)
HIV antivirals:	Itraconazole
Efavirenz	Ketoconazole
Nevirapine	Posaconazole
Nafcillin	Voriconazole
Phenobarbital	Calcium channel blockers:
Phenytoin	Diltiazem
Pioglitazone	Nicardipine
Rifabutin	Verapamil
Rifampin	Cimetidine
Troglitazone	Ciprofloxacin
St. John's Wort	Glucocorticoids
	HIV antivirals:
	Indinavir
	Nelfinavir
	Ritonavir
	Saquinavir
	Macrolides:
	Clarithromycin
	Erythromycin
	Telithromycin
	Grapefruit juice

likely to cause hypertension and hyperlipidemia. Tacrolimus can cause hair loss whereas cyclosporine can cause hair growth.

Tacrolimus and cyclosporine have been directly compared in several randomized controlled trials in lung transplant recipients[17-22]:

Griffith, et al compared tacrolimus with cyclosporine in 74 lung transplant recipients.17 Concomitant immunosuppression included methylprednisolone for the first 24 hours only and azathioprine. Long-term prednisone was added if patients experienced two episodes of acute rejection during the first six weeks. Rejection occurred within 120 days in 87% of patients on tacrolimus and 97% of patients on cyclosporine. The mean number of rejection episodes per 100 days was 1.2 for tacrolimus and 2 for cyclosporine ($p< 0.05$). Only 34% and 6% of patients in the tacrolimus and cyclosporine groups remained steroid-free, respectively ($p< 0.0001$). One- and six-month survival was similar between the two groups. Although it wasn't the study objective, these results underscore the importance of using a three-drug maintenance immunosuppressive regimen.

Keenan, et al compared tacrolimus with cyclosporine in 133 lung transplant recipients[18]. Concomitant immunosuppression included azathioprine and prednisone. Tacrolimus was adjusted to a goal trough of 10-20 ng/mL and cyclosporine adjusted to a goal of 750-1000 ng/mL. Acute rejection occurred in 86% of patients on tacrolimus and 88% of patients on cyclosporine (p= ns). The mean number of acute rejection episodes per 100 days was 0.85 for tacrolimus and 1.09 for cyclosporine (p= 0.07). Obliterative bronchiolitis occurred in fewer patients in the tacrolimus group (22% vs. 38%, p= 0.025). One- and two-year survival were similar between the two groups.

Treede and Zuckermann, et al compared tacrolimus with cyclosporine in 74 lung transplant recipients in Munich and Vienna.19-20 Concomitant immunosuppression included rabbit ATG induction for three days with mycophenolate and prednisone maintenance. Tacrolimus was adjusted to a goal trough of 12-15 ng/mL for the first month then 9-12 ng/mL thereafter and cyclosporine was adjusted to a goal trough of 250-300 ng/mL for the first month, then 200 ng/mL thereafter. Acute rejection occurred within the first 12 months in 54% of patients on tacrolimus and 65% of patients on cyclosporine (p= ns). The number of acute rejection episodes per 100 days was 0.22 for tacrolimus and 0.32 for cyclosporine (p= 0.097). One year survival was similar between the two groups.

Hachem, et al compared tacrolimus with cyclosporine in 90 lung transplant recipients[21]. Concomitant immunosuppression included basiliximab induction with azathioprine and prednisone maintenance. Tacrolimus was adjusted to a goal trough of 5-15 ng/ml and cyclosporine was adjusted to a goal trough of 175-325 ng/mL. Sirolimus was started to replace azathioprine in patients who experienced the rejection endpoint. In patients taking sirolimus, goal troughs were 5-15 ng/mL for sirolimus, 4-10 ng/mL for tacrolimus, or 100-175 ng/mL for cyclosporine. The study rejection endpoint occurred in 55% of patients on tacrolimus and 85% of patients on cyclosporine (p= 0.002). Severe rejections occurred in 41% and 63%, respectively (p= 0.036). Graft survival was similar between the two groups.

In the final randomized controlled trial, Treede et al compared tacrolimus with cyclosporine in 249 lung transplant recipients at 14 centers across five European countries[22]. Concomitant immunosuppression included mycophenolate and prednisone. Tacrolimus was adjusted to a goal trough of 10-15 ng/mL for the first 3 months then 8-12 ng/mL thereafter. Cyclosporine was adjusted to a goal trough of 200-300 ng/ml for the first three months then 150-200 ng/mL thereafter. Centers who preferred to manage cyclosporine by two-hour post-dose levels were allowed to do so. The primary endpoint of this study was the development of BOS at three years post-transplant. BOS occurred in 12% of patients on tacrolimus and 21% of patients on cyclosporine (p= 0.037). Three-year survival was similar between the two groups.

Overall, these trials demonstrate equivalent survival but less acute rejection and less BOS with tacrolimus. Although the incidence of infections and drug-related adverse events was not statistically different in any of the trials, a few trends were noted. Patients on cyclosporine tended to have more bacterial infections[17,18,20] and a higher incidence of hypertension[20] whereas patients on tacrolimus tended to have more fungal18-20 infections and a higher incidence of diabetes[20,21].

Antimetabolites: Antimetabolite immunosuppressants include azathioprine (Imuran®), mycophenolate mofetil (Cellcept®), and mycophenolate sodium (Myfortic®). Each of these medications prevents T cell proliferation by blocking the cell's ability to replicate its DNA. Azathioprine is converted into 6-mer-

captopurine, which blocks purine synthesis and DNA formation. Mycophenolate mofetil is a prodrug of mycophenolic acid whereas mycophenolate sodium is enteric coated; mycophenolate acid is the active component of both products. It inhibits inosine-5'-monophosphate-dehydrogenase, which results in an inability to synthesize guanosine nucleotides. Mycophenolate's mechanism is limited to de novo purine synthesis pathways, which are critical for lymphocyte cell division. Unlike azathioprine, mycophenolate does not impair salvage purine pathways. Without the necessary raw materials (e.g. purines), T and B lymphocytes cannot replicate their DNA and thus cannot divide and proliferate.

Azathioprine is given once a day whereas both mycophenolate products are given twice a day. Checking for deficiencies in thiopurine methyltransferase (TPMT) can identify patients unlikely to tolerate traditional weight-based azathioprine doses. Monitoring mycophenolate concentrations is possible, but the clinical utility should be carefully considered. Trough levels are not helpful as they do not correspond with efficacy. Collecting multiple precisely-timed samples then calculating a mini-AUC can be useful for mycophenolate mofetil in select patient settings[23]. Monitoring mycophenolate concentrations is not useful for mycophenolate sodium. One of the most relevant drug interactions to avoid is azathioprine with allopurinol or febuxostat; the combination can cause a fatal marrow suppression. An additional interaction to be aware of is azathioprine with ACE-inhibitors; the combination can increase the incidence of anemia and/or neutropenia. Common adverse effects of the antimetabolites include marrow suppression, including leukopenia and thrombocytopenia. Anemia can occur with any drug but is more common with azathioprine. Gastrointestinal adverse events like nausea or diarrhea can occur with any drug but are more common with the mycophenolate products.

Azathioprine and mycophenolate have been compared in two randomized controlled trials in lung transplantation[24,25]:

Palmer, et al compared mycophenolate mofetil 1g twice daily with azathioprine 2 mg/kg once daily in 81 lung transplant recipients[24]. Concomitant immunosuppression included cyclosporine titrated to a goal trough of 250-300 ng/mL and prednisone; no induction was used. Biopsy proven acute rejection graded 2A or higher occurred in 63% of patients on mycophenolate and 58% of patients on azathioprine (p= 0.82). Drug intolerance occurred in 30% of patients randomized to mycophenolate and 16% of patients randomized to azathioprine. Approximately two-thirds of all patients who did not tolerate their assigned drug were successfully switched to the other treatment arm. Six-month survival was similar between the two groups.

McNeil, et al compared mycophenolate mofetil 1.5 g twice daily for three months then 1 g twice daily with azathioprine 2 mg/kg once daily in 320 lung transplant recipients at 22 centers across Australia, Canada and Europe[25]. Concomitant immunosuppression included at least one dose of rabbit ATG induction, cyclosporine titrated to a goal trough of 300-500 ng/mL for three months then 200-400 ng/mL thereafter, and prednisone. The incidence of acute rejection at three years was 57% for patients on mycophenolate and 60% for patients on azathioprine (p= ns). The incidence, severity, and time to BOS was the same between the two groups.

mTOR Inhibitors: The mTOR inhibitors sirolimus (Rapaune® and everolimus (Zortress®) have a more selective place in lung transplant immunosuppressive regimens. These drugs should be used with caution early post-op, due to their tendency to impair wound healing and their risk of causing venous thromboembolism.

Sirolimus is given once a day whereas everolimus is given twice a day. Both drugs should be dosed to achieve target trough concentrations. Drug interactions, particularly those involving the cytochrome p-450 3A4 enzymes, are prevalent and clinically significant (Table 2). Important drug-related side effects include impaired wound healing, edema, oral ulcers, hyperlipidemia, and proteinuria.

The mTOR inhibitors have been evaluated in lung transplantation in two different ways. One role is to reduce calcineurin exposure[26-28] and the other is to replace the antimetabolite[29-36].

The Nordic Certican trial in heart and lung transplantation (NOCTET) evaluated the impact of introducing everolimus at one or more years post-transplant in order to decrease calcineurin inhibitor exposure by 30-70%. A total of 282 heart and lung recipients were enrolled; 92 were lung recipients. Outcomes in the lung cohort show a mean eGFR difference for everolimus vs control of +2.3 mL/min vs. -1.3 mL/min at one year[26] (p= 0.07), +2.5 vs. -3.5 at two years[27] (p= 0.02), and -5 vs. -5.4 at approximately five years[28] (p= 0.916). Rejection rates were not

different, though adverse events were significantly more frequent in the everolimus arm. Edema (29% vs. 9%, p< 0.001), diarrhea (17% vs. 6%, p= 0.003) and leukopenia (11% vs. 0%, p< 0.001) occurred significantly more often in the everolimus cohort versus the control[26].

The Assessment of Immunosuppressive Regimen in Suppressing Acute and Chronic Rejection (AIRSAC) study was a randomized controlled trial that compared azathioprine versus sirolimus in 181 lung recipients[29-31]. All patients received basiliximab induction, tacrolimus + azathioprine + prednisone for the first 90 days post-transplant, then they were randomized to continue azathioprine or switch to sirolimus. Acute rejection, BOS, and survival rates were similar between the two groups at one and three years post-transplant[29]. The sirolimus cohort experienced significantly more venous thromboembolism (17% vs. 3%, p< 0.01)[30] and less CMV that was significant at one year but not at three years post-transplant[31].

Everolimus has also been compared with azathioprine. One trial identified 3-12 ng/mL as an optimal everolimus target trough, when used in combination with cyclosporine and prednisone[32]. A second evaluated the cellular and cytokine milieu in 23 lung transplant recipients randomized to everolimus or azathioprine, in combination with cyclosporine and prednisone[33]. Snell, et al evaluated the clinical outcomes in 213 stable lung or heart-lung recipients[34]. At three or more months post-transplant, subjects were randomized to remain on azathioprine 1-3 mg/kg per day or convert to everolimus 1.5 mg twice daily. All subjects continued a background regimen of cyclosporine and prednisone. Efficacy failure defined as a composite of decline in FEV_1 > 15%, graft loss, death, or loss to follow-up favored everolimus at one year 22% vs. 34% (p= 0.046), but the advantage lost significance by two years post-transplant. The occurrence of treatment discontinuation, renal impairment, and serious adverse events were more common with everolimus.

Everolimus has been compared with mycophenolate in two trials. Glanville, et al enrolled 165 lung recipients, between one and three months post-transplant, with bronchial anastamotic healing confirmed by bronchoscopy[35]. Baseline immunosuppression was cyclosporine, mycophenolic acid, and prednisone. At randomization, subjects either remained on mycophenolic acid or switched to everolimus 1.5 mg twice daily with doses titrated to achieve troughs of 3-8 ng/mL. The two arms did not differ with regard to BOS at three years. The mycophenolate group experienced more biopsy proven acute rejection (p= 0.02), leukopenia (p< 0.01), diarrhea (p< 0.01), and CMV infection (p= 0.04) whereas the everolimus group experienced more venous thromboembolism (p= 0.02). Streuber, et al enrolled 190 lung transplant recipients[36]. Baseline immunosuppression was cyclosporine, mycophenolate mofetil, and prednisone. At 28 days post-transplant, patients were randomized to continue mycophenolate mofetil and cyclosporine at the same baseline doses or switch to everolimus 0.75 mg twice daily with doses titrated to achieve troughs of 4-7 ng/mL and reduced cyclosporine. The everolimus group experienced less BOS (p= 0.041), less acute rejection (p= 0.005), fewer lower respiratory tract infections (p= 0.003), and no leukopenia. Treatment discontinuations occurred in 55% of everolimus and 43% of the mycophenolate groups.

In sum, the mTOR inhibitors have a unique niche in lung transplantation. Their use in the first weeks post-transplant is not recommended, due to their impairment of wound healing. As a class, they may offer a unique antiviral or anticancer benefit. However, this must be countered by their venous thromboembolism risk and high rates of intolerance.

Costimulation blockers: Belatacept (Nulojix®) is a selective T cell costimulation blocker. Specifically, the drug is a fusion protein made of the Fc fragment of a human IgG1 immunoglobulin linked to the extracellular domain of CTLA-4. Belatacept binds to CD80 and CD86 receptors on antigen presenting cells, which prevents them from binding to the corresponding CD28 receptor on T cells. Blocking this costimulatory signal ultimately blocks T cell activation.

Belatacept is administered as a 30-minute intravenous infusion. This is unique from all other maintenance immunosuppressive therapies, which are oral. A major complication of belatacept therapy is the development of post-transplant lymphoproliferative disorder, particularly in patients who are not immune to Epstein Barr virus (EBV). It is for this reason that belatacept is contraindicated in any patient who is EBV seronegative or who has an unknown EBV serostatus[37]. In the kidney transplant literature, unique features of belatacept-based therapy when compared with calcineurin inhibitor-based therapy include improved renal function, a lower incidence of donor specific antibody formation, and improved patient and graft survival despite a higher incidence

of early acute rejection[38,39]. The benefit of belatacept in lung transplantation has not been formally evaluated. The literature to date includes two case series and several case reports.

Timofte, et al describe an eight-patient case series from the University of Maryland Medical Center[40]. Patients were a median of 585 (range 139-1414) days post-transplant when belatacept was started. Each patient was started on belatacept in the setting of acute or chronic renal insufficiency, in order to reduce calcineurin inhibitor exposure. Belatacept was dosed as 10 mg/kg IV on day 0, day 4, week 2, week 4, then monthly thereafter. Once belatacept was started, concomitant calcineurin inhibitors were reduced to goal troughs of 2-6 ng/mL for tacrolimus and 75-100 ng/mL for cyclosporine. The concomitant immunosuppressive regimen at the time of belatacept initiation was tacrolimus + mycophenolate + prednisone (n= 2), cyclosporine + mycophenolate + prednisone (n= 2), tacrolimus + prednisone (n= 2), or oral tacrolimus + inhaled cyclosporine + oral prednisone (n= 2). One patient experienced mild acute cellular rejection (A1) 3 weeks after starting belatacept, and was successfully treated with three doses of IV methylprednisolone. The remaining patients enjoyed stable lung function over 6 months post-conversion. The median (IQR) GFR was 24 (18-26) mL/min at baseline, 28 (20-60) mL/min at 1 month, 31 (27-39) mL/min at 3 months, and 36 (25-60) mL/min at 6 months after starting belatacept. Two of three patients on hemodialysis were successfully weaned off renal replacement therapy between 6 and 13 days after starting belatacept. One patient received only two belatacept doses prior to transfer to another center where belatacept was not continued. This patient died 4 months later of respiratory and multisystem organ failure. Infections occurred half as frequently in the belatacept group when compared with historic controls.

Iasella, et al describe an eleven-patient case series from the University of Pittsburgh[41]. Patients were a median of 492 (range 8-3276) days post-transplant when belatacept was started. All patients were converted to belatacept after calcineurin inhibitor failure, and the calcineurin inhibitor was discontinued in 9 patients. The reason for calcineurin inhibitor failure was thrombotic thrombocytopenic purpura (n= 4), posterior reversible encephalopathy syndrome (n= 3), recurrent rejection (n= 2), BOS (n= 1), or renal impairment (n= 1). Two different belatacept dosing strategies were used: nine patients received a conventional dosing strategy of belatacept 10 mg/kg on days 1, 5, 15, 29, 45, and 59 followed by 5 mg/kg monthly thereafter and two patients received a transitional dosing strategy of belatacept 5 mg/kg every 2 weeks for 6 doses followed by 5 mg/kg monthly thereafter. The concomitant immunosuppressive regimen used with belatacept was mycophenolate + steroid (n= 6), azathioprine + steroid (n= 2), everolimus + steroid (n= 2), and cyclosporine + mycophenolate + steroid + methotrexate (n= 1). Five patients experienced biopsy-proven rejection episodes, and two experienced progression of chronic lung allograft dysfunction. Of four patients with donor specific antibody (DSA) at baseline, only one continued to have DSA after starting belatacept. Mean GFR improved from 33 mL/min at baseline to 45 mL/min at the time of last follow-up (p= 0.03). Three patients expired from infections complications and one from progressive renal disease during the observation period.

In addition to the above two series, three case reports have been published. One describes the successful use of a belatacept + mycophenolate + prednisone regimen for two years in a lung transplant recipient who could not tolerate tacrolimus or sirolimus due to hemolytic uremic syndrome[42]. The second describes the unexpected development of invasive tracheobronchial aspergillosis at three years post-transplant in a lung recipient receiving an immunosuppressive regimen of belatacept + mycophenolate + prednisone[43]. This patient could not tolerate tacrolimus due to thrombotic thrombocytopenic purpura/hemolytic uremic syndrome nor cyclosporine due to posterior reversible encephalopathy syndrome. The third describes a fatal case of acute respiratory distress syndrome due to fulminant acute rejection occurring 25 days after converting from tacrolimus to belatacept for renal impairment.44 This case, and the above case series, suggest that the role of belatacept in lung transplantation may be calcineurin inhibitor minimization rather than calcineurin inhibitor replacement.

Regimens and Trends

The International Society for Heart and Lung Transplantation (ISHLT) maintains the International Thoracic Organ Transplant (TTX) Registry. The TTX Registry is an actively maintained registry that provides data on patient, donor, and recipient characteristics as well as post-transplant outcomes. The data represent the activities and outcomes from 390 lung

transplant centers worldwide[12]. The data are available online and featured in the Annual TTX Registry report[45].

Figures 1-4 illustrate TTX Registry data for immunosuppressive drug usage trends from January 2014 through June 2017. A majority of lung transplant centers utilize induction immunosuppression, with basiliximab selected as the predominant induction immunosuppressant agent. Survival data support the use of induction immunosuppression, with significantly improved survival for those receiving induction versus those who do not. The predominant regimen selected for maintenance immunosuppression is tacrolimus, mycophenolate, and prednisone. Interestingly, however, ISHLT data suggest that azathioprine-containing regimens may offer improved survival versus mycophenolate-containing regimens.

Treatment of Rejection

Acute rejection: Diagnostic criteria for lung allograft rejection have been published[46]. Minimal (grade 1) rejection may not require treatment whereas mild (grade 2) or higher warrants treatment. High-dose corticosteroids are the mainstay of treatment for cellular rejection, and most episodes of acute cellular rejection will respond. Common regimens include methylprednisolone 1000 mg IV daily for three days[17,18], methylprednisolone 500 mg IV daily for 3-5 days[8,19,24], or methylprednisolone 10-15 mg/kg IV daily for 3 days(47). Rejection that is resistant to a first course may respond to a second course, or may require escalation to T-cell depleting antibodies like ATG.

Antibody-mediated rejection: A consensus definition for antibody-mediated rejection (AMR) has been published[48]. No randomized clinical trials have evaluated AMR therapies in lung transplantation. Treatment targets include antibodies, B cells, plasma cells, and/or complement. Therapies and their expected benefit are largely borrowed from the kidney transplant literature. The most reliable therapy is plasma exchange and intravenous immunoglobulin (IVIG), though multimodal therapies are needed. Rituximab or obinutuzumab have been used to target B cells. Bortezomib or carfilzomib have been used to target plasma cells. Eculizumab could be used to target damaging complement deposition. It is important to remember that drug interactions exist between these therapies. For example, eculizumab will blunt the efficacy of rituximab and obinutuzumab, and IVIG may blunt the efficacy of bortezomib and carfilzomib.

Hachem, et al observed a cohort of 122 lung transplant recipients; 65 of whom developed de novo DSA after transplant[49]. All patients who developed DSA were treated with IVIG 500 mg/kg IV monthly for six months and rituximab 375 mg/m^2 IV once. Rituximab was held for any patient with chronic leukopenia, a history of recurrent infections, or colonized with multi-drug resistant bacteria. Of the 44 treated with IVIG and rituximab, 61% cleared their DSA. Of

Figure 1. Trends in induction immunosuppression usage for adult patients transplanted in 2004, in 2009, and January-June 2017. This analysis is limited to patients receiving prednisone(45).

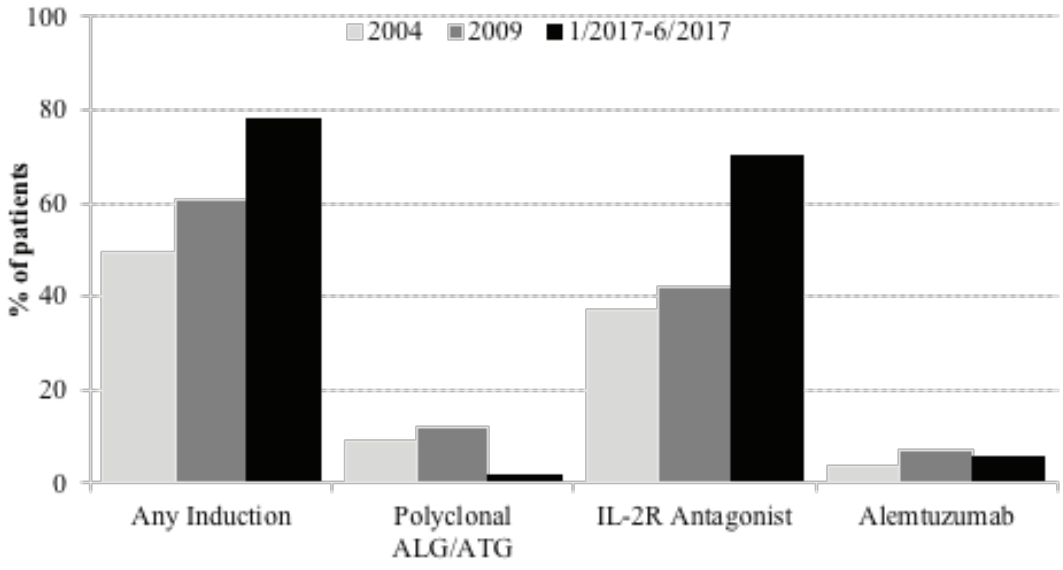


Figure 2. Kaplan-Meier survival by induction immunosuppression usage conditional on survival to 14 days post-transplant, for patients transplanted January 2004 to June 2016 (p <0.0001)[45].

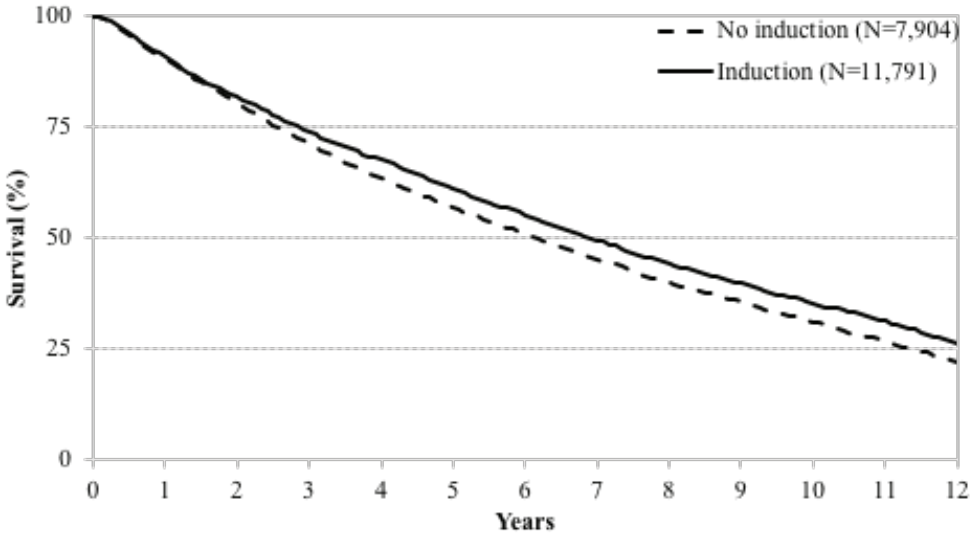
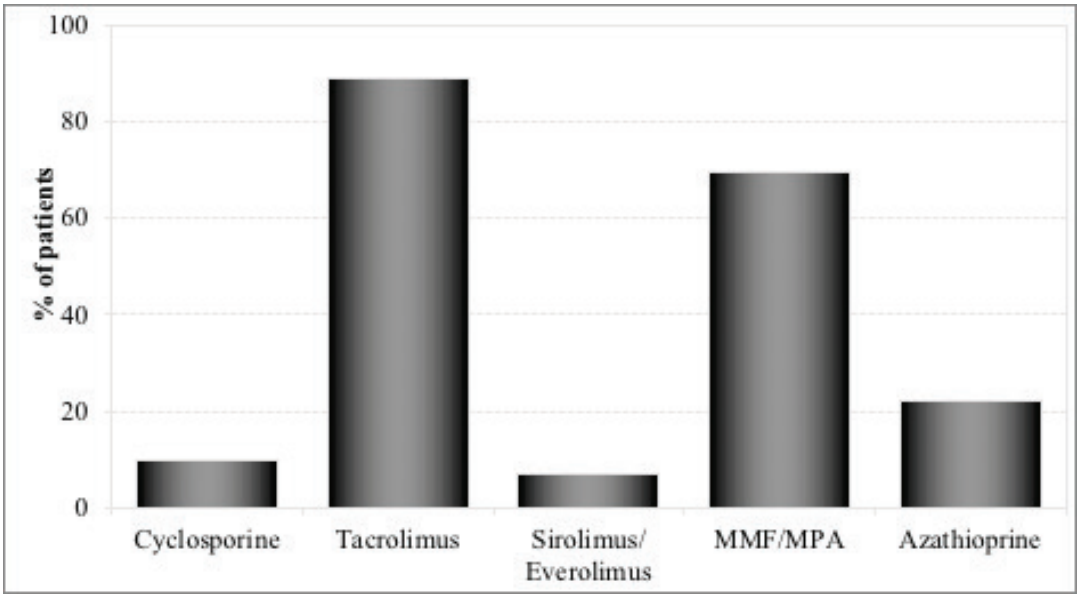


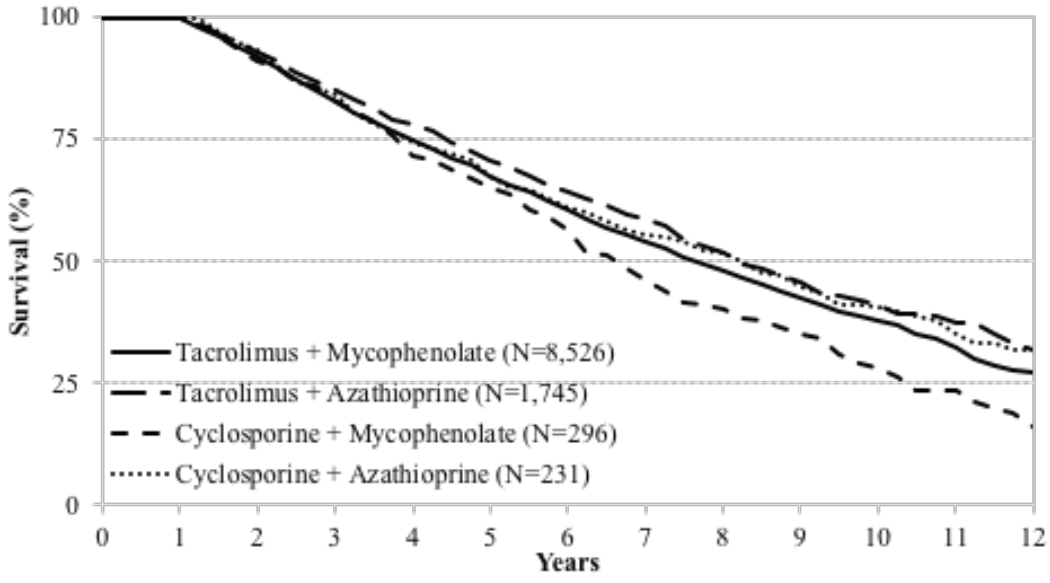
Figure 3. Maintenance immunosuppression at the time of 1-year follow-up, for patients transplanted January 2004 to June 2017 (n= 18.007). This analysis is limited to patients receiving prednisone[45].



the 17 treated with IVIG alone, 65% cleared their DSA. Patients who developed DSA experienced more grade A2 or higher rejection (55% vs. 39%, p= 0.07), but the timing of rejection and DSA development was not consistent. Recipients who had persistent DSA had a significantly worse survival than those who cleared DSA (p< 0.01).

Vacha, et al report on a cohort of 16 lung transplant recipeints who received AMR treatment with a regimen of plasma exchange, corticosteroids, bortezomib, rituximab, and IVIG(50). Three of 11 patients who survived to 6 months (27%) responded to therapy, with response defined as clearance of DSA by 6 months. One-year survival was 56%.

Figure 4. Kaplan-Meier survival by maintenance immunosuppression combinations conditional on survival to 1 year post-transplant, for patients transplanted January 2004 – June 016 (p = 0.0004)[45].



Ensor, et al observe a cohort of 14 lung transplant recipients who received AMR treatment with a regimen of plasma exchange, carfilzomib, and IVIG[51]. Ten (71%) responded to therapy, with response defined as suppressed complement-1q fixing ability. No deaths occurred within 120 days of therapy, but 50% of the patients died of allograft failure by one year after therapy. There was not a significant difference in mortality between patients who did or did not respond to therapy (p= 0.47).

Chronic lung allograft dysfunction: Chronic lung allograft dysfunction (CLAD) is a significant loss in lung function. It can be in the form of BOS, where the total lung capacity is preserved, or in the form of a restrictive allograft syndrome (RAS).

Diagnostic criteria for BOS have been published[52]. BOS occurs in approximately 40-50% of lung transplant recipients within 3 years of transplantation, and the median survival after the diagnosis of BOS is only 3 years[49]. Standard interventions include azithromycin, aggressive antireflux therapy, prokinetic medications if there is evidence of gastroparesis, infectious treatment where applicable, enhance the maintenance immunosuppressive regimen, and photophoresis. Azithromycin 250 mg three times a week has proven benefit in randomized controlled trials for both prevention and treatment of BOS[53-55]. Montelukast 10 mg daily has been evaluated, and may be helpful in recipients with late-onset BOS stage 1[56].

RAS has been described[57] but there are no current therapies, aside from re-transplantation, and this complication portends a particularly poor survival.

Challenges

Highly sensitized candidates: Patients who are sensitized present a unique challenge to transplant centers. It is a challenge to find compatible organs for transplantation. To help manage the pre-transplant antibody, various desensitization regimens for a waitlisted candidate have been attempted, though none with reliable success.

Snyder, et al published an experience with 18 transplant candidates receiving a desensitization regimen while on the transplant waiting list[58]). The regimen consisted of plasmapheresis, methylprednisolone, bortezomib, and rituximab given in combination over 19 days followed by IVIG. Eight of the original 18 candidates completed therapy. The reasons for the ten who did not complete therapy were transplant (n= 3), therapy-related adverse events (n= 5), and non therapy-related event (n= 2). This regimen did not offer any significant change in PRA or cPRA over time.

The Toronto group has published their experience with 53 patients undergoing a novel empirical protocol for sensitized patients receiving a transplant despite a known DSA(59). Patients receive plasma exchange (PLEX) of three volume exchanges intra-

operatively followed by five additional PLEX sessions of 1-1.5 volume exchanges each over the first two weeks post-transplant. IVIG 1g/kg is given after the final PLEX. Thymoglobulin is given at a dose of 3 mg/kg if the cytotoxic cross-match is negative and 5 mg/kg if the cross-match is positive and not reduced with dithiothreitol. All patients receive cyclosporine modified titrated to troughs of 250-350 ng/mL, mycophenolate sodium 360-540 mg twice daily, and prednisone. In this study cohort, 23 of 53 patients received PLEX, IVIG and ATG; 20 received PLEX, IVIG and basiliximab; 6 received PLEX alone. This paper describes longer ICU and hospital length of stay but equivalent rates of PGD 3, CLAD and lower rates of A2 rejection or worse.

Conclusions

In sum, substantial progress has been made in defining optimal immunosuppressive strategies for lung transplant recipients over the past 30 years. However, many clinical challenges remain to be solved. Many of the therapies used in lung transplantation borrow from kidney transplantation. Tested outcomes in the lung transplant populations are not consistent with tested outcomes in the kidney population, though, and illustrate the limitations in applying literature from another organ group to lung transplantation. The need for controlled trials in lung transplantation is most obvious and urgent for belatacept, AMR therapies, and desensitization regimens. Lung transplantation is a life-saving procedure, yet living with a lung transplant can be a challenge. Learning how to best meet those challenges is a large and unfinished research agenda.

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