Laryngeal Transplantation: Research, Clinical Experience, and Future Goals

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ABSTRACT

The loss of a functional voice because of trauma or laryngectomy can have a devastating impact on a patient's self-esteem and overall quality of life. Unfortunately, even with advances in organ preservation therapy, total laryngectomy is frequently necessary in the treatment of laryngeal carcinoma. Over the past several years, the senior author initiated research into laryngeal transplantation with the goal of restoring lung-powered speech for these patients. The research led to the development of an animal model and several groundbreaking studies in this area. Investigations into the use of irradiation, single-drug and multidrug immunosuppression, and the effects of mammalian target of rapamycin (mTOR) inhibitors have produced significant insight into laryngeal allograft preservation. The laboratory research culminated in the first successful total laryngeal transplant in 1998. The patient had suffered significant laryngeal trauma and strongly desired return of laryngeal phonation. The patient has been maintained on multidrug immunosuppression with minimal difficulties. Now more than 8 years after the procedure, the patient continues to have an excellent voice and dramatically improved quality of life. Recent data suggest that altered immunosuppression schedules and the use of mTOR inhibitors may allow patients to minimize immunosuppression-related adverse effects and ameliorate the risk of developing recurrent or de novo carcinoma. These data, when considered in combination with the progress made over the past 14 years, lead us to believe that the future of laryngeal transplantation is bright.

KEYWORDS: Laryngeal allograft, immunosuppression

Since the first successful solid organ transplant in 1954, the field of transplantation has steadily grown. In the years after the first transplant, an increasing number of solid organs have been transplanted with steadily improving survival rates. As a result, patients with chronic liver, renal, pulmonary, and cardiac disease have a potentially lifesaving treatment option as organ failure reaches end-stage. Any morbidity arising from

chronic immunosuppression has been viewed as acceptable risk associated with lifesaving treatment.

The larynx has been classically considered to be nonvital because survival after resection is routine; however, considering the functional and social losses associated with total laryngectomy, the designation of nonvital is relative. Reconstruction of the partially resected larynx has evolved over the past 50 years. Boles

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defined the objectives of laryngeal reconstruction in the 1960s as (1) phonation dependent on pulmonary airflow and vocal cord motion, (2) deglutition without aspiration, and (3) functioning oral and nasal passages enabling olfactory and gustatory sensation.¹ In patients who have undergone total laryngectomy, laryngeal transplantation is the only treatment that can help patients achieve these objectives.

Investigations into laryngeal transplantation began in the 1960s through work by Ogura, Takenouchi, and Silver.²⁻⁴ The work by these authors explored vascular reanastomosis, reimplantation, and canine orthotopic transplantation. This led to a subtotal laryngeal transplant performed by Kluyskens in 1969.⁵ The transplant was subtotal, preserving recipient perichondrium to revascularize the donor organ, and without reinnervation. Rapid recurrence of the extirpated tumor essentially halted investigation into laryngeal transplantation after this initial attempt.

ETHICAL CONSIDERATIONS

Potential ethical issues must be part of any discussion regarding laryngeal transplantation. The most obvious issue regards nonvital organ transplantation in an otherwise healthy individual. The postoperative immunosuppressive regimen is associated with adverse side effects when administered chronically. Prior to making ethical considerations, however, an understanding of purpose is required. Laryngeal transplantation aims to attain serviceable speech, swallowing, and cosmesis with minimal aspiration. Potential candidates must be counseled to weigh these potential benefits against the risks of graft rejection, chronic immunosuppression-associated morbidity, and de novo or recurrent carcinoma.

Many head and neck surgeons would agree that some patients, when faced with the prospect of laryngectomy, would prefer suboptimal treatment or even death to preserve their larynx. McNeil and colleagues interviewed 37 healthy firefighters and executives. When given the options of laryngectomy or radiation therapy for advanced laryngeal cancer, 20% chose radiation therapy despite being informed of a 20% improvement in survival with laryngectomy.⁶ The possibility of reversing the negative functional and aesthetic effects of laryngectomy has encouraged a significant percentage of postlaryngectomy patients to indicate a willingness to undergo laryngeal transplant with lifelong immunosuppression for the opportunity of obtaining a new larynx.⁸ These data strongly suggest that patients recognize the morbidity associated with laryngectomy and wish to avoid it. To do so, many will consider treatment alterations that could result in decreased survival. Furthermore, laryngeal transplantation is a desirable option in the eyes of patients who have undergone laryngectomy to achieve a significant improvement in quality of life.

LABORATORY STUDIES

The most important step in laryngeal transplantation research was the development of a rat model of vascularized laryngeal allograft.⁷ The rat model of laryngeal transplantation has allowed us to perform numerous studies aimed at understanding optimal immune suppression for laryngeal allografts. The technique has been published in detail elsewhere, but a brief description will be included here.⁶ The donor rat's neck is exposed and the strap muscles are divided. Both carotid sheaths are opened, and the internal and external carotid arteries are isolated. The external and internal carotid arteries are ligated bilaterally superiorly leaving the larynx pedicled only on the superior thyroid arteries. The esophagus and trachea are divided inferiorly, and a similar transection is performed above the thyroid cartilage. The proximal carotid arteries are divided after clamping and the composite graft containing the larynx, pharynx, thyroid, and parathyroid glands is removed.

The recipient rat's neck is then exposed in a similar fashion. The left common carotid artery is exposed and anastomosed in an end-to-side fashion with the donor right common carotid artery. The donor left common carotid artery is anastomosed in an end-to-side fashion. This allows blood to flow through the allograft via arteriovenous shunt such that the drainage is accomplished through the left carotid artery of the graft. The skin is then closed, and the animal is allowed to awaken from anesthesia.

This model has allowed us to successfully transplant more than 2000 animals and treat them with varying immunosuppressive protocols while examining the donor larynges for rejection at the time of sacrifice. A further advance in our technique came with the use of parathyroid hormone as a marker for graft viability. To accomplish this, a complete parathyroidectomy is performed in the recipient such that the only functioning glands are contained in the transplanted allograft. Weekly Parathyroid Hormone (PTH) assays allow the detection of rejection without histologic examination of the graft.

Having created an animal model that allows evaluation of immunosuppressive regimens on a transplanted larynx, we have performed several studies aimed at inducing tolerance.

Irradiation of the Murine Transplanted Larynx

Although irradiation of donor organs is known to decrease acute rejection, defining the most efficacious regimen has been difficult. Using the rat laryngeal transplant model, we performed a prospective study including 16 treatment arms using various techniques of irradiation including (1) recipient animal pretransplant irradiation at various time points, (2) donor larynx in vivo irradiation at various time points prior to harvest and transplantation, (3) irradiation of both the donor organ

in vitro and to the recipient animal at various time points prior to transplantation, and (4) in vitro irradiation of the donor larynx immediately prior to transplantation.⁹

The larynges were harvested 15 days after transplantation and sectioned for histologic analysis. Rejection scoring of these animals demonstrated no significant difference between any of the radiation-treated animals in the study; however, all of the radiation-treated animals demonstrated less acute rejection than the untreated controls. These data suggested that, in the clinical realm, irradiation of the donor larynx immediately prior to transplantation would provide as much benefit as radiation several days prior to transplant. Of note, this study also demonstrated that there is no significant deleterious effect to the transplanted organ when radiation is given. The latter was evaluated by comparing irradiated on nonirradiated isogenic animals.

Efficacy of Cyclosporine with and without Steroids and Radiation

Since its introduction in 1972, cyclosporine has developed into one of the cornerstones of immunosuppressive therapy. Whereas azathioprine inhibits cell division of all elements of the immune system, cyclosporine selectively alters adaptive immune responses by inhibiting activated T lymphocytes and preventing them from manufacturing or releasing IL-2.

Unfortunately, cyclosporine is not without toxicities including hepatotoxicity, neurologic effects, and, most commonly, nephrotoxicity. In almost all patients, cyclosporine causes a 20% reduction in renal function that can progress to fulminant renal failure.¹⁰ Cyclosporine-induced nephrotoxicity can occur at any dose but is less common at lower doses.¹¹ Starzl demonstrated that combining steroids with cyclosporine allowed lower doses of cyclosporine.^{12,13}

We therefore hypothesized that combination therapy of cyclosporine and prednisone would allow for lower cyclosporine doses in rat laryngeal transplantation. Using our established rat laryngeal transplant model, cyclosporine was administered alone, in combination with prednisone and with in vitro irradiation of the harvested larynx. Cyclosporine was given at two different low dosages: $1.5 \text{ mg kg}^{-1} \text{ day}^{-1}$ and $2.0 \text{ mg kg}^{-1} \text{ day}^{-1}$. Although there was no significant difference noted at 15 days posttransplant, animals treated with a combination of cyclosporine ($2 \text{ mg kg}^{-1} \text{ day}^{-1}$) and prednisone ($1 \text{ mg kg}^{-1} \text{ day}^{-1}$) showed the least rejection at 30 days posttransplant.¹⁴ Rejection rates were significantly higher in animals treated with cyclosporine ($2 \text{ mg kg}^{-1} \text{ day}^{-1}$) alone. The addition of radiation to these medications did not result in decreased rejection. As will be discussed later in this chapter, the results of this study mirror what we have seen in our human laryngeal transplant patient.

Combination Therapy with Mycophenolate Mofetil and Tacrolimus

The success of cyclosporine in solid organ transplantation took a leap forward with the development of tacrolimus in the late 1980s. Although tacrolimus is also a calcineurin inhibitor, the two medications have distinctly different initial intracellular targets. Whereas multiple studies had demonstrated the efficacy of cyclosporine, tacrolimus has shown improved prevention of acute rejection.¹⁵ Still, tacrolimus possesses a side-effect profile similar to that of cyclosporine. Again, combination therapy with this medication would seem to be advantageous in that it would enable a lowering of the necessary dose of tacrolimus.

Mycophenolate mofetil (MMF) is an ideal medication for use in combination with tacrolimus in that it possesses a different mechanism of action and a side-effect profile that does not overlap with that of calcineurin inhibitors. MMF is an inhibitor of the purine synthesis pathway used selectively during lymphocyte activation.¹⁶ The combination of tacrolimus and MMF immunosuppression has shown success in kidney, liver, heart, and lung transplantation.^{17–20}

To study the combination of tacrolimus and MMF in rat laryngeal transplantation, we designed a study with several different arms combining tacrolimus at 0.1 mg/kg or 0.2 mg/kg with MMF at 15, 30, or 40 mg/kg.²¹ Also included were animals treated with 0.1, 0.2, 0.3, and 0.6 mg/kg tacrolimus alone. Results of this study showed a significant association between increasing dose of tacrolimus and decreasing rejection scores at 15 and 30 days posttransplant. This difference was more pronounced at 30 days posttransplant compared with 15 days posttransplant. This study provided support for the use of tacrolimus and MMF in the clinical realm as a means of minimizing side effects while preventing acute rejection.

Studies Using Pulsed Immunosuppression

More recently, we have performed investigations into the use of intermittent or “pulsed” immunosuppression. These studies resulted from efforts to further reduce the potential adverse effects associated with chronic immunosuppression. By administering high-dose immunosuppression perioperatively, we hoped to be able to significantly space the time between immunosuppressive doses. Akst et al administered tacrolimus (1.2 mg/kg) and anti- $\alpha\beta$ -T-cell receptor (TCR) to recipients beginning 12 hours prior to transplant through 5 days posttransplant.²² All animals were sacrificed at 100 days posttransplant. Histologic analysis showed allograft preservation, but studies designed to detect true tolerance were negative.

A second study took this approach further. A similar dosing schedule was used but everolimus, a

rapamycin analogue, was substituted for tacrolimus.²³ Everolimus, like rapamycin, acts on mammalian target of rapamycin (mTOR), inhibiting the cellular response to positive cytokine signaling by inhibiting the translation of messenger RNA critical for cell cycle progression. This acts to delay T cell-dependent acute rejection. Compared with rapamycin, everolimus has a shorter half-life and better bioavailability. In this study, animals were treated with 7 days of perioperative treatment consisting of everolimus and anti- $\alpha\beta$ -TCR. At 90 days posttransplant, animals were given a pulse of treatment for 5 days using the same medications. Thereafter, animals were given (1) no treatment or (2) daily 1.0 mg/kg everolimus or (3) daily 2.5 mg/kg everolimus. Animals were sacrificed at 180 days posttransplant. Those animals receiving daily treatment between 90 and 180 days evidenced no rejection, and those who were untreated for that time period showed, at most, mild chronic rejection. These data established several important points. First, the animals responded to a pulse at 90 days, which was demonstrated by a decreased in T-cell count. This alleviated a theoretical concern that the animals would quickly become tachyphylactic to the anti- $\alpha\beta$ -TCR monoclonal antibody. Second, the animals treated with daily everolimus between 90 and 180 days demonstrated that everolimus may potentially be used as low-dose monotherapy in laryngeal transplantation. This would significantly lower the risk for adverse reactions. Lastly, the animals that were untreated after 90 days were near normal and therefore provide hope for success with multiple pulses every 90 days.

Antitumor Effects of Everolimus

The anticancer effects that have been demonstrated in studies on rapamycin have been preliminarily identified in everolimus. As discussed above, everolimus inhibits mTOR resulting in blocking of the translation of mRNA of critical cell cycle regulatory proteins. This leaves cells unable to progress from the G₁ phase to the S phase of the cell cycle.^{24,25} Partial characterization of the downstream effects of RAD have shown that RAD leads to decreased activity of p70^{S6} kinase (S6K1) and eukaryotic initiation factor 4E (eIF-4E)-phosphorylatable heat stable protein I (PHAS-I).²⁶ Upstream of mTOR, RAD inhibits the IL-2 and CD28 signaling pathways. This inhibition leads to several important immunologic effects, including (1) effector functions of CD4⁺ and CD8⁺ cells, (2) monocyte activation, and (3) proliferation and differentiation of B cells.^{24,27,28} An important property of RAD is its synergistic effects with calcineurin inhibitors which block transition from G₀ to G₁.²⁶

In recent years, the immunosuppressive agent rapamycin has shown antitumor properties.²⁹⁻³¹ Considering that most potential candidates for laryngeal

transplantation are those patients who have been treated for carcinoma, this property of mTOR inhibitors is of great interest. This is especially true in light of the volumes of data demonstrating tumor potentiation by most conventional immunosuppressants.³² We noted that the antitumor effects of mTOR inhibitors had not been demonstrated in a model of squamous cell carcinoma.

Therefore, we performed a mouse study utilizing a spontaneously occurring murine squamous cell carcinoma (SCC VII). We were interested in the effects of everolimus with regard to local tumor spread as well as distant disease. To study this, mice were inoculated with tumor cells intradermally as well as intravenously. The latter reliably results in pulmonary metastases, whereas the former leads to local disease only.³³ We then treated mice with everolimus (0.5 mg/kg or 1.0 mg/kg), cyclosporine (7.5 mg/kg), or no treatment. The animals were sacrificed at predetermined end points and examined for tumor growth and spread. Our results demonstrated significant tumor inhibition in animals treated with everolimus. This effect trended toward dose-dependence. The antitumor effects resulted in an ~50% decrease in local tumor growth as well as distant spread (Figs. 1 and 2).³⁴

This data has strong implications for the future of laryngeal transplantation. Laryngeal transplantation in patients treated for malignancy would carry a high risk of recurrent or second malignancy with conventional steroid or calcineurin inhibitor immunosuppression. Everolimus, however, provides excellent immunosuppression, favorable physicochemical properties, and potent tumor inhibition. These properties make it ideal for use in laryngeal transplantation. Furthermore, recent data suggest that everolimus may be used in low-dose combination with tacrolimus or as single-agent immunosuppression with excellent laryngeal allograft preservation.^{23,35}

THE HUMAN LARYNGEAL TRANSPLANT

The recipient was a 40-year-old man who had suffered a crush injury to his larynx and pharynx during a motorcycle accident 20 years earlier. Despite multiple attempts at another institution to reconstruct his larynx, he remained aphonic and tracheostoma dependent. The patient underwent extensive pretransplant counseling including psychiatric evaluation, speech pathology testing, and four interviews with members of the surgical team. All of those involved agreed that the patient understood the risks and his motivation was appropriate. The procedure was approved by the Institutional Review Board of the Cleveland Clinic Foundation. After a 6-month search, a 40-year-old man who was brain dead from a ruptured cerebral aneurysm was identified as a suitable donor. He met all of the predetermined

Figure 1 Mice after intradermal injection of squamous cell carcinoma: (A) untreated and (B) treated with everolimus.

criteria for acceptance with regard to HLA matching and serum virology.

During the donor organ harvest, the entire pharyngolaryngeal complex, including six tracheal rings and the thyroid and parathyroid glands, were removed (Fig. 3). The organ complex was perfused with University of Wisconsin solution during transport until revascularization 10 hours later. Prior to surgery, the recipient patient received cyclosporine, azathioprine, and methylprednisolone. After surgical exposure of the patient's severely deformed laryngeal

structures but prior to their removal, perfusion to the donor organ was reestablished. The donor's right superior thyroid artery was anastomosed to that of the patient, and the proximal end of the donor's right internal jugular vein was anastomosed to the patient's right common facial vein. Blood flow through the transplanted thyroid gland, six tracheal rings, larynx, and pharynx was observed within 30 minutes of clamp release.

A narrow field laryngectomy was performed leaving the thyroid lobes lateralized and the hyoid bone in

Figure 2 Mouse lungs after intravenous injection of squamous cell carcinoma: (A) untreated and (B) treated with everolimus.

Figure 3 Schematic of laryngeal transplantation microvascular anastomoses and suture lines.

place. Seventy-five percent of the donor's pharynx was used to widen the patient's stenotic pharyngo–upper esophageal complex. The donor laryngeal cartilage was sutured to the hyoid bone for laryngeal elevation. Five tracheal rings were needed to reach the patient's tracheostoma. The left-sided anastomoses, which included the donor superior thyroid artery to the recipient superior thyroid artery and the donor middle thyroid donor vein to the recipient internal jugular vein, were then completed. Both superior laryngeal nerves were located and reanastomosed, but only the recipient's right recurrent laryngeal nerve could be located for reinnervation.

In the immediate postoperative period, the patient was maintained on muromonab-CD3, cyclosporine, methylprednisolone, and MMF. Initial aspiration was controlled with glycopyrrolate and atropine, which were later discontinued. At the end of a 1-month period of observation in the hospital, the patient's transplanted trachea was normal on both endoscopy and biopsy. Fifteen months posttransplant, the patient experienced an episode of rejection that presented as a decrease in voice quality. After 3 daily doses of methylprednisolone 1 g/day, his larynx returned to normal. The patient is now more than 8.5 years posttransplant and is maintained on 7.5 mg prednisone per day, 1 g MMF daily, and 3 mg tacrolimus per day with stable blood pressure and renal function. A second episode of rejection occurred 6 years after transplant due to laboratory error in tacrolimus values measuring levels falsely high, which resulted in decreasing the patient's medication below therapeutic levels. Laryngeal edema quickly resolved once medication levels returned to the therapeutic range.

Three months posttransplant, the supraglottis and vocal folds were sensitive to touch and purposeful swallowing returned. Subsequent barium swallows revealed no aspiration, and the patient's sense of taste and smell have returned. The patient did experience three early episodes of tracheobronchitis that were successfully

treated with oral amoxicillin clavulanate. At 16 weeks posttransplant, the patient inadvertently stopped his trimethoprim-sulfamethoxazole and developed *Pneumocystis carinii* pneumonia, which cleared rapidly with intravenous antibiotics. To evaluate thyroid function, a 4-hour uptake of iodine-123 demonstrated 83% activity in the transplanted thyroid lobes and 17% in the patient's native thyroid. Thyroid function tests, serum calcium, and phosphate all remain within normal ranges.

The patient's first posttransplant voicing was on postoperative day 3. At 1 month, both true vocal folds were lateral, creating a breathy voice. By 4 months, the right fold (the side of the recurrent nerve anastomosis) was midline and at 6 months the left was paramedian. Recent electromyographic (EMG) measurements have confirmed reinnervation of both folds; we believe that the left is supplied by surrounding motor nerves or "field-reinnervation."³⁶ Volitional cricothyroid function has been confirmed by EMG as well (Fig. 4). Subjective and objective measures of phonation including pitch, jitter, intensity, and maximal phonation time were within the normal range at 36 months posttransplant. The patient has become a motivational speaker and reports that his quality of life has improved "immeasurably" now more than 8 years after transplantation. Laser cordotomy or sling tracheoplasty remain options for stomal management, which the patient continues to decline.³⁴ Of significance, this patient is, to our knowledge, the longest functioning transplant recipient after a first-time transplant followed by immunosuppression.

Currently, we believe that the outlook for laryngeal transplantation is excellent. With one human transplant performed successfully and research-based advances in the interim, we are highly optimistic of future successes. As this life-altering treatment advances, we hope to offer dramatic quality of life improvements for patients facing the difficulties of social integration after total laryngectomy.

Figure 4 EMG tracings of (A) resting and (B) volitional cricothyroid (CT) and thyroarytenoid (TA) muscles in the transplanted larynx.

REFERENCES

- Boles R. Surgical reimplantation of the larynx in dogs: a progress report. *Laryngoscope* 1966;76:1057-1067
- Ogura JH, Kawasaki M, Takenouchi S, et al. Replantation and Transplantation of the Canine Larynx. *Ann Otol Rhinol Laryngol* 1966;75:295-312
- Silver CE, Liebert PS, Som ML. Autologous transplantation of the canine larynx. *Arch Otolaryngol* 1967;86:95-102
- Takenouchi S, Ogura JH, Kawasaki M, et al. Autogenous transplantation of the canine larynx. *Laryngoscope* 1967;7:1644
- Kluyskens P, Ringoir S. Follow up of a human larynx transplantation. *Laryngoscope* 1970;80:1244-1250
- McNeil BJ, Weichselbaum R, Pauker S. Speech and survival. *N Engl J Med* 1981;305:982-987
- Strome S, Sloman-Moll E, Samonte B, Wu J, Strome M. A rat model for a vascularized laryngeal allograft. *Ann Otol Rhinol Laryngol* 1992;101:950-953
- Potter CP, Birchall MA. Laryngectomy's views on laryngeal transplantation. *Transpl Int* 1998;11:433-438
- Lorenz RR, Dan O, Fritz MA, et al. Immunosuppressive effect of irradiation in the murine laryngeal transplantation model: a controlled trial. *Ann Otol Rhinol Laryngol* 2003;112:712-715
- Lewis RM, Janney RP, Golden DL, et al. Stability of renal allograft function associated with long-term cyclosporine immunosuppressive therapy-five year follow-up. *Transplantation* 1989;47:266-272
- Ferguson RM, Rynasiewicz JJ, Sutherland DE, et al. Cyclosporin A in renal transplantation: a prospective randomized trial. *Surgery* 1982;92:175-182
- Starzl TE, Weil RIII, Iwatsuki S, et al. The use of cyclosporin A and prednisone in cadaver kidney transplantation. *Surg Gynecol Obstet* 1980;151:17-26
- Starzl TE, Klintmalm GBG, Weil RIII, et al. Cyclosporin A and steroid therapy in sixty-six cadaver kidney recipients. *Surg Gynecol Obstet* 1981;153:486-494
- Lorenz R, Dan O, Huang M, Strome M. Effects of adding steroids, in vitro irradiation, or both to cyclosporine immunosuppression in the murine laryngeal transplantation model. *Ann Otol Rhinol Laryngol* 2002;111:455-459
- U.S. Multicenter Liver Study Group. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression in liver transplantation. *N Engl J Med* 1994;331:1110-1115
- Suthanthiran M, Strom T. Immunobiology and immunopharmacology of organ allograft rejection. *J Clin Immunol* 1995;15:161-171
- Ahsan N, Johnson C, Gonwa T, et al. Randomized trial of tacrolimus plus mycophenolate mofetil or azathioprine versus cyclosporine oral solution (modified) plus mycophenolate mofetil after cadaveric kidney transplantation: results at 2 years. *Transplantation* 2001;72:245-250
- Eckoff DE, McGuire B, Frenette L, et al. Tacrolimus (FK506) and mycophenolate mofetil combination therapy vs tacrolimus in adult liver transplantation. *Transplantation* 1998;65:180-187
- Hunt SA. New immunosuppressive agents in clinical use: mycophenolate mofetil and tacrolimus. *Cardiol Rev* 2000;8:180-184
- Reichenspurner H, Kur F, Treede H, et al. Optimization of an immunosuppressive protocol after lung transplantation. *Transplantation* 1999;68:67-71
- Nelson M, Fritz M, Dan O, et al. Tacrolimus and mycophenolate mofetil provide effective immunosuppression in rat laryngeal transplantation. *Laryngoscope* 2003;113:1308-1313
- Akst LM, Siemionow M, Dan O, et al. Induction of tolerance in a rat model of laryngeal transplantation. *Transplantation* 2003;76:1763-1770
- Khariwala SS, Dan O, Lorenz R, et al. Pulsed immunosuppression with everolimus and anti- $\alpha\beta$ T-cell receptor: laryngeal allograft preservation at six months. *Ann Otol Rhinol Laryngol* 2006;115:74-80
- Schuler W, Sedrani R, Cottens S, et al. SDZ RAD, a new rapamycin derivative: pharmacological properties in vitro and in vivo. *Transplantation* 1997;64:36-42
- Kovarik JM, Kaplan B, Tedesco Silva H, et al. Exposure-response relationships for everolimus in de novo kidney transplantation: defining a therapeutic range. *Transplantation* 2002;73:920-925
- Neuhaus P, Klupp J, Langrehr JM. mTor inhibitors: an overview. *Liver Transpl* 2001;7:473-484
- Abraham RT, Wiederrecht GJ. Immunopharmacology of rapamycin. *Annu Rev Immunol* 1996;14:483-510
- Schmidbauer G, Hancock WW, Wasowska B, et al. Abrogation by rapamycin of accelerated rejection in sensitized rats by inhibition of alloantibody responses and selective suppression of intragraft mononuclear and endothelial cell activation, cytokine production and cell adhesion. *Transplantation* 1994;57:933-941
- Huang S, Houghton PJ. Inhibitors of mammalian target of rapamycin as novel antitumor agents: from bench to clinic. *Curr Opin Investig Drugs* 2002;3:295-304
- Muthukkumar S, Ramesh TM, Bondada S. Rapamycin, a potent immunosuppressive drug, causes programmed cell death in B lymphoma cells. *Transplantation* 1995;60:264-270
- Seufferlein T, Rozengurt E. Rapamycin inhibits constitutive p70^{s6k} phosphorylation, cell proliferation, and colony formation in small cell lung cancer cells. *Cancer Res* 1996;56:3895-3897
- Penn I. Occurrence of cancers in immunosuppressed organ transplant recipients. *Clin Transplant* 1998;147-158
- Khurana D, Martin EA, Kasperbauer JL, et al. Characterization of a spontaneously arising murine squamous cell carcinoma (SCC VII) as a prerequisite for head and neck cancer immunotherapy. *Head Neck* 2001;23:899-906
- Khariwala SS, Kjaergaard J, Lorenz R, et al. Everolimus (RAD) inhibits the growth of murine squamous cell carcinoma (SCC VII). *Laryngoscope* 2006;116:814-820
- Knott PD, Nelson M, Dan O, Van Lente F, Strome M. Immunosuppressive efficacy of everolimus and tacrolimus combination therapy in a rat laryngeal transplant model. (Submitted)
- Lorenz RR, Hicks DM, Shields RW, Fritz MA, Strome M. Laryngeal nerve function after total laryngeal transplantation. *Otolaryngol Head Neck Surg* 2004;131:1016-1018