Curriculum Vitae

Marniker Alex Wijesinha, MS, PhD
Doctoral Student, Department of Epidemiology and Public Health
University of Maryland, Baltimore

Date December 18, 2018

Contact Information

Email: mwijesinha@umaryland.edu , mwijesinha@gmail.com
Foreign languages: Spanish (working knowledge)

Education

2012-2018 Ph.D. in Epidemiology: University of Maryland Baltimore

2012-2014 M.S. in Epidemiology: University of Maryland Baltimore

2007-2011 B.S. in Bioinformatics (with minor in Applied Statistics): University of Pittsburgh *(Magna cum Laude)*

Employment History

Graduate Research Assistantship at University of Maryland Baltimore (20 hours/week):

2012-2014 Department of Epidemiology and Public Health

- SAS programming and statistical analysis for JHU Lupus cohort data and University of Maryland renal transplant cohort data
- SAS programming and statistical analysis to assess the association between known genetic variants in the Amish and certain phenotypic traits, such as lipid and micronutrient levels

2014-2015 Epidemiology of Aging Training Program

- Analyses in SAS, Excel, and R including: HAPI and AFCS study participant recollection of health information conveyed at study visits, association between Vitamin D and Insulin Resistance, and heritability of metabolites

2015-present Assistantship supported by mentors Aldo Iacono, MD and Michael Terrin, MD

- Data analysis in SAS for various lung transplant research projects in Dr. Iacono's group, with statistical and scientific advice contributed by Dr. Terrin
- Design, management, and weekly updating of a Microsoft Access relational database consisting of clinical data on UMMC lung transplant recipients who are current patients of Dr. Iacono
- Design, management, and weekly updating of a Microsoft Access relational database consisting of clinical data on recipients of

Extracorporeal Membrane Oxygenation (ECMO) at UMMC who are current patients of Dr. Iacono
- Data entry and organization of forms documenting Serious Adverse Events (SAEs) in the Non-Invasive Treatment of Abdominal Aortic Aneurysm Clinical Trial (N-TA^{3}CT), for Dr. Terrin

Honors and Awards

2001-2002	Scored #2 regionally in Pennsylvania Mathematics League Competition
2002-2003	Scored #2 regionally in Pennsylvania Mathematics League Competition
2003	Selected for JHU Center for Talented Youth Study of Exceptional Talent, Math
2006	National Merit Commended Scholar
2006	Pennsylvania Governor's School of Excellence for Health Care

Teaching Service

Teaching Assistant at University of Maryland Baltimore:

2013	Principles of Epidemiology (PREV 600) Course led by Drs. Mona Baumgarten and Nancy Ellish
2013	Principles of Biostatistics (PREV 620) Course led by Dr. Clayton Brown
2014	Research Practicum I (PREV 747) Course led by Drs. Surbhi Leekha, Laura Hungerford, and John Sorkin
2014	Biostatistical Methods (PREV 621) Course led by Drs. Olga Goloubeva and Larry Magder
2015	Genetic Epidemiology (PREV 711) Course led by Dr. Laura Yerges-Armstrong
2016, 2017	Biostatistical Methods (PREV 621) Course led by Dr. Olga Goloubeva
2018	Clinical Trials and Experimental Epidemiology (PREV 803) Course led by Dr. Michael Terrin

Publications

Peer-reviewed journal articles:

Lamos EM, Wijesinha MA, Ramhmdani S, Magder LS, Silver KD. **Role of glycemic control on hospital-related outcomes in patients with diabetes mellitus undergoing renal transplantation.** *Diabetes Therapy. 2017:10 13–17*

Jeffrey S. Wolf, Andrea Hebert, Pablo Sanchez, Marniker Wijesinha, Scott Shapiro, Robert Morales, Aldo T. Iacono. **Intrabronchial Catheter Resuscitation for Respiratory and Cardiorespiratory Arrest.** *Shock* (Augusta, Ga.). United States, Sept. 14, 2017. ISSN: 1540-0514.

Abstracts:

I. Timofte, M. Terrin, M. Wijesinha, P. Sanchez, R. Pierson, E. Barr, J. Kim, Z. Kon, S. Pham, A. Iacono. **Pulmonary Hypertension in COPD and IPF: a Survival Analysis of Patients Listed for Lung Transplantation.** *ISHLT 36th Annual Meeting and Scientific Sessions*. April 27-30, 2016, Washington, DC.

J. Kim, D. Kukuruga, M. Wijesinha, I. Timofte, A. Iacono, S. Pham. **A Case of Accelerated Antibody Mediated Rejection in the Modern Age of Single Antigen HLA Antibody and C1q Assays.** *ISHLT 36th Annual Meeting and Scientific Sessions*. April 27-30, 2016, Washington, DC.

J. Kim, E. Barr, M. Terrin, I. Timofte, P. Sanchez, Z. Kon, R. Pierson, M. Wijesinha, S. Pham, A. Iacono. **Change in Body Mass Index at Listing from Primary Transplant to Re-Transplantation Predicts Re-Transplant Survival.** *ISHLT 36th Annual Meeting and Scientific Sessions*. April 27-30, 2016, Washington, DC.

I. Timofte, M. Terrin, M. Wijesinha, P. Sanchez, R. Pierson, E. Barr, J. Kim, Z. Kon, S. Pham, A. Iacono. **6 Minute Walk Test and FVC Analysis: A New Method to Prioritize Organ Allocation for Patients with COPD.** *2016 American Transplant Congress (ATC).* June 11-15, 2016, Boston, MA.

A. Iacono, M. Wijesinha, K. Rajagopal, N. Murdock, T. Hughes, S. McGee, I. Timofte, J. Kim, J. Rinaldi, M. Terrin. **Stabilization of Lung Function and Survival Improvement By Aerosolized Liposomal Cyclosporine A (L-CsA) for Bronchiolitis Obliterans Syndrome.** *ISHLT 38th Annual Meeting and Scientific Sessions*. April 11-14, 2018, Nice, France.

I. Timofte, M. Wijesinha, R. Vesselinov, J. Kim, Z. Kon, R. Reed, K. Rajagopal, S. Scharf, R. Wise, A. Sternberg, D. Kaczorowski, B. Griffith, M. Terrin, A. Iacono. **Lower six minute walk distance or FEV1 predict a survival benefit of lung transplantation compared to medical management for end stage COPD.** *ISHLT 38th Annual Meeting and Scientific Sessions*. April 11-14, 2018, Nice, France.

M. Wijesinha, J. Hirshon, M. Terrin, L. Magder, C. Brown, K. Stafford, A. Iacono. **Sirolimus + Tacrolimus Maintenance with No Induction Therapy May Maximize Survival in Lung Transplant Recipients.** *ISHLT 39th Annual Meeting and Scientific Sessions*. April 3-6, 2019, Orlando, FL.

Abstract

Dissertation Title: Immunosuppressive Drug Regimens that May Help Improve Survival and Reduce the Risks of Rejection, Infection, and Malignancy after a Lung Transplant

Marniker Wijesinha, Doctor of Philosophy, 2018

Dissertation Directed By:

Jon Mark Hirshon, MD, MPH, PhD

Professor

Dept. of Emergency Medicine, Dept. of Epidemiology & Public Health

Background: Median survival among lung transplant recipients is below 6 years, and there is minimal knowledge regarding modifiable factors that may help improve long-term survival. Identifying induction and maintenance immunosuppressive regimens associated with optimal survival can potentially improve outcomes.

Methods: We classified lung transplant recipients in the United States from 2003-2016 according to their induction and prophylactic cell cycle inhibitor maintenance therapies, within a tacrolimus-based regimen. We compared the different therapies via multivariable Cox Proportional Hazards models, generating adjusted hazard ratios for death, rejection, infection, and malignancy, the latter three of which utilized semi-competing risks methods. Since prophylactic sirolimus initiation is delayed by up to 1 year post-transplant, adjustments were made to avoid immortal time bias. Multiple imputation was utilized to handle missing data.

Results: Sirolimus had the best survival among cell cycle inhibitor maintenance therapies: adjusted Hazard Ratio (HR)=0.71, p=0.003, compared to mycophenolate mofetil [MMF]; chronic rejection incidence was also reduced with sirolimus (HR=0.75, p=0.005). Azathioprine also had slightly better survival than MMF (HR=0.92, p=0.05), and reduced infection incidence (HR=0.62, p<0.0001). Among induction therapies, equine ATG had the best survival: HR=0.79, p=0.003, compared to no induction, as well as reduced rejection (HR=0.75, p=0.02) and infection (HR=0.57, p=0.008) incidence. The combination of induction and maintenance therapies associated with the most favorable survival was sirolimus + tacrolimus maintenance with no induction; HR=0.48, p=0.002 compared to MMF + tacrolimus with induction, and HR=0.41, p<0.0001 compared to MMF + tacrolimus with no induction.

Conclusions: Sirolimus initiated in the first year within a tacrolimus-based regimen may significantly improve long-term survival compared to MMF in lung transplant recipients. Out of all combinations of maintenance and induction therapies studied, sirolimus + tacrolimus maintenance with no induction therapy was associated with the best survival. In patients whom sirolimus cannot be utilized for any reason, azathioprine may modestly improve survival compared to MMF. Additional long-term studies in lung transplantation are needed to confirm these findings.

Immunosuppressive Drug Regimens that May Help Improve Survival and Reduce the Risks of Rejection, Infection, and Malignancy after a Lung Transplant

By
Marniker Wijesinha

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2018

DEDICATION

I dedicate this dissertation to my parents, Manel and Alexander Wijesinha, who have unwaveringly and unconditionally given me maximal love and support, and without whom this dissertation work would never have been possible. I believe this dissertation work became what it is, particularly as far as its focus towards helping patients, because of my parents instilling in me from a young age the importance of doing all you can to help others, and their leading by example in this regard.

ACKNOWLEDGEMENTS

I would like to acknowledge the invaluable input of each one of my dissertation committee members towards this study and also towards my growth as a researcher. To start, I am deeply appreciative of my committee chair, Dr. Jon Mark Hirshon, first of all for his willingness to serve in this role on a project in a new area in spite of his busy schedule, and for his strong mentorship throughout the progression of my dissertation work. I will always remember, among other things, his reminders not to "let the best be the enemy of the good," which were instrumental in helping me to be continuously productive and to deliver the highest quality of work that I could without spending excessive time on certain details.

I am exceedingly grateful to Dr. Aldo Iacono for his generosity in supporting my Graduate Research Assistantship for an extended period of time, and for making me easily become interested in the field of lung transplantation which was brand new to me. Dr. Iacono consistently made this project and every other project I worked with him on a great pleasure and an outstanding learning experience for me, due to his dedication towards excellence in patient care and research, his tremendous expertise, and yet his very open-minded nature on the multiple occasions when our studies led to findings that appear to challenge existing beliefs and paradigms in his field.

I owe tremendous gratitude to Dr. Michael Terrin, first for believing in me at a critical time and going far out of his way to help me just after I got to know him, and for providing steadfast support and mentorship ever since. He was the first person to make this dissertation possible, as he is the one who connected me with Dr. Iacono, and throughout

the course of this project (and other projects that I've had the privilege to work with him on), Dr. Terrin always gave thoughtful feedback and asked profound questions that would lead to an improved product. I also greatly appreciate the indispensable contributions of Dr. Laurence Magder and Dr. Clayton Brown, who in addition to solidifying the analytical depth of my project, were always ready and willing to discuss intricate statistical matters whenever I would stop by their offices, and immediately provided excellent suggestions and ideas every time. I am also extremely grateful to Dr. Kristen Stafford, who on countless occasions made me realize the importance of certain subtle-but-crucial epidemiological and interpretative details, which facilitated seeing "the forest" as well as "the trees" and aided in clarifying what the numerous findings from this project really mean.

Outside my committee, I'd like to acknowledge the many faculty, staff, and students who were helpful and supportive during my PhD. I particularly recall the kindness of Dr. Min Zhan, Dr. John Sorkin, Dr. Samer El-Kamary, Dr. Sania Amr, and Ms. Jessica Kelley, who each went out of their way to help me, and I'm sure there are others who deserve mention.

My parents, Manel and Alex, have been much more to me than I would have even thought parents could be: perhaps just one example is that I hardly ever noticed I was an "only child", though almost everyone I knew had siblings. I also acknowledge my grandparents, who made my parents who they are: the late Harriet Cooray, the late Sri Wijesinha, and Pearl Wijesinha (fortunately still with us at 103), the three of whom I was privileged to grow up around, and the late Justin Cooray, who left us too soon and before I really knew him. I'd also like to pay tribute to my late aunt Nelum Muttupulle who left us far too soon.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

A1AD	Alpha-1 antitrypsin deficiency
ATG	Anti-thymocyte globulin
BMI	Body Mass Index
CF	Cystic Fibrosis
CMV	Cytomegalovirus
COPD	Chronic Obstructive Pulmonary Disease
D_{LCO}	Diffusing capacity of the lungs for carbon monoxide
DNA	Deoxyribonucleic acid
FEV_1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
GERD	gastro-esophageal reflux disease
HLA	Human Leukocyte Antigen
HHS	U.S. Department of Health and Human Services
IL-2	Interleukin 2
IPF	Idiopathic Pulmonary Fibrosis
ISHLT	International Society of Heart and Lung Transplantation
LAS	Lung Allocation Score
mTOR	Mammalian Target of Rapamycin
MMF	Mycophenolate Mofetil
MPA	Mycophenolic Acid

MPS	Mycophenolate Sodium
NYHA	New York Heart Association
OPTN	Organ Procurement and Transplantation Network
PAP	Pulmonary Arterial Pressure
pCO2	partial pressure of carbon dioxide
pO2	partial pressure of oxygen
PPH	Primary Pulmonary Hypertension
PRA	Panel Reactive Antibody
RNA	Ribonucleic acid
SRTR	Scientific Registry of Transplant Recipients
UNOS	United Network for Organ Sharing

Chapter 1: Introduction and Background

A. INTRODUCTION

Role of lung transplantation and overview of lung transplant candidate selection

Lung transplantation offers the only hope of preventing imminent mortality and restoring quality of life for individuals with severe lung disease that is expected to be imminently fatal and/or that interferes with basic activities of daily living. Recent data show that approximately 3,500 lung transplants are performed each year[1], around 2,000 of which are in the United States[2]. In general, the International Society of Heart and Lung Transplantation (ISHLT) advises considering a lung transplant for: 1) individuals who are likely to die of lung disease within two years without a transplant and 2) individuals who experience function-limiting respiratory symptoms during minimal activity or at rest (corresponding to New York Heart Association functional classes III or IV, respectively)[3]. More specifically, ISHLT provides measurable, objective physiological criteria for the consideration of a lung transplant[3] (see Appendix A), based on quantitative factors such as lung function and exercise capacity, as well as the presence of other comorbidities indicating a worse prognosis, or disabilities that can be alleviated by a lung transplant. However, these criteria depend on the type of lung disease, since the common lung diseases are heterogeneous and tend to have different prognostic and practical implications, even at given levels of lung function and other health indicators. The four lung disease categories for classifying patients under consideration for lung transplantation, used by ISHLT as well as the United Network for Organ Sharing (UNOS), the organization which manages organ transplants in the United States, are: A) *Obstructive lung disease* which includes Chronic

Obstructive Pulmonary Disease (COPD) and Alpha-1 antitrypsin deficiency (A1AD), B) *Pulmonary vascular disease* which includes Primary Pulmonary Hypertension (PPH), C) *Cystic Fibrosis (CF) and immunodeficiency disorders*, and D) *Restrictive lung disease* which includes Idiopathic Pulmonary Fibrosis (IPF) and sarcoidosis[2,4]. Given the immediate and lifelong risks associated with undergoing a lung transplant, it is important that patients considered for lung transplantation are relatively healthy aside from their lung disease. Accordingly, ISHLT recommends that patients should be likely to survive at least 5 years post-transplant provided that their transplanted lung(s) continue to function adequately[3], and ISHLT has also devised absolute and relative contraindications to lung transplantation[3] (see Appendix B).

Patients who appear to meet the aforementioned criteria may be self-referred or referred by a health care professional to undergo evaluation at a lung transplant center, for the final step in determining whether they will become candidates for lung transplantation. These evaluations are meant to focus on a second set of objective, disease-specific criteria provided by ISHLT for assessing transplant suitability among those who have been referred based on the first set of criteria; this second set of criteria[3] (see Appendix C) is generally more stringent. Ultimately, however, the decision on acceptance or rejection of each patient is made according to the judgment of a transplant center, based on whether the patient is ill enough to need a transplant yet healthy enough to endure the transplant procedure and its long-term medical management demands, taking other details of each case into consideration. Patients become candidates for lung transplantation if a center accepts them; presently approximately 2,500 patients become lung transplant candidates each year in the

United States, and they are then placed on the national waitlist for lung transplantation that is managed by UNOS.

Overview of the U.S. lung transplant recipient population

After placement on the UNOS waitlist for lung transplantation in the U.S., the likelihood and speed of receiving a transplant is mainly influenced by a priority metric called the Lung Allocation Score (LAS), which quantifies each candidate's predicted degree of benefit from transplantation. The LAS is two-thirds based upon a candidate's medical urgency and prioritizes candidates who are more severely ill based on characteristics such as: lower lung function, lower exercise capacity, higher oxygen use, non-obstructive lung disease, and older age, among several others, while the remaining one-third is based upon a candidate's chances of surviving at least 1 year after a transplant, as estimated using many of these same factors. The full details of the LAS calculation, which has been developed by UNOS[5], are provided in Appendix D. In addition to predicted survival benefit from transplantation as reflected in the LAS, other factors that impact a candidate's likelihood and speed of receiving a transplant include the availability of a compatible donor in terms of size/height and blood type, and the geographic location of the compatible donor relative to the candidate.

The LAS was implemented in May 2005. From 1995-2005, candidates were prioritized mainly based on how long they had been waitlisted (i.e. on a "first come, first served" basis) but patients with IPF were given slight precedence over those with other diseases due to its comparatively worse prognosis.[3] Before 1995, candidates were prioritized solely on a "first come, first served" basis[4]. Particularly since the

implementation of the LAS, the population of lung transplant recipients can be generally characterized as a sicker, older, and more functionally impaired subset of the full population of waitlisted lung transplant candidates. However, candidates with extreme illness severity that portends a poor post-transplant prognosis are less likely to receive a transplant, as they are not favored per the LAS, and they are also at greater risk of dying while waiting for lung(s) or of being removed from the waitlist due to becoming too sick to undergo transplantation.

In 2015, 2,072 lung transplants were performed in the U.S., the highest number in any year thus far. Approximately 75% of U.S. lung transplant recipients in 2015 were aged 50 or older, and over 25% were aged 65 or older; 59% were male and 81% were white. 58% of recipients were in the restrictive lung disease category, followed by 28% in the obstructive lung disease category, 11% in the cystic fibrosis and immunodeficiency disorder category, and 4% in the pulmonary vascular disease category[2]. The median time from listing to transplant was 3.4 months, and 67% of candidates received a transplant within 1 year of being listed. Nevertheless, many more patients could benefit from transplantation if donor lung availability were greater, as hundreds of waitlisted candidates continue to die each year while waiting for donor lungs[2]. In addition to the major problem of insufficient donor lung availability, perhaps the greatest impediment to the overall utility of lung transplantation is poor long-term survival prospects following the procedure. The most recent estimates of median survival after lung transplantation in the U.S. (and in other countries) are between 5 and 6 years[6,7].

Significance of problem: poor long-term survival after lung transplantation, and minimal knowledge of ways to improve survival

Although lung transplantation is a life-saving procedure, its benefits are severely limited by high mortality in the years soon afterwards. Over 50% of patients die within 6 years after transplant, and approximately two-thirds die within 10 years after transplant[2]. The majority of deaths among lung transplant recipients are attributable to one of three causes: chronic lung rejection, malignancy, and infection[8]. Chronic lung rejection occurs as a consequence of the recipient's immune system treating the transplanted lung(s) as "foreign", and mounting a response which destroys the lung(s) over time. This manifests as a decline in lung function that is usually progressive and irreversible, leading to respiratory failure which results in death. To reduce the risk of chronic lung rejection, patients are placed on a lifelong regimen of immunosuppressive drugs after transplantation. Patients face two major quandaries related to these immunosuppressive drugs: first, the drugs are often unable to prevent chronic lung rejection, and second, suppression of the immune system tends to increase susceptibility to multiple life-threatening conditions, most notably malignancies and infections[9,10,11]. Therefore, in addition to chronic lung rejection, malignancies and infections are also responsible for a considerable proportion of deaths among lung transplant recipients.

Unfortunately, almost no methods to prevent these three main causes of death, or to improve survival overall, are currently known for lung transplant recipients. Information on factors associated with major causes of death after lung transplantation is especially limited, as hardly any studies of lung transplant patients have considered chronic lung rejection, malignancy, or infection as outcomes of interest. Furthermore, almost all of the well-established predictors of improved survival for lung transplant recipients are non-

modifiable, such as younger age, and less injurious type of native lung disease: COPD instead of IPF or PPH[2,12].

However, one of the most easily modifiable and important factors in the management of a lung transplant recipient, choice of maintenance immunosuppressive medication regimen, has been hardly studied in regard to its effect on survival. Prior studies of maintenance immunosuppression in lung transplantation had short follow-up duration (≤3 years) or very small sample size (<50 patients). Furthermore, certain newer maintenance immunosuppressive drugs have been studied only based on very small sample sizes, or not at all among lung transplant patients, despite fairly common use in this population. These newer drugs are of particular interest since they have shown multiple promising attributes in other organ transplant studies or small lung transplant studies, but large-scale studies are needed to further validate the findings for lung transplant patients.

In over half of the lung transplant patient population, maintenance immunosuppression is preceded by an optional brief course of high-intensity immunosuppression in the peri-operative period, referred to as induction immunosuppression. Different results have ensued from the few national cohort studies on induction immunosuppression for U.S. lung transplant recipients, and there is no consensus regarding which induction immunosuppressive drug, if any, has the most beneficial effect on survival. Also, some induction immunosuppression drugs have limited study in lung transplant recipients, in spite of promising attributes and findings in studies thus far. Additionally, it is important to note that none of the prior national cohort studies of induction immunosuppression in lung transplant recipients have considered potential confounding by transplant center, even though center preference is a major determinant of

induction immunosuppression decisions. Similarly, the use of certain induction drugs is likely to be correlated with the use of certain maintenance regimens, because the patient's physician(s) are typically responsible for deciding both, and this can potentially lead to spurious associations between either factor and survival.

Therefore, further study of associations between the aforementioned modifiable factors and survival among lung transplant recipients may reveal novel strategies to maximize survival in this population which has a particularly precarious prognosis. It is also useful to identify modifiable factors that are specifically associated with each major cause of death among lung transplant recipients, as in addition to helping reduce overall mortality in this population, this information could enable more individualized patient management, by making it possible to strategically reduce the risk of the event or events that a patient is particularly prone to experiencing based on his or her characteristics.

B. SPECIFIC AIMS AND HYPOTHESES

Overview of study objectives: Median survival after a lung transplant is limited to approximately 5-6 years, and there is minimal knowledge of ways to improve survival. Therefore, it is useful to study modifiable factors to identify potential strategies for improving survival among lung transplant patients. Survival after lung transplantation is primarily limited by 3 events or "causes of death": chronic lung rejection, infection, and malignancy. Little is known regarding which factors may affect the risks of each of these events. Examining associations between modifiable factors and the risk of each event can help identify potential strategies to reduce the likelihood of each event occurring, which in

turn can reduce mortality caused by each event. Discovery of such strategies can also aid in optimizing patient management based on a patient's personal susceptibility to each event.

Aim 1: Identify maintenance and induction immunosuppression regimens that are associated with improved survival in lung transplant recipients.

Hypotheses for Aim 1:

1) **[**Sirolimus + tacrolimus] or [mycophenolate sodium (MPS) + tacrolimus] maintenance immunosuppressive will be associated with better post-transplant survival than the most common regimen [mycophenolate mofetil (MMF) + tacrolimus].

2) Basiliximab, daclizumab, or alemtuzumab induction will be associated with better survival compared to no induction.

Aim 2: Identify maintenance and induction immunosuppression regimens that are associated with a reduced risk of each event that is a major cause of death after lung transplantation.

Aim 2.1: Identify maintenance and induction immunosuppression regimens associated with a reduced risk of chronic rejection in lung transplant recipients.

Aim 2.2: Identify maintenance and induction immunosuppression regimens associated with a reduced risk of infection in lung transplant recipients.

Aim 2.3: Identify maintenance and induction immunosuppression regimens associated with a reduced risk of malignancy in lung transplant recipients.

Hypotheses for Aim 2:

Aim 2.1: [Mycophenolate sodium (MPS) + tacrolimus] or [sirolimus + tacrolimus] maintenance immunosuppression will be associated with a lower risk of chronic lung rejection than [mycophenolate mofetil (MMF) + tacrolimus]. Alemtuzumab or ATG induction will be associated with a lower risk of rejection compared to no induction.

Aim 2.2: [Sirolimus + tacrolimus] or [MPS + tacrolimus] maintenance immunosuppression will be associated with a lower risk of infection than [MMF + tacrolimus]. Alemtuzumab or ATG induction will be associated with a higher risk of infection compared to no induction immunosuppression.

Aim 2.3: [Sirolimus + tacrolimus] maintenance immunosuppression will be associated with a lower risk of malignancy than [MMF + tacrolimus]. Induction immunosuppression with any drug will be associated with a higher risk of malignancy, compared to no induction immunosuppression.

C. BACKGROUND

Maintenance immunosuppression in lung transplantation

Almost all lung transplant recipients are on a daily regimen of multiple immunosuppressive drugs for the remainder of their lives, known as a "maintenance" immunosuppressive regimen, in order to lower their risk of lung rejection. The most common regimens in current use consist of a **calcineurin inhibitor** (such as tacrolimus or

cyclosporine) combined with either an **antimetabolite** a.k.a. **cell cycle inhibitor** (such as mycophenolate mofetil (MMF), mycophenolate sodium (MPS), or azathioprine) OR a **mammalian target of rapamycin (mTOR) inhibitor** (such as sirolimus), which is a newer class of drugs. There is a severe deficiency in knowledge regarding the impact of the choice of maintenance immunosuppressive regimen on survival, as there have been no large-scale studies of maintenance immunosuppression with follow-up beyond 3 years. Furthermore, in spite of sirolimus and MPS each being used in hundreds of lung transplant recipients to date, almost all lung transplant studies of sirolimus have been at single centers and had notably small sample sizes, while MPS has not been studied at all in this population.

Although the choice of immunosuppressive regimen varies according to the transplant center as well as any physician preferences, the most popular maintenance immunosuppressive regimen today and the *de facto* standard-of-care, is the combination of tacrolimus and mycophenolate mofetil (MMF). Tacrolimus + MMF has been the predominant maintenance immunosuppressive regimen among lung transplant patients for at least a decade. Since median survival after a lung transplant is unfortunately stagnated at approximately 5-6 years, it is worthwhile to examine the associations of other common maintenance immunosuppression regimens with survival duration, particularly regimens including the newer drugs.

Tacrolimus is a member of the calcineurin inhibitor class of immunosuppressants, as is cyclosporine, an older drug that has been generally replaced by tacrolimus but is still used in a small proportion of patients. Calcineurin inhibitors block the transcription of cytokines such as Interleukin 2 (IL-2)[13], a signaling protein that has a major role in the

activation and proliferation of T-cells, which are implicated in the inflammatory response underlying the pathogenesis of rejection[7,14]. Tacrolimus and cyclosporine are distinguished by the type of immunophilin they bind in order to achieve calcineurin inhibition; tacrolimus binds to FK-binding proteins, while cyclosporine binds to cyclophilins[13,14,15].

Three randomized trials have compared tacrolimus and cyclosporine[16,17,18], and while none of them found a significant difference in patient survival over the follow-up periods of up to 3 years, two of the three found that tacrolimus was associated with lower incidence of chronic rejection, and infections, compared to cyclosporine. Therefore, tacrolimus tends to be favored in practice today. Both tacrolimus and cyclosporine are linked to nephrotoxicity, neurotoxicity, and hyperlipidemia; tacrolimus is more likely to cause the onset of diabetes, while cyclosporine is more likely to cause hypertension[7].

MMF belongs to a class of immunosuppressants which may be referred to by one of the following: antimetabolite[14,19,20], cell cycle inhibitor[7,13,21], or nucleotide-blocking agent[15]. Azathioprine is an older drug within the same class, and although MMF is preferred at most centers, azathioprine continues to be used in a significant minority of patients. Both MMF and azathioprine inhibit the proliferation of T cells (which directly mount a response against the donor lung) and B cells (which produce antibodies that mount a response against the donor lung)[13]. The active ingredient of MMF, mycophenolic acid (MPA), achieves this inhibition selectively, theoretically reducing undesired inhibitory effects, by blocking a pathway that is crucial for the proliferation of T cells and B cells but can be bypassed by other types of cells[22]. In contrast, azathioprine exerts a non-selective inhibition by halting DNA and RNA synthesis[7,13,14], which is thought to lead to major side effects, including the suppression of bone marrow activity.

A randomized controlled trial comparing MMF and azathioprine found no significant differences in survival up to 3 years, and no differences in the incidences of acute or chronic rejection. However, treatment withdrawals due to adverse side effects were more common in patients on azathioprine compared to those on MMF[23]. This finding likely had a major role in the development of the widespread preference of MMF over azathioprine in practice today, as another randomized controlled trial limited to 6 months of follow-up found no significant differences between the two drugs in terms of survival, acute rejection, infections, or adverse events[24]. Although MMF may have a superior safety profile compared to azathioprine, it is nevertheless associated with a relatively high incidence of adverse events (particularly gastrointestinal) that often lead to interruptions in anti-rejection prophylaxis[25,26,27,28,29].

Mycophenolate sodium (MPS) is an enteric-coated antimetabolite medication which has the same active ingredient as MMF, mycophenolic acid (MPA). However, MPS was designed to reduce the side effects of MMF by delivering MPA directly to the small intestine, rather than having it metabolized in the stomach. Multiple studies in other populations, including kidney and liver transplant recipients, and autoimmune disease patients, have found that MPS is associated with lower risks of serious infections[28,29] and gastrointestinal side effects[26,27,28,29], and better renal function compared to MMF[25], while providing similar anti-rejection efficacy. MPS, compared to MMF, also demonstrated better pharmacokinetic absorption of MPA in several studies of kidney transplant recipients[25,29,30]; one explanation is that absorption of MPA may be improved when it is delivered to the small intestine, since absorption can be suboptimal in the stomach if acidity is inadequate[25,29]. This may be particularly relevant for the lung transplant patient

population due to the significant prevalence of gastro-esophageal reflux disease (GERD), which frequently needs to be treated with acid-reducing medication; at least one such medication has been found to impair MMF absorption but not MPS absorption[31]. The apparently superior absorption, in addition to the potential lessening of side effects, which are likely to reduce lapses in immunosuppression, suggest that MPS might lower the risk of chronic lung rejection compared to MMF, thereby possibly leading to increased survival. Use of MPS has slowly but significantly increased in recent years among lung transplant patients, though MMF remains the most widely used antimetabolite in this population.

Finally, one other drug that has had moderately significant usage in lung transplant patients is sirolimus, a member of the mammalian target of rapamycin (mTOR) inhibitor category of immunosuppressants. mTOR inhibitors, like calcineurin inhibitors, interfere with IL-2 driven T-cell proliferation[32]. In fact, sirolimus binds to FK-binding proteins, like tacrolimus does; however, rather than blocking IL-2 transcription, sirolimus inhibits DNA and protein synthesis in the mTOR pathways downstream of IL-2, thereby preventing IL-2 driven T-cell proliferation[14]. In addition to its anti-proliferative effect, reasons why sirolimus could hypothetically aid in the prevention of chronic lung rejection or bronchiolitis obliterans syndrome include: inhibition of proinflammatory cytokines, increasing regulatory B lymphocytes (which have been shown to attenuate the immune response)[33], as well as anti-fibrotic effects[34,35]. In addition to its immunosuppressive properties, sirolimus has unique potential advantages in that anti-cancer[9,36,37] and anti-aging[37,38] effects have been attributed to mTOR inhibitors in general, which may be particularly beneficial for the lung transplant population given its high risk of cancer and other age-related diseases. However, due to apparent effects of sirolimus on wound healing,

administering sirolimus immediately after lung transplant was found to be associated with bronchial anastomotic dehiscence that led to airway compromise and fatality in severe cases[39], and it is therefore recommended for sirolimus initiation to be delayed by 3 months after transplant in order to allow adequate time for airway healing[40]. Sirolimus has been shown to be an effective "rescue medication" after treatment failures on other immunosuppressant(s), including the onset of severe diseases such as chronic lung rejection[35], skin cancer[41], nephroxicity[42], etc., so it is currently more popular in this context than as a prophylactic medication that would be initiated as soon as the airway healing period is complete (at approximately 3 months). Hence, the majority of studies on sirolimus in lung transplantation were based on using it as a rescue medication, i.e. in patients who have already developed a serious condition post-transplant and have an impaired prognosis at the time they start the drug[35,40,43,44].

The limited extent to which prophylactic use of sirolimus has been studied in lung transplant patients has led to differing conclusions. A multi-center randomized trial comparing sirolimus (initiated at 3 months post-transplant) to azathioprine in a tacrolimus-based regimen, in which over half the study participants discontinued the intention-to-treat medication, found no significant differences in survival or chronic rejection incidence. Nevertheless, the incidence of CMV infection was significantly lower in the sirolimus arm within the first year[34]. A single-center study of approximately 40 patients with follow-up exceeding 10 years found that lung transplant recipients on sirolimus in a tacrolimus-based regimen (initiated within the first year of transplantation) had significantly better survival compared to MMF (67% sirolimus vs. 37% MMF at 9 years), as well as reduced incidences of chronic lung rejection and infection[32]. Another single-center study found that patients

converted to sirolimus within 6 months post-transplant had better lung function 3 years afterwards[42]. Finally, a recent small single-center study reported very low chronic rejection incidence and favorable short-term and long-term survival when sirolimus was initiated at 1 month post-transplant, within a cyclosporine-based regimen, in patients judged to have a low risk of dehiscence after completion of healing of the bronchial anatomoses[61].

Based on this information, it is plausible that utilizing sirolimus or MPS in the maintenance immunosuppressive regimen may improve survival for lung transplant patients. For this reason, a large-scale study of survival associated with maintenance immunosuppressive regimens that include sirolimus or MPS is of significant potential clinical utility. It is also of interest to study a triple-drug regimen containing the most promising drugs within each class: sirolimus, tacrolimus, and MMF (as MPS is not commonly used in a triple drug regimen). A triple-drug regimen such as this can theoretically provide more comprehensive immunosuppression and a lower risk of adverse effects compared to two-drug regimens. A 56-patient randomized study of renal transplant recipients observed a reduction in gastrointestinal adverse effects and significant improvement in renal function with the addition of a third immunosuppressant mizoribine to a halved-dose regimen of MMF + tacrolimus, compared to full dose MMF + tacrolimus only[45]. No organ transplant studies have assessed the Sirolimus + MMF + Tacrolimus regimen specifically, but a renal transplant study observed poor survival with a sirolimus + MMF regimen compared to a regimen containing a calcineurin inhibitor (tacrolimus or cyclosporine) and MMF[46]. It is not clear whether this is due to the lack of a calcineurin inhibitor in the regimen, or due to a potential negative interaction between MMF and sirolimus. Nevertheless, it has been stated that sirolimus increases MMF exposure and vice

versa when the two are co-administered[47], and a rat study demonstrated that adding MMF to sirolimus and tacrolimus results in nephrotoxicity[48]. These unwanted effects could potentially counterbalance a possible benefit that the triple-drug combination of Sirolimus + MMF + Tacrolimus may have had.

In studying regimens containing sirolimus or MPS, tacrolimus is a more relevant calcineurin inhibitor than cyclosporine, for multiple reasons. First, the vast majority of patients taking either sirolimus or MPS are also on tacrolimus rather than cyclosporine. Furthermore, when sirolimus and cyclosporine are co-administered, levels of both drugs may inadvertently increase, and nephrotoxicity may also occur[20]. On the other hand, tacrolimus and sirolimus have been found to have synergistic effects when used together[49]. Perhaps most importantly, most studies have found tacrolimus to have higher anti-rejection efficacy than cyclosporine[16,17,18,50]. Therefore, it appears that the maximum potential benefits of using either sirolimus or MPS are most likely to manifest when each drug used in combination with tacrolimus.

Induction immunosuppression in lung transplantation

Unlike maintenance immunosuppression, which almost always continues for a transplant recipient's lifetime, induction immunosuppression is an optional, brief, high-intensity, typically single-drug immunosuppression routine employed in the peri-operative period, with the goal of further reducing the risk of eventual chronic lung rejection. Induction immunosuppressive medications fall into two main categories: T-lymphocyte depleting and non T-lymphocyte depleting. IL-2 receptor antagonists, including the drugs basiliximab and daclizumab, are the most common non T-lymphocyte depleting induction

medications. Basiliximab contains 75% human antibodies, while daclizumab contains 90%. Among T-lymphocyte depleting drugs, alemtuzumab is a newer agent which has been shown to induce prolonged T-cell depletion for >1 year, while ATG (Anti Thymocyte Globulin) is an older agent that targets multiple cell surface antigens (i.e. a polyclonal agent), unlike the aforementioned three drugs (monoclonal agents) which only target a single antigen. There is currently no consensus regarding which induction immunosuppressive drug is associated with the best survival.

A retrospective study of nearly 17,000 U.S. lung transplants from 1987 to 2013 found that basiliximab was the only induction drug (out of alemtuzumab, anti-thymocyte globulin [ATG], basiliximab, and corticosteroids) associated with improved survival compared to no induction[51]. However, this study omitted daclizumab, which demonstrated slightly better survival than ATG in a 2007 randomized controlled trial of 50 patients that was limited to 1 year of follow-up[52]. Another retrospective study by the same authors on U.S. lung transplants from 1997-2013 found that alemtuzumab, and to a much lesser extent, basiliximab, were associated with a significantly reduced risk of chronic lung rejection compared to no induction[53]. Similarly, a retrospective study restricted to double lung transplants, including over 6000 patients transplanted in the U.S. between 2006 and 2013, found that both alemtuzumab and basiliximab were associated with significantly improved survival compared to no induction; the two drugs had similar survival compared to each other[54].

Based on the limited evidence to date, basiliximab and alemtuzumab appear to have shown the most promising results out of the induction immunosuppression drugs presently used in lung transplant recipients. There is currently no clear indication of which drug

should be preferred; out of two studies, one found basiliximab to be associated with better survival than alemtuzumab but the other study did not, while a third study found that alemtuzumab may have an advantage over basiliximab in the prevention of chronic lung rejection. Furthermore, neither drug has been compared to daclizumab, which seems worthy of further investigation based on favorable results from at least one clinical trial, and the fact that it contains more human antibodies compared to basiliximab. Finally, it is important to note that none of the prior national studies of induction immunosuppression in lung transplant recipients have considered potential confounding by transplant center, given that center preference is a major determinant of induction immunosuppression decisions.

Innovation of study

To our knowledge, this is the first study utilizing the national UNOS dataset to compare long-term outcomes (including survival and the incidence of common mortality-causing events) between common maintenance immunosuppressive regimens in lung transplantation. Prior studies on maintenance immunosuppression, which have not covered all commonly used agents, were predominantly conducted at single centers. This study also includes the a thorough, national-level comparison of outcomes between all induction immunosuppression drugs commonly used in lung transplant patients; prior large-scale studies making similar comparisons have not studied all common drugs, and also have not considered the likely confounding effect of transplant center. Furthermore, this is one of the only studies to focus on the main causes of death after lung transplant as some of its primary outcomes of interest; in particular, this appears to be the first study which aims to

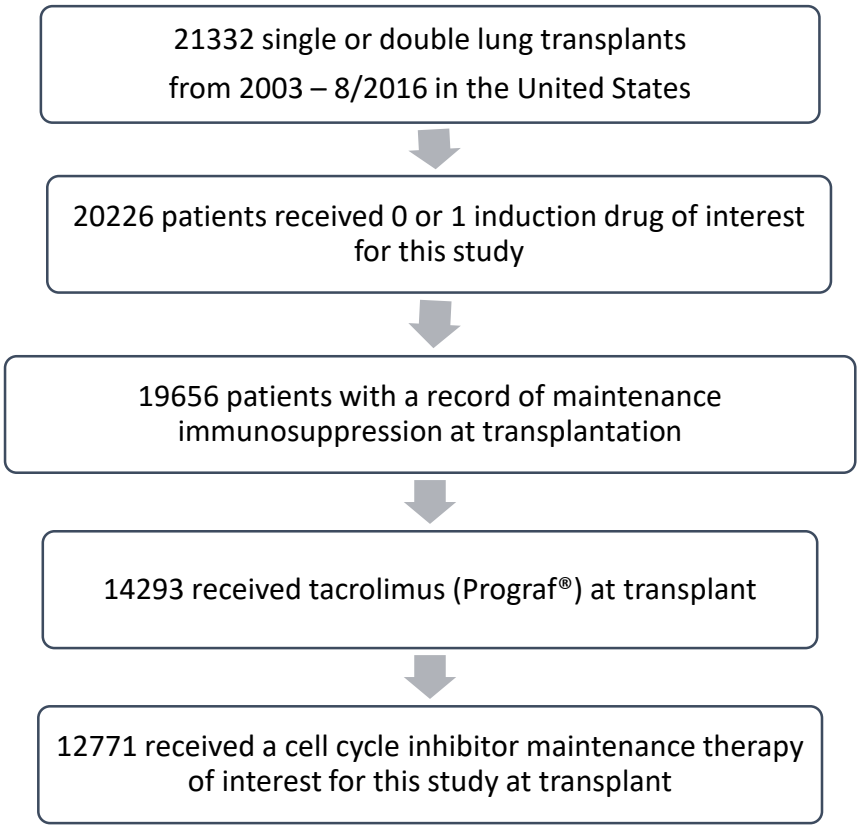
identify modifiable factors associated with malignancies and infections in the lung transplant population. Our study employs a thorough and comprehensive control for confounding through the use of the extensive set of variables which are included in the dataset.

Chapter 2: Study Methods

A. STUDY DESIGN AND PARTICIPANTS

This is a retrospective cohort study consisting of patients transplanted in the U.S. from 2003-2016 (as the first use of alemtuzumab in these lung transplant patient data was in 2003, and follow-up within our dataset ends in August 2016). The diagram below describes the patient inclusion criteria for our study.

Figure 2.1: Flow Diagram to Describe Study Population



B. DATA SOURCE

This study will involve secondary data analyses of the UNOS (United Network for Organ Sharing) lung transplant dataset. UNOS is the organization which coordinates organ

transplant activities in the U.S.; therefore, this dataset includes all U.S. lung transplant recipients.

UNOS transplant data are collected by the OPTN (Organ Procurement and Transplantation Network), which is operated by UNOS, under contract with the U.S. Department of Health and Human Services (HHS). The main purpose of collecting these data is to monitor the performance of each transplant program based on its patient outcomes, and also to track overall trends in outcomes according to patient characteristics, donor characteristics, type of transplant performed, treatments administered, etc. Survival is the main outcome of interest for tracking, but other important outcomes include chronic transplant rejection, hospitalizations for acute rejection episodes or infections, malignancy, other organ failure, or other chronic disease development, etc.

The main sources of the data include hospitals (transplant centers), organ procurement organizations, and immunology and histocompatibility laboratories. The data from these sources are updated monthly for each transplant patient, including new information as well as any revisions to existing information on each patient. These updates are made mainly through an Internet-based system called UNet. Data from the Social Security Administration Death Master File (SSADMF) are updated monthly as well, for accurate tracking of patient deaths, while hospitals (transplant centers) are the sources of information on the cause of death for each patient. The Scientific Registry of Transplant Recipients (SRTR), which also operates under a contract from the HHS, processes the data collected by the OPTN, and then conducts a quality validation process consisting of "numerous tests…to identify potential errors in the data", sending reports to the OPTN towards the investigation and resolution of any such issues. Various entities rely on these

data collected by OPTN, such as: public and private insurers, researchers, and the general public.[55]

C. MEASURES/VARIABLES

Exposure Variables for Aim 1:

1) Maintenance immunosuppression regimen*
    - **MMF + Tacrolimus (reference level)**
    - MPS + Tacrolimus
    - Azathioprine + Tacrolimus
    - Sirolimus + Tacrolimus
    - MMF + Sirolimus + Tacrolimus

*Maintenance immunosuppressive regimen classifications are based on the regimen that each patient received prophylactically, and can generally be defined according to the initial maintenance regimen received immediately after transplant (i.e. the regimen documented at their first immunosuppression record in the database). One exception is that since patients who are intended to receive sirolimus prophylactically usually do not begin the drug until at least 3 months after transplant due to the possibility that it may impair wound healing post-operatively, the second immunosuppression record in the database for each patient (which is usually at 1 year following transplant, but can be 3 months or 6 months or at death) must be used to identify most patients who received sirolimus prophylactically.

2) Induction immunosuppression regimen
    - **None (reference level)**
    - Alemtuzumab
    - Basiliximab

- Daclizumab
- Equine ATG
- Rabbit ATG

Induction immunosuppressive regimen classifications are defined according to the induction drug, if any, that each patient received in the peri-operative period. Patients who received more than one induction drug will not be included in the study.

Outcome Variable for Aim 1: Death

Exposure Variables for Aim 2 (Aims 2.1-2.3):

1) Maintenance immunosuppression regimen*
    - **MMF + Tacrolimus (reference level)**
    - MPS + Tacrolimus
    - Azathioprine + Tacrolimus
    - Sirolimus + Tacrolimus
    - MMF + Sirolimus + Tacrolimus
2) Induction immunosuppression regimen
    - **None (reference level)**
    - Alemtuzumab
    - Basiliximab
    - Daclizumab
    - Equine ATG
    - Rabbit ATG

Outcome Variables for Aim 2:

- Aim 2.1: Incidence of chronic lung rejection
- Aim 2.2: Incidence of malignancy
- Aim 2.3: Incidence of infection (involving hospitalization)

Potential Confounders:

- Age
- Gender
- Race
- BMI
- Education
- Diabetes
- Poor renal function
- Lung disease type
- Medical condition at transplant
- Prior transplant
- Donor-recipient HLA matching
- Donor-recipient CMV status
- Donor age
- Donor gender
- Donor smoking (>20 pack-years)
- Single vs. Double lung transplant
- Transplant era
- Transplant center
- Induction regimen

**For donor variables, donor-recipient matching will be considered if relevant, via interaction terms or numerical differences between the corresponding donor and recipient variables, or via categorical variables which contain a level to represent each possible combination of the corresponding donor and recipient variables.

A variable can be considered a "potential confounder" if the following conditions are plausible: 1) the variable is not an effect modifier, 2) the variable is associated with the

exposure, 3) the variable affects the probability of the outcome, and 4) the variable is not on the causal pathway between the exposure and the outcome. Variables were included in the above list of potential confounders if they met one of two criteria: a) they are an important predictor of outcome according to a priori knowledge such as in literature describing factors associated with post-lung transplant outcomes, or b) if they are expected to be very likely associated with the exposure of interest, such as being a factor that might drive the decision to use one immunosuppressive regimen instead of another.

Potential Effect Modification:

We will test for interaction between the induction and maintenance immunosuppression variables, and will assess outcomes associated with each possible combination of induction and maintenance immunosuppressive regimen in our study that has at least 25 patients, in order to identify the combination(s) of induction and maintenance regimens associated with the best survival.

D. ANALYSIS

A time-to-event analysis, utilizing a semi-parametric Cox Proportional Hazards Model, will be conducted to assess the associations between each of the exposures (maintenance immunosuppressive regimen, induction immunosuppressive regimen) and outcomes (death, chronic lung rejection, infection, malignancy) of interest.

A) Confounding control

1. For exploratory and descriptive purposes, each potential confounder (out of those specified *a priori*) will be assessed in terms of its association with the exposure, using chi-square tests for categorical variables and Wilcoxon rank sum or Kruskal-Wallis tests for continuous variables.

2. Multiple imputation, involving the following process, will be used to handle missing data:

    a. Creation of m=20 complete datasets based on different plausible imputations for each value that is missing. It has been shown that the creation of 20 datasets will lead to a power loss of <1% compared to creation of 100 datasets, given a fraction of missing information (FMI) of 30%[56,57]. All of our variables of interest have less than 30% missing data. The FCS (fully conditional specification) imputation method will be used, since many categorical variables are missing values, and it has been demonstrated that FCS is preferable to MVN (multivariable normal) imputation for categorical variables[58].

    b. Running the multivariable Cox model(s) of interest on each of the imputed datasets.

    c. Pooling of results from the analyses of the different imputed datasets. Rubin's rules will be used, such that the final regression coefficients will be the averages of the coefficients based on the models run for the 20 datasets, and the total variance for regression coefficients will be the sum of: the average within-imputation variance *W*, the between-imputation variance *B*, and *B*/20.[59]

3. To account for expected confounding induced by variation in outcomes between transplant centers, while appropriately estimating the associated standard errors, a

random effect for transplant center will be added to the Cox model (this is referred to as a “frailty model” or “mixed effects Cox model”). The random effect for each center will represent the deviation of that center from the overall baseline risk of outcome.

B) Non-terminal events of interest: chronic lung rejection, infection, and malignancy

4. When determining the Hazard Ratio for each non-terminal event of interest (chronic lung rejection, infection, and malignancy), the role of death in precluding the event will be accounted for, by using an illness-death semi-competing risks model. This illness-death model accounts for the dependence between the risk of each non-terminal event and risk of death from another cause, using a patient-specific frailty that is analogous to a random effect in a mixed-effect model for longitudinal analyses, representing the residual patient-specific differences beyond those explained by available covariates. From the illness-death model, 3 hazard ratios (HR) can be obtained, indicating each one of the following for one exposure group compared to the reference group (using cancer as an example of the event of interest):

    1. the relative hazard of experiencing cancer given that death has not occurred
    2. the relative hazard of death given that cancer has not occurred
    3. the relative hazard of death after cancer has occurred

5. For each event type (chronic lung rejection, infection, malignancy), we will analyze the joint distributions of the event and event-free deaths, in terms of cumulative incidences. We will examine for each exposure level over time, the cumulative

incidence of the event of interest (e.g. cancer) along with the cumulative incidence of event-free death (e.g. death without cancer). In summary, the following joint distributions of cumulative incidences will be examined:

- Chronic lung rejection & Rejection-free death
- Infection & Infection-free death
- Malignancy & Malignancy-free death

C) Approaches to prevent immortal time bias pertaining to sirolimus

6. Since prophylactic initiation of sirolimus is typically delayed until at least 3 months after lung transplant (and can be delayed up to 1 year)**,** most patients receiving sirolimus have an "immortal time" of 3-12 months. Therefore, multiple approaches will be utilized to avoid immortal time bias in survival analyses involving sirolimus, and we will examine whether results are generally consistent between approaches:
   a. Standard survival analyses will be performed starting at a landmark time of either 3 or 12 months (all patients included in each group are alive at the landmark time). The main analysis will have a landmark time of 12 months since many patients only have their second follow-up immunosuppression record in the database at 1 year, but an alternate landmark time of 3 months will also be used, to determine whether results remain consistent.
   b. A time-dependent covariate Cox Proportional Hazards model will be run, with the start time of sirolimus considered as either 3 or 12 months.
   c. Multiple imputation will be used to infer which patients were most likely planned to receive sirolimus prophylactically, but died before they could initiate

it (within the first year). Standard survival analyses will be performed, starting at the time of transplantation, based on a sirolimus group that is comprised of the multiple imputation-identified patients as well as the patients who actually received sirolimus in the first year.

E. SAMPLE SIZE AND POWER CALCULATIONS

All but one of our exposure levels have at least 219 subjects. We calculated the hazard ratio representing the smallest effect size that there is 80% power to detect, based on an exposure group size of 219.

The proportions of patients in our dataset experiencing each outcome of interest are: 44% for death (Aim 1), and 46%, 56%, and 22% for chronic lung rejection, infection, and malignancy, respectively (Aim 2). A rearrangement of the equation $events = \frac{(z_{a/2}+z_{\beta})^2}{\pi_1\pi_2(\ln HR)^2}$ to $HR = e^{\sqrt{\frac{(z_{a/2}+z_{\beta})^2}{\pi_1\pi_2 * events}}}$ allows calculation of the effect size that there is 80% power to detect, based on counts in the dataset[60].

Parameters for equation above

- $z_{a/2} = 1.96\ (for \propto = 0.05)$
- $z_{\beta} = 0.842\ (for\ \beta = 0.20\ or\ 80\%\ power)$
- $\pi_1 =$ proportion in exposed group
- $\pi_2 =$ proportion in control group
- events = number of events in both groups combined

- HR = Hazard Ratio representing the smallest effect size that is expected to be detected

Calculations for Death (Aim 1):

Based on an event rate of 44%, and a count of 219 patients in an exposure group, and 5782 patients in the reference group (MMF + tacrolimus maintenance immunosuppression), 2640 total events are estimated. By entering this number, along with the proportions of subjects in these two groups, and the z values specified above, into the equation for calculating HR, it is calculated that there is 80% power to detect a HR **≥1.34** or **≤0.75.**

Calculations for Chronic Lung Rejection (Aim 2.1):

Based on an event rate of 46%, and a count of 219 patients in an exposure group, there is 80% power to detect a HR **≥1.33** or **≤0.75**.

Calculations for Infection (Aim 2.2):

Based on an event rate of 56%, and a count of 219 patients in an exposure group, there is 80% power to detect a HR **≥1.29** or **≤0.77**.

Calculations for Malignancy (Aim 2.3):

Based on an event rate of 22%, and a count of 219 patients in an exposure group, there is 80% power to detect a HR **≥1.51** or **≤0.66**.

Chapter 3: Sirolimus + Tacrolimus Maintenance with No Induction Therapy May Maximize Survival in Lung Transplant Recipients

A. ABSTRACT

Background: Sirolimus has purported advantages as the cell cycle inhibitor for maintenance immunosuppression in lung transplantation, including anti-fibrotic, anti-proliferative, and anti-aging effects, which may help improve survival.

Methods: Among U.S. lung transplant recipients from 2003-2016 in the United Network for Organ Sharing (UNOS) dataset, we compared prophylactic use of sirolimus (n=219) to mycophenolate mofetil [MMF] (n=5482) within a tacrolimus-based regimen. Other cell cycle inhibitor therapies compared were MMF + sirolimus combined (n=54), mycophenolate sodium [MPS] (n=408), and azathioprine (n=2556). As prophylactic sirolimus initiation is usually delayed by up to 1 year post-transplant to avoid risks of bronchial anastomotic dehiscence and other complications, multiple approaches were used to prevent immortal time bias. The primary survival comparisons were based on patients alive at 1 year, and free of chronic rejection and malignancy, to exclude these common rescue indications of sirolimus. Survival comparisons were based on Cox multivariable regression utilizing multiple imputation to handle missing data, and Kaplan-Meier estimates. The combined effects of common induction therapies and cell cycle inhibitor agents were also evaluated in terms of survival, to assess how sirolimus compares to other agents depending on the induction therapy used.

Results: Sirolimus was associated with better survival than MMF: adjusted Hazard Ratio (HR)=0.71, p=0.003, median survival (MS) was 8.9 years vs. 7.1 years post-transplant, in patients alive at 1 year. Sirolimus + MMF concomitantly (MS=5.8 years; HR=1.14,

p=0.49) and MPS (MS=7.5 years; HR=0.95, p=0.61) were not associated with better survival than MMF, while azathioprine had a trend towards better survival (MS=7.9 years; HR=0.93, p=0.13). Among all combinations of induction and maintenance therapies analyzed, sirolimus + tacrolimus with no induction was associated with the most favorable survival (MS=10.7 years), which was significantly better (HR=0.48, p=0.002) compared to MMF + tacrolimus with any induction (MS=7.4 years), or without induction (MS=6.8 years).

Conclusions: Sirolimus, initiated within 1 year after lung transplantation in a tacrolimus-based regimen, may improve survival compared to MMF. National data representing the U.S. lung transplant population suggest that sirolimus + tacrolimus maintenance with no induction therapy may achieve the best survival among common immunosuppressive strategies.

B. INTRODUCTION

Lung transplantation is a life-saving procedure for patients with end-stage lung disease; however, its benefits are severely limited by high mortality in the years soon afterwards. Long-term survival after lung transplantation is primarily impeded by 3 common causes of death: chronic rejection, infection, and malignancy. Unfortunately, the immunosuppressive medications that patients take to prevent chronic rejection often become ineffective eventually, and they also predispose patients to infections, malignancies, and other life-threatening complications.

The most common immunosuppressive regimens that patients receive in the long-term consist of a **calcineurin inhibitor** (tacrolimus, or less commonly cyclosporine) combined with an **antimetabolite** (mycophenolate mofetil [MMF], or less commonly, mycophenolate sodium [MPS] or azathioprine) that serves as a cell cycle inhibitor. An alternative regimen involves using a **mammalian target of rapamycin (mTOR) inhibitor** (such as sirolimus or everolimus) as the cell cycle inhibitor, instead of an antimetabolite.

Sirolimus is the predominant mTOR inhibitor used in lung transplant recipients to date; particularly in the U.S., everolimus has been introduced in this population fairly recently. Sirolimus acts similarly to tacrolimus by binding to FK-binding proteins; however, rather than blocking IL-2 transcription like tacrolimus, it inhibits DNA and protein synthesis in the mTOR pathways downstream of IL-2, thereby preventing IL-2 driven T-cell proliferation[14]. Along with its antiproliferative effect, sirolimus could aid in preventing chronic lung rejection or bronchiolitis obliterans syndrome in other ways, such as by inhibiting proinflammatory cytokines, increasing regulatory B lymphocytes (which have been shown to attenuate the immune response)[33], and exerting anti-fibrotic effects[34,35].

In addition to their immunosuppressive properties, mTOR inhibitors have shown other important potential benefits such as anti-cancer[9,36,37] and anti-aging[37,38] effects. Therefore, they may be particularly advantageous for the lung transplant population given its relatively high risk of cancer and other age-related diseases. However, a limitation of the use of mTOR inhibitors is that if they are administered immediately after lung transplantation, they may impede wound healing, resulting in a potentially fatal condition called bronchial anastomotic dehiscence[39], hence the recommendation to delay their initiation by at least 3 months after transplantation. Consequently, patients intended to receive sirolimus prophylactically usually begin on either mycophenolate or azathioprine as the cell cycle inhibitor, for 3-12 months post-transplant until sirolimus can be initiated.

In lung transplantation, sirolimus has shown effectiveness as a "rescue medication" after treatment failures on other immunosuppressant(s), such as the onset of serious conditions such as chronic lung rejection[35], skin cancer[41], renal insufficiency[42], etc., so it is currently more popular in this context than as a prophylactic medication. The limited extent to which prophylactic use of sirolimus has been studied in lung transplant patients has led to differing conclusions. A multi-center randomized open-label 3-year trial comparing sirolimus (initiated at 3 months post-transplant) to azathioprine in a tacrolimus-based regimen, in which over half the study patients had the intention-to-treat discontinued, found no significant differences in survival or chronic rejection incidence, although incidence of CMV infection was lower in the sirolimus arm[34]. A single-center long-term study of lung transplant recipients found that within a tacrolimus-based regimen, sirolimus initiated in the first year post-transplant was superior to MMF in terms of survival (67% alive for sirolimus vs. 37% alive for MMF at 9 years) as well as chronic lung rejection and

infection incidence[32]. Another single-center study found that patients switched to sirolimus within 6 months post-transplant experienced improved lung function after 3 years[42]. Finally, a recent small single-center study reported very low chronic rejection incidence and favorable short-term and long-term survival when sirolimus was initiated at 1 month post-transplant, within a cyclosporine-based regimen, in patients judged to have a low risk of dehiscence whose bronchial anastomoses were completely healed[61]. Since no large-scale studies have examined long-term survival associated with prophylactic sirolimus use, the primary goal of this study was to compare long-term survival between sirolimus and the most popular cell cycle inhibitor, mycophenolate mofetil (MMF), by analyzing national United States lung transplant data in the United Network for Organ Sharing (UNOS) dataset.

C. METHODS

This was a retrospective study of lung transplant recipients from 2003-2016, utilizing the United Network for Organ Sharing (UNOS) dataset, which contains all United States lung transplants. All patients in this study were on a tacrolimus-based immunosuppressive regimen; that is, they received tacrolimus (Prograf®) as their calcineurin inhibitor immunosuppressant, as in the UNOS lung transplant dataset, cyclosporine use in combination with sirolimus is infrequent.

Patient classifications in this study were based on the cell cycle inhibitor received for prophylactic maintenance immunosuppression, i.e. the cell cycle inhibitor received prior to any drug switches that may have been motivated by treatment failures, for example chronic rejection or cancer development. Cell cycle inhibitor therapies evaluated in our

study were sirolimus (Rapamune®), mycophenolate mofetil [MMF] (Cellcept®), mycophenolate sodium [MPS] (Myfortic®), azathioprine (Imuran®), and the combination of MMF + sirolimus (Cellcept® + Rapamune®). The prophylactic cell cycle inhibitor for each patient is generally found in the immunosuppression record corresponding to transplantation time; that is, the first immunosuppression record for each patient in the dataset. One exception is that since most patients who receive sirolimus prophylactically do not start the drug until ≥3 months post-transplant due to the risk of post-operative wound healing complications, the majority of such patients can only be identified based on their second immunosuppression record in the dataset, which can be at 3 months, 6 months, or a maximum of 1 year following transplant, or at death for patients who died within the first year.

Since the delayed initiation of sirolimus introduces potential for immortal time bias, we used multiple approaches to avoid this bias, and compared the results between approaches to determine whether they were generally consistent. Additionally, because we cannot determine the exact time of sirolimus initiation within the first year, we considered the possibilities at either extreme: 1) as if patients initiated sirolimus as early as possible, at 3 months post-transplant, or 2) as if patients initiated sirolimus as late as possible, at 12 months post-transplant. The first analytical approach involved standard survival analyses using Cox Proportional Hazards regression and Kaplan-Meier methods, starting at a "landmark time" of either 3 months or 12 months, such that all patients included in the analyses were alive at the landmark time. The second approach utilized a time-dependent covariate for sirolimus in a Cox regression model, with either 3 months or 12 months considered as the initiation time of sirolimus. This approach enables all patients, including

those who died in the first 12 months, to be included in the analyses; however, it cannot accommodate comparisons of absolute survival such as Kaplan-Meier survival curves or median survival times, etc. The third approach utilized multiple imputation to identify, among patients who died within the first 12 months, those who were most likely planned to receive sirolimus prophylactically (but died before they could receive it). For this approach, the sirolimus group consisted of the patients identified via multiple imputation, in addition to the patients who actually received sirolimus within the first year. Based on the multiple imputation-derived patient classifications, standard Cox regression and Kaplan-Meier analyses were performed, starting at the time of transplantation since these classifications are not susceptible to immortal time bias.

An important consideration is that not all patients receiving sirolimus within the first year are prophylactic users; a very common indication for sirolimus is as a "rescue medication" after treatment failure events (i.e. chronic rejection, malignancies, etc.) occur on other medications. As the 3-month landmark time postulates that patients began sirolimus at the early time of 3 months post-transplant, it is reasonable to assume that treatment failure events recorded within the first year probably arose while the patient was on sirolimus, so its use as a rescue medication would be less likely in this scenario. However, as the 12-month landmark time postulates that patients only began sirolimus at 12 months, it is impossible that treatment failure events recorded within the first year could have arisen while the patient was on sirolimus (in other words, sirolimus use was initiated *after*, and most likely as a result of, the treatment failure event), so such patients were not prophylactic users of sirolimus, but rather, rescue users. Therefore, to ensure comparable groups at the start time of the analyses, and to avoid significant potential confounding by

indication, analyses based on the 12-month landmark time point excluded, from all groups, patients with chronic rejection or malignancy prior to this time point (i.e. within the first year post-transplant), These are two of the most common indications for "rescue" use of sirolimus[35,41,22], and their incidences are recorded annually in the dataset. Though a 3-month landmark time allows more patients to be included in analyses than a 12-month landmark time, the 3-month landmark time does not completely protect against immortal time bias, since patients could have initiated sirolimus as late as 12 months given that most patients only have their second follow-up record at 12 months (1 year). Therefore, the 12-month landmark time was used for our main analyses as this was the safer approach, and the 3-month landmark time was used to determine whether the results were similar either way.

MMF, the most commonly administered cell cycle inhibitor in a tacrolimus-based regimen, was used as the reference group for comparisons. First, baseline patient, donor, and transplant characteristics were compared between the groups. Continuous variables were compared in terms of medians and interquartile ranges, with Wilcoxon rank sum tests used for formal comparisons in order to accommodate non-normal distributions. Categorical variables were compared in terms of frequency distributions, using chi-square tests for formal comparisons. Variables compared were age, gender, race, body mass index (BMI) category, education level, smoking history (>10 pack-years), diabetes, poor renal function (creatinine≥1.3 mg/dL), type of lung disease, medical condition, prior transplant, donor-recipient HLA matches, donor-recipient CMV status, donor age, donor gender, donor smoking (>20 pack-years), transplant type (single or double), era of transplant (2003 to 2005 pre-LAS, 2005 post-LAS to 2010, or 2011 to August 2016), Lung Allocation Score

(LAS), and induction drug (if any). All of these variables except Lung Allocation Score (LAS) were included in multivariable Cox regression analyses, as the LAS is primarily based on several of the aforementioned variables, and some patients in our study were transplanted before the LAS was introduced. A random effect representing transplant center was also included in the multivariable Cox regression model. To enable regression analyses to include patients with missing data on one or more covariates, multiple imputation was utilized. 20 datasets with imputations for missing values were generated using the fully conditional specification (FCS) imputation method, and results based on these 20 datasets were averaged.

Survival was compared between groups using multivariable Cox Proportional Hazards models and Kaplan-Meier survival estimates. Percentages of patients dead from each common cause (chronic rejection/pulmonary, infection, malignancy, other organ failure, or other/unknown) were examined between the sirolimus and MMF groups, based on cumulative incidence functions. For the 3 most common mortality-causing events (chronic rejection, infection, malignancy), in order to compare event risks and post-event death risks between the two drugs, semi-competing risks multivariable Cox regression analyses were performed via the *SemiCompRisks* package in R. The semi-competing risks analysis framework is intended to address the possibility that censoring of a non-fatal event by a fatal event of another type may be informative; such as if patients experiencing the fatal event also had an increased risk of a different non-fatal event of interest at the time they died, compared to patients remaining alive at that time. This scenario creates a dependence between the non-fatal event and the fatal event, which the semi-competing risks framework accounts for by incorporating a patient-specific frailty into the standard

proportional hazards regression model (analogous to a random effect in a mixed-effects model for longitudinal analyses). This frailty represents the residual patient-specific differences beyond those explained by available covariates, thereby serving to account for the dependence between the non-fatal event of interest and the fatal event (death from any other cause). A thorough description of the semi-competing risks framework is provided by Haneuse and colleagues[62,63], the authors of the *SemiCompRisks* package, and a reference manual is available at https://cran.r-project.org; an example of its application in the context of cancer research is described by Jazic et. al[64].

Finally, we examined survival associated with each possible combination of a common induction therapy (alemtuzumab, ATG, basiliximab, daclizumab, or no induction) and cell cycle inhibitor maintenance therapy (sirolimus, MMF, or azathioprine), in order to assess how sirolimus compares to MMF and azathioprine depending on the induction therapy used (if any), and to determine which induction-maintenance combination was associated with the best survival.

SAS version 9.4 and R version 3.4.3 were used for all analyses.

D. RESULTS

Table 3.1 presents a comparison of major baseline patient, donor, and transplant characteristics between the sirolimus and MMF groups. The two groups were similar with respect to most of the baseline characteristics compared, and the aforementioned characteristics were all adjusted for in multivariable analyses.

Table 3.1: Patient Characteristics at Time of Transplant, sirolimus vs. MMF

		Sirolimus	MMF	p-value
		n = 219	**n = 5782**	
Age	**Median (IQR), years**	58 (48-63)	58 (46-64)	0.53
Gender				0.79
	Female	91 (42%)	2456 (42%)	
	Male	128 (58%)	3326 (58%)	
Race				0.05
	Black	27 (12%)	456 (8%)	
	White	178 (81%)	4788 (83%)	
	Hispanic	9 (4%)	407 (7%)	
	Other	5 (2%)	131 (2%)	
BMI category				0.34
	Underweight	38 (17%)	1115 (19%)	
	Normal	62 (28%)	1764 (30%)	
	Overweight	76 (35%)	1982 (35%)	
	Obese	42 (19%)	859 (15%)	
	Missing	1 (0.5%)	62 (1%)	
Lung Disease (primary)				0.70
	Cystic Fibrosis	31 (14%)	881 (15%)	
	Pulmonary Fibrosis	84 (38%)	1944 (39%)	
	COPD	63 (29%)	1535 (26%)	
	Alpha-1 Antitrypsin Deficiency	3 (1%)	175 (3%)	
	Pulmonary Hypertension	6 (3%)	181 (3%)	
	Sarcoidosis	9 (4%)	163 (3%)	
	Other	23 (11%)	596 (11%)	
Prior transplant				0.007
	Yes	17 (8%)	233 (4%)	
	No	202 (93%)	5549 (96%)	
Lung Allocation Score (LAS)	**Median (IQR)**	41 (35-48)	40 (35-50)	0.97
	Missing or N/A (pre-LAS era)	70 (32%)	581 (10%)	
Transplant Type				0.12
	Single	89 (41%)	2056 (36%)	
	Double	130 (59%)	3726 (64%)	
Induction Therapy				<0.0001
	Alemtuzumab	15 (17%)	504 (19%)	
	Equine ATG	4 (2%)	323 (6%)	
	Rabbit ATG	2 (1%)	266 (5%)	
	Basiliximab	71 (32%)	1890 (33%)	
	Daclizumab	60 (27%)	467 (8%)	
	No induction	67 (31%)	2332 (40%)	
HLA matching				0.75
	0-3 matches	171 (97%)	4927 (96%)	
	4-6 matches	6 (3%)	198 (4%)	
	Missing	*42 (19%)*	*657 (11%)*	
Donor age	**Median (IQR), years**	30 (20-44)	31 (21-45)	0.48
Donor smoking (≥20 pack-yrs.)				0.008
	Yes	37 (17%)	637 (11%)	
	No	182 (83%)	5082 (89%)	
	Missing	*0 (0%)*	*63 (1%)*	

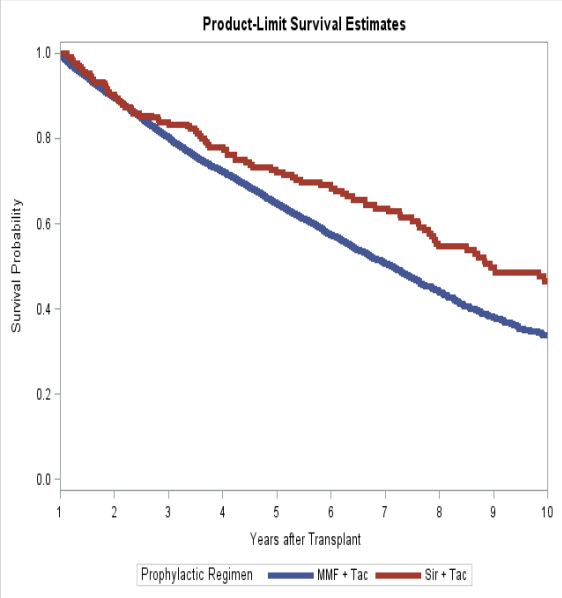
All the different analytical approaches utilized to avoid immortal time bias indicated that sirolimus had better survival than MMF. In multivariable-adjusted analyses based on landmark times of either 12 months or 3 months, the Hazard Ratios for sirolimus vs. MMF were 0.71 (95% CI: 0.56-0.89), p=0.003 for 12 months, and 0.70 (95% CI: 0.58-0.85), p=0.0003 for 3 months. The corresponding Kaplan-Meier survival curves for sirolimus and MMF are shown in Figure 3.1; median survival times were sirolimus 8.9 years (IQR: 4.4-12.7) and MMF 7.1 years (IQR: 3.6-12.1) for a 12-month landmark time, and sirolimus 7.9 years (IQR: 3.5-12.7) and MMF 6.2 years (IQR: 2.7-11.5) for a 3-month landmark time. In analyses using a time-dependent covariate, the HR for sirolimus vs. MMF was 0.66 (95% CI: 0.53-0.82), p=0.0002 for 12 months as the sirolimus initiation time, and 0.67 (95% CI: 0.55-0.81), p<0.0001 for 3 months as the sirolimus initiation time. In analyses starting at transplantation time, after including in the sirolimus group the multiple imputation-identified patients who were likely planned to receive sirolimus but died in the first year, sirolimus remained associated with better survival than MMF: HR=0.71 [95% CI: 0.58-0.87], p=0.001; median survival was 7.8 years [IQR: 2.8-11.9] for sirolimus and 5.8 years [IQR: 2.3-11.1] for MMF, and Kaplan-Meier survival curves for the multiple imputation-derived groups are shown in Figure 3.2.

For the other 3 tacrolimus-based therapies analyzed (MMF + sirolimus combined, mycophenolate sodium [MPS], and azathioprine), survival comparisons are shown based on each of the 3 approaches: landmark time (Figure 3.1), time-dependent covariate (Table 3.2), and multiple imputation-derived identification of planned sirolimus therapy (Figure 3.2), respectively. Based on all approaches, sirolimus had the most favorable adjusted

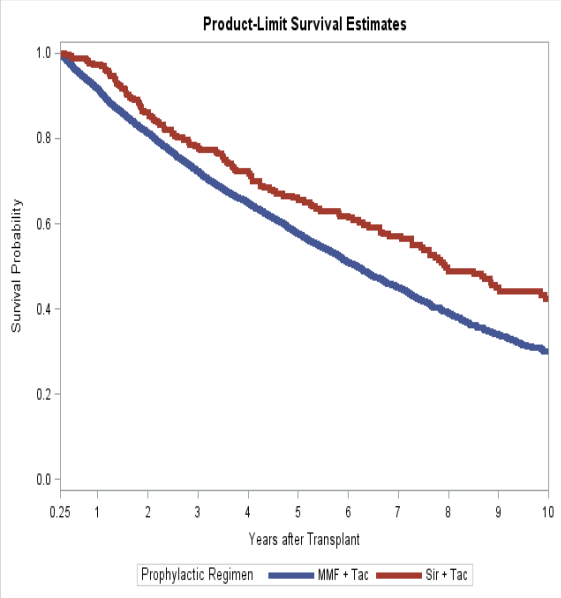
hazard ratio out of all therapies analyzed. No other therapy exhibited a statistically significant improvement over MMF, though azathioprine was associated with a trend towards better survival.

Figure 3.1: Landmark time analyses

12-month landmark time



3-month landmark time



12-month landmark time

	Sir + Tac	Sir + MMF + Tac	Aza + Tac	MPS + Tac	MMF + Tac
# of Patients	219	54	2556	408	5782
# of Centers	33	19	49	26	62
Median Survival (IQR)	8.9 years (4.4–12.7)	5.8 years (3.8– -)	7.9 years (4.2–12.6)	7.5 years (4.1–10.0)	7.1 years (3.6–12.1)
10-year survival	47%	26%	39%	29%	34%
Adjusted HR (95% CI)	**0.71** (0.56–0.89)	**1.14** (0.79–1.65)	**0.93** (0.84–1.02)	**0.95** (0.77–1.17)	**1.00** Reference
	p = 0.003	**p = 0.49**	**p = 0.13**	**p = 0.61**	

3-month landmark time

	Sir + Tac	Sir + MMF + Tac	Aza + Tac	MPS + Tac	MMF + Tac
# of Patients	267	74	3196	535	7751
# of Centers	41	21	52	33	62
Median Survival (IQR)	7.9 years (3.5–12.7)	5.1 years (2.5–11.1)	7.2 years (3.2–11.6)	6.9 years (3.2–10.0)	6.2 years (2.7–11.5)
10-year survival	42%	28%	35%	26%	30%
Adjusted HR (95% CI)	**0.70** (0.58–0.85)	**1.05** (0.77–1.44)	**0.92** (0.85–1.00)	**0.97** (0.82–1.16)	**1.00** Reference
	p = 0.0003	**p = 0.74**	**p = 0.06**	**p = 0.76**	

Sir = Sirolimus; Aza = Azathioprine; Tac = Tacrolimus

Table 3.2: Time-dependent covariate analyses

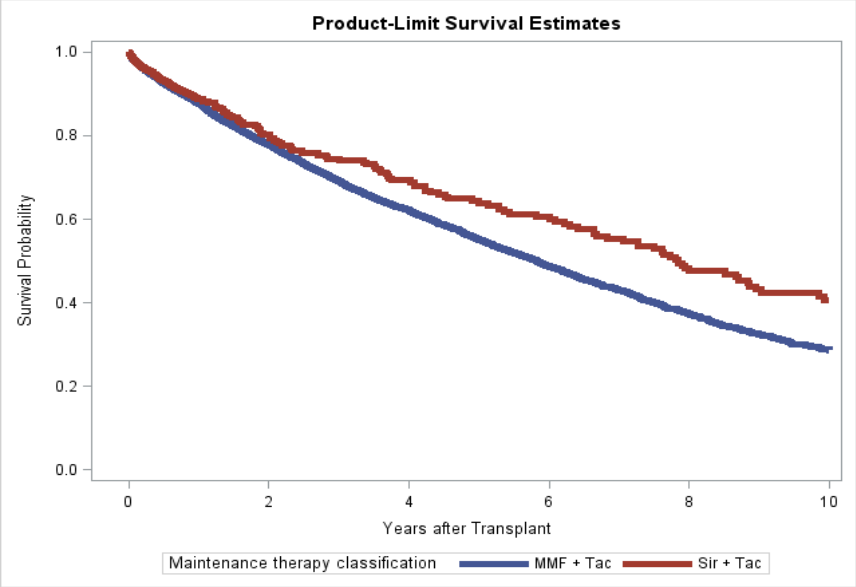
12-month sirolimus initiation time

	Sir + Tac	MMF + Sir + Tac	MPS + Tac	Aza + Tac	MMF + Tac (Standard of Care)
Adjusted HR (95% CI)	**0.66** (0.53–0.82)	**1.22** (0.87–1.72)	**0.91** (0.76–1.08)	**0.93** (0.85–1.01)	**1.00** Reference
	p = 0.0002	**p = 0.24**	**p = 0.27**	**p = 0.07**	

3-month sirolimus initiation time

	Sir + Tac	MMF + Sir + Tac	MPS + Tac	Aza + Tac	MMF + Tac (Standard of Care)
Adjusted HR (95% CI)	**0.67** (0.55–0.81)	**1.09** (0.80–1.47)	**0.90** (0.76–1.06)	**0.93** (0.86–1.01)	**1.00** Reference
	p < 0.0001	**p = 0.60**	**p = 0.21**	**p = 0.07**	

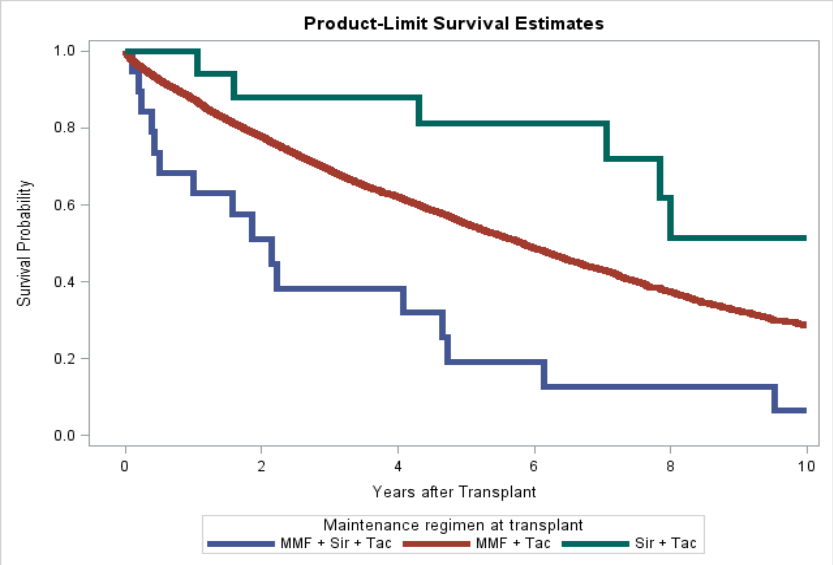
Figure 3.2: Multiple imputation-based classification of planned sirolimus treatment



	Sir + Tac	Aza + Tac	MPS + Tac	MMF + Tac
Median Survival (IQR)	7.8 years (2.8–11.9)	6.8 years (2.7–11.4)	6.4 years (2.9–10.0)	5.8 years (2.3–11.1)
10-year survival	41%	34%	26%	29%
Adjusted HR (95% CI)	**0.71** (0.58–0.87)	**0.93** (0.86–1.01)	**0.90** (0.78–1.17)	**1.00** Reference
	p = 0.001	**p = 0.07**	**p = 0.21**	

Despite the risk of bronchial anastomotic dehiscence if sirolimus is administered too soon after transplantation, the immunosuppression records at transplantation time indicate that 18 patients received sirolimus + tacrolimus and 19 patients received sirolimus + MMF + tacrolimus. The corresponding survival curves are shown in Figure 3.3. Compared to MMF + tacrolimus at transplantation, survival was significantly better for patients receiving sirolimus + tacrolimus (HR=0.42 [95% CI: 0.19–0.94], p=0.04), but significantly worse for patients receiving the MMF + sirolimus + tacrolimus combination (HR=2.01 [95% CI: 1.23-3.41], p=0.006).

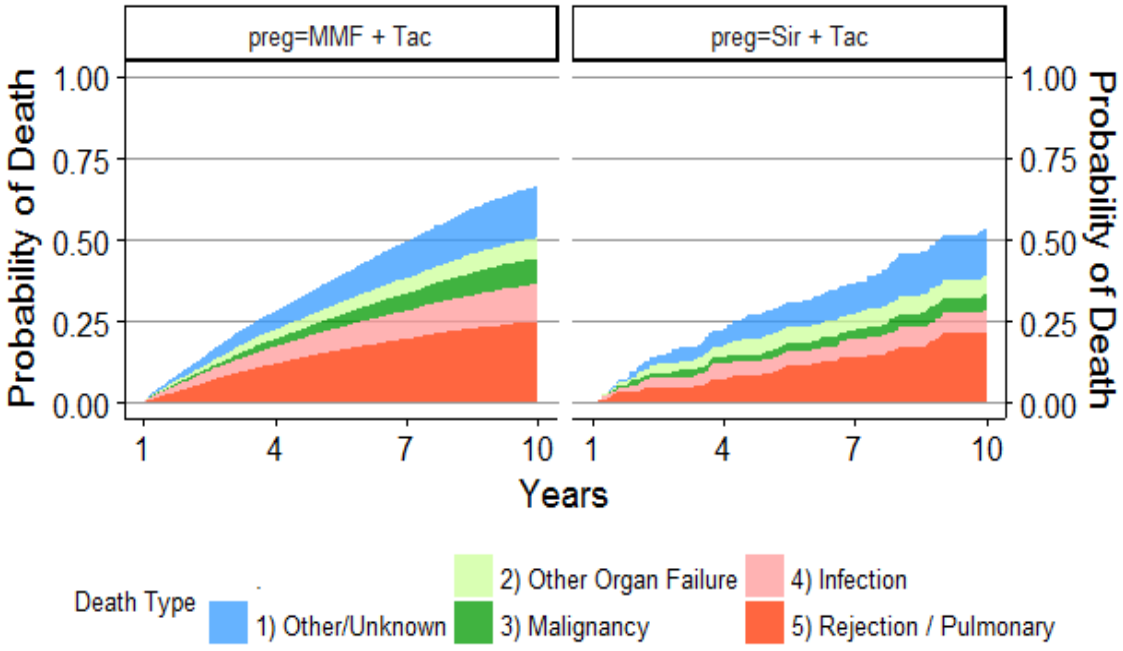
Figure 3.3: Survival comparison between sirolimus, MMF + sirolimus, and MMF administered de novo in a tacrolimus-based regimen



	Sir + Tac	MMF + Sir + Tac	MMF + Tac
# of Patients	18	19	8453
# of Centers	10	11	62
Median Survival (IQR)	- years (7.0– -)	2.1 years (0.4–4.7)	5.8 years (2.3–11.1)
Adjusted HR (95% CI)	**0.42 (0.19–0.94) p = 0.03**	**2.01 (1.20–3.35) p = 0.008**	**1.00 Reference**

For the sirolimus + tacrolimus and MMF + tacrolimus groups, the probabilities of death from each major cause (rejection or respiratory failure, infection, malignancy, other organ failure, or other/unknown) are shown in Figure 3.4, at 3-year intervals beginning at 1 year post-transplant (when all patients in each group are alive).

Figure 3.4: Percentages of patients dead, by major causes (sirolimus vs. MMF)



Death Type	4 years		7 years		10 years	
	MMF	**Sir**	**MMF**	**Sir**	**MMF**	**Sir**
Rejection / Pulmonary	12	7	19	14	25	21
Infection	5	5	9	5	11	7
Malignancy	2	2	5	3	8	5
Other Organ Failure	3	3	5	5	6	5
Other / Unknown	6	5	11	9	16	15
Total Deaths	**28**	**22**	**49**	**36**	**66**	**53**

Analyses of all events, including non-fatal instances, of the 3 most common causes of death (chronic rejection, infection, and malignancy) based on semi-competing risks methods are presented in Table 3.3. The adjusted hazard ratios representing the relative risk of each event (comparing sirolimus vs. MMF) are referred to as HR_1 in the semi-competing risks framework. The corresponding absolute cumulative incidences of each event at 4, 7, and 10 years are also shown in Table 3.3 for the sirolimus and MMF groups. Table 3 also displays: HR_2, which represents the relative risk of death without experiencing the event if interest, along with the corresponding absolute cumulative incidences of event-free death; and HR_3, which represents the relative risk of death after experiencing each event type. The sirolimus group had a lower incidence of chronic rejection (HR=0.75 [95% CI: 0.61-0.92], p=0.005) and a lower risk of death after chronic rejection (HR=0.52 [95% CI: 0.31-0.81], p=0.009). Hospitalized infection incidence was similar (HR=0.91 [95% CI: 0.55-1.36], p=0.68) between groups, but the risk of death after infection was lower in the sirolimus group (HR=0.33 [95% CI: 0.21-0.53], p<0.0001). There was a trend towards reduced malignancy incidence in the sirolimus group (HR=0.71 [95% CI: 0.47-1.03], p=0.09), but no significant differences in the risk of death after malignancy (HR=0.70 [95% CI: 0.37-1.23], p=0.26).

Table 3.3: Incidences of common mortality-causing events (rejection, infection, and malignancy), sirolimus vs. MMF

Event	Adjusted HR (95% CI), Sirolimus vs. MMF	4 years		7 years		10 years	
		MMF	**Sir**	**MMF**	**Sir**	**MMF**	**Sir**
Rejection	HR_1: 0.75 (0.61 – 0.92) p = 0.005	34%	27%	55%	49%	67%	61%
Death without Rejection	HR_2: 0.79 (0.57 – 1.09) p = 0.15	*11%*	*11%*	*17%*	*14%*	*21%*	*19%*
Death after Rejection	HR_3: 0.52 (0.31 – 0.81) p = 0.009						

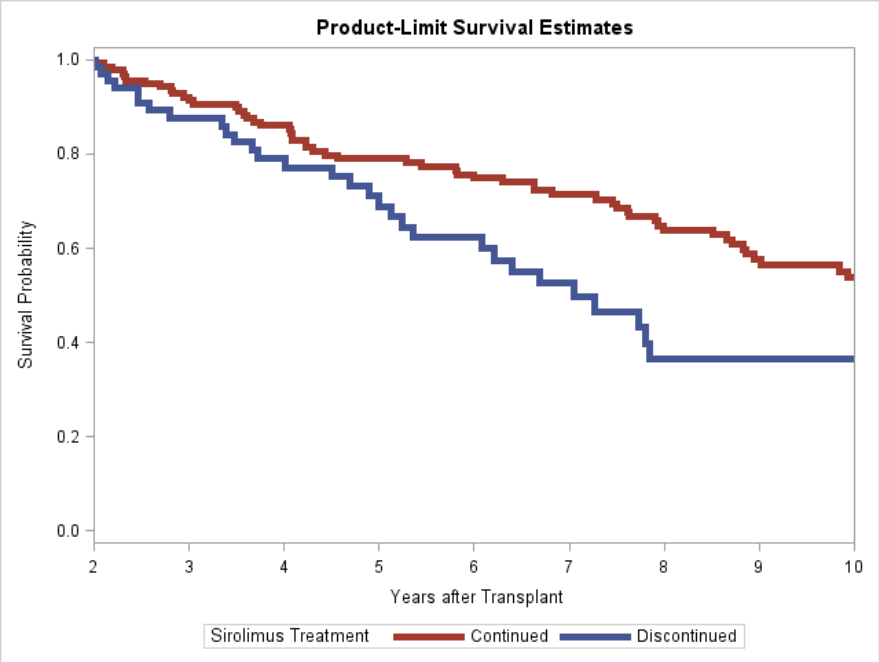
Event	Adjusted HR (95% CI), Sirolimus vs. MMF	4 years		7 years		10 years	
		MMF	**Sir**	**MMF**	**Sir**	**MMF**	**Sir**
Infection	HR_1: 0.91 (0.55 – 1.36) p = 0.68	45%	45%	55%	49%	67%	61%
Death without Infection	HR_2: 0.86 (0.51 – 1.56) p = 0.60	*8%*	*7%*	*17%*	*14%*	*21%*	*19%*
Death after Infection	HR_3: 0.33 (0.21 – 0.53) p < 0.0001						

Event	Adjusted HR (95% CI), Sirolimus vs. MMF	4 years		7 years		10 years	
		MMF	**Sir**	**MMF**	**Sir**	**MMF**	**Sir**
Malignancy	HR_1: 0.71 (0.47 – 1.03) p = 0.09	13%	11%	55%	49%	67%	61%
Death without Malignancy	HR_2: 0.70 (0.55 – 0.89) p = 0.004	*22%*	*18%*	*17%*	*14%*	*21%*	*19%*
Death after Malignancy	HR_3: 0.70 (0.37 – 1.23) p = 0.26						

In the sirolimus group, 66% of patients remained on sirolimus a year later (at the year 2 follow-up record). As shown in Figure 3.5, survival was significantly better in patients remaining on sirolimus compared to those who were no longer on the drug (median survival 10.5 vs. 7.0 yr., p=0.01), among patients alive at 2 years. Within the MMF group, those remaining on MMF at the same time point also had better survival than those no longer on MMF (median 8.1 vs. 7.2 years, p<0.0001). However, the sirolimus-remaining

patients had better survival than the MMF-remaining patients (median 10.5 years vs. 8.1 years, p=0.006).

Figure 3.5: Continued vs. Discontinued Sirolimus Treatment a Year Later



Among sirolimus-treated patients, survival did not differ significantly by the antimetabolite drug (MMF, azathioprine, or MPS) initially used at transplantation prior to sirolimus initiation, although a weak trend towards favorability of azathioprine over MMF was apparent (HR=0.57, 95% CI [0.25-1.29], p=0.18). Additionally, among sirolimus-treated patients, the hazard ratios for use of each induction immunosuppressive drug (Equine ATG, Rabbit ATG, alemtuzumab, basiliximab, and daclizumab) in comparison to no induction were all greater than 1, suggesting that survival was not better, and possibly worse, if any induction therapy was given. However, as three of the five induction drug categories had ≤15 patients, basiliximab was the only induction drug significantly associated with worse survival than no induction (HR=2.79 [95% CI: 1.17-6.61], p=0.02). These results are shown in Table 3.4a.

When assessing survival associated with possible combinations of the aforementioned induction and cell cycle inhibitor maintenance immunosuppressive therapies within a tacrolimus-based regimen, sirolimus with no induction had the most favorable survival: MS=10.7 years; adjusted HR=0.41 [95% CI: 0.26–0.64], p<0.0001 compared to the most common combination, MMF with no induction (MS=6.8 years), in patients alive at 1 year. Table 3.4b shows the adjusted hazard ratios for each combination.

Figure 3.6 displays Kaplan-Meier survival curves (among patients alive at 1 year) for sirolimus and MMF within a tacrolimus-based regimen, with or without induction. Compared to MMF with induction (the most popular immunosuppressive strategy, which over half the patients received), sirolimus with no induction had significantly better survival: adjusted HR=0.48 [95% CI: 0.31–0.76], p=0.002; median 10.7 years [IQR: 7.3–12.7] vs 7.4 years [IQR: 3.9–12.6]. There was a significant interaction between the maintenance and induction variables, p=0.002. The analyses were also performed starting at the time of transplantation after including the multiple imputation-identified patients likely planned to receive sirolimus who died in the first year, and these results are shown in Figure 3.7. In these analyses, sirolimus with no induction remained associated with better survival than MMF with induction: adjusted HR=0.52 [95% CI: 0.34–0.80], p=0.003; median 10.2 years [IQR: 3.4–12.6] vs 6.0 [IQR: 2.4–11.5] years. The interaction between the maintenance and induction variables remained significant in the multiple imputation-based classification analyses, p=0.005.

Table 3.4a: Survival comparisons by induction therapy, among sirolimus-treated patients

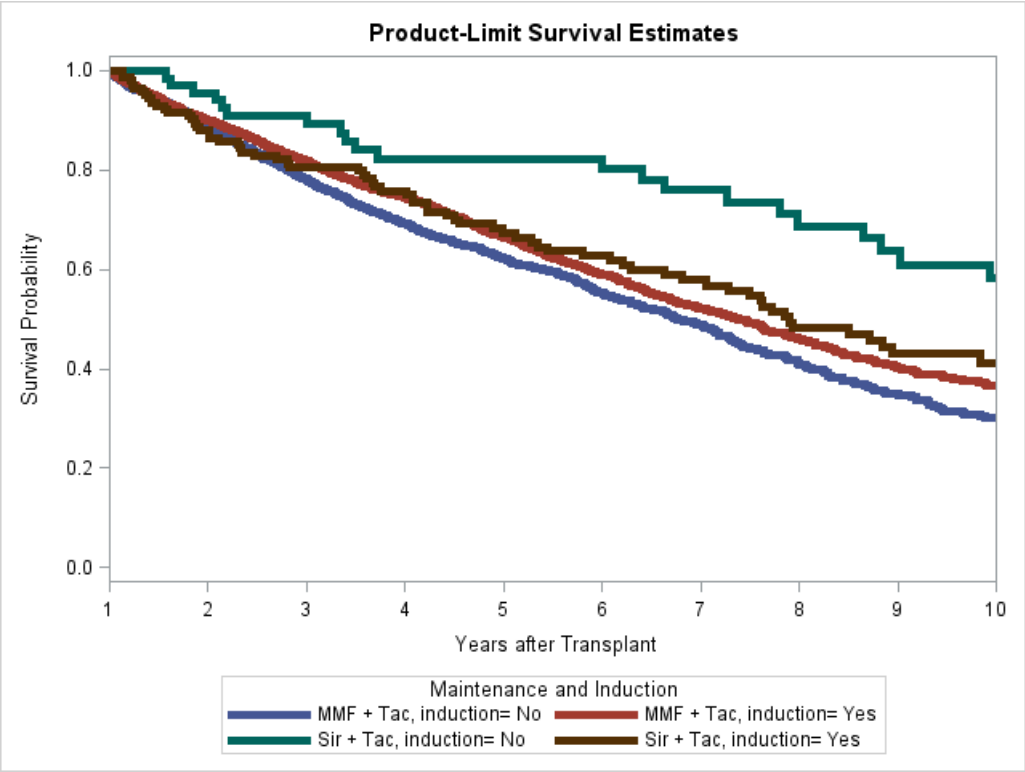
	No induction	Basiliximab	Daclizumab	Alemtuzumab	Equine ATG	Rabbit ATG
Adjusted HR for Death (95% CI) p-value	**1.00** Reference	**2.79** (1.17–6.61) **p = 0.02**	**1.21** (0.45–3.26) **p = 0.71**	**1.47** (0.42–5.20) **p = 0.55**	**8.21** (0.91–73.64) **p = 0.06**	**3.72** (0.16–84.19) **p = 0.41**
Patients	67	71	60	15	4	2

Table 3.4b: Survival comparisons between maintenance and induction therapy combinations

Rank	Maintenance therapy (cell cycle inhibitor within a tacrolimus-based regimen)	Induction therapy	Adjusted Hazard Ratio for Death (95% CI), compared to MMF with no induction	p-value	# of patients	# of centers
1	Sirolimus	None	**0.41 (0.26 – 0.64)**	<0.0001	67	25
2	Sirolimus	Daclizumab	**0.57 (0.38 – 0.88)**	0.01	60	8
3	Azathioprine	Equine ATG	**0.75 (0.54 – 1.03)**	0.08	149	9
4	MMF	Daclizumab	**0.79 (0.66 – 0.94)**	0.009	467	25
5	MMF	Alemtuzumab	**0.79 (0.62 – 1.02)**	0.07	504	7
6	MMF	Equine ATG	**0.80 (0.64 – 1.01)**	0.06	323	12
7	Azathioprine	Daclizumab	**0.82 (0.66 – 1.02)**	0.08	269	15
8	Azathioprine	Basiliximab	**0.82 (0.70 – 0.97)**	0.02	972	30
9	Sirolimus	Basiliximab	**0.87 (0.60 – 1.27)**	0.48	71	18
10	MMF	Basiliximab	**0.89 (0.78 – 1.01)**	0.07	1890	52
11	Azathioprine	None	**0.90 (0.79 – 1.02)**	0.09	1136	42
12	*MMF*	*None*	***1.00 (Ref Group)***	*N/A*	*2332*	58
13	MMF	Rabbit ATG	**1.15 (0.90 – 1.46)**	0.27	266	26

*The following combinations had small numbers of patients and are not shown in Table 4b: 1) Azathioprine and Rabbit ATG, 2) Sirolimus and Alemtuzumab, 3) Azathioprine and Alemtuzumab, 4) Sirolimus and Equine ATG, 5) Sirolimus and Rabbit ATG

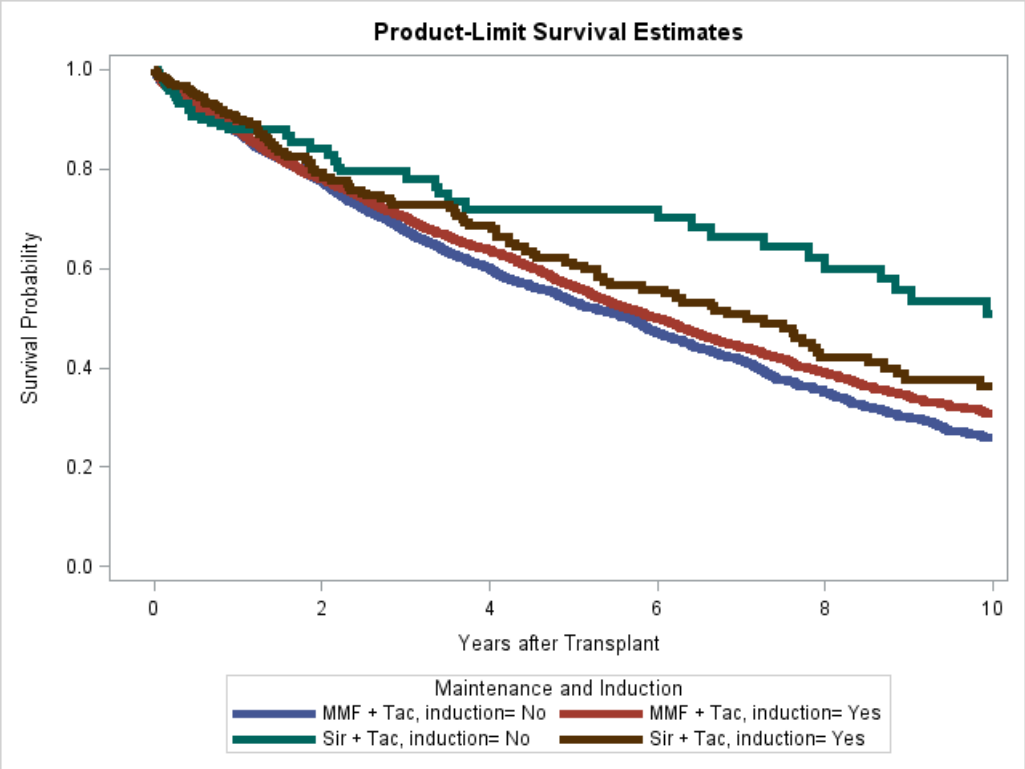
Figure 3.6: Survival for Sirolimus vs. MMF, with or without induction



	Maintenance and Induction			
	Sir + Tac, without induction	**Sir + Tac, with induction**	**MMF + Tac, without induction**	**MMF + Tac, with induction**
Median Survival (IQR)*	10.7 years (7.3–12.7)	7.9 years (4.1– -)	6.8 years (3.3–11.6)	7.4 years (3.9–12.6)
10-year survival*	58%	41%	30%	37%
Adjusted HR (95% CI)	**0.48 (0.31-0.76)**	**0.90 (0.69-1.18)**	**1.15 (1.04-1.28)**	1.00 Reference
	p = 0.002	p = 0.45	p = 0.01	

*These survival estimates are conditional on surviving 1 year post-transplant

Figure 3.7: Multiple imputation-based classifications- Sirolimus vs. MMF, with or without induction

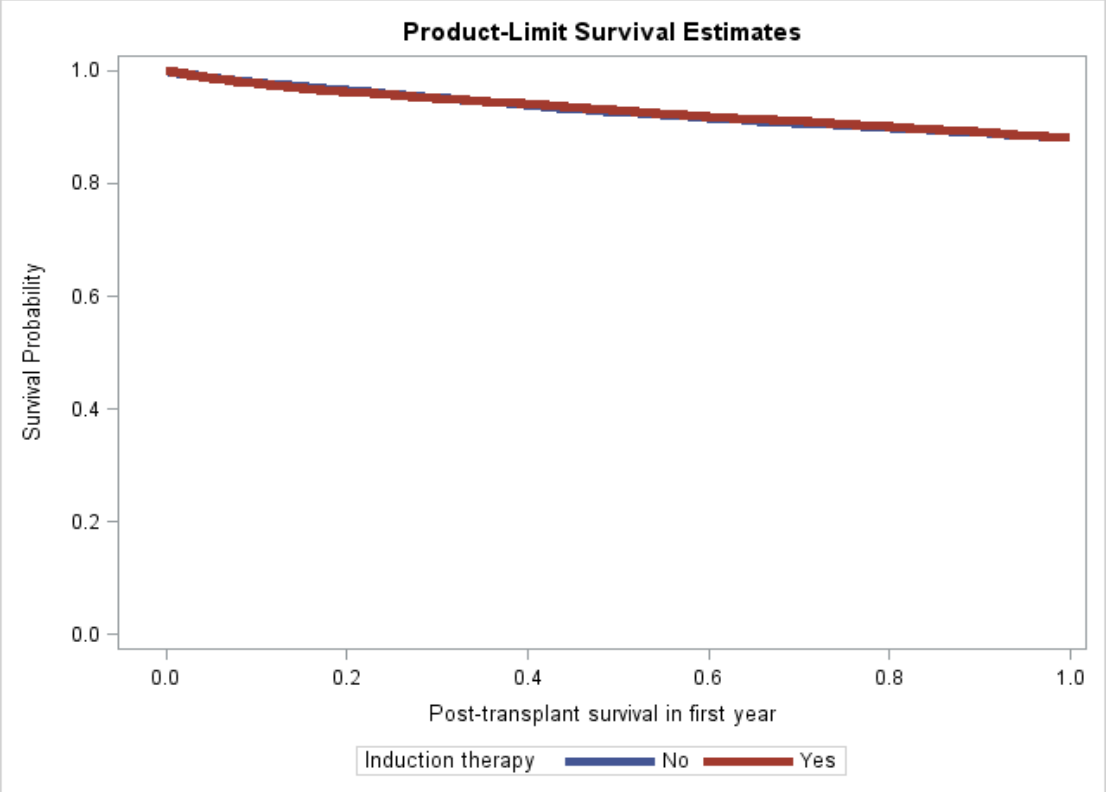


	Maintenance and Induction			
	Sir + Tac, without induction	**Sir + Tac, with induction**	**MMF + Tac, without induction**	**MMF + Tac, with induction**
Median Survival [IQR]	10.2 years (3.4–12.6)	7.0 years (2.5– -)	5.7 years (2.2–10.5)	6.0 years (2.4–11.5)
10-year survival	51%	36%	26%	31%
Adjusted HR (95% CI)	**0.52 (0.31-0.77)**	**0.85 (0.67-1.09)**	**1.09 (1.00-1.19)**	1.00 Reference
	p = 0.003	p = 0.19	p = 0.05	

Since out of all possible induction and maintenance combinations, sirolimus + tacrolimus maintenance with no induction immunosuppression was associated with the best survival among patients alive at 1 year, we assessed whether the presence of

induction therapy was associated with survival in the first year post-transplant (i.e. survival before the time when sirolimus is initiated), among patients receiving tacrolimus in combination with MMF, MPS, or azathioprine at transplantation. 1-year survival was the same (88%) for patients who did or did not receive induction, including in an adjusted model (p=0.93). A Kaplan-Meier survival plot is shown in Figure 3.8.

Figure 3.8: Survival in the first year, with or without induction therapy



E. DISCUSSION

To our knowledge, this study provides the first report on long-term survival associated with prophylactic (or nearly prophylactic) use of sirolimus based on national U.S. lung transplant data. This study has the advantage of representing the overall experience of sirolimus use at 33 centers throughout the United States. Based on multiple

analytical approaches which served to prevent immortal time bias due to the delayed initiation of sirolimus (while also adjusting for available potential confounders), our results consistently indicate that prophylactic use of sirolimus is associated with better survival than MMF within a tacrolimus-based regimen. Additionally, survival for sirolimus was also the most favorable out of all other cell cycle inhibitor maintenance therapies (azathioprine, mycophenolate sodium [MPS], and the combination of MMF + sirolimus) assessed within a tacrolimus-based regimen. The absolute survival improvement with sirolimus over MMF corresponds to a median survival increase of nearly 2 years, among patients alive at 1 year post-transplant. Compared to MMF, 10-year survival probability among patients alive at 1 year was 38% higher for sirolimus.

Among sirolimus-treated patients, we found that patients who received no induction therapy had better survival than patients who received any induction therapy (with basiliximab, daclizumab, anti-thymocyte globulin, or alemtuzumab): median survival 10.7 vs. 7.9 years among patients alive at 1 year post-transplant. Additionally, patients who received sirolimus + tacrolimus with no induction had better survival than patients who received MMF + tacrolimus with induction therapy (median 7.4 years), Notably, sirolimus + tacrolimus with no induction had the most favorable survival out of all possible combinations of induction and maintenance therapies analyzed. These findings remained very similar between multiple analytical approaches we used.

Two prior lung transplant studies have compared sirolimus to more common cell cycle inhibitors for prophylactic use. The first study, a randomized multi-center 181-patient open-label trial by Bhorade et. al.[34], compared sirolimus initiated at 3 months post-transplant to continued use of azathioprine within a tacrolimus-based regimen, and found

no significant differences in 1 or 3 year survival or chronic rejection incidence, but fewer CMV infections with sirolimus. A subsequent single-center 41-patient cohort study by Sacher et. al.[32], with approximately 10 years of follow-up, found that sirolimus initiated at 1-year post-transplant had significantly better long-term survival and lower chronic rejection incidence compared to continued use of MMF, within a tacrolimus-based regimen. In addition to the different study designs, initiation times of sirolimus, and follow-up durations, another important difference between the studies is that in the Bhorade trial there was a high discontinuation rate in both treatment arms, with nearly half the patients having sirolimus discontinued within approximately 1 year of initiation, compared to only 21% of patients in the Sacher cohort study. Sacher et. al. attribute the superior retention among their sirolimus-treated patients to the fact that they delayed sirolimus initiation until 1-year post transplant, since by that time concomitant medications such as valganciclovir and voriconazole that are toxic to bone marrow have been discontinued, and the kidneys have also had sufficient time to recover after the transplant[32], considering that the most common reason for sirolimus discontinuation in the Bhorade trial was renal dysfunction. Interestingly, the Sacher cohort study found significantly better renal function over time in the sirolimus group compared to the MMF group. In agreement with prior studies, this suggests that sirolimus usually has a renal-sparing effect within a tacrolimus-based regimen, though the experience of the Bhorade trial indicates that in the first few months post-transplant, sirolimus use can contribute to nephrotoxicity during this period of high tacrolimus levels and lingering perioperative acute kidney injury.

The short follow-up of the Bhorade trial may partly explain its different findings compared to our study and the Sacher cohort study. In fact, our results suggest that the

survival benefit of prophylactic sirolimus use may only start to manifest by year 2 or 3 post-transplant (as some patients only began sirolimus at 1 year post-transplant), even though this benefit is considerable in the long term. In the short term, mortality after lung transplantation is relatively low and largely related to complications from the procedure itself or the patient's inherent medical condition, so it is logical that any survival differences between immunosuppressive regimens may only become apparent later. In addition, as our data suggest a trend of azathioprine being associated with modestly better survival than MMF, the potential benefit of sirolimus may be more apparent when comparing to MMF, as was done in our study and the Sacher cohort study. Another important point is that the Bhorade trial utilized induction therapy with basiliximab or daclizumab, while our study found that use of induction was associated with decreased survival among patients who received sirolimus. Additionally, the Bhorade trial had consistently lower trough levels of sirolimus and tacrolimus than the Sacher study, at approximately 1 year into each study, and also at the final follow-up reported for each study.

In addition to our finding of improved survival, we found that the incidence of chronic rejection was lower with sirolimus than MMF, and in addition, the risk of death after chronic rejection was also lower. The risk of death after chronic rejection is important as well, because the incidence and grade of rejection do not necessarily predict mortality, since the diagnosis of chronic rejection is based on a percentage decline from the best lung function test in the patient's post-transplant history, so the absolute lung function of one patient with chronic rejection may be much higher than that of another patient, even if they have the same rejection severity or "grade" (which only implies a similar *percentage*

decline from their maximum lung function test value). Therefore, if the peak lung function tends to be higher for patients on one drug compared to another, when patients on both drugs meet the numerical criteria for a rejection diagnosis, patients on the first drug may be less likely to die from rejection or respiratory failure than those on the second drug, because their absolute lung function is better.

Although the incidence of hospitalized infections was not significantly different between the two groups, the risk of death after infection was significantly lower in the sirolimus group compared to MMF, and the absolute frequency of deaths from infection was 36% lower at 10 years. One possible explanation is that sirolimus weakens immunological defenses against infection development as severely as MMF does, but does not reduce the immune system's ability to fight an existing infection as drastically as MMF does. Another possibility is that the types of infections to which sirolimus predisposes patients are, overall, less life-threatening than those to which MMF predisposes patients.

Our results suggest a trend towards a moderate reduction in malignancy incidence with sirolimus compared to MMF, although there was no statistically significant difference between the groups. Additionally, the absolute frequency of deaths from malignancy was 38% lower at 10 years in the sirolimus group. As the effect size is reasonably strong but statistical significance was not achieved, this may be partly explained by inadequate power to detect a difference with respect to malignancy since it is the least frequently occurring of the 3 events, and the sirolimus group was relatively small in size. Similarly as for infection, one possible explanation for the reduced deaths is that in addition to a potentially lower incidence of malignancies with sirolimus, the types of malignancy which commonly

occur in patients on sirolimus may be generally not as deadly as those commonly occurring in patients on MMF.

A fundamental consideration is that although we do not ha e drug dosages or trough levels for patients in our study, all prior studies have reported that patients on sirolimus are maintained with significantly lower tacrolimus levels compared to those on MMF or azathioprine[34,32,43]. Therefore, the reduced deaths from infection or malignancy in the sirolimus group could be simply due to lower tacrolimus levels, or as hypothesized, due to substituting sirolimus for MMF. However, the reduction in chronic rejection incidence and related deaths is almost certainly due to substituting sirolimus for MMF, given that patients on sirolimus generally have much lower tacrolimus levels.

When subdividing by induction therapy, sirolimus-treated patients who received no induction had the best survival out of all possible induction and maintenance combinations in our study, which was somewhat unexpected. The group that received sirolimus with no induction included patients from 25 transplant centers, so the very good survival of this group was not dependent on one or two centers or physicians with outstanding results. Although induction therapy is given for a short time in the peri-operative period, its effects are expected to persist years later; in fact, the goal of induction is to improve long-term outcomes[65]. One explanation for why survival may be reduced with induction therapy among sirolimus-treated patients is that the long-lasting effects of induction, in combination with sirolimus maintenance therapy, could result in an unnecessary level of immunosuppression which leads to increased deaths from infection, malignancy, etc. without a worthwhile decrease in rejection, since sirolimus + tacrolimus immunosuppression seems quite effective even with no induction. It has been mentioned

that thymoglobulin (rabbit ATG) induction is a risk factor for side effects of sirolimus treatment in renal transplant recipients[66], though it is unknown whether this should apply to other induction agents as well. It is also possible that levels of sirolimus and tacrolimus typically maintained are optimal for patients who have not been induced, but too high for patients who have been induced, resulting in excessive immunosuppression; however, our study does not have drug level data to investigate this. A somewhat opposite explanation for the reduced survival with induction therapy would be that patients who received induction were generally maintained on lower levels of sirolimus and tacrolimus, and those lower levels were inadequate for preventing rejection. This possibility might seem to be supported by the differences in management and outcomes between the Bhorade trial and the Sacher cohort study that are mentioned in each article.

It should be made clear that sirolimus use is not detrimental in patients who have received induction therapy, as the survival for sirolimus was at least as good as MMF among induced patients. In fact, sirolimus with daclizumab induction had better survival than every other possible induction-maintenance combination except sirolimus with no induction. Therefore, the main point is that survival appears to be maximized if sirolimus is given with no induction therapy, and the benefit of sirolimus appears to exceed the benefit of any induction drug even with another maintenance therapy. It is also important to note that survival within the first year (i.e. survival until the time when sirolimus might be initiated) does not appear to be compromised by a lack of induction, as 1-year survival was indistinguishable between patients who did or did not receive induction, even in an adjusted model.

On another note, the phenomenon of excessive immunosuppression, which could explain why induction therapy is associated with worse survival among sirolimus-treated patients, is also a likely explanation for our finding that the sirolimus + MMF + tacrolimus regimen has worse survival than sirolimus + tacrolimus alone. This finding suggests that MMF should generally be discontinued at the time sirolimus is initiated, although it is possible that the drug doses typically administered were simply too high for using all three drugs concurrently, and lower doses could result in favorable outcomes with combined use of all three drugs. The sirolimus + MMF + tacrolimus triple-drug regimen appears especially dangerous if used at the time of transplantation, with 5 of 19 patients (over 25%) dying within 0.4 years post-transplant, and a median survival of approximately 2 years. On the other hand, even though the 18 patients on sirolimus + tacrolimus immediately after transplantation had much better survival than those on sirolimus + MMF + tacrolimus, this provides no assurance that sirolimus administration immediately after lung transplantation can be safe, even if it is with tacrolimus only and without MMF. In fact, the study which initially reported the high risk of bronchial anastomotic dehiscence (and associated fatalities) attributed to sirolimus use in *de novo* lung transplant recipients was based on patients receiving sirolimus + tacrolimus only[39]. Therefore, the recommendation to delay sirolimus initiation until the bronchial anastomoses are healed should be followed unquestionably to prevent potentially fatal outcomes.

As this is a non-randomized study, its susceptibility to confounding must be noted, although our regression analyses adjusted for an extensive list of important potential confounders. The sirolimus and MMF groups were quite comparable with respect to the numerous variables we assessed, and in fact, the few imbalances detected were generally

more likely to pose disadvantages to the sirolimus group (this group had more previously transplanted patients, more heavy smoking history donors, and a trend towards more single lung transplant patients). Therefore, it does not appear that the sirolimus group was intrinsically advantaged or healthier at baseline. Our adjusted analyses also controlled for potential confounding by transplant center, to address the possibility that the centers which administered sirolimus tended to have different outcomes from other centers regardless of drug. It remains possible that on average, physicians who chose to administer sirolimus may have been inherently more effective in managing their patients and this could have resulted in better outcomes. However, nearly half the patients in the sirolimus group were from over 20 centers that each had 10 or less patients on this regimen, so the sirolimus group is not comprised of large clusters of patients managed by the same center or physician. Alternatively, it is possible that patients who agreed to try sirolimus as an unconventional but potentially superior therapy, or requested sirolimus themselves due to knowledge about its purported advantages, may have been more conscious about their health in general and this helped them to have better outcomes.

There is also potential confounding that could have biased our findings *against* sirolimus, since it is fairly likely that a nontrivial proportion of patients in the sirolimus group are not truly prophylactic users. This is evident from the fact that over 20% of patients in the sirolimus group are from centers that had 5 or fewer patients on sirolimus, strongly suggesting that sirolimus is not the default prophylactic therapy at those centers or even under a given physician at those centers. In other words, the likely reason why these patients started sirolimus within the first year was as a “rescue” from some complication that arose while on their prior treatment, but is not captured in the dataset. If

the complications prompting the rescue switches to sirolimus were commonly life-threatening and irreversible, then our results may be biased against sirolimus, since many patients would have already had an impaired prognosis when starting the drug. Even at centers which commonly use sirolimus for long-term prophylactic therapy, there is the potential phenomenon of some reluctance to switch patients who are doing especially well in the first year (on mycophenolate or azathioprine) over to sirolimus, while for patients doing poorly in the first year, switching to sirolimus may be a favored strategy, thereby resulting in a disproportionate number of patients with an impaired prognosis in the sirolimus group. Nevertheless, chronic rejection and malignancy are perhaps the most life-threatening irreversible conditions that develop after transplantation, and these are captured in the dataset, so our data seem adequate to provide a reasonably balanced comparison of sirolimus to other drugs. Even if the results are somewhat biased against sirolimus, it is clearly noteworthy that the sirolimus group had better survival in spite of being potentially disadvantaged by indications.

Our study design of classifying patients (as best as possible) by the maintenance immunosuppressive regimen they received prophylactically, rather than by any "rescue" regimens that may have been initiated following a treatment failure of the prophylactic regimen, appears to be the most reasonable way to compare regimens in terms of outcomes. However, the main limitation of this approach is that any subsequent drug switches are not considered; in other words, some patients would have eventually received a different regimen than that which they are classified under. We found that for both sirolimus and MMF, patients who switched off their prophylactic therapy had significantly worse subsequent survival compared to those remaining on the therapy at the same time point.

This indicates that patients usually switch therapies due to an event worsening their prognosis such as a serious complication or a lack of effectiveness, and this suggests that it is best to analyze outcomes according to the prophylactic treatment that each patient received (i.e. analyze in a manner analogous to intention-to-treat principles), as done in this study. Another important advantage of this approach is that the immunosuppression received in the early years after transplantation is likely to be more consequential than that received several years later. This is because tolerance of the transplanted lung(s), which is critical in helping to prevent transplant rejection and is the primary goal of immunosuppression, is important to establish relatively early in the course of a patient's post-transplant lifetime[65]; for instance, this is part of the rationale for induction immunosuppression. This is also why doses of maintenance immunosuppressive drugs are usually highest in the first few years, and consequently, the impact of drug choice in terms of anti-rejection efficacy as well as life-threatening adverse effects, is likely to be greater early compared to later on post-transplant.

The strengths of this study, starting with those related to the data source, include the use of a national dataset that contains all lung transplant recipients in the United States, as well as adjustments for a large number of potential confounders available in the dataset. Notably, our Cox regression models incorporated a random effect representing transplant center, which accounts for expected confounding induced by variation in outcomes between transplant centers, while appropriately estimating the associated standard errors (unlike the usual fixed effect). Additionally, we explored multiple approaches to address the issue of potential immortal time bias due to delayed sirolimus initiation, as detailed in the Methods and Results sections, with the hope of confirming that the results would be

similar regardless of approach, which we were able to confirm. Finally, the semi-competing risks regression models, which we used for assessing associations with events of interest such as chronic rejection, infection, and malignancy, address possible informative censoring of non-fatal events by a fatal event of another type; for example, if patients experiencing the fatal event generally had an increased risk of the non-fatal event of interest at the time they died, compared to patients remaining alive at that time. In addition to comparing the risks of each event type between regimens, the semi-competing risks framework also compares between regimens the risk of death after a given non-fatal event occurs. This is relevant since chronic rejection, infections, and cancer all have significant variation in severity, and the typical severity of each event type could differ between regimens.

Given that sirolimus should not be administered immediately after transplantation, a fundamental decision is by how many months post-transplant to delay the initiation of sirolimus. This study unfortunately cannot provide insight in this regard due to the infrequent intervals at which immunosuppression data are collected in the dataset. The Sacher cohort study, in which patients initiated sirolimus between 9-12 months, found a remarkable survival benefit of sirolimus over MMF, while the Bhorade trial, which started patients on sirolimus at 3 months post-transplant, did not find a significant benefit over azathioprine (perhaps partly due to high treatment discontinuation rates). However, as previously mentioned, there were other important differences besides the sirolimus initiation time between these two studies, which could also be responsible for the conflicting results. Another single-center study suggested superior lung function when sirolimus was initiated in the first 6 months post-transplant rather than later on. Considering

these findings together, it appears that around 6-12 months post-transplant may be the best time to initiate sirolimus. Nevertheless, a recent single-center study of 13 patients on a cyclosporine-based regimen evaluated to be low-risk for dehiscence who initiated sirolimus by 1 month post-transplant following complete healing of their bronchial anastomoses, reported that short-term and long-term survival were favorable and only one patient developed chronic rejection[61]. This suggests that initiating sirolimus as early as 1 month after transplantation may be safe in carefully selected patients, and may benefit certain patients, such as those at relatively high risk of rejection.

For patients who are several years post-transplant and stable on another regimen, our data do not indicate whether there will be a benefit of switching to sirolimus + tacrolimus. The data clearly suggest that a switch to sirolimus within the first year from mycophenolate or azathioprine in a tacrolimus-based regimen is likely to be beneficial, but it is uncertain whether this applies to a switch several years after transplant. Likely benefits of switching to sirolimus even after the first year include a decreased risk of chronic rejection and associated deaths, and possible reductions in the risks of death from infection or malignancy, but these are based on extrapolation of the outcomes for first-year switches to sirolimus.

Because of the significant potential benefit of sirolimus in lung transplant recipients, strong efforts should be made to enable patients to tolerate and stay on the drug. It has been stated that most side effects linked to sirolimus are either moderate or mild and dose-related; there is a publication dedicated to strategies for minimizing the risks of most common adverse effects associated with mTOR inhibitors, and for managing these adverse effects if they do occur[67]. Though optimal drug dosing and trough level targets are critically

important, our data do not provide any information on drug dosages or trough levels. In the cohort study by Sacher et. al., which reported very good survival with sirolimus + tacrolimus, a sirolimus dose of 2 mg/day was administered initially at 1 year post-transplant and adjusted as necessary, and prednisone doses were 7.5 ± 2.9 mg/day at 2 years post-transplant and 4.5 ± 2.3 mg/day at the final follow-up. Actual trough levels of sirolimus and tacrolimus were: at 2 years post-transplant (1 year after sirolimus initiation), 9.1 ± 2 ng/ml for sirolimus and 8.6 ± 2.2 ng/ml for tacrolimus, and at the final follow-up, 7.7 ± 1.8 ng/ml for sirolimus and 6.8 ± 1.7 ng/ml for tacrolimus. It should be noted that the patient population was relatively young (mean 43 ± 13 years), so optimal levels may be different for older patients. The Bhorade trial, which did not observe a benefit of sirolimus, reported somewhat lower trough levels of both sirolimus and tacrolimus: at 1 year post-transplant (9 months after sirolimus initiation), sirolimus median 6.6 ng/ml (interquartile range 5.0-9.1) and tacrolimus median 7.0 ng/ml (interquartile range 5.5-9.4), and at 3 years, sirolimus median 6.2 ng/ml (interquartile range 4.8-7.4) and tacrolimus median 6.0 ng/ml (interquartile range 3.2-6.5). It is plausible that the higher sirolimus and tacrolimus levels in the Sacher study were an important contributor to the encouragingly low chronic rejection incidence (39% at 9 years), which would have helped to enable the very good survival in their sirolimus-treated patients.

In conclusion, our study based on the national U.S. lung transplant dataset suggests that sirolimus, initiated in the first year post-transplant within a tacrolimus-based regimen, improves survival compared to MMF. The data indicate that maximal survival may be achieved with sirolimus by not administering induction therapy, although sirolimus use in patients who have received induction therapy is still associated with favorable survival.

Future long-term prospective studies in lung transplantation will be needed to confirm the promising findings observed in this study. In the meantime, in order to potentially improve long-term survival, elective switching to sirolimus as the cell cycle inhibitor in the first year if possible, within a tacrolimus-based regimen preferably without induction therapy for future transplant recipients, should be given strong consideration for all lung transplant patients.

Chapter 4: Azathioprine Is Associated with Better Survival than Mycophenolate Mofetil in a Tacrolimus-Based Regimen among U.S. Lung Transplant Recipients

A. ABSTRACT

Background

Long-term survival after lung transplantation remains limited, although easily modifiable factors including induction and maintenance immunosuppressive therapies lack thorough survival comparisons in this population. We report elsewhere that sirolimus initiated within the first year in a tacrolimus-based maintenance regimen has better survival than antimetabolites including mycophenolate or azathioprine, with the highest survival observed in patients who received sirolimus + tacrolimus and no induction therapy, based on national U.S. lung transplant data. However, long-term survival has not been compared between the antimetabolites, which this study aims to do, in addition to comparing common induction therapies.

Methods

This study of U.S. lung transplants from 2003-2016 in the United Network for Organ Sharing (UNOS) dataset compared common induction and *de novo* antimetabolite maintenance therapies within a tacrolimus-based regimen. Induction therapies included were: alemtuzumab, equine anti-thymocyte globulin [ATG], rabbit ATG, basiliximab, daclizumab, and no induction. Maintenance therapies included were: mycophenolate mofetil [MMF], mycophenolate sodium [MPS], and azathioprine. Survival was compared via Cox multivariable regression models utilizing multiple imputation for missing data. No induction and MMF maintenance were the respective reference levels for all comparisons.

Results

Azathioprine (HR=0.92, p=0.05) maintenance therapy had better survival than MMF. Among induction therapies, equine anti-thymocyte globulin (ATG) was associated with the best survival: adjusted hazard ratio (HR)=0.79, p=0.003 compared to no induction, and daclizumab was also associated with better survival (HR=0.88, p=0.03). Alemtuzumab (HR=0.83, p=0.06) had a trend towards better survival, while rabbit ATG (HR=1.18, p=0.07) had a trend towards worse survival.

Conclusions

Since azathioprine is associated with slightly better survival than MMF in a tacrolimus-based maintenance regimen, it is a promising alternative to MMF given its cost-effectiveness. However, based on the same dataset, we have found that sirolimus + tacrolimus maintenance with no induction therapy has the most favorable survival of all common immunosuppressive strategies, so azathioprine may be most appropriate if sirolimus cannot be utilized for any reason. Although the optimal immunosuppressive strategy appears to be no induction therapy with maintenance therapy consisting of sirolimus initiated within the first year in a tacrolimus-based regimen, equine ATG induction may improve survival when antimetabolites including azathioprine or mycophenolate are planned to be used instead of sirolimus for long-term maintenance therapy.

B. INTRODUCTION

Although lung transplantation is a life-saving procedure for patients with end-stage lung disease, long-term survival unfortunately remains poor. Over 50% of patients die

within 6 years after transplant, and approximately two-thirds die within 10 years after transplant[2]. The majority of deaths among lung transplant recipients are attributable to one of three causes: chronic lung rejection, infection, and malignancy[8]. To reduce the risk of chronic lung rejection, patients are placed on a lifelong regimen of immunosuppressive drugs after transplantation. There are two major quandaries related to these immunosuppressive drugs: first, the drugs often cannot prevent chronic lung rejection in the long-term, and second, immunosuppression tends to increase susceptibility to multiple life-threatening conditions, most notably infections and malignancies[9,10,11].

Prior to beginning their lifelong regimen of immunosuppressive medications, referred to as maintenance immunosuppression, over 50% of lung transplant patients receive a brief course of high-intensity immunosuppression in the peri-operative period, referred to as induction immunosuppression. Induction immunosuppressive medications fall into two main categories: T-lymphocyte depleting and non T-lymphocyte depleting agents. IL-2 receptor antagonists, including the drugs basiliximab and daclizumab, are the most common non T-lymphocyte depleting induction agents. Basiliximab contains 75% human antibodies, while daclizumab contains 90%. Among T-lymphocyte depleting drugs, alemtuzumab is a newer drug which has been shown to induce prolonged T-cell depletion for >1 year, while ATG (Anti-Thymocyte Globulin) is an older drug that targets multiple cell surface antigens (i.e. a polyclonal agent), unlike the three aforementioned drugs (monoclonal agents) which only target a single antigen.

There is currently no consensus regarding which induction immunosuppressive drug, if any, has the most beneficial effect on survival, since different results have ensued from the few previous studies[65,68]. No randomized trials have established that any induction

drug improves survival over no induction[52,69]. Prior national cohort studies[51,54] have suggested that basiliximab and alemtuzumab are associated with better survival than no induction among lung transplant recipients; however, the studies have not considered potential confounding by transplant center, even though center preferences are a fundamental determinant of induction immunosuppression decisions. Additionally, the use of certain induction drugs is likely to be correlated with the use of certain maintenance regimens, because the patient's physician(s) are typically responsible for deciding both, so spurious associations can potentially arise between these treatment factors and survival.

The typical maintenance immunosuppressive regimens in current use consist of a calcineurin inhibitor (such as tacrolimus or cyclosporine) combined with a cell cycle inhibitor. The cell cycle inhibitor is usually an antimetabolite (such as mycophenolate mofetil (MMF), mycophenolate sodium (MPS), or azathioprine), but much less commonly, can be a mammalian target of rapamycin (mTOR) inhibitor (such as sirolimus or everolimus). Three randomized trials have compared tacrolimus and cyclosporine[16,17,18], and while none of them found a significant difference in patient survival over the follow-up periods of up to 3 years, they were in general agreement that tacrolimus was associated with lower incidences of acute and chronic rejection, and infections, compared to cyclosporine. A randomized controlled trial comparing MMF and azathioprine also found no significant differences in survival up to 3 years, and no differences in the incidences of acute or chronic rejection. However, treatment withdrawals due to adverse side effects were more common in patients on azathioprine compared to those on MMF[23]. This finding likely had a major role in the development of the widespread preference of MMF over azathioprine in practice today, as another randomized controlled trial limited to 6 months

of follow-up found no significant differences between the two drugs in terms of survival, acute rejection, infections, or adverse events[24]. Mycophenolate sodium (MPS) is an enteric-coated antimetabolite medication which has the same active ingredient as MMF: mycophenolic acid (MPA). However, MPS was designed to reduce the side effects of MMF by delivering MPA directly to the small intestine, rather than having it metabolized in the stomach. Multiple studies in other populations, including kidney and liver transplant recipients, and autoimmune disease patients, have found that MPS is associated with lower risks of serious infections[28,29] and gastrointestinal side effects[26,27,28,29] and better renal function, compared to MMF[25], while providing similar anti-rejection efficacy. MPS, compared to MMF, also demonstrated better pharmacokinetic absorption of MPA in several studies of kidney transplant recipients[25,29,30]; one explanation is that absorption of MPA may be improved when it is delivered to the small intestine, since absorption can be suboptimal in the stomach if acidity is inadequate[25,29]. This may be particularly relevant for the lung transplant patient population due to the significant prevalence of gastro-esophageal reflux disease (GERD), which is frequently treated with acid-reducing medications such as proton pump inhibitors; at least one proton pump inhibitor has been found to impair MMF absorption but not MPS absorption[31]. The apparently superior absorption, in addition to the potential lessening of side effects, which is likely to reduce lapses in immunosuppression, suggest that MPS might lower the risk of chronic lung rejection compared to MMF, thereby possibly leading to increased survival. Use of MPS has slowly increased in recent years among lung transplant patients, though MMF remains by far the most widely used antimetabolite in this population.

We described earlier our findings that, based on the UNOS (United Network for Organ Sharing) national U.S. lung transplant data, patients receiving sirolimus in the first year as the cell cycle inhibitor within a tacrolimus-based regimen, replacing antimetabolites such as MMF, MPS, or azathioprine, had better long-term survival. The most favorable survival out of all possible induction and maintenance combinations was observed with no induction immunosuppression and sirolimus + tacrolimus maintenance immunosuppression; the median survival for this group was over 10 years, among patients alive at 1 year post-transplant, and the findings remained similar in adjusted analyses. However, a limitation of sirolimus use is that it must be delayed by at least 3 months post-transplant to avoid the risk of a potentially fatal complication called bronchial anastomotic dehiscence[39], and is sometimes delayed up to 1 year to prevent other complications including nephrotoxicity[32]. Therefore, an antimetabolite must be used in its place as the cell cycle inhibitor for the first 3-12 months. Although our prior analyses determined that sirolimus is associated with better survival than antimetabolites, they were not intended for directly comparing the antimetabolites to each other, since due to the way these analyses were generally structured to avoid immortal time bias arising from the delayed initiation of sirolimus, they may not properly reflect any survival differences between antimetabolites within the first year. A comparison of long-term survival between antimetabolites has never been done in lung transplantation, to our knowledge, and would be particularly useful to patients in whom sirolimus cannot or will not be administered for any reason.

This study serves to compare survival between common induction therapies (alemtuzumab, basiliximab, daclizumab, equine ATG, rabbit ATG, and no induction) and

between antimetabolite maintenance therapies (MMF, MPS, and azathioprine) administered at the time of transplantation.

C. METHODS

This retrospective cohort study consisted of 12,425 U.S. lung transplant recipients from 2003-2016, contained in the United Network for Organ Sharing (UNOS) dataset. Patients were classified according to the induction therapy they received: alemtuzumab (Campath®), equine anti-thymocyte globulin [equine ATG] (Atgam®), rabbit anti-thymocyte globulin [rabbit ATG] (Thymoglobulin®), basiliximab (Simulect®), daclizumab (Zenapax®), or no induction, and according to the antimetabolite they received for maintenance therapy at transplantation: mycophenolate mofetil [MMF] (Cellcept®), mycophenolate sodium [MPS] (Myfortic®), or azathioprine (Imuran®). Patients who received more than one induction drug were excluded. All patients received tacrolimus (Prograf®) at transplantation as their calcineurin inhibitor for maintenance therapy.

Kaplan-Meier survival estimates were compared between the different induction and maintenance therapies, and Cox Proportional Hazards multivariable regression was utilized to perform adjustment for confounding. In addition, we assessed the presence of an interaction between induction and maintenance therapies, representing whether the effect of one or more maintenance therapies differed according to the induction therapy used, or vice versa. We also examined, via tests for interaction, whether the survival comparisons between induction therapies or between maintenance therapies varied according to age group. Based on cumulative incidence functions, we examined the probabilities of death from each major cause, within each of the different induction and

maintenance therapy categories. For the 3 most common mortality-causing events (chronic rejection, infection, malignancy), in order to compare event risks and post-event death risks between therapies, we used semi-competing risks multivariable Cox regression models via the *SemiCompRisks* package in R. The purpose of the semi-competing risks analysis framework is to address the possibility that censoring of a non-fatal event by a fatal event of another type may be informative; for example, if patients who experienced a fatal event also had an increased risk of a different non-fatal event at the time they died, compared to patients remaining alive at that time. Resources providing more detailed explanations of the semi-competing risks framework include thorough descriptions as provided by Haneuse and colleagues[62,63], the authors of the *SemiCompRisks* package, and an article by Jazic et. al. describing an example of its application in cancer research.[64]

For induction therapy comparisons, no induction served as the reference group, and for antimetabolite maintenance therapy comparisons, MMF served as the reference group, since these were the most popular induction and antimetabolite maintenance therapies, respectively. First, patient, donor, and transplant characteristics at baseline were compared between the 6 induction groups and between the 3 maintenance groups. Continuous variables were compared in terms of medians and interquartile ranges, with Kruskal-Wallis tests used for formal comparisons in order to accommodate non-normal distributions, and categorical variables were compared in terms of frequency distributions, using chi-square tests for formal comparisons. Variables compared were age, gender, race, body mass index (BMI) category, education level, smoking history (>10 pack-years), diabetes, poor renal function (creatinine≥1.3 mg/dL), type of lung disease, medical condition, prior transplant, donor-recipient HLA matches, donor-recipient CMV status, donor age, donor gender,

donor smoking (>20 pack-years), transplant type (single or double), era of transplant (2003 to 2005 pre-LAS, 2005 post-LAS to 2010, or 2011 to August 2016), Lung Allocation Score (LAS), and induction drug (if any). These variables were all included in multivariable Cox regression analyses, except for Lung Allocation Score (LAS), since some patients in our study were transplanted before the LAS was introduced, and the aforementioned variables comprise a large proportion of the factors that determine the LAS. A random effect representing transplant center was also added to the multivariable Cox regression model, to account for this source of likely confounding. To enable patients with missing data on one or more covariates to be included in regression analyses, multiple imputation was utilized by generating 20 imputed datasets using the fully conditional specification (FCS) imputation method, and results based on these 20 datasets were averaged. The same model was used to generate results for both induction and maintenance immunosuppressive therapies, so that when analyzing either factor as the exposure of interest, the other factor was accounted for. SAS version 9.4 and R version 3.4.3 were used for all analyses.

D. RESULTS

A comparison of major baseline characteristics is shown between induction therapies in Table 4.1a, and between antimetabolite maintenance therapies in Table 4.1b. Baseline differences between groups, though usually small in magnitude with few exceptions, were mostly statistically significant due to large group sizes. Survival comparisons, including adjusted analyses, for induction and maintenance therapies are shown in Figures 4.1a and 4.1b, respectively.

Among induction therapies, equine ATG was associated with the best survival (median (MS)=7.1 [IQR: 3.1– -] years, adjusted Hazard Ratio (HR)=0.79 [95% CI: 0.67–0.92], p=0.003; n=599) compared to no induction (MS=5.8 [IQR: 2.3–10.9] years; n=5038). To a lesser degree, daclizumab was also associated with better survival (MS=6.2 [IQR: 2.3–11.4] years, HR=0.88 [95% CI: 0.79–0.99], p=0.03; n=1047). Alemtuzumab was weakly associated with better survival (MS=6.6 [IQR: 3.0–11.0] years, HR=0.83 [95% CI: 0.69–1.01], p=0.06; n=699) while rabbit ATG was weakly associated with worse survival (MS: 5.4 [IQR: 1.9–9.1] years, HR=1.18 [95% CI: 0.98–1.41], p=0.07; n=429). Survival was not significantly different for basiliximab (MS=6.3 [IQR: 2.5–11.7] years, HR=0.95 [95% CI: 0.88–1.02], p=0.18; n=4613) compared to no induction. We did not detect a significant interaction between induction therapy and age (p=0.44), suggesting that the effects of various induction therapies are similar across age groups.

Among *de novo* antimetabolite maintenance therapies, azathioprine had the best survival (MS=6.8 [IQR: 2.7-11.4] years; n=3407); HR=0.92 [95% CI: 0.85-1.00], p=0.05) compared to mycophenolate mofetil [MMF] (MS=5.8 [IQR: 2.3-11.1] years; n=8453). Mycophenolate sodium [MPS] had similar survival (MS=6.4 [IQR: 2.9-10.0] years, HR=0.90 [95% CI: 0.76-1.07], p=0.22; n=565) to MMF. There was no interaction between maintenance therapy and age (p=0.98), or between the induction and maintenance immunosuppression variables (p=0.74).

Table 4.1a: Comparison of patient characteristics between induction therapy groups

		Alemtuzumab	Basiliximab	Daclizumab	Equine ATG	Rabbit ATG	None	p
		n = 699	**n= 4613**	**n= 1047**	**n= 599**	**n= 429**	**n= 5038**	
Age	**Median (IQR), years**	60 (51-66)	58 (47-64)	56 (44-62)	58 (47-63)	58 (43-63)	58 (47-63)	<0.001
Gender								0.09
	Female	309 (44%)	1844 (40%)	451 (43%)	253 (42%)	168 (39%)	96 (42%)	
	Male	390 (56%)	2769 (60%)	596 (57%)	346 (58%)	261 (61%)	133 (58%)	
Race								<0.001
	Black	69 (10%)	401 (9%)	120 (11%)	24 (4%)	33 (8%)	400 (8%)	
	White	606 (87%)	3814 (83%)	837 (80%)	527 (88%)	345 (80%)	4250 (84%)	
	Hispanic	18 (3%)	294 (6%)	71 (7%)	36 (6%)	38 (9%)	286 (6%)	
	Other	6 (1%)	104 (2%)	19 (2%)	12 (2%)	13 (3%)	102 (2%)	
BMI category								<0.001
	Underweight	103 (15%)	880 (19%)	240 (23%)	123 (21%)	86 (20%)	967 (20%)	
	Normal	212 (30%)	1491 (32%)	330 (32%)	201 (34%)	134 (31%)	1509 (30%)	
	Overweight	228 (33%)	1592 (35%)	348 (34%)	202 (34%)	140 (33%)	1764 (36%)	
	Obese	155 (22%)	641 (14%)	119 (11%)	72 (12%)	69 (16%)	716 (14%)	
	Missing	*1 (0.1%)*	*9 (0.2%)*	*10 (1%)*	*1 (0.2%)*	*0 (0%)*	*82 (2%)*	
Lung Disease (primary)								<0.001
	Cystic Fibrosis	79 (11%)	723 (16%)	152 (15%)	97 (16%)	69 (16%)	730 (14%)	
	Pulmonary Fibrosis	268 (38%)	1943 (42%)	294 (28%)	245 (41%)	184 (43%)	1967 (39%)	
	COPD	237 (34%)	1070 (23%)	349 (33%)	160 (27%)	79 (18%)	1402 (28%)	
	Alpha-1 Antitrypsin Deficiency	16 (2%)	138 (3%)	38 (4%)	27 (5%)	12 (3%)	158 (3%)	
	Pulmonary Hypertension	12 (2%)	106 (2%)	65 (6%)	8 (1%)	11 (3%)	143 (3%)	
	Sarcoidosis	20 (3%)	140 (3%)	45 (4%)	20 (3%)	12 (3%)	157 (3%)	
	Other	67 (10%)	493 (11%)	104 (10%)	42 (7%)	62 (14%)	481 (10%)	
Prior transplant								<0.001
	Yes	22 (3%)	174 (4%)	58 (6%)	9 (2%)	32 (7%)	225 (4%)	
	No	677 (97%)	4439 (96%)	989 (94%)	590 (99%)	397 (93%)	4813 (96%)	

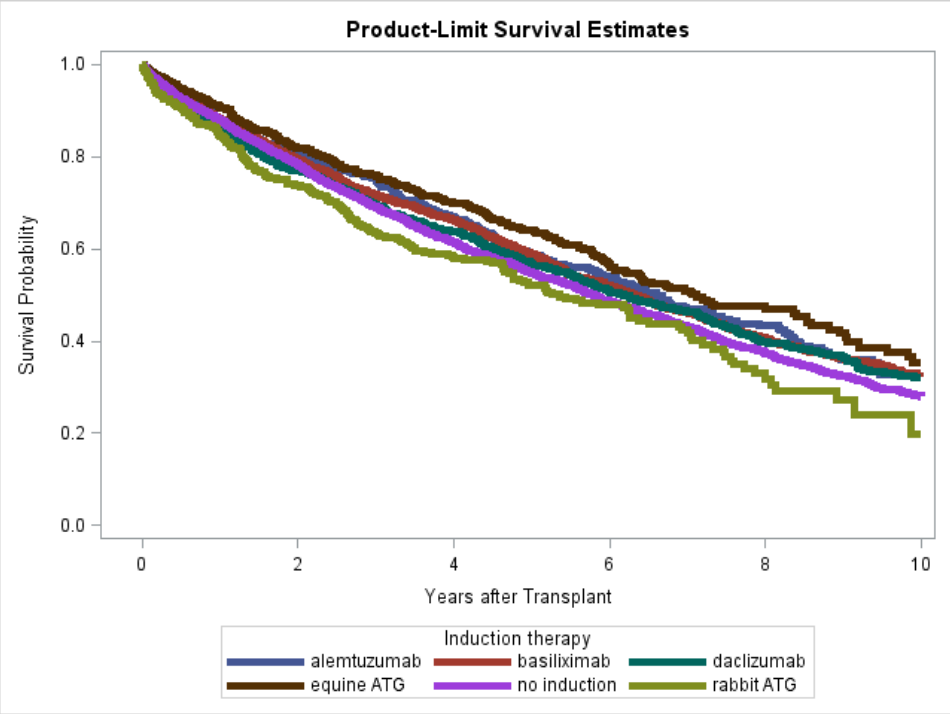
Table 4.1a continued: Comparison of patient characteristics between induction therapy groups

Lung Allocation Score (LAS)	**Median (IQR)**	39 (34-49)	41 (35-52)	37 (34-44)	39 (35-48)	41 (36-55)	39 (35-49)	<0.001
	Missing or N/A (pre-LAS era)	*22 (3%)*	*324 (7%)*	*220 (21%)*	*81 (14%)*	*28 (7%)*	*621 (12%)*	
Transplant Type								<0.001
	Single	192 (27%)	1211 (26%)	479 (46%)	210 (35%)	113 (26%)	2073 (41%)	
	Double	503 (73%)	3402 (74%)	568 (54%)	389 (65%)	316 (74%)	2965 (59%)	
HLA matching								0.72
	0-3 matches	662 (96%)	4040 (96%)	676 (97%)	454 (97%)	310 (96%)	4393 (96%)	
	4-6 matches	30 (4%)	156 (4%)	21 (3%)	15 (3%)	14 (4%)	182 (4%)	
	Missing	*7 (1%)*	*417 (9%)*	*350 (33%)*	*130 (1%)*	*105 (24%)*	*463 (9%)*	
Donor age	**Median (IQR), years**	34 (21-49)	31 (21-45)	27 (19-43)	34 (21-49)	30 (20-46)	32 (21-46)	<0.001
Donor smoking (≥20 pack-yrs.)								0.007
	Yes	97 (14%)	465 (10%)	132 (13%)	69 (12%)	41 (10%)	590 (12%)	
	No	593 (86%)	4106 (90%)	908 (87%)	524 (88%)	387 (90%)	4390 (88%)	

Table 4.1b: Comparison of patient characteristics between de novo maintenance therapy groups

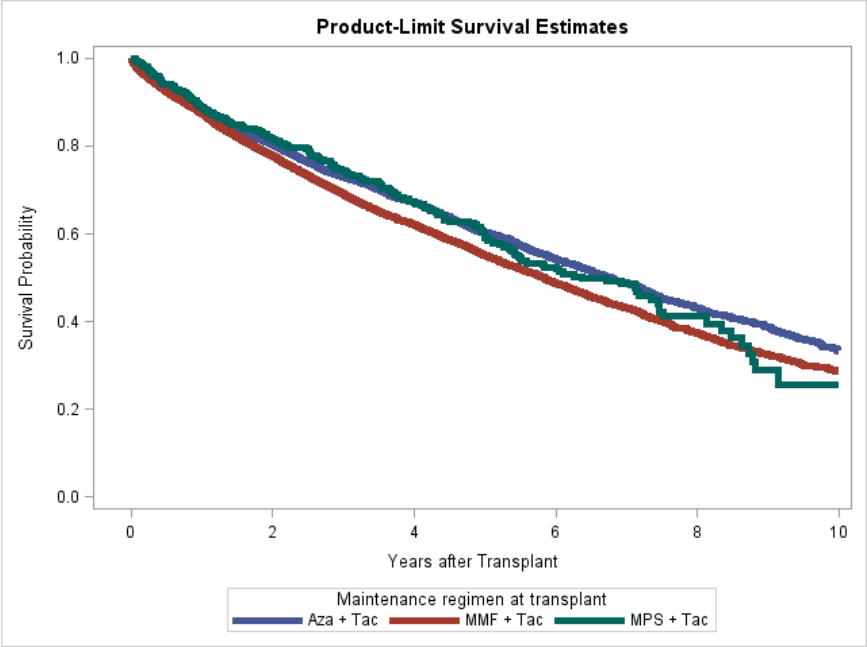
		Aza + Tac	MMF + Tac	MPS + Tac	p-value
		n = 3407	n = 8453	n = 565	
Age	**Median (IQR), years**	57 (47-63)	58 (47-64)	59 (47-64)	0.0006
Gender					0.03
	Female	1442 (42%)	3498 (41%)	206 (36%)	
	Male	1965 (58%)	4955 (59%)	359 (64%)	
Race					<0.0001
	Black	342 (10%)	660 (8%)	45 (8%)	
	White	2875 (84%)	7023 (83%)	481 (85%)	
	Hispanic	130 (4%)	582 (7%)	31 (5%)	
	Other	60 (2%)	188 (2%)	8 (1%)	
BMI category					<0.0001
	Underweight	669 (20%)	1631 (19%)	99 (18%)	
	Normal	1137 (34%)	2553 (31%)	187 (33%)	
	Overweight	1181 (35%)	2912 (35%)	181 (32%)	
	Obese	42 (19%)	859 (15%)	99 (18%)	
	Missing	*19 (1%)*	*82 (1%)*	*2 (0.4%)*	
Lung Disease (primary)					<0.0001
	Cystic Fibrosis	504 (15%)	1264 (15%)	82 (15%)	
	Pulmonary Fibrosis	1275 (37%)	3389 (40%)	237 (42%)	
	COPD	1010 (30%)	2149 (25%)	138 (24%)	
	Alpha-1 Antitrypsin Deficiency	116 (3%)	254 (3%)	19 (3%)	
	Pulmonary Hypertension	100 (3%)	235 (3%)	10 (2%)	
	Sarcoidosis	124 (4%)	246 (3%)	24 (4%)	
	Other	278 (8%)	9166 (11%)	55 (10%)	
Prior transplant					<0.0001
	Yes	107 (3%)	361 (4%)	38 (7%)	
	No	3300 (93%)	8078 (96%)	527 (93%)	
Lung Allocation Score (LAS)	**Median (IQR)**	39 (35-49)	40 (35-51)	40 (35-50)	0.04
	Missing or N/A (pre-LAS era)	*554 (16%)*	*739 (9%)*	*3 (1%)*	
Transplant Type					0.0002
	Single	1093 (32%)	2976 (35%)	209 (37%)	
	Double	2314 (68%)	5477 (65%)	356 (63%)	
HLA matching					0.66
	0-3 matches	2774 (96%)	7236 (96%)	525 (97%)	
	4-6 matches	114 (4%)	287 (4%)	17 (3%)	
	Missing	*519 (15%)*	*930 (11%)*	*23 (4%)*	
Donor age	**Median (IQR), years**	30 (21-45)	31 (21-46)	33 (22-46)	0.0018
Donor smoking (≥20 pack-yrs.)					0.25
	Yes	399 (12%)	923 (11%)	72 (13%)	
	No	2980 (88%)	7439 (89%)	489 (87%)	
	Missing	*28 (1%)*	*91 (1%)*	*4 (1%)*	

Figure 4.1a: Kaplan-Meier survival curves comparing induction therapies



	Alemtuzumab	Equine ATG	Rabbit ATG	Basiliximab	Daclizumab	No induction
# of Patients	699	599	429	4613	1047	5038
# of Centers	10	17	33	57	29	59
Median Survival (IQR)	6.6 years (3.0–11.0)	7.1 years (3.1– -)	5.4 years (1.9–9.1)	6.3 years (2.5–12.6)	6.2 years (2.3–10.2)	5.8 years (2.3–11.1)
Adjusted HR (95% CI)	**0.83 (0.69-1.01)**	**0.79 (0.67-0.92)**	**1.18 (0.98-1.41)**	**0.95 (0.88-1.02)**	**0.88 (0.79-0.99)**	**1.00 Reference**
	p = 0.06	p = 0.003	p = 0.07	p = 0.18	p = 0.03	

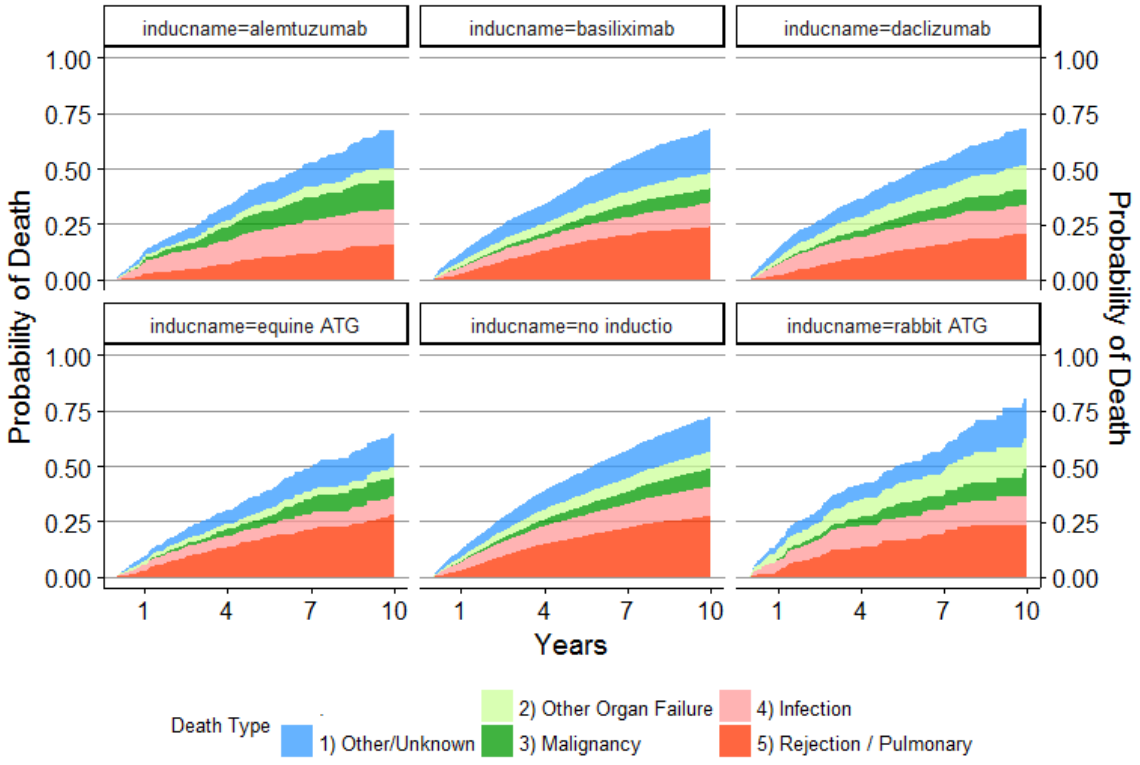
Figure 4.1b: Kaplan-Meier survival curves comparing maintenance therapy at transplant



	Azathioprine + Tacrolimus	**MPS + Tacrolimus**	**MMF + Tacrolimus**
# of Patients	3407	565	8453
# of Centers	53	34	62
Median Survival (IQR)	6.8 years (2.7–11.4)	6.4 years (2.9–10.0)	5.8 years (2.3–11.1)
Adjusted HR (95% CI)	**0.92 (0.85-1.00)**	**0.90 (0.76-1.07)**	**1.00 Reference**
	p = 0.05	p = 0.22	

For each of the induction and maintenance therapies, Figures 4.2a and 4.2b, respectively, show the probabilities of death from each major cause (chronic rejection or respiratory failure, infection, malignancy, other organ failure, or other/unknown). Tables 4.2a and 4.2b compare induction and maintenance therapies, respectively, in terms of event risks and post-event death risks for the 3 most common mortality-causing events: chronic rejection, infection, and malignancy.

Figure 4.2a: Percentages dead from each major cause, by induction therapy

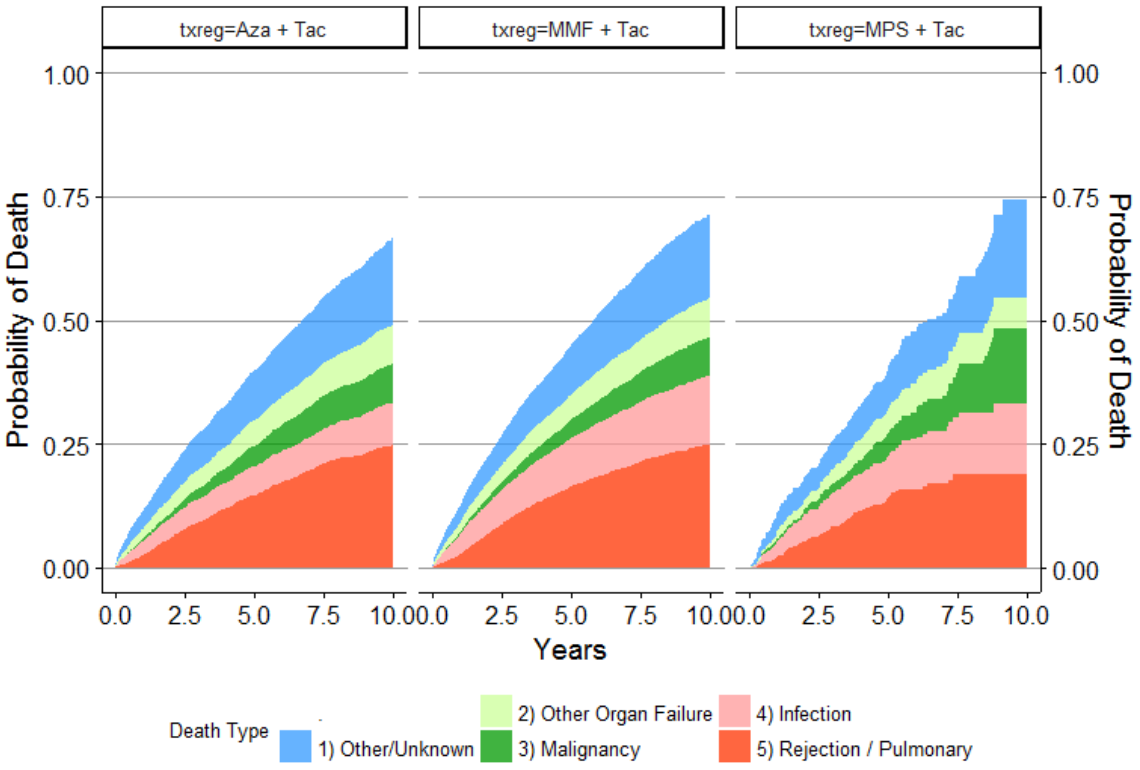


	5 years						10 years					
Death Type	**Al**	**Eq**	**Ra**	**Ba**	**Da**	**No**	**Al**	**Eq**	**Ra**	**Ba**	**Da**	**No**
Rejection / Pulmonary	9	16	16	16	12	17	16	28	23	24	20	27
Infection	12	5	10	7	10	9	16	8	13	11	13	13
Malignancy	8	3	5	3	4	4	13	9	12	6	7	8
Other Organ Failure	4	3	9	5	7	5	5	4	14	7	10	8
Other / Unknown	8	8	8	11	10	10	17	15	18	20	17	16
Total Deaths	**41**	**36**	**48**	**41**	**43**	**45**	**67**	**65**	**80**	**68**	**68**	**72**

Al = Alemtuzumab; Eq = Equine ATG; Ra = Rabbit ATG;

Basil = Basiliximab; Dacli = Daclizumab; No = No Induction

Figure 4.2b: Percentages dead from each major cause, by maintenance therapy



Death Type	5 years			10 years		
	Aza	**MMF**	**MPS**	**Aza**	**MMF**	**MPS**
Rejection / Pulmonary	15	16	14	25	25	19
Infection	6	10	8	9	14	14
Malignancy	4	4	5	8	8	15
Other Organ Failure	5	5	5	8	8	6
Other / Unknown	10	10	9	18	17	20
Total Deaths	**40**	**45**	**41**	**67**	**71**	**74**

MMF = Mycophenolate Mofetil; MPS = Mycophenolate Sodium; Aza = Azathioprine

Among induction therapies, alemtuzumab had the lowest risk of chronic rejection (HR=0.58 [95% CI: 0.40-0.84], p=0.004) and risk of death after chronic rejection (HR=0.50 [95% CI: 0.35-0.72], p=0.0002), compared to no induction. Equine ATG (HR=0.75 [95% CI: 0.59-0.96], p=0.02) and daclizumab (HR=0.75 [95% CI: 0.61-0.92], p=0.007) also had a lower chronic rejection incidence than no induction. Among antimetabolite maintenance therapies, no significant differences were detected in rejection risk, although azathioprine was associated with a lower risk of death after rejection than MMF (HR=0.86 [95% CI: 0.76-0.97], p=0.01). The risk of infection was lowest with equine ATG induction (HR=0.57 [95% CI: 0.38-0.86], p=0.008), as was the risk of death after infection (HR=0.81 [95% CI: 0.68-0.98], p=0.03). For maintenance therapy, azathioprine had the lowest risk of infection (HR=0.62 [95% CI: 0.51-0.75], p<0.0001). The risk of malignancy was lowest with basiliximab induction (HR=0.80 [95% CI: 0.62-1.03], p=0.09), though not significantly different from no induction. Alemtuzumab (HR=1.86 [95% CI: 1.22-2.84], p=0.004) and Rabbit ATG (HR=1.61 [95% CI: 1.00-2.59], p=0.05) induction had an increased risk of malignancy. No statistically significant differences in malignancy risk were found between maintenance therapies, although MPS had a trend towards an increased risk over MMF (HR=1.40 [95% CI: 0.90-2.17], p=0.13).

Table 4.2a: Incidences of common mortality-causing events (rejection, infection, and malignancy), by induction therapy

Event	Alemtuzumab		Equine ATG		Rabbit ATG		Basiliximab		Daclizumab		None	
Rejection	HR_1: 0.58 (0.40 – 0.84) p = 0.004		HR_1: 0.75 (0.59 – 0.96) p = 0.02		HR_1: 1.12 (0.82 – 1.52) p = 0.49		HR_1: 0.94 (0.81 – 1.08) p = 0.35		HR_1: 0.75 (0.61 – 0.92) p = 0.007		HR_1: 1.00 Reference	
	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y
	29%	63%	47%	70%	47%	66%	48%	66%	46%	70%	48%	68%
Death without Rejection	HR_2: 1.06 (0.83 – 1.38) p = 0.67		HR_2: 0.80 (0.61 – 1.05) p = 0.11		HR_2: 1.18 (0.85 – 1.53) p = 0.40		HR_2: 0.80 (0.69 - 0.91) p = 0.001		HR_2: 0.86 (0.71 – 1.03) p = 0.10		HR_2: 1.00 Reference	
	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y
	25%	*33%*	*12%*	*19%*	*18%*	*27%*	*14%*	*21%*	*17%*	*22%*	*18%*	*23%*
Death after Rejection	HR_3: 0.50 (0.35 – 0.72) p = 0.0002		HR_3: 0.90 (0.72 – 1.17) p = 0.33		HR_3: 1.23 (0.93 – 1.62) p = 0.15		HR_3: 0.98 (0.87 – 1.11) p = 0.78		HR_3: 1.00 (0.85 – 1.19) p = 0.98		HR_3: 1.00 Reference	

Event	Alemtuzumab		Equine ATG		Rabbit ATG		Basiliximab		Daclizumab		None	
Infection	HR_1: 1.07 (0.73 – 1.57) p = 0.72		HR_1: 0.57 (0.38 - 0.86) p = 0.008		HR_1: 1.10 (0.73 – 1.65) p = 0.64		HR_1: 0.83 (0.68 – 1.01) p = 0.06		HR_1: 0.95 (0.73 – 1.22) p = 0.67		HR_1: 1.00 Reference	
	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y
	37%	48%	45%	49%	56%	58%	50%	54%	53%	56%	53%	58%
Death without Infection	HR_2: 0.95 (0.64 – 1.40) p = 0.78		HR_2: 0.82 (0.59 – 1.12) p = 0.20		HR_2: 1.08 (0.74 – 1.58) p = 0.68		HR_2: 0.80 (0.64 – 0.94) p = 0.01		HR_2: 0.88 (0.69 – 1.12) p = 0.31		HR_2: 1.00 Reference	
	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y
	11%	*30%*	*10%*	*20%*	*11%*	*23%*	*9%*	*22%*	*9%*	*20%*	*10%*	*23%*
Death after Infection	HR_3: 1.31 (1.03 – 1.66) p = 0.03		HR_3: 0.81 (0.68 – 0.98) p = 0.03		HR_3: 1.06 (0.85 – 1.32) p = 0.62		HR_3: 0.87 (0.79 – 0.96) p = 0.008		HR_3: 0.87 (0.76 – 1.00) p = 0.05		HR_3: 1.00 Reference	

Table 4.2a continued: Incidences of common mortality-causing events (rejection, infection, and malignancy), by induction therapy

Event	Alemtuzumab		Equine ATG		Rabbit ATG		Basiliximab		Daclizumab		None	
Malignancy	HR_1: 1.86 (1.22 – 2.84) p = 0.004		HR_1: 1.10 (0.72 – 1.68) p = 0.66		HR_1: 1.61 (1.00 – 2.59) p = 0.05		HR_1: 0.80 (0.62 – 1.03) p = 0.09		HR_1: 0.96 (0.69 – 1.33) p = 0.79		HR_1: 1.00 Reference	
	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y
	22%	41%	19%	37%	28%	42%	19%	34%	18%	33%	21%	36%
Death without Malignancy	HR_2: 0.71 (0.56 – 0.90) p = 0.006		HR_2: 0.71 (0.58 – 0.86) p = 0.001		HR_2: 1.22 (0.98 – 1.52) p = 0.07		HR_2: 0.87 (0.78 – 0.96) p = 0.006		HR_2: 0.86 (0.75 – 0.99) p = 0.03		HR_2: 1.00 Reference	
	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y	5 y	10 y
	27%	*42%*	*25%*	*37%*	*33%*	*47%*	*28%*	*42%*	*30%*	*44%*	*32%*	*45%*
Death after Malignancy	HR_3: 1.14 (0.80 – 1.63) p = 0.46		HR_3: 1.07 (0.80 – 1.44) p = 0.64		HR_3: 0.96 (0.67 – 1.37) p = 0.80		HR_3: 1.00 (0.84 – 1.20) p = 0.98		HR_3: 0.98 (0.78 – 1.24) p = 0.88		HR_3: 1.00 Reference	

Table 4.2b: Incidences of common mortality-causing events (rejection, infection, and malignancy), by antimetabolite maintenance therapy

Event	Azathioprine		MMF		MPS	
Rejection	HR_1: 0.95 (0.83 – 1.08) p = 0.42		HR_1: 1.00 Reference		HR_1: 0.86 (0.64 – 1.17) p = 0.34	
	5 yr.	10 yr.	5 yr.	10 yr.	5 yr.	10 yr.
	50%	71%	46%	66%	36%	57%
Death without Rejection	HR_2: 0.79 (0.70 – 0.89) p = 0.0001		HR_2: 1.00 Reference		HR_2: 0.94 (0.73 – 1.19) p = 0.60	
	5 yr.	10 yr.	5 yr.	10 yr.	5 yr.	10 yr.
	13%	*19%*	*18%*	*24%*	*20%*	*33%*
Death after Rejection	HR_3: 0.86 (0.76 – 0.97) p = 0.01		HR_3: 1.00 Reference		HR_3: 1.00 (0.76 – 1.30) p = 0.97	

Table 4.2b continued: Incidences of common mortality-causing events (rejection, infection, and malignancy), by antimetabolite maintenance therapy

Event	**Azathioprine**		**MMF**		**MPS**	
Infection	HR_1: 0.62 (0.51 – 0.75) p < 0.0001		HR_1: 1.00 Reference		HR_1: 0.91 (0.63 – 1.32) p = 0.63	
	5 yr.	10 yr.	5 yr.	10 yr.	5 yr.	10 yr.
	45%	49%	54%	59%	56%	61%
Death without Infection	HR_2: 1.00 (0.80 – 1.20) p = 0.97		HR_1: 1.00 Reference		HR_2: 0.95 (0.65 – 1.39) p = 0.79	
	5 yr.	10 yr.	5 yr.	10 yr.	5 yr.	10 yr.
	9%	*25%*	*10%*	*21%*	*7%*	*17%*
Death after Infection	HR_3: 0.98 (0.89 – 1.08) p = 0.63		HR_3: 1.00 Reference		HR_3: 0.92 (0.76 – 1.12) p = 0.43	

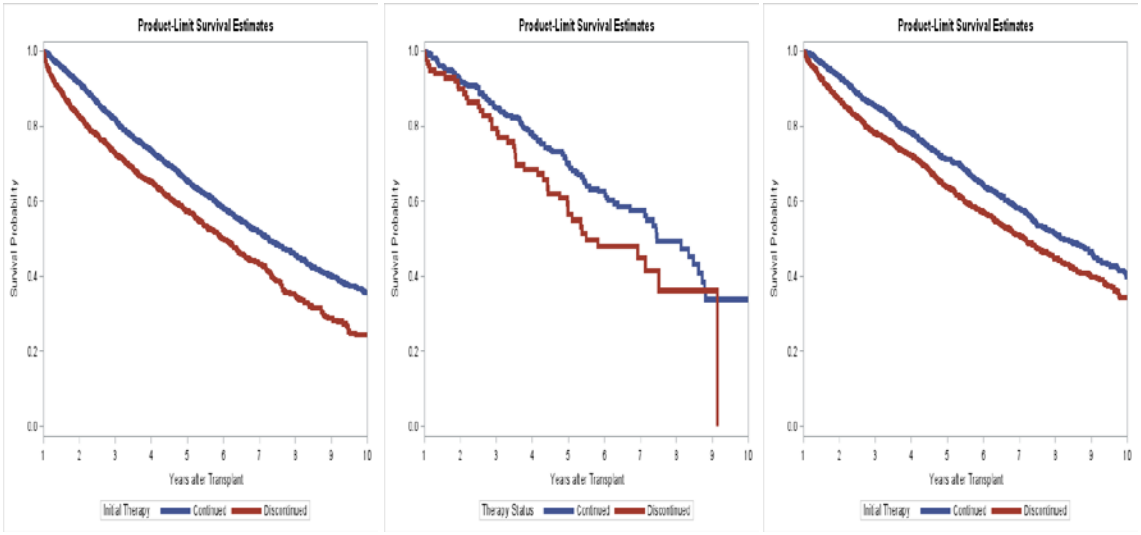
Event	**Azathioprine**		**MMF**		**MPS**	
Malignancy	HR_1: 1.18 (0.94 – 1.47) p = 0.15		HR_1: 1.00 Reference		HR_1: 1.40 (0.90 – 2.17) p = 0.13	
	5 yr.	10 yr.	5 yr.	10 yr.	5 yr.	10 yr.
	20%	35%	20%	36%	25%	42%
Death without Malignancy	HR_2: 0.89 (0.81 – 0.98) p = 0.02		HR_2: 1.00 Reference		HR_2: 0.99 (0.74 – 1.15) p = 0.94	
	5 yr.	10 yr.	5 yr.	10 yr.	5 yr.	10 yr.
	26%	*40%*	*32%*	*45%*	*30%*	*43%*
Death after Malignancy	HR_3: 0.99 (0.85 – 1.16) p = 0.91		HR_3: 1.00 Reference		HR_3: 0.89 (0.63 – 1.26) p = 0.50	

For all three antimetabolite maintenance therapies initially administered at transplantation, survival was significantly better for patients who remained on the therapy 1 year later compared to those who did not, among patients alive at 1 year post-transplant. 68% continued MMF at 1 year (median survival 7.2 years vs. 6.0 years for those who discontinued, p<0.0001); 57% continued azathioprine (median survival 8.2 years vs. 7.2

years for those who discontinued, p<0.0001); 76% continued MPS (median survival 7.5 years vs. 5.5 years for those who discontinued, p=0.02) Among patients who remained on their initial therapy at 1 year, survival trends were similar to those observed from transplantation time: survival was better for azathioprine-remaining patients than MMF-remaining patients (median 8.2 vs. 7.2 years, p<0.0001), but MPS-remaining patients had similar survival (median 7.5 years) to MMF-remaining patients (p=0.24). These results are shown in Figure 4.3.

Figure 4.3: Survival in patients who continued vs. discontinued each initial maintenance therapy, at 1 year post-transplant

Mycophenolate Mofetil (MMF) Mycophenolate Sodium (MPS) Azathioprine



E. DISCUSSION

This study utilized national U.S. lung transplant data to compare outcomes between common induction therapies, and also between antimetabolite maintenance therapies administered at the time of transplantation, namely mycophenolate (MMF or MPS) and

azathioprine, within a tacrolimus-based regimen. Although induction therapies have been assessed previously using the same national data source, this study expands upon prior knowledge by taking into consideration transplant center as a likely confounder, and also accounting for *de novo* maintenance therapy. To our knowledge this is the first study comparing long-term outcomes between antimetabolite maintenance therapies using the national U.S. lung transplant data. In particular, MPS has not been studied in lung transplant recipients to date.

This study found that out of all common induction therapies, equine ATG was associated with the most favorable survival, and daclizumab was also associated with improved survival over no induction. Among antimetabolite maintenance therapies, azathioprine was associated with better survival than MMF, while MPS was not. We did not detect any significant interaction between age and either induction or antimetabolite maintenance therapies; that is, the survival comparisons between induction therapies or between antimetabolite maintenance therapies do not appear to differ according to age.

As we described earlier, using this same national U.S. lung transplant dataset, we also compared prophylactic use of the mTOR inhibitor sirolimus to antimetabolites such as mycophenolate and azathioprine within a tacrolimus-based regimen. We found that sirolimus was associated with the most favorable survival of all the cell cycle inhibitors. Notably, the combination of sirolimus with no induction therapy had better survival than any other induction-maintenance combination (including sirolimus itself with induction therapy), and this finding persisted with adjustments for confounding (including transplant center) and prevention of immortal time bias since sirolimus initiation is typically delayed 3-12 months post-transplant. Median survival in the sirolimus and no induction group was

over 10 years among patients alive at 1 year. Sirolimus use in patients who received induction therapy was still associated with favorable survival, generally at least as good as mycophenolate or azathioprine, although significantly inferior compared to the sirolimus-treated patients who received no induction. Therefore, although we have found that equine ATG has the best survival among induction therapies when comparing them to each other as a single factor, our analyses nevertheless indicate that the benefit of sirolimus when used with no induction therapy is larger than the benefit of equine ATG or any other induction therapy (regardless of the maintenance therapy used). Consequently, it would seem advisable to reserve use of induction with equine ATG for patients in whom sirolimus is planned to be avoided in favor of mycophenolate or azathioprine for whichever reason. For such patients, azathioprine appears to be the next-best choice of cell cycle inhibitor based on the results of this study, as it has the best survival out of the antimetabolites, and modestly better survival than mycophenolate mofetil [MMF], which doesn't differ significantly from mycophenolate sodium [MPS].

Two randomized trials have compared azathioprine and MMF in lung transplant recipients, with follow-up lengths of 6 months[24] and 3 years[23], and both found no significant difference in survival or chronic rejection incidence. However, some trials in other organ transplants have shown superiority of MMF over azathioprine. Though our study has increased potential for confounding due to its retrospective, non-randomized design, it should be noted that that almost all differences detected in baseline characteristics between the MMF and azathioprine groups were small in magnitude, and any such differences were controlled for in adjusted analyses. Additionally, given that MMF is widely perceived to be superior to azathioprine, and it is also significantly more expensive,

there is no apparent reason why patients who received azathioprine would be otherwise advantaged at baseline. Other than the study designs, an important distinctions between our study and the two trials, both of which enrolled patients from 1997-1999, is that all patients in our study received the contemporary agent tacrolimus as the calcineurin inhibitor, while both trials utilized cyclosporine instead. This could partly explain the differences in findings, as it has been suggested that the initially apparent benefits of MMF over azathioprine were negated once tacrolimus (a more potent agent that has been demonstrated to have lower rejection incidence) superseded cyclosporine as the calcineurin inhibitor to be used in combination with these drugs[70]. Additionally, possible use of methods to optimize dosing of azathioprine such as thiopurine methyltransferase pharmacogenetics and monitoring of thioguanine nucleotides, which were reportedly not used in older clinical trials[70], might have enabled an improvement over time in outcomes associated with azathioprine. However, a predominant factor behind the different results is likely to be the different durations of follow-up, especially considering that the effect size detected in our study was relatively small. A fundamental mechanism by which azathioprine might be expected to improve survival over MMF is a reduction in cytomegalovirus (CMV) infections, as has been described in prior studies of renal transplantation[71,72]. This was supported in our study, as we found the incidence of infections to be significantly reduced with azathioprine compared to MMF. Notably, our study showed no evidence of an increase in chronic rejection incidence nor deaths from chronic rejection with azathioprine compared to MMF, which is consistent with the two prior randomized trials in lung transplantation. In fact, though the incidences of chronic rejection were similar between

groups, we found that the risk of death after chronic rejection development was significantly lower for the azathioprine group.

In addition, our study did not show an overall survival benefit of MPS over MMF. Though both medications have the same active ingredient, mycophenolic acid, MPS is an enteric-coated version which has been associated with reduced gastrointestinal side effects in kidney and liver transplant recipients[27,25]; therefore, we hypothesized that this reduction in side effects would translate into better prevention of chronic rejection due to less interruptions in anti-rejection prophylaxis. However, there was no statistically significant reduction in rejection incidence with MPS compared to MMF; furthermore, MPS had a trend towards increased incidence of malignancies, presumably due to a higher level of immunosuppression achieved. Therefore, it does not appear that MPS poses a clear advantage over MMF in the general lung transplant patient population.

Among induction agents, our finding that equine ATG is associated with the best survival is novel, particularly because this agent has not been individually studied in lung transplantation, to our knowledge. A large cohort study which utilized the UNOS lung transplant recipient dataset, analyzing equine ATG and rabbit ATG as a combined group, found no significant survival differences compared to no induction[51]. This could possibly be explained by the fact that in stark contrast to equine ATG, we found rabbit ATG to be associated with the lowest survival out of all induction therapies, with a trend towards worse survival than no induction. Long-term follow-up of a randomized trial comparing rabbit ATG to no induction in lung transplant recipients found no significant differences in survival or chronic rejection incidence[69]. However, a heart transplant study found significantly improved survival with ATG (either rabbit or equine) compared to

basiliximab[73]. Though no organ transplant studies have compared equine and rabbit ATG, the superiority of equine ATG over rabbit ATG as suggested by our results concurs with a randomized trial in severe acquired aplastic anemia[74], in which survival was significantly better with equine ATG than with rabbit ATG. In general, our finding that equine ATG is associated with more favorable survival than IL-2 receptor antagonists such as basiliximab and daclizumab is somewhat contrary to conventional wisdom, as the IL-2 receptor antagonists have been considerably preferred over ATG in recent practice, due to the notion that ATG, as a polyclonal antibody T-lymphocyte depleting agent, would be linked to an increase in serious complications such as infections and malignancies compared to the IL-2 receptor antagonists which are non-T-lymphocyte depleting monoclonal antibodies. It seems that these notions might correctly apply to rabbit ATG, but not necessarily to equine ATG. In fact, the improved survival with equine ATG appears to have been partly driven by a significant decrease in the incidence and mortality from infections, although this decrease was not observed for rabbit ATG. As there is no apparent biological explanation linking equine ATG induction to prevention of infections, the most likely explanation is that levels of maintenance immunosuppressive drugs were lower in patients who received equine ATG than in those who received no induction, and the reduction in infections was driven by the lower levels of the maintenance drugs, although this must imply that equine ATG induction itself did not increase the risk of infections appreciably. If this is the correct explanation, the key role of equine ATG induction in this scenario might be to enable good anti-rejection prophylaxis even with reduced maintenance drug levels.

Out of the monoclonal antibody induction agents, we found that daclizumab, a non T-lymphocyte depleting agent in the Interleukin-2 Receptor Antagonist (IL-2RA) class,

was also associated with better survival than no induction, although to a lesser extent than equine ATG. Surprisingly, basiliximab, by far the most popular induction agent and also a member of the IL-2RA class but with less human antibodies than daclizumab, was not significantly associated with better survival than no induction. Alemtuzumab had a trend towards improved survival, which appears to be driven by a lower incidence and mortality from chronic rejection compared to no induction or other induction agents. However, there appears to be a corresponding increase in deaths from infections and particularly malignancies with alemtuzumab, which seems to weaken its overall survival benefit.

Considering the retrospective, non-randomized nature of our study, there are a few notable limitations. Although we controlled for numerous available covariates that are likely to be important, our study is susceptible to unmeasured confounding. For example, potential sources of confounding include factors such as overall differences between physicians who prefer a particular induction or maintenance agent over another, even though we controlled for transplant center to address the possibility that centers which use certain agents may have intrinsically better or worse outcomes than other centers.

Since we assessed outcomes for antimetabolite maintenance immunosuppressive drugs by classifying patients according to the antimetabolite they received *de novo* (at the time of transplantation), our study has the shortcoming that any subsequent drug switches are not considered, even though some patients eventually replaced or discontinued their initial antimetabolite therapy. We found that regardless of the specific therapy, patients who switched off their initial antimetabolite by 1 year post-transplant had significantly worse subsequent survival, compared to those remaining on the therapy, among patients alive at 1 year. This indicates that patients most commonly switch therapies due to an event

arising while on the original therapy that adversely affects their prognosis, such as a life-threatening complication or a lack of anti-rejection efficacy. This strongly suggests that analyzing outcomes according to the prophylactic treatment for each patient, or in other words, using the intention-to-treat analysis approach, is the best strategy for comparing outcomes between treatments. Additionally, it is important to note that the drugs received early in the course of a patient's post-transplant lifetime are likely to be more consequential than those received several years after transplantation, because the greatest opportunity to establish tolerance of the transplanted lung(s), which is critical to prevent transplant rejection, tends to be relatively early post-transplant[65]. Also, for this reason, doses of immunosuppressive drugs are usually highest within the first few years, so the drug impact in terms of anti-rejection efficacy as well as the development of serious complications such as infections, malignancies, renal failure, etc. is likely to be greater in this time frame than later on post-transplant. Unfortunately, we do not have data on maintenance drug dosages for the patients in our study (nor trough levels although they are rarely collected for antimetabolites), so our results should be interpreted as being based on the "typical" or "average" levels at which patients tend to receive each drug. The lack of maintenance drug dosages also culminates in a limitation pertaining to our analyses of induction therapy, as we have no way of knowing differences in the dosages of maintenance immunosuppressive drugs between the induction groups. Therefore, as we observed reductions in the risks of infections or malignancies with the use of certain induction agents, we cannot determine whether this is due to actual protective effects of the drugs against these conditions, or simply due to lower levels of maintenance immunosuppressive drugs among patients who received those induction drugs.

In summary, this study of over 10,000 U.S. lung transplant recipients has led to novel findings that equine ATG is associated with the best survival among induction agents, and that azathioprine is associated with slightly better survival than MMF in a tacrolimus-based regimen. The survival benefits of both equine ATG induction and azathioprine maintenance appear to be largely mediated by reductions in infection risk and associated deaths, relative to no induction and MMF maintenance, respectively. Therefore, in the case of equine ATG induction, it needs to be established whether this infection prevention is a direct effect of the drug, or simply due to lower levels of maintenance immunosuppression that are enabled through the use of equine ATG. Importantly, although equine ATG appears to be the best choice if induction therapy is to be used, the U.S. national lung transplant data suggest fairly strongly that sirolimus + tacrolimus maintenance with no induction therapy is the most promising immunosuppressive strategy for maximizing survival at this time. Equine ATG induction therefore seems most appropriate in patients for whom it is believed at the time of transplantation that sirolimus will not be used for maintenance therapy. In these patients, as well as those who cannot take sirolimus due to intolerance or complications after attempting it, azathioprine appears to be the best choice of cell cycle inhibitor for maintenance immunosuppression, as our results indicate that its survival is surpassed only by sirolimus and is slightly better than MMF.

The finding of slightly better survival with azathioprine relative to MMF is particularly encouraging given the significantly lower cost of azathioprine. It has been suggested that the success of azathioprine use may depend on factors such as optimization of dosing via strategies including thiopurine methyltransferase pharmacogenetics and

thioguanine nucleotide monitoring. Nevertheless, although the findings from this study of *de novo* lung transplant recipients suggest that azathioprine is likely to be an ideal alternative to MMF, our results do not necessarily imply that patients who appear to be doing well on MMF should be switched over to azathioprine solely on the promise of survival improvement, especially considering the relatively small effect size we observed. Future studies can aid in answering the question of whether there is any benefit (or disadvantage) in switching from MMF to azathioprine for patients who are stable on MMF, and also in confirming the findings observed in this study.

Chapter 5: Discussion

Long term survival after lung transplantation is limited, with a median survival of approximately 5-6 years. However, easily modifiable factors such as induction and maintenance immunosuppressive therapies have not been thoroughly studied in lung transplant recipients. This study aimed to fill gaps in the literature pertaining to long-term outcomes associated with different immunosuppressive therapies in lung transplantation. The predominant goals of the study were to determine whether there were significant differences in outcomes between induction therapies and between cell cycle inhibitor maintenance therapies, and if so, to identify the immunosuppressive strategy associated with the best survival in this population. For induction immunosuppression, this study included all common induction agents, as some have not been previously studied in this population as far as long-term outcomes. This study also controlled for likely confounding by transplant center, which has not been considered in previous national cohort studies focusing on induction[51,54]. For maintenance immunosuppression, to our knowledge, this is the first study to utilize national U.S. lung transplant data, as well as the first multi-center study to assess long-term outcomes.

Prior randomized studies in lung transplantation have generally not been able to show superior survival with any given induction or maintenance therapy over another[34,23,24]. However, these trials have all had short follow-up (≤3 years), and considering that in the short term, survival after lung transplantation is relatively high, it may be quite unlikely that significant survival differences appear between agents within a short time frame post-transplant, even if significant differences exist in the long-term that may translate into years of extra survival with one therapy instead of another. Overall, the

most promising finding in the existing lung transplant literature is from Sacher et. al., who reported based on their single-center retrospective data, very good survival associated with prophylactic use of sirolimus + tacrolimus for maintenance therapy (67% alive at 9 years), which was significantly better compared to the *de facto* standard of care in lung transplantation, MMF + tacrolimus (37% alive at 9 years in their study, which is similar to national survival statistics). Therefore, a goal of major interest in our study was to assess outcomes associated with prophylactic use of the sirolimus + tacrolimus regimen among lung transplant recipients throughout the U.S., using the UNOS dataset which represents all lung transplant centers in the nation.

In brief, our study assessed outcomes according to the induction immunosuppression that each patient received, and according to the cell cycle inhibitor maintenance immunosuppression that each patient received prophylactically; that is, before any maintenance drug switches prompted by treatment failures, for example chronic rejection or cancer. In most cases, the prophylactic maintenance regimen is simply the regimen recorded in the first immunosuppression record for each patient, at the time of transplantation. However, in the case of sirolimus, identifying prophylactic users is more complicated, since initiation of sirolimus must be generally delayed by a minimum of 3 months post-transplant to avoid the risk of bronchial anastomotic dehiscence. Therefore, most patients who received sirolimus prophylactically can only be identified from their second immunosuppression record in the dataset (which is at 3 months, 6 months, or a maximum of 1 year post-transplant, or at death for patients who died within the first year), and care must be taken not to misclassify “rescue users” of sirolimus within the first year (for conditions such as chronic rejection or cancer) as prophylactic users. Also, due to the

delayed initiation of sirolimus in most patients, there is potential for immortal time bias since by definition, patients receiving sirolimus prophylactically had to survive at least 3-12 months post-transplant in general, while patients receiving other cell cycle inhibitors prophylactically could have died even immediately after transplantation. We utilized 3 different approaches to avoid this bias, in order to determine whether the overall findings would be consistent between approaches.

The first approach to avoid immortal time bias was based on standard survival analyses, requiring all patients to be alive in all groups at a "landmark time" of either 12 months (the latest time when sirolimus could have been initiated within the first year) or 3 months (the earliest time when sirolimus initiation is clinically indicated). This approach enables the identification of a clearly defined "sirolimus group" and "MMF group", such that comparisons can be made between the groups in terms of demographic characteristics as well as survival time estimates via Kaplan-Meier curves, and multivariable-adjusted comparisons can be made using standard Cox regression. The second approach utilized a time-dependent covariate in a Cox regression model; for patients who switched to sirolimus within the first year (hypothetically at either extreme of 3 or 12 months), the survival time prior to the hypothetical switch time is counted towards the treatment that the patient started on at transplantation time. This approach enables all patients to be included in the analyses and allows the analyses to start at the time of transplantation, but it has no distinct patient groups defined according to treatment received, since sirolimus-receiving patients switch treatment classifications during follow-up, and therefore no absolute survival time comparisons can be made. The third approach utilized multiple imputation to infer which patients were most likely planned to receive sirolimus, out of those who died within the

first year (before they could have initiated sirolimus). Each imputed dataset will contain a "sirolimus group" that includes, in addition to patients who actually received sirolimus in the first year, patients who presumably were planned to receive sirolimus but died before they could initiate it. Due to the inclusion of the latter, the analyses can start at the time of transplantation without being susceptible to immortal time bias, although the patient classifications are hypothetical since they rely on an imputation model. Unlike the first two approaches, which directly assess the treatment effect of sirolimus from the time when it is initiated (i.e. 3 months to 1 year post-transplant), this approach estimates the effect of sirolimus in the full population starting from the time of transplantation, taking into account that it cannot have an effect in some patients (about 10%) since they will not survive long enough to initiate it. As we found that the results were very similar based on all 3 approaches, this strengthens our confidence in the findings based on comparing sirolimus to other therapies that are started immediately after transplantation.

Survival was the outcome of primary interest in our study, and the main survival comparisons utilized multivariable-adjusted Cox regression models. Other outcomes of interest in our study were the 3 most common mortality-causing events in lung transplant recipients: chronic rejection, infection, and malignancy. To analyze these events, we used semi-competing risks multivariable regression models, which are intended to address the possibility that censoring of a non-fatal event of interest by death from another cause may be informative; this is often referred to as "dependent censoring", implying a dependence between the non-fatal event and death. For example, the patients who died at a certain time may have also been at a higher risk of experiencing the non-fatal event than patients who survived longer, so the non-fatal event incidence will be underestimated based on the

surviving patients. The semi-competing risks framework addresses dependent censoring by incorporating a patient-specific frailty into the standard Cox proportional hazards regression model, which is analogous to a random effect in a mixed-effect model for longitudinal analyses. This frailty represents the residual patient-specific differences beyond those explained by available covariates, thereby accounting for the dependence between the fatal event and the non-fatal event of interest. Though the main purpose of the semi-competing risks framework is to estimate the risk of a non-fatal event for one treatment relative to another (this is Hazard Ratio #1 in this framework), the methods provide two other hazard ratios. Hazard Ratio #2 compares the risk of event-free death between treatments, which aids in assessing the extent of competing risks with respect to each non-fatal event. Hazard Ratio #3 compares between treatments the risk of death after the occurrence of a given non-fatal event, which is useful in the context of this study since chronic rejection, infections, and cancer all vary considerably in severity, and the typical severity of events may differ between treatments, even if the incidence does not differ significantly.

The most noteworthy findings from this study were that sirolimus was associated with the most favorable survival among cell cycle inhibitors within a tacrolimus-based maintenance regimen, as well as lower chronic rejection incidence and lower risks of death after chronic rejection and infections, compared to MMF, the most popular cell cycle inhibitor. Azathioprine was also associated with slightly better survival than MMF, as well as lower incidence of infections. Among induction agents, equine ATG was associated with the best survival, and daclizumab was also associated with better survival than no induction. The survival benefit of equine ATG appears to be driven by reduced risks of

chronic rejection and infections, compared to no induction. Out of all possible combinations of maintenance and induction therapies, sirolimus + tacrolimus maintenance with no induction therapy was associated with the best survival (median=10.7 years among patients alive at 1 year), which was a substantial improvement over MMF + tacrolimus with induction (median survival=7.4 years) or MMF + tacrolimus with no induction (median survival=6.8 years), and these findings remained in multivariable adjusted analyses.

There were a few main limitations of our study. First, as this was a retrospective, non-randomized study, the susceptibility to confounding must be noted. The extensive UNOS dataset contains the vast majority of important prognostic variables, including most of those which might influence the decision for a patient to be placed on a particular immunosuppressive regimen, and this aided in reducing confounding by indication in multivariable analyses. Importantly, our study adjusted for likely confounding by transplant center. Nevertheless, unmeasured confounding remains possible from factors such as potential differences within centers between physicians who prefer one medication and those who prefer another medication. However, as almost all therapies assessed in this study were administered at over 30 transplant centers, it is fairly unlikely that major differences between physicians that correlate with treatment preferences happened to be consistently similar throughout most centers, such that considerable confounding would result.

Additionally, we do not have data on dosages or trough levels of immunosuppressive medications. Although all medications are likely to have some degree of variation in dosages or levels of the drug received, we do not expect any particular

medication to be more prone to such variation than others; nevertheless, this is still important to consider when interpreting our results. However, it should be noted that since our study data are based on the national U.S. lung transplant population, and most medications in our study had over 500 patients taking them, the average levels at which patients on a given regimen actually received each medication should be generally very well representative of the "typical" and "realistic" levels at which the medication tends to be received by patients on that regimen. Therefore, our results should be informative even in the presence of drug level variations or deficiencies in adherence. However, it is true that the success (or lack thereof) with a given immunosuppressive therapy is at least partially dependent on optimal administration of that therapy (which encompasses dosages as well as many other factors), and although the outcome data used for this study are among the most informative available since they represent the experience with each immunosuppressive therapy at dozens of lung transplant centers throughout the United States, it is possible that some therapies (particularly the less popular therapies) were generally not used as optimally as others, leading to apparently inferior outcomes with those therapies even though the results may be considerably different if they had been used optimally.

Furthermore, some subjects are missing data for certain variables in the UNOS dataset, though the frequency is <10% for most variables. We utilized multiple imputation to handle this relatively small frequency of missing data; this allows important potential confounding variables to be controlled for even if they are not available for all patients, while still including in the analyses all patients with missing data. In addition, the possibility that some patients were misclassified in the dataset with respect to

immunosuppressive therapies must be considered. Even if this misclassification was present to some degree, it is unlikely that the patients who were misclassified under any therapy characteristically had better or worse outcomes than those who were correctly classified on that therapy. Therefore, such misclassification would most likely be non-differential with respect to outcome, and would probably result in our study providing an underestimate of the true effect. Nevertheless, bias resulting in either exaggeration or underestimation of true effects remains possible if misclassification of therapies was present, depending on how the patients with misclassified data differed from the rest of the patients.

Although our study utilized the national dataset representing the U.S. lung transplant population, there are a few important considerations with regard to generalizability. Due to the fact that tacrolimus is the most fundamental and commonly used agent for maintenance immunosuppression in lung transplantation at this time, and has almost completely replaced the older calcineurin inhibitor cyclosporine after demonstrating superiority for rejection prevention in two randomized trials, our study population was restricted to tacrolimus-receiving patients, as there were hardly any patients in the dataset receiving cyclosporine in combination with several agents of interest for our study. For this reason, the intended target population to which the findings should directly apply is lung transplant recipients on a tacrolimus-based regimen. It should be noted that the results may not be generalizable to the minority of patients not on tacrolimus, such as those on cyclosporine, or on calcineurin inhibitor-free regimens. Also, as previously mentioned that there may have been differences in the ways that certain therapies were administered in this study, which could have affected outcomes associated with each

therapy, it is important to consider that the findings from this study will only be reproducible in clinical practice if the manner of administration of each therapy is generally similar to that in the study participants overall (which may not have been the optimal manner in all cases). This issue is also pertinent to the applicability of our findings to lung transplant patients in other countries. Although we would expect that our overall findings should be generalizable internationally, it is possible that differences in the way that medications are administered, or in other patient management factors, could lead to different outcomes from those we report based on the U.S. national lung transplant data. Other factors that could hinder generalizability to patients in other countries include differences in responses to certain agents according to race, or environmental or lifestyle factors, although such differences are not known at this time.

Our study also has several notable strengths. To our knowledge, this study provides the first report on long-term survival outcomes associated with common maintenance immunosuppressive therapies in lung transplantation, based on U.S. national-level data. Among national cohort studies on induction immunosuppression in lung transplantation, this is the first to account for transplant center as a likely confounder, and to include all common induction drugs for comparison. This study also assesses outcomes associated with each possible combination of induction and maintenance immunosuppressive therapies, in order to account for any potential synergistic or antagonistic effects that may occur when certain induction and maintenance immunosuppressive medications are used in the same patient. Additionally, this study provides novel information on associations between maintenance immunosuppressive regimens and the three most common causes of death (chronic lung rejection, malignancy, and infection) in the lung transplant patient

population. Since the dataset used for this study contains all U.S. lung transplant patients, the study population should not be prone to selection bias, although the findings should be generalized carefully as previously described. Although the study is retrospective in nature, the UNOS dataset contains a large breadth of important confounding variables, which enables good confounding control for most of the important factors that are likely to differ between exposure groups. Finally, this study employs multiple methods of addressing potential immortal time bias arising from the delayed initiation of prophylactic sirolimus treatment, and also utilizes several advanced analytical approaches in order to improve the validity of the results in general. These include: multiple imputation to handle missing data, a random effect in the Cox models to represent a transplant center effect (which accounts for potential confounding by transplant center while accurately estimating standard errors), and an illness-death semi-competing risks model utilizing a patient-specific frailty to account for the dependence between each event and death (which addresses the possibility that censoring of a non-fatal event by a fatal event of another type may be informative).

From this study, we conclude that sirolimus, initiated within the first year in a tacrolimus-based regimen, may significantly improve long-term survival for lung transplant recipients compared to MMF. The immunosuppressive strategy of sirolimus + tacrolimus maintenance with no induction was associated with the best survival among all induction-maintenance combinations assessed, representing a survival improvement of multiple years compared to MMF, the most commonly used agent, with or without induction. In patients whom sirolimus cannot be utilized for any reason, azathioprine may modestly improve survival compared to MMF in a tacrolimus-based regimen. Additional long-term studies in lung transplantation are needed to confirm these findings.

Appendix A: ISHLT disease-specific referral criteria for evaluation at a lung transplant center

A) COPD

- Disease is progressive, despite maximal treatment including medication, pulmonary rehabilitation, and oxygen therapy.
- Patient is not a candidate for endoscopic or surgical LVRS (Lung volume reduction surgery). Simultaneous referral of patients with COPD for both lung transplant and LVRS evaluation is appropriate.
- BODE (Body-mass index, airflow Obstruction, Dyspnea, and Exercise) index of 5 to 6.
- Pa_{CO2}>50 mm Hg or 6.6 kPa and/or Pa_{O2}<60 mm Hg or 8 kPa.
- FEV_1<25% predicted.

B) Pulmonary vascular diseases

- NYHA Functional Class III or IV symptoms during escalating therapy.
- Rapidly progressive disease (assuming weight and rehabilitation concerns not present).
- Use of parenteral targeted pulmonary arterial hypertension (PAH) therapy regardless of symptoms or NYHA
  Functional Class.
- Known or suspected pulmonary veno-occlusive disease (PVOD) or pulmonary capillary hemangiomatosis.

C) Cystic fibrosis

- FEV1 that has fallen to 30% or a patient with advanced disease with a rapidly falling FEV1 despite optimal therapy (particularly in a female patient), infected with non-tuberculous mycobacterial (NTM) disease or *B cepacia* complex (see previous comment on *B cenocepacia* and subsequently) and/or with diabetes.
- A 6-minute walk distance <400 m.
- Development of pulmonary hypertension in the absence of a hypoxic exacerbation (as defined by a systolic pulmonary arterial pressure (PAP) >35 mm Hg on echocardiography or mean PAP >25 mm Hg measured by right heart catheterization).
- Clinical decline characterized by increasing frequency of exacerbations associated with any of the following:

  ▪ An episode of acute respiratory failure requiring non- invasive ventilation.

  ▪ Increasing antibiotic resistance and poor clinical recovery from exacerbations.

  ▪ Worsening nutritional status despite supplementation.

  ▪ Pneumothorax.

  ▪ Life-threatening hemoptysis despite bronchial embolization.

D) Interstitial lung disease

- Histopathologic or radiographic evidence of usual interstitial pneumonitis (UIP) or fibrosing non-specific interstitial pneumonitis (NSIP), regardless of lung function.
- Abnormal lung function: forced vital capacity (FVC) <80% predicted or diffusion capacity of the lung for carbon monoxide (DLCO) <40% predicted.
- Any dyspnea or functional limitation attributable to lung disease.
- Any oxygen requirement, even if only during exertion.
- For inflammatory interstitial lung disease (ILD), failure to improve dyspnea, oxygen requirement, and/or lung function after a clinically indicated trial of medical therapy.

Appendix B: ISHLT absolute and relative contraindications to lung transplant

Absolute contraindications

- Lung transplantation should not be offered to adults with a recent history of malignancy. A 2-year disease-free interval combined with a low predicted risk of recurrence after lung transplantation may be reasonable, for instance, in non- melanoma localized skin cancer that has been treated appropriately. However, a 5-year disease-free interval is prudent in most cases, particularly for patients with a history of hematologic malignancy, sarcoma, melanoma, or cancers of the breast, bladder, or kidney. Unfortunately, for a portion of patients with a history of cancer, the risk of recurrence may remain too high to proceed with lung transplantation even after a 5-year disease-free interval.
- Untreatable significant dysfunction of another major organ system (e.g., heart, liver, kidney, or brain) unless combined organ transplantation can be performed.
- Uncorrected atherosclerotic disease with suspected or confirmed end-organ ischemia or dysfunction and/or
  coronary artery disease not amenable to revascularization.
- Acute medical instability, including, but not limited to, acute sepsis, myocardial infarction, and liver failure.
- Uncorrectable bleeding diathesis.
- Chronic infection with highly virulent and/or resistant microbes that are poorly controlled pre-transplant.
- Evidence of active *Mycobacterium tuberculosis* infection.
- Significant chest wall or spinal deformity expected to cause severe restriction after transplantation.
- Class II or III obesity (body mass index [BMI] 35.0 kg/m^2).
- Current non-adherence to medical therapy or a history of repeated or prolonged episodes of non-adherence to medical therapy that are perceived to increase the risk of non-adherence after transplantation.
- Psychiatric or psychologic conditions associated with the inability to cooperate with the medical/allied health care team and/or adhere with complex medical therapy.
- Absence of an adequate or reliable social support system.

- Severely limited functional status with poor rehabilitation potential.
- Substance abuse or dependence (e.g., alcohol, tobacco, marijuana, or other illicit substances). In many cases, convincing evidence of risk reduction behaviors, such as meaningful and/or long-term participation in therapy for substance abuse and/or dependence, should be required before offering lung transplantation. Serial blood and urine testing can be used to verify abstinence from substances that are of concern.

Relative contraindications

- Age >65 years in association with low physiologic reserve and/or other relative contraindications. Although there cannot be endorsement of an upper age limit as an absolute contraindication, adults >75 years old are unlikely to be candidates for lung transplantation in most cases. Although age by itself should not be considered a contraindication to transplant, increasing age generally is associated with comorbid conditions that are either absolute or relative contraindications.
- Class I obesity (BMI 30.0–34.9 kg/m^2), particularly truncal (central) obesity.
- Progressive or severe malnutrition.
- Severe, symptomatic osteoporosis.
- Extensive prior chest surgery with lung resection.
- Mechanical ventilation and/or extracorporeal life support (ECLS). However, carefully selected candidates without other acute or chronic organ dysfunction may be successfully transplanted.
- Colonization or infection with highly resistant or highly virulent bacteria, fungi, and certain strains of mycobacteria (e.g., chronic extrapulmonary infection expected to worsen after transplantation).
- For patients infected with hepatitis B and/or C, a lung transplant can be considered in patients without significant clinical, radiologic, or biochemical signs of cirrhosis or portal hypertension and who are stable on appropriate therapy. Lung transplantation in candidates with hepatitis B and/or C should be performed in centers with experienced hepatology units.
- For patients infected with human immunodeficiency virus (HIV), a lung transplant can be considered in patients with controlled disease with undetectable HIV- RNA, and compliant on combined anti-retroviral therapy. The most suitable candidates should have no current acquired immunodeficiency syndrome–defining illness. Lung transplantation in HIV-positive candidates should be performed in centers with expertise in the care of HIV-positive patients.
- Infection with *Burkholderia cenocepacia*, *Burkholderia gladioli,* and multi-drug–resistant *Mycobacterium abscessus* if the infection is sufficiently treated preoperatively and there is a reasonable expectation for adequate control postoperatively. For patients with these infections to be considered suitable transplant candidates, the patients should be evaluated by centers with significant experience managing these infections in the transplant setting, and patients should be aware of the increased risk of transplant because of these infections.

Appendix C: ISHLT disease-specific lung transplant candidate selection criteria

A) COPD (presence of one criterion is sufficient)

- BODE index ≥7.
- FEV_1 <15% to 20% predicted.
- Three or more severe exacerbations during the preceding year.
- One severe exacerbation with acute hypercapnic respiratory failure.
- Moderate to severe pulmonary hypertension.

B) Pulmonary vascular diseases

- NYHA Functional Class III or IV despite a trial of at least 3 months of combination therapy including prostanoids.
- Cardiac index of <2 liters/min/m2.
- Mean right atrial pressure of >15 mm Hg.
- 6-minute walk test of <350 m.
- Development of significant hemoptysis, pericardial effusion, or signs of progressive right heart failure (renal insufficiency, increasing bilirubin, brain natriuretic peptide, or recurrent ascites).

C) Cystic fibrosis

- Chronic respiratory failure.
  - With hypoxia alone (partial pressure of oxygen [Pa_{O2}] <8 kPa or <60 mm Hg).
  - With hypercapnia (partial pressure of carbon dioxide [Pa_{CO2}] >6.6 kPa or >50 mm Hg).
- Long-term non-invasive ventilation therapy.
- Pulmonary hypertension.
- Frequent hospitalization.
- Rapid lung function decline.
- World Health Organization Functional Class IV.

D) Interstitial lung disease

- Decline in FVC ≥10% during 6 months of follow-up (note: a 5% decline is associated with a poorer prognosis and may warrant listing).
- Decline in D_{LCO} ≥15% during 6 months of follow-up.
- Desaturation to <88% or distance <250 m on 6-minute-walk test or >50 m decline in 6-minute-walk distance over a 6-month period.
- Pulmonary hypertension on right heart catheterization or 2-dimensional echocardiography.
- Hospitalization because of respiratory decline, pneumothorax, or acute exacerbation.

Appendix D: Variables used to calculate Lung Allocation Score (LAS)

- Age at offer
- Bilirubin (mg/dL)
- Bilirubin increase of at least 50%*
- BMI (weight (kg)/height $(m)^2$)
- Cardiac index prior to any exercise
- Central venous pressure (CVP) (mmHg) at rest, prior to any exercise
- Continuous mechanical ventilation, if candidate is hospitalized*
- Creatinine (serum) (mg/dL)
- Diabetes (regardless of insulin dependency)*
- Diagnosis (type of lung disease)
- Forced vital capacity (FVC) % predicted
- Functional status
- Oxygen need to maintain adequate oxygen saturation (88% or greater) at rest (L/min)
- pCO2
- pCO2 increase of at least 15%*
- Pulmonary artery (PA) systolic pressure at rest, prior to any exercise (mmHg)
- Six-minute walk distance (feet) obtained while the candidate was receiving supplemental oxygen required to maintain an oxygen saturation of 88% or greater at rest.

*Indicates a dichotomous (yes/no) variable

References

1. Yusen RD, Edwards LB, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Lung and Heart-Lung Transplant Report --2016; Focus Theme: Primary Diagnostic Indications for Transplant. *J Hear Lung Transplant*. 2016;35(10):1170-1184. doi:10.1016/j.healun.2016.09.001

2. Valapour M, Skeans MA, Smith JM, et al. *OPTN / SRTR 2015 Annual Data Report : Lung*.; 2015. doi:10.1111/ajt.14129

3. Weill D, Chairs C, Benden C, et al. ISHLT CONSENSUS A consensus document for the selection of lung transplant candidates : 2014 — An update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. *J Hear Lung Transplant*. 2015;34(1):1-15. doi:10.1016/j.healun.2014.06.014

4. Murthy, Sudish C., Marshall MB. *Lung Transplantation, An Issue of Thoracic Surgery Clinics. Volume 25, Issue 1 of The Clinics: Surgery*. Philadelphia, PA: Elsevier; 2014.

5. United Network for Organ Sharing. A Guide to Calculating the Lung Allocation Score. 2015:1-14.

6. Rana A, Gruessner A, Agopian VG, et al. Survival benefit of solid-organ transplant in the United States. *JAMA Surg*. 2015;150(3):252-259. doi:10.1001/jamasurg.2014.2038

7. Vigneswaran, Wickii; Garrity, Edward; Odell J. *Lung Transplantation: Principles and Practice*. Boca Raton, FL: CRC Press; 2016.

8. Yusen RD, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation : Thirty-second Official Adult Lung and Heart-Lung Transplantation Report — 2015 ; Focus Theme : Early Graft Failure. *J Hear Lung Transplant*. 2015;34(10):1264-1277. doi:10.1016/j.healun.2015.08.014

9. Nair N, Gongora E, Mehra MR. Long-term immunosuppression and malignancy in thoracic transplantation : Where is the balance ? *J Hear Lung Transplant*. 2014;33(5):461-467. doi:10.1016/j.healun.2014.03.002

10. Chapman JR, Webster AC, Wong G. Cancer in the transplant recipient. *Cold Spring Harb Perspect Med*. 2013;3(7):1-16. doi:10.1101/cshperspect.a015677

11. Singh NKCGG. Infection in Organ Transplant Recipients. *Infect Dis Antimicrob Agents*. 2010;(19):1741-1751. doi:10.1016/S0003-9993(98)90407-5

12. Weiss ES, Allen JG, Merlo CA, Conte J V, Shah AS. Factors indicative of long-term survival after lung transplantation : A review of 836 10-year survivors. *J Hear Lung Transplant*. 2010;29(3):240-246. doi:10.1016/j.healun.2009.06.027

13. Ij S, Akinyede O, Ka R. Immunosuppressive drug therapy for preventing rejection following lung transplantation in cystic fibrosis (Review). 2015;(11).

doi:10.1002/14651858.CD009421.pub3.www.cochranelibrary.com

14. Bhorade SM, Stern E. Immunosuppression for Lung Transplantation. *Proc Am Thorac Soc*. 2009;(10). doi:10.1513/pats.200808-096GO

15. Jaksch P. IMMUNOSUPRESSIVE THERAPY IN THE LUNG TRANSPLANT RECIPIENT. *Rad 522 Med Sci*. 2015;(April):47-55.

16. Treede H, Glanville AR, Klepetko W, et al. Tacrolimus and cyclosporine have differential effects on the risk of development of bronchiolitis obliterans syndrome : Results of a prospective , randomized international trial in lung transplantation. *J Hear Lung Transplant*. 2012;31(8):797-804. doi:10.1016/j.healun.2012.03.008

17. Hachem RR, Yusen RD, Chakinala MM, et al. A Randomized Controlled Trial of Tacrolimus Versus Cyclosporine After Lung Transplantation. *J Hear Lung Transplant*. 2007;26(10):1012-1018. doi:10.1016/j.healun.2007.07.027

18. Zuckermann A, Reichenspurner H, Birsan T, et al. Cyclosporine A versus tacrolimus in combination with mycophenolate mofetil and steroids as primary immunosuppression after lung transplantation: One-year results of a 2-center prospective randomized trial. *J Thorac Cardiovasc Surg*. 2003;125(4):891-900. doi:10.1067/mtc.2003.71

19. Snell GI, Westall GP. Immunosuppression for lung transplantation: Evidence to date. *Drugs*. 2007;67(11):1531-1539. doi:10.2165/00003495-200767110-00002

20. Mcdonnell M, Lordan J. Review of Immunosuppressive Treatment in Lung Transplantation. 2014:94-101. doi:10.5152/ttd.2014.4265

21. Snell GI, Westall GP, Paraskeva M a. Immunosuppression and allograft rejection following lung transplantation: Evidence to date. *Drugs*. 2013;73(16):1793-1813. doi:10.1007/s40265-013-0136-x

22. Scheffert JL, Raza K. Immunosuppression in lung transplantation. *J Thorac Dis*. 2014;6(2):1039-1053. doi:10.3978/j.issn.2072-1439.2014.04.23

23. McNeil K, Glanville AR, Wahlers T, et al. Comparison of mycophenolate mofetil and azathioprine for prevention of bronchiolitis obliterans syndrome in de novo lung transplant recipients. *Transplantation*. 2006;81(7):998-1003. doi:10.1097/01.tp.0000202755.33883.61

24. Palmer SM, Baz M a, Sanders L, et al. Results of a randomized, prospective, multicenter trial of mycophenolate mofetil versus azathioprine in the prevention of acute lung allograft rejection. *Transplantation*. 2001;71(12):1772-1776.

25. Sánchez Fructuoso A, Calvo N, Moreno MA, Giorgi M, Conesa J, Barrientos A. Better Mycophenolic Acid 12-Hour Trough Level After Enteric-Coated Mycophenolate Sodium in Patients With Gastrointestinal Intolerance to Mycophenolate Mofetil. *Transplant Proc*. 2007;39(7):2194-2196. doi:10.1016/j.transproceed.2007.06.033

26. Manger B, Hiepe F, Schneider M, et al. Impact of switching from mycophenolate

mofetil to enteric-coated mycophenolate sodium on gastrointestinal side effects in patients with autoimmune disease: A Phase III, open-label, single-arm, multicenter study. *Clin Exp Gastroenterol*. 2015;8:205-213. doi:10.2147/CEG.S81922

27. Sterneck M, Settmacher U, Ganten T, et al. Improvement in gastrointestinal and health-related quality of life outcomes after conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium in liver transplant recipients. *Transplant Proc*. 2014;46(1):234-240. doi:10.1016/j.transproceed.2013.09.026

28. Feng JJ, Zhang LW, Zhao P, Bow LM, Tian J. Enteric-coated mycophenolate sodium given in combination with tacrolimus has a lower incidence of serious infections in Asian renal-transplant recipients compared with mycophenolate mofetil. *Int J Clin Pract*. 2015;69(S183):1-7. doi:10.1111/ijcp.12660

29. Xu L, Cai M, Shi BY, Li ZL, Li X, Jin HL. A Prospective analysis of the effects of enteric-coated mycophenolate sodium and mycophenolate mofetil co-medicated with a proton pump inhibitor in kidney transplant recipients at a single institute in china. *Transplant Proc*. 2014;46(5):1362-1365. doi:10.1016/j.transproceed.2014.01.012

30. Yin H, Qiu K, Hu XP, et al. Lower dosing of enteric-coated mycophenolate sodium (Myfortic) can achieve target mycophenolic acid exposure rapidly in most Chinese renal transplant patients: a pilot study. *Int J Clin Pract Suppl*. 2014;68(181):31-37. doi:10.1111/ijcp.12404

31. Kees MG, Steinke T, Moritz S, et al. Omeprazole impairs the absorption of mycophenolate mofetil but not of enteric-coated mycophenolate sodium in healthy volunteers. *J Clin Pharmacol*. 2012;52(8):1265-1272. doi:10.1177/0091270011412968

32. Sacher VY, Fertel D, Srivastava K, et al. Effects of prophylactic use of sirolimus on bronchiolitis obliterans syndrome development in lung transplant recipients. *Ann Thorac Surg*. 2014;97(1):268-274. doi:10.1016/j.athoracsur.2013.07.072

33. Zhao Y, Gillen JR, Meher AK, Burns JA, Kron IL, Lau CL. Rapamycin prevents bronchiolitis obliterans through increasing infiltration of regulatory B cells in a murine tracheal transplantation model Read at the 95th Annual Meeting of the American Association for Thoracic Surgery, Seattle, Washington, April 25-29. *J Thorac Cardiovasc Surg*. 2016;151(2):487-493e3. doi:10.1016/j.jtcvs.2015.08.116

34. Bhorade S, Ahya VN, Baz MA, et al. Comparison of sirolimus with azathioprine in a tacrolimus-based immunosuppressive regimen in lung transplantation. *Am J Respir Crit Care Med*. 2011;183(3):379-387. doi:10.1164/rccm.201005-0775OC

35. Laporta Hernández R, Ussetti Gil P, García Gallo C, De Pablo Gafas A, Carreño Hernández MC, Ferreiro Álvarez MJ. Rapamycin in lung transplantation. *Transplant Proc*. 2005;37(9):3999-4000. doi:10.1016/j.transproceed.2005.09.191

36. Peddi VR, Wiseman A, Chavin K, Slakey D. Review of combination therapy with mTOR inhibitors and tacrolimus minimization after transplantation. *Transplant Rev*. 2013;27(4):97-107. doi:10.1016/j.trre.2013.06.001

37. Blagosklonny M V. Rapalogs in cancer prevention Rapalogs in cancer prevention Anti-aging or anticancer ? *Cancer Biol Ther*. 2012;4047(November). doi:10.4161/cbt.22859

38. Lamming DW, Ye L, Sabatini DM, Baur JA. Review series Rapalogs and mTOR inhibitors as anti-aging therapeutics. *J Clin Invest*. 2013;123(3). doi:10.1172/JCI64099.980

39. King-Biggs MB, Dunitz JM, Park SJ, Kay Savik S, Hertz MI. Airway anastomotic dehiscence associated with use of sirolimus immediately after lung transplantation. *Transplantation*. 2003;75(9):1437-1443. doi:10.1097/01.TP.0000064083.02120.2C

40. Stuckey LJ, Bartos CE, McCullough HA, et al. Use of Sirolimus in Lung Transplantation: A Single Center Experience. *J Hear Lung Transplant*. 2013;32(4):S89. doi:10.1016/j.healun.2013.01.990

41. Hillen U, Griewank K, Sommerwerck U, Baba H, Schadendorf D. Tumor regression and sirolimus-based therapy in lung transplantation. *J Hear Lung Transplant*. 2013;32(9).

42. Mariski M, Feist A, Yung G, Awdishu L. Sirolimus Improves Renal Function and May Prevent BOS Progression After Lung Transplant. *J Hear Lung Transplant*. 2014;33(4):S190. doi:10.1016/j.healun.2014.01.515

43. Villanueva J, Boukhamseen A, Bhorade SM. Successful use in lung transplantation of an immunosuppressive regimen aimed at reducing target blood levels of sirolimus and tacrolimus. *J Hear Lung Transplant*. 2005;24(4):421-425. doi:10.1016/j.healun.2004.01.014

44. Shitrit D, Rahamimov R, Gidon S, et al. Use of sirolimus and low-dose calcineurin inhibitor in lung transplant recipients with renal impairment: Results of a controlled pilot study. *Kidney Int*. 2005;67(4):1471-1475. doi:10.1111/j.1523-1755.2005.00224.x

45. Yan T-Z, Wu X-Q, Rong L. A four-drug combination therapy consisting of low-dose tacrolimus, low-dose mycophenolate mofetil, corticosteroids, and mizoribine in living donor renal transplantation: A randomized study. *SAGE Open Med*. 2016;4. doi:10.1177/2050312116643672

46. Srinivas TR, Schold JD, Guerra G, Eagan A, Bucci CM, Meier-Kriesche HU. Mycophenolate mofetil/sirolimus compared to other common immunosuppressive regimens in kidney transplantation. *Am J Transplant*. 2007;7(3):586-594. doi:10.1111/j.1600-6143.2006.01658.x

47. Al-Khafaji A. *ICU Care of Abdominal Organ Transplant Patients*. Oxford University Press; 2013. https://books.google.com/books?id=HC9pAgAAQBAJ.

48. Klawitter J, Klawitter J, Schmitz V, et al. Mycophenolate mofetil enhances the negative effects of sirolimus and tacrolimus on rat kidney cell metabolism. *PLoS One*. 2014;9(1):3-8. doi:10.1371/journal.pone.0086202

49. McAlister V, Gao Z, Peltekian K, Domingues J, Mahalati K, MacDonald A. Sirolimus-tacrolimus combination immunosuppression. *Lancet*. 2000;355:377-378. doi:10.1016/S0140-6736(99)05143-0

50. Penninga L, Ei P, Ch M, Iversen M, Da S, Gluud C. Tacrolimus versus cyclosporin as primary immunosuppression for lung transplant recipients (Review) SUMMARY OF FINDINGS FOR THE MAIN COMPARISON. *Cochrane Libr*. 2013;(5). doi:10.1002/14651858.CD008817.pub2.Copyright

51. Hayes D, Whitson BA, Ghadiali SN, Tobias JD, Mansour HM, Black SM. Influence of HLA Mismatching on Survival in Lung Transplantation. *Lung*. 2015;193(5):789-797. doi:10.1007/s00408-015-9768-9

52. Mullen JC, Oreopoulos A, Lien DC, et al. A Randomized, Controlled Trial of Daclizumab vs Anti-thymocyte Globulin Induction for Lung Transplantation. *J Hear Lung Transplant*. 2007;26(5):504-510. doi:10.1016/j.healun.2007.01.032

53. Hayes D, Black SM, Tobias JD, et al. Influence of human leukocyte antigen mismatching on bronchiolitis obliterans syndrome in lung transplantation. *J Hear Lung Transpl*. 2016;35(2):186-194. doi:10.1016/j.healun.2015.08.022

54. Furuya Y, Jayarajan SN, Taghavi S, et al. The Impact of Alemtuzumab and Basiliximab Induction on Patient Survival and Time to Bronchiolitis Obliterans Syndrome in Double Lung Transplantation Recipients. *Am J Transplant*. 2016;16(8):2334-2341. doi:10.1111/ajt.13739

55. Leppke S, Leighton T, Zaun D, et al. Scientific Registry of Transplant Recipients: Collecting, analyzing, and reporting data on transplantation in the United States. *Transplant Rev*. 2013;27(2):50-56. doi:10.1016/j.trre.2013.01.002

56. Graham JW, Olchowski AE, Gilreath TD. How many imputations are really needed? Some practical clarifications of multiple imputation theory. *Prev Sci*. 2007;8(3):206-213. doi:10.1007/s11121-007-0070-9

57. Rubin DB. Multiple Imputation after 18+ Years. *J Am Stat Assoc*. 1996;91(434):473-489. doi:10.1080/01621459.1996.10476908

58. Kropko J, Goodrich B, Gelman A, Hill J. Multiple imputation for continuous and categorical data: Comparing joint multivariate normal and conditional approaches. *Polit Anal*. 2014;22(4):497-519. doi:10.1093/pan/mpu007

59. Rubin DB. An Overview of Multiple Imputation. *Proc Surv Res methods Sect Am Stat Assoc*. 1988:79-84.

60. Wittes J. Sample size calculations for randomized controlled trials. *Epidemiol Rev*. 2002;24(1):39-53. doi:10.1093/epirev/24.1.39

61. Wojarski J, Żegleń S, Ochman M, Karolak W. Early Sirolimus-Based Immunosuppression is Safe for Lung Transplantation Patients: Retrospective, Single Arm, Exploratory Study. *Ann Transplant*. 2018;23:598-607. doi:10.12659/AOT.908471

62. Alvares D, Haneuse S, Lee C, Lee KH. SemiCompRisks: An R Package for

Independent and Cluster-Correlated Analyses of Semi-Competing Risks Data. 2018. http://survey.hshsl.umaryland.edu/?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsarx&AN=edsarx.1801.03567&site=eds-live.

63. Haneuse S, Lee KH. Semi-competing risks data analysis. *Circ Cardiovasc Qual Outcomes*. 2016;9(3):322-331. doi:10.1161/CIRCOUTCOMES.115.001841

64. Jazic I, Schrag D, Sargent DJ, Haneuse S. Beyond composite endpoints analysis: Semicompeting risks as an underutilized framework for cancer research. *J Natl Cancer Inst*. 2016;108(12):1-6. doi:10.1093/jnci/djw154

65. Corris PA. Induction Therapy in Lung Transplantation? A Frustrating Message of Persisting Uncertainty. *Am J Transplant*. 2016;16(8):2250-2251. doi:10.1111/ajt.13787

66. Campistol JM, Cockwell P, Diekmann F, et al. Practical recommendations for the early use of m-TOR inhibitors (sirolimus) in renal transplantation. *Transpl Int*. 2009;22(7):681-687. doi:10.1111/j.1432-2277.2009.00858.x

67. Kaplan B, Qazi Y, Wellen JR. Strategies for the management of adverse events associated with mTOR inhibitors. *Transplant Rev*. 2014;28(3):126-133. doi:10.1016/j.trre.2014.03.002

68. Whitson BA, Lehman A, Wehr A, et al. To induce or not to induce: A 21st century evaluation of lung transplant immunosuppression's effect on survival. *Clin Transplant*. 2014;28(4):450-461. doi:10.1111/ctr.12339

69. Hartwig MG, Snyder LD, Appel III JZ, et al. Rabbit Anti-thymocyte Globulin Induction Therapy Does Not Prolong Survival After Lung Transplantation. *J Hear Lung Transplant*. 2008;27(5):547-553. doi:10.1016/j.healun.2008.01.022

70. Chocair P, Duley J. Mycophenolate has replaced azathioprine in immunosuppression regimens . But is it better ? *Rev Assoc Med Bras*. 2012;55(11).

71. Song ATW, Abdala E, Bonazzi PR, Bacchella T, Machado MCC. Does mycophenolate mofetil increase the risk of cytomegalovirus infection in solid organ transplant recipients?--A mini-review. *Braz J Infect Dis*. 2006;10:132-138.

72. Wang K, Zhang H, Li Y, et al. Safety of mycophenolate mofetil versus azathioprine in renal transplantation: A systematic review. *Transplant Proc*. 2004;36(7):2068-2070. doi:10.1016/j.transproceed.2004.07.057

73. Ansari D, Höglund P, Andersson B, Nilsson J. Comparison of basiliximab and anti-thymocyte globulin as induction therapy in pediatric heart transplantation: A survival analysis. *J Am Heart Assoc*. 2016;5(1):1-9. doi:10.1161/JAHA.115.002790

74. Scheinberg PP, Nunez O, Weinstein B, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N Engl J Med*. 2011;365(5):430-438. doi:10.1056/NEJMoa1103975