INCIDENCE AND RISK FACTORS FOR CANCER AFTER LIVER, HEART AND LUNG TRANSPLANTATION IN AUSTRALIA

Renhua Na

A thesis in fulfilment of the requirements for the degree of

Doctor of Philosophy

Prince of Wales Clinical School, Faculty of Medicine

The University of New South Wales

March, 2015

PLEASE TYPE

THE UNIVERSITY OF NEW SOUTH WALES
Thesis/Dissertation Sheet

Surname or Family name: **Na**

First name: **Renhua** Other name/s:

Abbreviation for degree as given in the University calendar: **PhD (Medicine)**

School: **Prince of Wales Clinical School** Faculty: **Faculty of Medicine**

Title: **Incidence and risk factors for cancer after liver, heart and lung transplantation in Australia**

Abstract 350 words maximum: (PLEASE TYPE)

Background: Iatrogenic immunosuppression increases the risk of cancer after solid organ transplantation, but little is known about the site-specific risk of cancer in non-kidney transplant recipients, or the features of immunosuppressive therapy responsible for cancer occurrence. Methods: I conducted a population-based cohort study of Australian liver, heart and lung transplant recipients (1984-2006) and ascertained deaths and incident cancers by record linkage. I collected comprehensive longitudinal clinical data, including the type and dose of immunosuppression, from 18 transplant units. I estimated the site-specific risk of cancer and cancer-related mortality in transplant recipients relative to the general population. I quantified the type and dose of individual immunosuppressive agents by time since transplantation and organ type. I examined the association between the type, dose and duration of immunosuppression and risk of the two most common cancers, non-Hodgkin lymphoma (NHL) and lip cancer, accounting for competing risk of death. Results: I observed an excess risk for sixteen cancer types, predominantly cancers with a viral cause. Overall the cancer risk profile was similar by organ type, but notable exceptions suggested a role for organ-specific pre-existing diseases, carcinogenic behaviours, and differences in the extent of immunosuppression. Risk of *de novo* cancer-related mortality was significantly elevated and as expected the profile mirrored that for cancer incidence. I observed significant changes in the type and dose of individual immunosuppressive agents by time since transplantation, transplant era, and organ type. I found that a high dose of azathioprine was independently associated with a moderate dose-related risk of NHL and lip cancer. I showed that differences in the risk of NHL and lip cancer by organ type are attributed to differences in the dose of immunosuppression. I confirmed the increased risk of early NHL with use of muromonab-CD3 induction antibody, the increased risk of late NHL and lip cancer with both increasing duration of immunosuppression and increasing age, and the increased risk of lip cancer with smoking. Earlier transplant era also increased lip cancer risk. Conclusions: The type, dose (extent) and duration of iatrogenic immunosuppression contribute to the excess risk of cancer in liver, heart and lung transplant recipients.

Declaration relating to disposition of project thesis/dissertation

I hereby grant to the University of New South Wales or its agents the right to archive and to make available my thesis or dissertation in whole or in part in the University libraries in all forms of media, now or here after known, subject to the provisions of the Copyright Act 1968. I retain all property rights, such as patent rights. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

I also authorise University Microfilms to use the 350 word abstract of my thesis in Dissertation Abstracts International (this is applicable to doctoral theses only).

Renhua Na ..... Signature

..... Witness

09/03/2015 ..... Date

The University recognises that there may be exceptional circumstances requiring restrictions on copying or conditions on use. Requests for restriction for a period of up to 2 years must be made in writing. Requests for a longer period of restriction may be considered in exceptional circumstances and require the approval of the Dean of Graduate Research.

FOR OFFICE USE ONLY Date of completion of requirements for Award:

THIS SHEET IS TO BE GLUED TO THE INSIDE FRONT COVER OF THE THESIS

Originality statement

ORIGINALITY STATEMENT

'I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at UNSW or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by others, with whom I have worked at UNSW or elsewhere, is explicitly acknowledged in the thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.'

Signed Renhua Na .....

Date **09/03/2015** .....

Copyright and Authenticity Statements

COPYRIGHT STATEMENT

'I hereby grant the University of New South Wales or its agents the right to archive and to make available my thesis or dissertation in whole or part in the University libraries in all forms of media, now or here after known, subject to the provisions of the Copyright Act 1968. I retain all proprietary rights, such as patent rights. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.
I also authorise University Microfilms to use the 350 word abstract of my thesis in Dissertation Abstract International (this is applicable to doctoral theses only).
I have either used no substantial portions of copyright material in my thesis or I have obtained permission to use copyright material; where permission has not been granted I have applied/will apply for a partial restriction of the digital copy of my thesis or dissertation.'

Signed 

Date 09/03/2015

AUTHENTICITY STATEMENT

'I certify that the Library deposit digital copy is a direct equivalent of the final officially approved version of my thesis. No emendation of content has occurred and if there are any minor variations in formatting, they are the result of the conversion to digital format.'

Signed 

Date 09/03/2015

Table of contents

Table of contents

Table of contents

Table of contents

Acknowledgements

I would like to express my sincere gratitude to Associate Professor Claire Vajdic, my supervisor, for offering me this opportunity and for consistently supporting me throughout the period of my PhD candidature. Thank you also to Dr Maarit Laaksonen for giving me thoughtful guidance and spiritual support. I am very grateful to both of my supervisors for providing critical comments and for the enormous help they have given me for my study. Thank you for being so patient with me.

I am truly grateful to all the staff members at the Australian liver and cardiothoracic transplant registries and all the officers at each transplant unit for providing me with such comprehensive clinical data. I would also like to thank Professor Andrew Grulich, Nicki Meagher, Professor Geoff McCaughan, and Professor Anne Keogh for giving me advice regarding the data management and interpretation.

I wish to acknowledge the University International Postgraduate Award and the PhD scholarship top-up award provided by the Translational Cancer Research Network; I could not have completed my PhD training without their financial support. Finally, I am very grateful to my family for their unconditional love and understanding.

Publications

Na R, Grulich AE, Meagher NS, McCaughan GW, Keogh AM, Vajdic CM (2013). Cancer risk after liver, heart and lung transplantation in Australia. ***Am J Transplant*** 13(1):174-183.

Na R, Grulich AE, Meagher NS, McCaughan GW, Keogh AM, Vajdic CM (2013). *De novo* cancer-related death in Australian liver and cardiothoracic transplant recipients. ***Am J Transplant*** 13(5):1296-1304.

Contribution of Authors

The work included in this thesis has been conducted by the author under the supervision of Associate Professor Claire Vajdic and co-supervision of Dr Maarit Laaksonen at Prince of Wales Clinical School, University of New South Wales Australia.

The details of the contributions of all the authors of the thesis and manuscripts that have arisen from my thesis are listed below.

Na R contributed to the study design, data collection, data management and data analyses, also interpretation of results, drafting and revision of the thesis and preparing manuscripts for submission to peer-reviewed journals.

Vajdic CM planned the research, designed the study, submitted ethics committee applications, and contributed to data management, data analysis, interpretation of results and to the drafting and revision of the thesis and manuscripts.

Laaksonen MA contributed to the study design, data analyses, interpretation of results and to the drafting and revision of the thesis and manuscripts.

Grulich AE contributed to the study design, interpretation of results and manuscript drafting and revision.

Contribution of Authors

McCaughan GW contributed to the study design, interpretation of results and manuscript drafting and revision.

Keogh AM contributed to the study design, interpretation of results and manuscript drafting and revision.

Meagher NS participated in the study design, submission of ethics, data management, interpretation of results and manuscript drafting and revision.

Chapters 3 to 7 involved data linkage between the Australia and New Zealand Liver Transplant Registry (ANZLTR), Australia and New Zealand Cardiothoracic Organ Transplant Registry (ANZCOTR), Australian Cancer Database (ACD) and National Death Index (NDI). The data linkage was conducted by the Australian Institute of Health and Welfare.

Abbreviations

6-TG	6-thioguanine
ABS	Australian Bureau of Statistics
ACD	Australian Cancer Database
AIDS	Acquired Immunodeficiency Syndrome
AIHW	Australian Institute of Health and Welfare
ALG	Anti-lymphocyte globulin
ANZDATA	Australia and New Zealand Dialysis and Transplant Registry
ANZCOTR	Australia and New Zealand Cardiothoracic Organ Transplant Registry
ANZLTR	Australia and New Zealand Liver Transplant Registry
ATG	Anti-thymocyte globulin
AZA	Azathioprine
BCC	Basal cell carcinoma
CI	Confidence interval
CMV	Cytomegalovirus
CNI	Calcineurin inhibitor
CNS	Central nervous system
CRC	Colorectal cancer
CTL	Cytotoxic T-cell
CTS	Collaborative Transplant Study
EBNA	Epstein-Barr nuclear antigen
EBV	Epstein-Barr virus
ESKD	End-stage kidney disease

List of Abbreviations

HAART	Highly active antiretroviral therapy
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
KSHV	Kaposi sarcoma herpesvirus
HIV	Human immunodeficiency virus
HL	Hodgkin lymphoma
HLA	Human leukocyte antigen
HPV	Human papillomavirus
HR	Hazard ratio
HSV-1	Herpes simplex virus type-1
IARC	International Agency for Research on Cancer
IBD	Inflammatory bowel disease
ICD	International Classification of Diseases
ICD-O	International Classification of Diseases for Oncology
Ig	Immunoglobulin
IL	Interleukin
IL-2Ra	Interleukin 2 receptor antagonists
IMPDH	Inosine monophosphate dehydrogenase
IQR	Interquartile range
IRR	Incidence rate ratio
ISHLT	International Society for Heart and Lung Transplantation
KS	Kaposi sarcoma
MCC	Merkel Cell carcinoma

List of Abbreviations

MCPyV	Merkel cell polyomavirus
MCV	Merkel cell polyomavirus
MMF	Mycophenolate mofetil
mTOR	mammalian target of rapamycin
NDI	National Death Index
NFAT	Nuclear factor of activated T-cells
NHL	Non-Hodgkin lymphoma
NK cell	Natural killer cell
NMSC	Non-melanoma skin cancer
OPTN	Organ Transplant Procurement Network
OR	Odds ratio
PSA	Prostate specific antigen
PSC	Primary sclerosing cholangitis
PTLD	Post-transplant lymphoproliferative disorder
PY	Person-years
RA	Rheumatoid arthritis
SCC	Squamous cell carcinoma
SEER	Surveillance, Epidemiology, and End Results
SIR	Standardised incidence ratio
SLE	Systemic lupus erythematosus
SMR	Standardised mortality ratio
TGF	Transforming growth factor
TPMT	Thiopurine methyltransferase
UC	Ulcerative colitis

UNOS	United Network for Organ Sharing
UNSW	University of New South Wales
US	United States
UVA	Ultraviolet A
UVB	Ultraviolet B
UVR	Ultraviolet radiation
VEGF	Vascular endothelial growth factor
WHO	World Health Organisation

List of Figures

List of Tables

Chapter 1 Introduction and scope of thesis

This thesis explores *de novo* cancer incidence, mortality and risk factors in Australian liver, heart, and lung transplant recipients. Chapter 2 reviews the practice of immunosuppressive therapy in clinical transplantation and the associated complications in different transplanted organs; of particular interest is the potential link between cancer risk and the dose of immunosuppression. The subsequent chapters detail my work examining cancer after transplantation in Australia. Cancer is a rare event but causes significant morbidity and mortality, posing a considerable unsolved challenge to transplant recipients and clinicians. Therefore, my first objective was to examine *de novo* cancer incidence and mortality in a population-based cohort of liver and cardiothoracic transplant recipients in Australia in relation to the general population, and to investigate *de novo* cancer risk in different recipient subgroups. These findings are presented in Chapters 3 and 4.

My second objective was to quantify the association between immunosuppression and the most frequent cancers in this cohort. As the practice of immunosuppressive therapy varies among different transplanted organs and different countries, Chapter 5 describes and quantifies the Australian practice of immunosuppressive therapy in liver, heart, and lung transplant recipients over two decades. An additional aim of Chapter 5 was to inform the later analysis of the relationship between immunosuppressive therapy and cancer risk. With the knowledge gained in Chapter 5, I examined the role of immunosuppression in the development of NHL and lip cancer, the most frequent cancers in this cohort, in Chapters 6 and 7.

Chapter 2 Literature review

2.1 Introduction

Solid organ transplantation is a procedure of transferring an organ or part-organ from one human to another (Humar and Dunn 2010). With only a 60-year history from an experimental procedure to a widely accepted therapeutic option for patients with end-stage organ failure, the field of solid organ transplantation has truly made remarkable progress. This success is largely due to the introduction of and improvements in immunosuppressive therapy which has substantially improved short-term graft survival. Complications including *de novo* cancer, however, have become the major cause of late morbidity and mortality.

This chapter provides an overview of immunosuppressive therapy and the associated complications in solid organ transplant recipients. Following a brief historical perspective of modern solid organ transplantation worldwide and in Australia, this chapter introduces current immunosuppressive therapy in solid organ transplantation. Major complications related to immunosuppressive therapy are then discussed; the epidemiology of *de novo* cancer is further reviewed in detail. The studies included in the literature review were restricted to English-language articles but were not restricted on the basis of the recipients' age at transplantation (paediatric or adult), race or country in which the study was conducted. The scope of this chapter is liver, heart and lung transplantation; however, evidence is also described for kidney transplantation as it was the first introduced and is the most studied transplanted organ. The literature search was conducted on MEDLINE (1966 to July 2014) and

EMBASE (1980 to July 2014) where full-text articles were available. I included the following publication types: “clinical guidelines”, “meta-analysis”, “review”, “systematic review” and “journal articles”, but not “case report”. In sections of cancer incidence, risk factor and mortality, I included studies that examined the relative risk or reported deaths in a population-based cohort of solid organ transplant recipients (kidney, liver, heart and lung). I excluded single-centre based studies. The bibliographies of the retrieved articles were also examined to identify additional articles relevant to the topic in each section. I used the most current versions when duplicate guidelines from the same societies were identified.

2.2 Solid organ transplantation: past and present

The first human-to-human deceased-donor transplant was a kidney transplant in 1930s which was unsuccessful because of unmatched donor-recipient blood type leading to immunologic-mediated allograft failure (Figure 2- 1, page 6). It was not until 1954 when the first long-term successful living-donor kidney transplant was performed between identical twins and the recipient survived for eight years (Humar and Dunn 2010). This extraordinary allograft survival relative to the failure of transplants between genetically non-identical individuals led to our initial understanding of modern transplant immunobiology. Rejection, defined as deterioration in allograft function related to specific pathologic changes in the graft, is based on the theory of 'immunological tolerance' provided by Sir Peter Medawar who identified the critical role of immune system in the failure of allografts.

The great success of kidney transplantation between non-identical individuals was contributed to by the evolutionary discovery of the immunosuppressive agent azathioprine. Azathioprine was introduced in 1962 to prevent and to treat transplant rejection (Starzl et al. 1963). The combination of azathioprine with corticosteroid has made kidney transplantation a widely-accepted therapy for end-stage renal failure. More importantly, this success inspired the pursuit of transplanting other solid organs. The first liver transplant was performed by Thomas Starzl in 1963, followed by the first heart transplant in 1967. Other solid organ transplants, such as intestine, lung and islet, have also been attempted since the 1970s. However, at that time, almost all recipients survived for only a few months due to either surgical failure or rejection. Another breakthrough was the introduction of cyclosporine in 1976 which substantially

improved recipients' survival (Borel et al. 1976). For the first time, the 1-year survival of kidney recipients exceeded 80% (Hakala et al. 1983, Rosenthal et al. 1983).

Recipient survival has been further improved by the use of effective and powerful antimicrobial, antifungal and antiviral agents as an adjunct to immunosuppressive therapy. In addition, more potent immunosuppressive agents were also discovered, such as polyclonal antilymphocyte globulin (ALG) first introduced in 1966, the precursor of immunosuppressive induction therapy and rescue therapy for rejection. Moreover, continuous innovations in surgical techniques have minimised perioperative complications and have maximised the use of graft material, for example deceased-donor split-liver transplant (Cazes 1963, Starzl et al. 1963, Reitz and Stinson 1982, Reitz et al. 1982). Together, these advances have been critical for the current success of solid organ transplantation.

In Australia, the first successful solid organ transplant was a living kidney transplant in 1965. The first heart transplant was performed in 1984, and the first liver transplant was performed in 1985, followed by the first successful heart/lung transplant in 1986 and lung transplant in 1990. During the past five-decades, Australia has made good progress in solid organ transplantation. Currently, the overall survival rate for solid organ transplant recipients is around 80-90% 1 year after transplantation (ANZDATA 2009, ANZCOTR 2012, ANZLTR 2012).

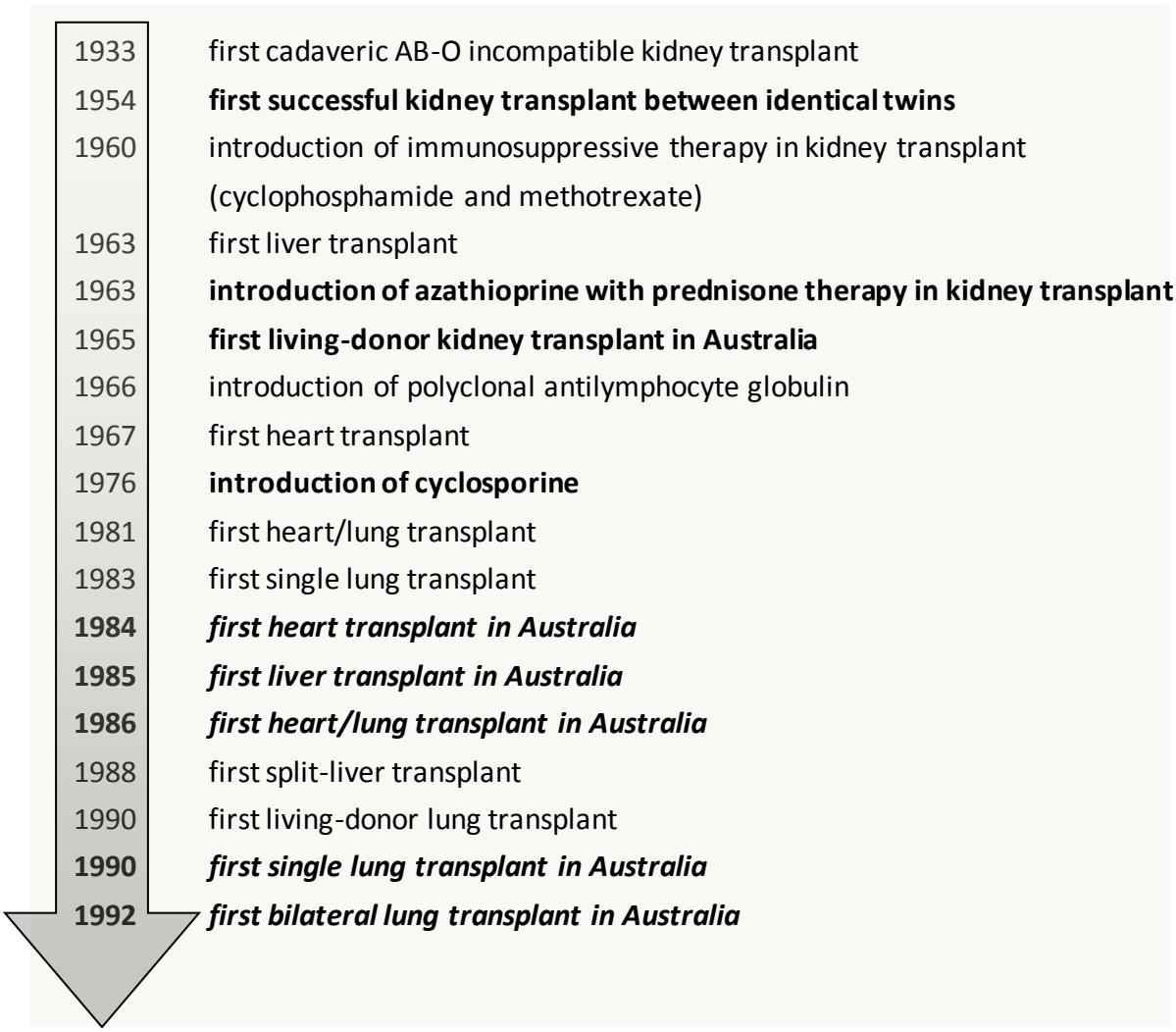


Figure 2- 1. Key milestones of solid organ transplantation

2.3 Clinical rejection: basic immunology

Rejection is the primary cause of organ failure after transplantation. It is a process in which the recipient's immune system recognises foreign antigens in the transplanted organ, leading to subsequent cell-mediated and antibody-mediated immunity against the graft (Humar and Dunn 2010). Specifically, this process is initiated by antigens coded by a group of genes namely the human leukocyte antigen (HLA) system which can be classified into two groups. The first group includes HLA-A, -B, and –C and the second group includes HLA-DR, -DP, and –DQ presented by antigen-presenting cells

(APCs), such as B lymphocytes, monocytes, and dendritic cells (Guttmann 1979, Denton et al. 1999).

Both cellular and humoral rejection can be involved in the process of transplant rejection. Humoral rejection is triggered when recipients produce antibodies specific to donor HLA molecules. Cellular rejection, however, is more common. Following recognition of donor HLA molecules, T-cells bind to the donor HLA molecules at the T-cell receptor-CD3 complex on the surface of lymphocytes. This process however is not able to fully activate the T-cells. The second signal, for example, binding CD25 or CD28 with the ligand B7 on the surface of APCs is necessary to activate gene expression of interleukin-2 (IL-2). Subsequently, T-cell proliferation and differentiation leads to graft injury and damage (Denton et al. 1999). Although T-cell activation is the essential mechanism of rejection, B-cell activation and production of antibodies such as cytokines can also be involved in this process.

Clinically, rejection can be divided into hyperacute, accelerated acute, acute and chronic rejection according to the timing and mechanisms. Hyperacute and accelerated acute rejection occur immediately or during the first few days after transplantation mainly due to mismatch of ABO blood type. Acute rejection, the most common subtype, occurs mainly during the first year and can be managed by immunosuppressive therapy (Denton et al. 1999, Hachem 2009). With improvement in short-term outcomes, chronic rejection occurring months to years after transplantation has become more problematic in recent decades.

2.4 Immunosuppressive therapy in solid organ transplantation: state of the art

The administration of immunosuppressive therapy includes three phases. Induction therapy, around the perioperative period of transplantation, remains an important phase of immunosuppression since the risk of graft loss is highest within the first month of transplantation. Higher doses of immunosuppressive agents, sometimes together with antibodies, are given in this period. After induction therapy, maintenance immunosuppression becomes a life-long journey for almost all transplant recipients. In this period, attempts to minimise the dose and the number of immunosuppressive agents are recommended. The third distinct phase of immunosuppression is the treatment of acute rejection with high-dose intravenous corticosteroid therapy, and antibody therapy for individuals with steroid resistant rejection.

2.4.1 Antibody induction

Mechanisms of action of antibodies

In general, induction agents are classified as depleting (antithymocyte globulins, and muromonab-CD3, alemtuzumab) or non-depleting (basiliximab and daclizumab) agents. The mechanisms of depleting antibodies involve the depletion of lymphocytes, mainly T-cells, B-cells or both T- and B-cells (Halloran 2004, Mahmud et al. 2010). Table 2 - 1 briefly summarises the mechanisms of the most common immunosuppressive agents, including antibodies (page 10). Antithymocyte globulin (ATG/ALG) is a polyclonal antibody widely used for all transplanted organs since the 1970s. The administration of ATG/ALG leads to a rapid T-cell depletion within the thymus and spleen (Kirk 2006, Mahmud et al. 2010). The non-specific effect of polyclonal antibody such as ATG/ALG

includes induction of lymphocyte apoptosis, interference with dendritic cell functional properties as well as induction of regulatory T-cells and natural killer T-cells (Mohty 2007). The recovery of T-cells after the use of ATG/ALG usually takes several months (Mahmud et al. 2010).

Muromonab-CD3, a T-cell depletion antibody, was the first monoclonal agent approved to treat transplant recipients (Norman 1995). The mechanisms involve a process of depletion of activated circulating T-cells, leading to the release of cytokines such as tumour necrosis factor and interferon gamma, IL-2, IL-3, IL-6 (Norman 1995, Sgro 1995). This can ultimately result in cytokine release syndrome, a side-effect which has significantly hindered this agent's clinical utility.

Another T-cell depletion antibody is the humanised monoclonal antibody alemtuzumab. This antibody specifically depletes CD52 expressing lymphocytes from the circulation and peripheral lymph nodes (Kirk 2006). However, since its initial Food and Drug Administration approval in 2001 for the treatment of chronic lymphocytic leukaemia, there has been limited data on the efficacy of alemtuzumab in solid organ transplantation.

Non-depletion antibodies include the interleukin-2 receptor (IL-2R) monoclonal antibodies, daclizumab and basiliximab. Both agents are specific for the chain of the IL-2R (CD25) expressed only on activated T-cells. Unlike the other antibodies, these two agents do not substantially deplete T-cells but inhibit T-cell proliferation and differentiation (Kirk 2006, Gutierrez-Dalmau and Campistol 2007).

Table 2 - 1. Main mechanisms of immunosuppressive agents used in solid organ transplantation

Agent	Type	Main mechanisms of immunosuppressive action
Biological immunosuppressive agents		
Antithymocyte globulin (ATG/ALG)	Polyclonal	Depletes circulating lymphocytes
Muromonab-CD3	Monoclonal	Depletes circulating lymphocytes
Alemtuzumab	Monoclonal	Binds the α-chain of the IL-2 receptor, reducing proliferation of T-cells and B-cells
Basiliximab or daclizumab	Monoclonal	Binds to the α-chain of the IL-2 receptors, reducing proliferation of T-cells and B-cells
Pharmacological immunosuppressive agents		
Cyclosporine	Calcineurin inhibitor (CNI)	Binds to cyclophilin, inhibits calcineurin, prevents activation of NFAT and expression of IL-2, stimulates TGF-β expression
Tacrolimus	Calcineurin inhibitor (CNI)	Binds to FK-binding protein, inhibits calcineurin, prevents activation of NFAT and expression of IL-2, stimulates TGF-β expression
Azathioprine	Antiproliferative	Converts to 6-mercaptopurine, inhibits purine biosynthesis
Mycophenolate (CellCept/Myfortic)	Antiproliferative	Inhibits inosine monophosphate dehydrogenase and *de novo* purine biosynthesis
Sirolimus or everolimus	Rapamycin (mTOR inhibitor)	Inhibits mTOR pathway by binding mTOR complex-1, reducing the proliferation of cytokines and growth factors

Notes: NFAT: nuclear factor of activated T-cells; TGF-β: transforming growth factor beta.

Clinical practice of antibody induction after solid organ transplantation

The use of induction therapy varies by transplanted organ type. In general, antibody induction is suggested for kidney recipients and cardiothoracic transplant recipients at high immunologic risk of acute rejection and to avoid or delay the use of calcineurin inhibitors (CNIs) in recipients with significant renal or hepatic dysfunction (Costanzo et al. 2010, Chadban et al. 2012). More than 70% of kidney recipients in the US and about

half of Australian kidney recipients received antibody induction in 2004 (Meier-Kriesche et al. 2006, Chang et al. 2008). Data from the International Society for Heart and Lung Transplantation (ISHLT) transplant registry indicates that more than 40% of heart and lung recipients received antibody induction between 2000 and 2004 (Brennan et al. 2006, Taylor et al. 2006). In contrast, about 10% of US liver recipients received antibody induction therapy between 1999 and 2008 (Wiesner and Fung 2011, OPTN/SRTR 2012).

With regard to individual antibodies, ATG/ALG remains a major part of antibody induction therapy whereas muromonab-CD3 has been removed from the market due to its significant side-effects (Wiesner and Fung 2011, Christie et al. 2012, Dipchand et al. 2013). On the other hand, the use of interleukin 2 receptor antagonists (IL-2Ra), specifically basiliximab, has markedly increased in recent years because of its efficacy in reducing acute rejection and side-effect profile (Henry and Rajab 2002, Christie et al. 2012, Ponticelli 2014). The overall survival of recipients receiving IL-2R antagonists is similar to that achieved with ATG/ALG (Brennan et al. 2006). In 2012, 20% of US kidney, liver and heart recipients and 40% of US lung recipients received IL-2R antagonists as part of their induction therapy (OPTN/SRTR 2012). In Australia, over 50% of kidney recipients received IL-2R antagonists as induction therapy (Chang et al. 2008). No data is available for Australian liver, heart and lung recipients. Although daclizumab was shown to be a potent and effective antibody, it was withdrawn from the market in 2009.

2.4.2 Maintenance immunosuppression regimens

Maintenance immunosuppressive regimens include CNIs, antiproliferative agents, and mammalian target of rapamycin (mTOR) inhibitors, with or without corticosteroids. These agents either inhibit T-cell activation or proliferation or they deplete T-cells (Table 2 - 1, page 10). In general, a combination of at least two different classes of immunosuppressive agents is recommended for most transplant recipients.

Therapeutic action of immunosuppressive agents

Corticosteroid

Corticosteroid is the primary agent used in transplant recipients. In addition to its anti-inflammatory properties, corticosteroid is also effective in the inhibition of circulating T-cells (Adcock et al. 2006).

Calcineurin inhibitors (CNIs: cyclosporine, tacrolimus)

There are two types of CNIs, cyclosporine and tacrolimus, which are widely used immunosuppressive agents. The mechanism of CNIs as potent immunosuppressive agents primarily relies on the inhibition of T-cell activation. T-cells activate calcineurin de-phosphorylation leading to the subsequent release of pro-inflammatory cytokines such as IL-2, IL-3 and IL-4 (Lindenfeld et al. 2004). Cyclosporine inhibits calcineurin by binding to its cytoplasmic receptor cyclophilin. Tacrolimus binds to the 12kDa FK506-binding protein (FKBP-12) and thereby inhibits calcineurin. After the administration of CNIs, the production of cytokines such as IL-2 is blocked and T-cell activation and proliferation is inhibited (Matsuda and Koyasu 2000, Taylor et al. 2005). Of note, the effect and toxicity of CNIs is closely related to total drug exposure which is monitored by blood 12 hour trough levels.

Antiproliferatives (azathioprine, mycophenolate)

Unlike CNIs, antiproliferatives (antimetabolites) mainly inhibit the cell cycle. The active component of azathioprine as an antiproliferative agent is 6-mercaptopurine (6-MP) via reduction by glutathione. 6-MP enters the purine salvage pathway, whereas lymphocytes lack this pathway. 6-MP is then converted to 6-thiuric acid, thiopurine methyltransferase (TPMT) and 6-thioguanine (6TG), the latter being the active product responsible for the inhibition of DNA synthesis. Another pathway is the TPMT-driven methylation of thioinosine monophosphate to methylthioinosine monophosphate, a potent inhibitor of purine biosynthesis (Mertens et al. 1981, Aarbakke et al. 1997). Notably, the therapeutic indication of azathioprine as an immunosuppressive agent is not restricted to solid organ transplantation. It is used for a number of chronic inflammatory and autoimmune diseases such as inflammatory bowel disease (IBD), rheumatoid arthritis (RA) and systemic lupus erythematosus.

Mycophenolate is an antiproliferative agent first approved in 1995 and officially registered in Australia in 1997 for solid organ transplantation. The active component of mycophenolate is mycophenolic acid targeting inosine monophosphate dehydrogenase (IMPDH) which is a rate-limiting enzyme essential for DNA synthesis. The majority of cells generate guanosine nucleotides via two pathways, the IMPDH pathway and the salvage pathway. Since lymphocytes lack a salvage pathway, the immunosuppressive effect of mycophenolate is dependent upon blocking the IMPDH pathway, leading to a blockade of both T- and B-cell lymphocyte proliferation (Allison and Eugui 1996).

mTOR inhibitors (sirolimus and everolimus)

The mammalian target of rapamycin (mTOR) inhibitors sirolimus and everolimus are relatively new drugs used mainly in kidney transplantation. Sirolimus (rapamycin) was found to be an effective immunosuppressive agent in the late 1990s and became available for solid organ transplantation in Australia in 2002. Everolimus was registered in Australia in 2005. mTOR inhibitors also bind to FKBP12 like tacrolimus, however, they do not inhibit calcineurin activity. mTOR inhibitors specifically target mammalian target of rapamycin complex 1 (mTORC1), blocking IL-2 and inhibiting T-cell proliferation by preventing progression of the cell cycle from the G1 to the S phase (Mita et al. 2003). The use of mTOR inhibitors however is limited by side-effects including delayed graft function, impaired wound healing and proteinuria (Scherer et al. 2007, Kawahara et al. 2011).

Clinical practice of maintenance immunosuppressive therapy

The combination of a CNI and an antiproliferative agent (with or without corticosteroid) is the standard maintenance immunosuppression regimen in solid organ transplantation. However, clinical practice has evolved over time with the emergence of new immunosuppressive agents. The use of cyclosporine at 1-year post-transplantation decreased between 1998 and 2012 from 60% to less than 5% in kidney recipients, from 30% to 5% in liver and from 60%-80% to less than 5% in heart and lung recipients in the US (OPTN/SRTR 2012). Over the same period, the use of azathioprine at 1-year also declined from 10% to about 3% in kidney and liver, from 40% to 1% in heart and from over 60% to 18% in lung recipients (OPTN/SRTR 2012). In contrast, over

the same period, the use of the combination therapy of tacrolimus with mycophenolate increased from 30% to 90% in kidney recipients, from 60% to more than 80% in liver and from 10%-20% to more than 80% in heart and lung recipients at 1-year post-transplantation (OPTN/SRTR 2012). mTOR inhibitors are used infrequently; less than 10% of US transplant recipients received an mTOR inhibitor 1-year post-transplantation between 2000 and 2012 (OPTN/SRTR 2012). Similar trends have been observed for Australian kidney transplant recipients (Chang et al. 2008). However, no data is available with regard to the trends of immunosuppressive therapy in Australian liver, heart and lung transplant recipients.

Despite the general rule of reducing the intensity of immunosuppression by minimising the dose and number of drugs over time, the practice of maintenance immunosuppressive therapy varies between transplanted organ types. In general, liver transplant recipients require less aggressive immunosuppressive therapy (Lucey et al. 2013) because the liver is less immunogenic than the heart and lung (Sánchez-Fueyo and Strom 2011). Indeed, the unique microenvironment of the liver can lead to spontaneous immune tolerance and remove the need for immunosuppression in some patients (Sánchez-Fueyo and Strom 2011). Whilst a head-to-head comparison of the type and dose of immunosuppressive agents between different transplanted organ types has not been performed, data from the US OPTN/SRTR (2000-2004) has shown that the proportion of transplant recipients using a single agent (monotherapy; mainly tacrolimus) has grown from 34% at 1-year to 50% at 3-years post-transplantation in liver recipients (Meier-Kriesche et al. 2006). In contrast, the combination of a CNI and an antiproliferative remains the primary immunosuppressive regimen for kidney, heart

and lung recipients according to the OPTN/SRTR and ISHLT reports (Meier-Kriesche et al. 2006, Christie et al. 2012). In addition, corticosteroid avoidance was achieved by at least 60% of liver recipients compared to 40% of kidney and 10-20% of heart and lung recipients at 1-year post-transplantation in the US in 2012 (OPTN/SRTR 2012). In Australia, 95% of kidney recipients were still on a corticosteroid-based regimen one year after transplantation in 2010 (Clayton et al. 2013). However, again there is no such data for Australian liver, heart and lung transplant recipients.

2.4.3 Treatment of acute rejection

A marked reduction in the incidence of acute rejection over time has been reported for all transplanted organs, a trend that has been attributed to advancements in immunosuppressive therapy (Meier-Kriesche et al. 2006, Stehlik et al. 2010, Clayton et al. 2013). In general, an initial short-term high dose of corticosteroids is suggested once acute rejection is confirmed. The majority of transplant recipients will respond to intravenous-bolus corticosteroid. This T-cell depleting antibody is given to those who are steroid resistant and for recurrent acute cellular rejections. In addition, changes in the type and dose of maintenance immunosuppressive therapy can be considered (McGuire et al. 2009, Costanzo et al. 2010, Chadban et al. 2012, Lucey et al. 2013). The proportion of US kidney recipients treated with anti-rejection drugs in the first post-transplant year has reduced from 50% in 1996 to 10% in 2012 (Meier-Kriesche et al. 2006). A similar trend was observed for Australian kidney recipients; the proportion with acute rejection during the first six months was around 30% in 2002 and about 18% in 2011 (Clayton et al. 2013). The proportion of treated acute rejection during the first year in US liver recipients declined from 24% to about 18% in 2002-2012 (Meier-

Kriesche et al. 2006, OPTN/SRTR 2012). Data from the ISHLT showed that the proportion of adult heart recipients treated for rejection within the first year decreased from 42% in 1994-1999 to 29% in 2000-2004 and 22% in 2005-2010 (Stehlik et al. 2012). Similar trends have been reported for lung recipients, with about 34% of adult lung recipients experiencing acute rejection between 2004 and 2011 (Christie et al. 2012).

2.5 Complications of immunosuppression

Soon after the introduction of immunosuppressive therapy it was observed that a significant proportion of recipients experienced a variety of opportunistic infections and the occurrence of lymphomas was first reported in 1969 (Hill et al. 1967, Penn et al. 1969). The occurrence of infections, cancers, and other chronic diseases has become an important challenge in the clinical management of transplant recipients.

2.5.1 Infection

Transplant recipients are at an increased risk for either acquired or reactivated infections, causing substantial morbidity and mortality. A broad spectrum of pathogens has been observed, such as bacterial and fungal infections as a result of neutropenia, as well as intracellular infections such as tuberculosis and viral infections such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV) (Fishman and Issa 2010). Whilst each organ may have some unique predisposing factors, the spectrum of infection and the principle of infection management are applicable to all transplanted organs. Community-acquired pathogens such as influenza and common bacterial pathogens are not uncommon and can be aggressive in transplant recipients.

In addition, a high incidence of reactivated viral infections has been observed, including herpes simplex virus (HSV), EBV, CMV, varicella-zoster virus (VZV, shingles), hepatitis B (HBV), hepatitis C (HCV), human papillomavirus (HPV), and BK polyomavirus. The reactivation of these viral infections is mainly attributed to the iatrogenic immunosuppression of the recipient (Fishman 2007, Fishman et al. 2007, Fishman and Issa 2010).

Several predisposing factors related to recipients or donors contribute to the risk of infection after transplantation. After transplantation, the transplanted organs are the most common site of infections; abdominal, respiratory, and urinary tract infections are more likely to occur in liver, cardiothoracic and renal transplant recipients respectively. These may appear soon after transplantation due to the surgical procedure or inflammation in the transplanted organ (Fishman 2007). In addition, age at transplantation is a significant risk factor for infections post-transplantation. Paediatric transplant recipients are particularly susceptible to the acquisition of EBV or CMV infections from an adult graft. On the other hand, increasing age at transplantation has a significant impact on the acquisition and progression of infection partly due to the effect of immune senescence (Meier-Kriesche et al. 1999). Recipients may acquire active or latent infections from transplanted organs. Acquired EBV and CMV infections are the most common donor-derived infections although other donor-derived infections are possible, such as HBV, HCV and HIV. Although there are guidelines for screening donors for pathogens and prophylaxis or pre-emptive

treatment (Kotton et al. 2013, Seem et al. 2013, Levi et al. 2014), data on donor-derived infections are scarce (Fishman and Grossi 2014).

The outcome of infections depends upon the type of pathogen, the duration and the severity of the infection. In addition to a direct impact, such as CMV-related disease and HBV- and HCV-related hepatitis, the indirect impact of infections may contribute to graft failure (Rosen 2003). Certain types of infection by themselves, such as CMV, may increase the risk of rejection (Reinke et al. 1994). On the other hand, more intense immunosuppressive therapy is necessary for rejection treatment and this may increase the risk of opportunistic infections. Infection by oncogenic viruses and bacteria can also increase the risk of cancer, as reviewed in Section 2.6.

2.5.2 Other complications

The toxicity of immunosuppressive agents is closely related to their therapeutic action, most of which is dose-dependent. Cyclosporine and tacrolimus share similar toxicity profiles; both can cause nephrotoxicity (Liptak and Ivanyi 2006), neurotoxicity and hepatotoxicity (Taylor et al. 2005, Pillai and Levitsky 2009). Dose-related bone marrow suppression is one of the most common side-effects of azathioprine and mycophenolate, and azathioprine carries an increased risk of hepatotoxicity (Taylor et al. 2005). Sirolimus is associated with hepatotoxicity and marrow suppression (Taylor et al. 2005). Complications such as hypertension, diabetes, obesity, dyslipidaemia and cardiovascular disease have become significant causes of death in transplant recipients (McGuire et al. 2009, Costanzo et al. 2010, Chadban et al. 2012). In addition, transplant recipients can develop complications with the transplanted organ. For example, biliary

complications and recurrence of primary liver disease are commonly seen in liver recipients.

2.6 De novo cancer incidence after transplantation

The success of solid organ transplantation is complicated by an increased risk of cancer that has been documented since the 1960s (Penn et al. 1969) The kidney is the most commonly transplanted organ and therefore our understanding of cancer after transplantation is mainly based on studies of kidney recipients. Evidence of the site-specific cancer risk profile for other transplanted organs, such as liver, heart and lung is less robust.

2.6.1 Incidence of de novo cancer

Population-based studies show that the overall cancer risk for solid organ transplant recipients is two to three-fold that of the matched general population (Grulich et al. 2007, Engels et al. 2011) (Table 2-3, page 29-30). With regards to site-specific cancers, studies have consistently shown an increased risk of the most common cancer, non-melanoma skin cancer (NMSC), and infection-related cancers such as non-Hodgkin lymphoma (NHL), but the evidence is conflicting for some solid cancers such as kidney, colorectal, and lung cancer, even within population-based studies (Grulich et al. 2007, Jiang et al. 2008, Collett et al. 2010, Jiang et al. 2010, Krynitz et al. 2013).

The varying cancer incidence findings after transplantation may be due to the use of inconsistent and non-optimal research methods. Some studies were based on a single institution, and most were limited by a small cohort size or short follow-up time. To

maximise comparability between studies, it is necessary to utilise standardised approaches. Population-based cohort and cancer-registry based data are superior to single institution studies with cancer incidence based on hospital or transplantation registry records because they represent the entire transplant population, removing the possibility of selection bias and enabling generalisability. Furthermore, cancers in the transplant cohort and the general population are ascertained using an identical approach, population-based cancer registration, providing non-differential ascertainment and classification. Deaths should be ascertained from population registers for the same reason. Conclusions drawn from such data are the most reliable.

The standardised incidence ratio (SIR), an internationally accepted measure of the relative risk of cancer, is widely used in cancer epidemiology studies. It is defined as the ratio of the observed to the expected number of cancers in a population sub-group with a specific exposure compared with the matched general population (Boyle and Parkin 1991). Population-based studies of solid organ transplant recipients have identified an increased risk of cancer at more than 30 different cancer sites, and most of the cancers are associated with a known or suspected infectious cause (Grulich et al. 2007) as shown in Figure 2- 2 (page 22). Herein, the most frequent types of cancers are discussed. It should be noted that a direct comparison of SIRs by transplant organ type is not valid because of the heterogeneity in the age and sex distributions of the transplanted populations (Rothman et al. 2008; p.254).

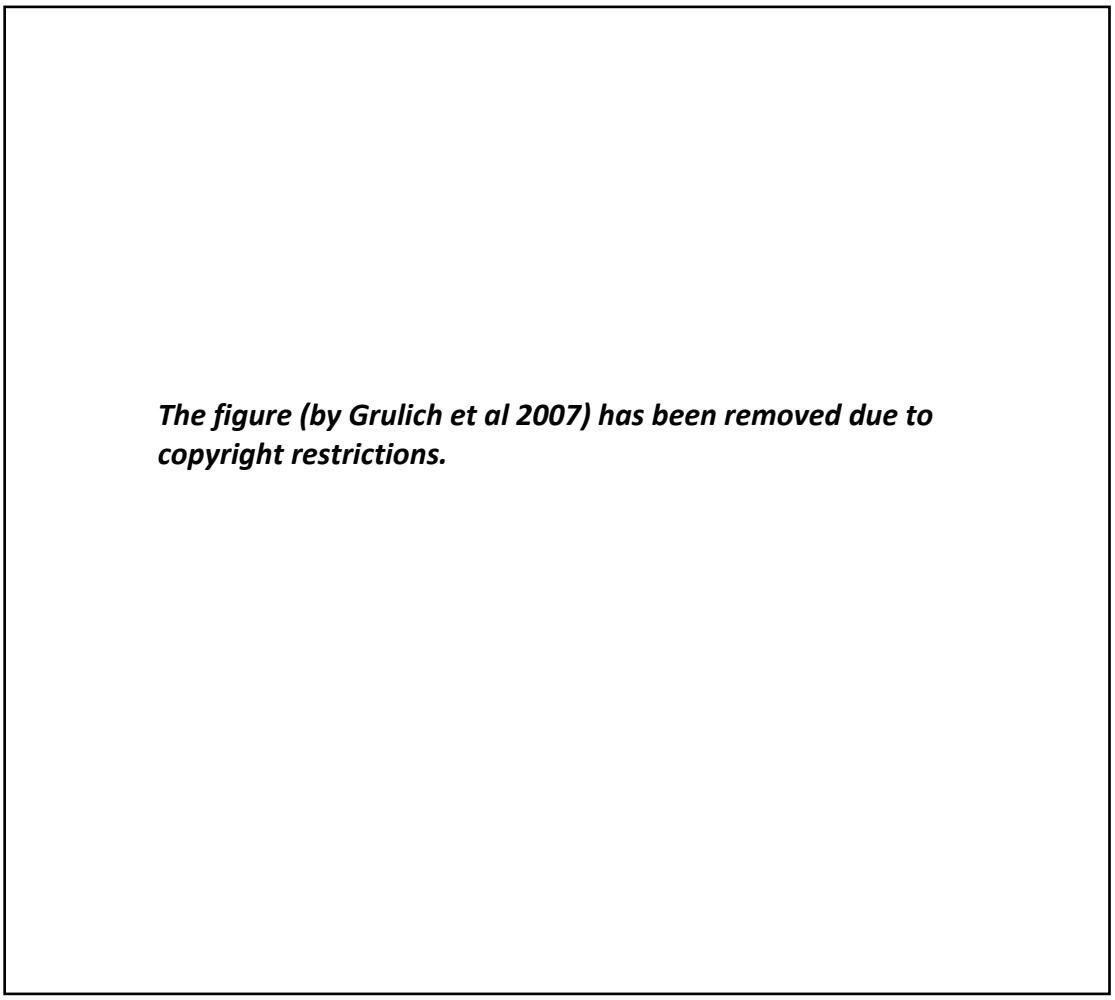


Figure 2- 2. Meta-analysis of standardised incidence ratios for cancers after solid organ transplantation by Grulich *et al* 2007

Non-melanoma skin cancer (NMSC)

NMSC, specifically squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), are the most common cancers diagnosed in solid organ transplant recipients. However, most population-based cancer registries do not routinely ascertain these malignancies and thus the true background incidence is unknown. In those countries that do record NMSC diagnoses, the ratio of BCC to SCC in transplant recipients is reversed relative to the general population, with BCC the most common subtype. The reported SIR is approximately 50-250 for SCC and at least 10 for BCC in kidney transplant recipients (Hartevelt et al. 1990, Moloney et al. 2006, Krynitz et al. 2013). Data are limited for the

other transplanted organs; a population-based study in Sweden reported a 200-fold increase risk of SCC for heart and lung recipients, and a 30-fold risk for liver recipients (Krynitz et al. 2013). In Australia, cutaneous SCC and BCC are not ascertained by the population-based cancer registries but their incidence is estimated to be amongst the highest in the world. A pronounced increased risk of NMSC has been reported in Australian kidney (Ramsay et al. 2002) and heart transplant recipients (Ong et al. 1999).

Non-Hodgkin Lymphoma (NHL)

Post-transplant lymphoproliferative disorder (PTLD) is a spectrum of lymphoproliferative disorders ranging from benign infectious mononucleosis to polyclonal lymphoid hyperplasia and highly invasive monomorphic proliferations occurring in the setting of solid organ and allogeneic haematopoietic cell transplantation (Swerdlow et al. 2008). As summarised in Table 2-2 (page 24), the WHO classification includes early lesions (plasmacytic hyperplasia, and infectious mononucleosis-like PTLD), polymorphic PTLD, monomorphic PTLD (B or T/NK-cell lymphoma), and classical Hodgkin lymphoma type PTLD (Swerdlow et al. 2008). NHL, the monomorphic PTLD subtype, is one of the most life-threatening complications after transplantation and is discussed here.

The incidence of NHL in transplant recipients is strikingly high regardless of the transplanted organ. In a meta-analysis, the pooled-SIR for NHL in organ transplant recipients, of which 97% were kidney, was 8.07 (95% CI 6.4-10.2) (Grulich et al. 2007). Population-based studies have shown a 7-20-fold increased risk for NHL in liver recipients (Aberg et al. 2008, Jiang et al. 2008, Engels et al. 2011, Krynitz et al. 2013)

and a 20-30-fold increased risk for heart and lung recipients (Collett et al. 2010, Jiang et al. 2010, Engels et al. 2011, Krynitz et al. 2013). The conclusive increased risk of NHL across different transplanted organs and in people with HIV infection is evidence that immune deficiency is a major risk factor for NHL.

Table 2 - 2. WHO classification of PTLD

WHO term¶	ICD-O-3 code
Early lesions	
Plasmacytic hyperplasia	9971/1
Infectious mononucleosis-like	9971/1
Polymorphic PTLD*	9971/3
Monomorphic PTLD*	According to the lymphoma they resemble:
B-cell neoplasms	
Diffuse large B-cell lymphoma	9680/3
Burkitt lymphoma	9687/3
Plasma cell myeloma	9732/3
Plasmacytoma-like lesions	
T-cell neoplasms	
Peripheral T-cell lymphoma, not otherwise specified	9702/3
Hepatosplenic T-cell lymphoma	9716/3
Other	
Classical Hodgkin lymphoma-type PTLD*	9650/3

Abbreviations: PTLD post-transplant lymphoproliferative disorder; ¶ (Swerdlow et al. 2008); *Both monomorphic PTLD and classical Hodgkin lymphoma-type PTLD are further sub-categorised by lineage and coded based on their corresponding leukaemia or lymphoma as presented in the general population.

The risk of developing NHL is highest during the first or two years post-transplantation (Adami et al. 2003, Faull et al. 2005, Villeneuve et al. 2007, van Leeuwen et al. 2009, Caillard et al. 2012). A bi-modal pattern of incidence of NHL has been observed, with a second peak occurring 5-10 years after transplantation (Faull et al. 2005, van Leeuwen et al. 2009, Caillard et al. 2012). As will be discussed, 'early' and 'late' NHL manifest different pathologic characteristics supporting two mechanisms of lymphomagenesis in the context of immunosuppression (Ghobrial et al. 2005, Schober et al. 2013).

Studies have found that the NHL risk is highest among heart and lung (as compared to liver and kidney) transplant recipients based on the relative magnitude of the SIRs, and investigators have concluded that this is explained by the higher intensity of immunosuppression in cardiothoracic recipients (Collett et al. 2010, Jiang et al. 2010, Engels et al. 2011). Of note, the high risk in lung transplant recipients may also be due to the large amount of lymphoid tissue in the allograft, facilitating lymphoproliferation by donor lymphocytes (Cockfield 2001).

Lip cancer

The risk of lip cancer is as high as 20-60 fold in kidney transplant recipients compared to the general population (Grulich et al. 2007, van Leeuwen et al. 2009, Krynitz et al. 2013). However, data are limited and somewhat inconsistent for the other transplanted organ types. The magnitude of lip cancer risk was 20-fold in liver and 60-fold in heart recipients in the UK, which is in line with a Swedish study (Schulz 2009, Collett et al. 2010, Krynitz et al. 2013), but an increased risk was not observed in another two Scandinavian population-based studies (Adami et al. 2003, Aberg et al. 2008). This variability may be due to the relatively small number of non-kidney recipients or the short follow-up time as lip cancer risk increases with increasing time since transplantation (van Leeuwen et al. 2009). In a population-based study of Australian kidney transplant recipients the incidence rate ratio (IRR) of lip cancer was 3.96 (95% CI 1.63-9.64) for 10 or more years compared to 1-year after transplantation (van Leeuwen et al. 2009). No study has examined the risk factors for lip cancer in liver, heart or lung transplant recipients.

Cutaneous melanoma

The risk of cutaneous melanoma is more than 2-fold in solid organ transplant recipients, based on studies in which the majority of the study populations were kidney recipients (Grulich et al. 2007, Vajdic et al. 2009, Collett et al. 2010, Engels et al. 2011). An increased risk has not been reported for liver, heart or lung recipients in population-based studies in Finland, Canada and Sweden (Aberg et al. 2008, Jiang et al. 2010, Krynitz et al. 2013). This variation in risk may be explained by the relatively small cohort sizes for the non-kidney transplant recipients. The relatively low ambient solar UV radiation (UVR) in some northern countries may also be a factor given the causal role of UVR exposure for melanoma in the general population (Miller and Mihm 2006, Garibyan and Fisher 2010).

Breast, prostate and colorectal cancer

A large-scale meta-analysis of population-based studies found that the risk of breast and prostate cancer in solid organ transplant recipients is comparable with the general population (Grulich et al. 2007). More recently a large US population-based study reported that the risk of both breast cancer (SIR 0.85, 95% CI 0.77-0.93) and prostate cancer (SIR 0.92, 95% CI 0.87-0.98) was significantly lower than for the general population (Engels et al. 2011). This could be a result of a close medical follow-up in transplant recipients, subsequently increasing the detection of intraepithelial neoplasia of the breast and prostate. If transplant recipients are more or less inclined than the general population to be screened for these malignancies then these risk estimates may be biased. However, in support of these being an unbiased findings, the

risk of both breast and prostate cancer is also either significantly reduced or null in another immune deficient population, people with HIV infection (Grulich et al. 2007). Furthermore, one study has reported that the reduced incidence of prostate cancer in HIV infected individuals is not due to differences in the rate of PSA screening between these two populations (Marcus et al. 2014).

Whether the immune deficient population is at a higher risk of colorectal cancer (CRC), another common malignant epithelial tumour, compared to the general population is controversial. Some studies have suggested that immunosurveillance might be critical in the oncogenesis of CRCs (Hamelin et al. 2004, de la Cruz-Merino et al. 2011), but this is challenged by epidemiological studies of immune deficient populations. Although the risk of CRC is increased in kidney (Grulich et al. 2007, Schulz 2009, Collett et al. 2010) and in liver recipients (Jiang et al. 2008, Collett et al. 2010, Sint Nicolaas et al. 2010), this pattern has not been observed in heart and lung recipients (Collett et al. 2010, Jiang et al. 2010, Krynitz et al. 2013), nor in individuals with HIV/AIDS infection (Grulich et al. 2007).

It is noteworthy that primary sclerosing cholangitis (PSC) with associated ulcerative colitis (UC), a type of IBD, is an indication for liver transplantation, and that both PSC and UC are established risk factors for CRC (Lieberman 2009, Danese and Fiocchi 2011). A Dutch cohort study suggested that the incidence of CRC is only elevated in PSC but not in non-PSC liver recipients (Sint Nicolaas et al. 2010). However, a meta-analysis including 29 studies concluded that non-PSC liver transplant recipients also have an increased CRC risk, although an intensified screening strategy is not warranted

(Sint Nicolaas et al. 2010). Of note, end-stage alcohol liver disease is also an indication for liver transplantation and may be responsible for an increased risk of CRC in non-PSC liver recipients. Therefore, a detailed analysis controlling for potential risk factors is needed to better understand the association between immunosuppression and CRC risk.

Cancer in the transplanted organ

Previous studies have found cancer occurrence is more frequent in the allograft, that is, kidney recipients have a 6-7-fold risk of kidney cancer, predominantly in the native kidney (Grulich et al. 2007, Engels et al. 2011, Krynitz et al. 2013), lung recipients have a 6-fold risk of lung cancer (Collett et al. 2010, Engels et al. 2011) and liver recipients have a higher risk of hepatocellular carcinoma (HCC) (Engels et al. 2011, Krynitz et al. 2013). However, this may be explained by pre-existing predisposing risk factors rather than immunosuppression *per se*. For example, glomerular diseases and polycystic kidney disease are the primary indications for kidney transplantation and they are closely related to kidney cancer (Vajdic et al. 2006, Vajdic and van Leeuwen 2009, Goh and Vathsala 2011). The high risk of HCC after liver transplantation is likely to be contributed to by higher than background rates of infection by the oncogenic viruses HBV and HCV and complicated by the inadvertent inclusion of HCC that is only registered by cancer registries after examination of the explanted organ (Vajdic et al. 2012). Of note, donor-derived cancer in the transplanted organ is also possible, particularly for cancers that occur soon after transplantation (Nalesnik et al. 2011).

Table 2 - 3. Standardised incidence ratio (SIR) for cancers in solid organ transplant recipients in population-based studies

Cancer site	Year, country	SIR (95% CI) Transplanted organ				
		All organs combined	Kidney	Liver	Heart	Lung
Overall	2013, Sweden	-	6.5 (6.3-6.8)	3.4 (2.9-4.0)	10.0 (9.0-11.0)	10.0 (9.0-11.0)
	2011, US	2.1 (2.0-2.1)	-	-	-	-
	2010, UK	-	2.4 (2.3-2.5)	2.2 (2.0-2.4)	2.5 (2.2-2.7)	3.6 (3.0-4.4)
	2008, Canada	-	-	2.5 (2.1-3.0)	-	-
	2008, Finland	-	-	2.6 (1.8-3.5)	-	-
	2006, Australia	-	3.3 (3.1-3.7)	-	-	-
NMSC	2013, Sweden	-	54.0 (52.0-56.0)	16 (12-20)	83 (73-94)	83 (73-94)
	2010, UK	6.6 (5.8-7.5)	16.0 (15.9-17.3)	6.6 (5.8-7.5)	18.5 (16.9-20.3)	16.1 (13.1-19.6)
	2008, Finland	-	-	38.5 (18.5-70.8)	-	-
	2000, Finland	-	39.2 (29.3-51.0)	-	-	-
NHL	2013, Sweden	-	4.8 (3.8-5.9)	14.0 (8.9-21.0)	17.0 (13.0-24.0)	17.0 (13.0-24.0)
	2011, US	7.5 (7.2-7.9)	6.1 (5.6-6.5)	7.8 (6.9-8.6)	7.8 (6.9-8.8)	18.7 (15.6-22.3)
	2010, UK	12.5 (11.2-13.8)	12.5(11.2-13.9)	13.3 (10.6-16.6)	19.8 (16.0-24.0)	30.0 (20.6-42.1)
	2008, Finland	-	-	13.9 (6.0-27.4)	-	-
	2008, Canada	-	-	20.8 (14.9-28.3)	22.7 (17.3-29.3)	-
Lip	2013, Sweden	-	46.0 (35.0-59.0)	19.0 (2.3-68.0)	84.0 (36.0-166.0)	84.0 (36.0-166.0)
	2011, US	16.8 (14.0-19.9)	-	-	-	-
	2010, UK	20.2 (5.4-51.2)	65.6 (49.9-84.6)	20.0 (5.4-51.2)	60.0 (31.0-104.0)	-
	2008, Finland	-	31.8 (20.8-46.6)	21.3 (0.5-118.0)	-	-
	2007, Canada	-	23.0 (12.6-38.5)	-	-	-
	2006, Australia	-	47.1 (39.1-75.1)	-	-	-
	2003, Sweden	-	53.3 (38.0-72.5)	-	-	-
CRC	2013, Sweden	-	-	2.2 (0.8-4.8)	1.4 (0.3-4.2)	-
	2011, US	-	1.24 (1.2-1.3)	-	-	-
	2010, UK	2.3 (1.7-3.0)	1.8 (1.2-2.1)	2.3 (1.7-3.0)	1.1 (0.7-1.7)	1.1 (0.7-1.7)
	2008, Canada	-	1.4 (1.0-1.8)	2.6 (1.4-4.4)	0.6 (0.2-1.5)	-
	2006, Australia	-	2.4 (1.9-2.9)	-	-	-

	2000, Finland	-	3.9 (2.1-6.7)	-	-	-
Lung	2013, Sweden	-	-	1.8 (0.7-4.0)	5.4 (3.0-8.9)	-
	2011, US	2.0 (1.7-2.2)	2.0 (1.9-2.1)	-	2.7 (2.4-3.0)	6.1 (5.2-7.2)
	2010, UK	-	1.4 (1.2-1.6)	1.6 (1.2-2.2)	2.1 (1.6-2.8)	5.9 (3.7-8.8)
	2008, Canada	-	2.1 (1.7-2.5)	1.4 (0.7-2.6)	2.0 (1.2-3.0)	-
Liver	2013, Sweden	-	2.7 (1.6–4.1)	14.0 (7.0-27.0)	3.7 (0.4-13)	3.7 (0.4-13)
	2011, US	11.6 (10.8-12.3)	1.1 (0.8-1.43)	43.8 (40.9-46.9)	1.0 (0.5-1.7)	2.0 (0.6-5.2)
	2010, UK	-	2.4 (1.5-3.8)	-	1.2 (0.2-4.5)	10.0 (2.1-29.2)
Kidney	2013, Sweden	-	6.2 (4.8–7.9)	1.8 (1.4-2.3)	1.1 (0.0-6.2)	1.1 (0.0-6.2)
	2011, US	4.6 (4.3-5.0)	6.7 (6.1-7.2)	1.8 (1.4-2.3)	2.9 (2.3-3.6)	1.5 (0.6-2.9)
	2010, UK	-	7.9 (6.7-9.3)	1.8 (0.8-3.6)	4.4 (2.5-7.0)	2.5 (0.3-9.0)
	2008, Canada	-	-	-	2.9 (1.0-6.2)	-
Breast (female)	2013, Sweden	-	1.2 (0.9–1.5)	1.0 (0.4–2.1)	0.6 (0.1-1.8)	0.6 (0.1-1.8)
	2011, US	0.9 (0.8-0.9)	-	-	-	-
	2010, UK	-	1.0 (0.8-1.2)	0.8 (0.5-1.1)	0.8 (0.3-1.7)	0.3 (0.0-1.2)
	2008, Canada	-	1.3 (1.0-1.7)	-	1.1 (0.2-3.2)	-
	2006, Australia	-	1.0 (0.8-1.4)	-	-	-
	2003, Sweden	-	1.0 (0.6-1.5)	-	-	-
Prostate (male)	2013, Sweden	-	1.1 (0.9–1.3)	0.5 (0.1–1.2)	1.3 (0.6–2.3)	1.3 (0.6–2.3)
	2011, US	0.9 (0.9-1.0)	-	-	-	-
	2010, UK	1.1 (0.9-1.4)	-	-	-	-
	2008, Canada	-	0.9 (0.6-1.3)	-	1.3 (0.7-2.2)	-
	2006, Australia	-	1.0 (0.7-1.3)	-	-	-
	2003, Sweden	-	1.1 (0.7-1.7)	-	-	-

Abbreviations: NMSC, nonmelanoma skin cancer; NHL, Non-Hodgkin lymphoma; CRC, colorectal cancer; Notes: Studies included in the table are population-based, registry-based cohort studies.

2.6.2 Risk factors for de novo cancer after solid organ transplantation

Recipient risk factors

The impact of recipient age on cancer risk has been confirmed in epidemiological studies. PTLD/NHL is the most common malignancy in paediatric and adolescent recipients, mainly due to primary EBV infection. On the other hand, increasing recipient age at transplantation has also been identified as an independent risk factor for *de novo* cancer occurrence after transplantation (Danpanich and Kasiske 1999, Haagsma et al. 2001, Kasiske et al. 2004). Impaired immunosurveillance and age-related accumulative genomic damage may be particularly important for cancer initiation and progression in the setting of immunosuppression (Heinbokel et al. 2013).

Other factors such as male gender, fair skin and UVR exposure are related to an increased risk of NMSC and lip cancer in both the general population and the transplanted population (Euvrard et al. 2003, Perea-Milla Lopez et al. 2003, van Leeuwen et al. 2009). Alcohol consumption and tobacco smoking are established carcinogens for humans (IARC 2012). However, the association between these and other lifestyle risk factors and the development of *de novo* cancer in transplant recipients is not well understood as most population-based registers of transplant recipients do not collect such exposures (Grulich et al. 2007, Collett et al. 2010, Engels et al. 2011, Krynitz et al. 2013). Nevertheless, the prevalence of alcohol consumption at transplantation is probably higher in liver transplant recipients than the general population as alcohol liver disease is one of the common primary indications for liver transplantation. These recipients are also likely to have a history of smoking (Lucey 2014). Similarly, a high prevalence of smoking is seen in heart and lung recipients at

transplantation, and smoking remains a significant problem after transplantation (Botha et al. 2008, Corbett et al. 2012, Olland et al. 2014, Ruttens et al. 2014). Recipient comorbidities are also likely to be associated with increased risk of cancer after transplantation. As discussed previously, cancer occurring in the allograft is probably associated with pre-existing chronic diseases. In addition, a history of cancer prior to transplantation contributes to recurrent and *de novo* cancer post-transplantation (Rubin et al. 2012, Chapman et al. 2013).

Donor risk factors

Several donor factors including HLA mismatch, age and cancer history have been identified to confer increased *de novo* cancer risk for transplant recipients. Increasing numbers of HLA mismatches and HLA-DR mismatches have been linked to a high risk of *de novo* cancer (particularly NHL) most likely due to their subsequent higher degree of immunosuppression to minimise the risk of rejection (Caillard et al. 2005, Morath et al. 2010, Opelz and Dohler 2010, Susal and Opelz 2013). However, other cohort studies did not find such an association (Cherikh et al. 2003, Faull et al. 2005) and the evidence is predominantly based on risk of PTLD/NHL in kidney recipients.

Although rare, cancer of donor origin has been reported for several cancer sites including melanoma and cancer of the lung, prostate, pancreas, and some extremely rare cancers (Baehner et al. 2000, Birkeland and Storm 2002, Myron Kauffman et al. 2002, Kanitakis and Euvrard 2013).

Viral infection and cancer after transplantation

A number of cancers occurring with increased incidence in the setting of immunosuppression are related to oncogenic virus infection, as shown in Figure 2- 2 (page 22) (Grulich et al. 2007). Oncogenic viruses such as EBV, Kaposi sarcoma herpesvirus (KSHV), HPV, HBV, HCV, and the Merkel cell polyomavirus (MCV) (IARC 2012) are causally associated with a number of different cancers. Some of these viruses are ubiquitous in the general population, but the virus-associated cancers are rare. The initiation of virus-associated cancer therefore requires cofactors to promote tumourigenesis. Impaired immunosurveillance as a result of iatrogenic immunosuppression is believed to play a critical role in the development of virus-associated cancers (Moore and Chang 2010, Heinbokel et al. 2013).

The aetiological role of EBV in NHL development

EBV is a human herpesvirus within the gamma-herpesviruses subfamily, infecting over 90% of adults and persisting for a lifetime. EBV mainly exhibits latent infections in memory B-cell lymphocytes after primary infection via saliva from infected individuals, although the virus itself has a broad infectious scope including T-lymphocytes, squamous and glandular epithelia and smooth muscle cells (Cohen 2000, IARC 2012). Despite its high prevalence in healthy individuals, EBV is IARC-classified as an oncogenic virus in immune deficient populations (IARC 2012). In immunocompetent individuals a strong humoral and cellular immune response will facilitate infection control following primary EBV infection, depleting most infected cells via latent antigen-specific T-lymphocyte response. During this process, natural killer cells and CD4+ and CD8+ cytotoxic T-cells (CTLs) are essential to control the proliferation of infected B-cells (Cohen 2000, Loren et al. 2003). However, the T-cell immune response

is highly depressed in immune deficient hosts and this can lead to uncontrolled EBV-driven lymphoproliferation (Kuppers 2003).

As previously mentioned, there are two recognised types of post-transplantation NHL, namely early and late NHL, and they have distinctly different clinical, histological and epidemiological characteristics. Early NHL, occurring within one or two years of transplantation, is of B-cell origin and closely associated with EBV infection or reactivation due to potent immunosuppression particularly anti-lymphocyte antibody therapy (Ghobrial et al. 2005, van Leeuwen et al. 2009, Quinlan et al. 2011, Caillard et al. 2012). Late NHL, on the other hand, features a subset of T-cell origin lymphomas (Dotti et al. 2000, Ghobrial et al. 2005, Quinlan et al. 2011) and is less likely to be related to EBV infection (Olagne et al. 2011, Caillard et al. 2012, Kinch et al. 2014). EBV-negative NHL tends to be more aggressive and is more likely to present as nodal disease (Dotti et al. 2000, Dotti et al. 2002).

Most NHL occurring in adult transplant recipients is caused by EBV reactivation (Loren et al. 2003) although a proliferation of donor cells is possible (Capello et al. 2009). EBV seronegative transplant recipients are at highest risk of NHL due to primary EBV infections from EBV positive donor (EBV mismatch) (Opelz et al. 2009, van Leeuwen et al. 2009, Caillard et al. 2012). This explains the high incidence of NHL in paediatric transplant recipients (Katz et al. 2007), a large proportion of whom are EBV seronegative at transplantation (Cohen 2000, Young and Rickinson 2004, Taylor et al. 2005).

KSHV and Kaposi's sarcoma

The risk for Kaposi's sarcoma (KS), an angioproliferative disorder caused by KSHV infection, is remarkably high in kidney transplant recipients and people with HIV/AIDS (Grulich et al. 2007, Lebbe et al. 2008, Einollahi et al. 2009, Piselli et al. 2009). The majority of KS occurring after transplantation is due to viral reactivation and risk is closely related to the degree of immunosuppression (Serraino et al. 2005, Lebbe et al. 2008). The role of immunosuppression in KS is further supported by the regression of KS after the cessation of immunosuppressive agents in kidney recipients (Barozzi et al. 2008). Variation in KS risk estimates among different transplanted organs might be explained by variation in the degree of immunosuppression but also by variation in the prevalence of the virus itself. The prevalence of KSHV and thus KS is relatively low in the US and northern and western Europe, whereas it is relatively common in some Mediterranean and Middle Eastern countries (Mbulaiteye and Engels 2006, Lebbe et al. 2008).

HPV infection and cancer risk

Persistent infection by oncogenic HPV genotypes is causal for virtually all cervical cancers and differing proportions of anal, vaginal, vulva, penile and oropharyngeal cancers (IARC 2012). Genital HPV infection is not uncommon in the general population and the majority of healthy individuals are able to clear the virus within 1-2 years, but immune deficient individuals are less able to eliminate the infection (IARC 2012). Immunosuppression is thus a significant cofactor for increased risk of precursor lesions and invasive cancers (IARC 2012). HPV-related cancers occur at increased risk in both solid organ transplant recipients and in individuals with HIV/AIDS, with the exception

of cervical cancer (Grulich et al. 2007, Collett et al. 2010, Madeleine et al. 2013). The latter observation is evidence of the success of cervical cancer screening (Kasiske et al. 2000, European best practice guidelines for renal transplantation 2002, Kaplan et al. 2009).

HBV and HCV infection

Both HBV and HCV are established as oncogenic for HCC (IARC 2012). HCC risk is increased in kidney and liver transplant recipients and in individuals with HIV/AIDS (Grulich et al. 2007, Engels et al. 2011, Krynitz et al. 2013). However, the excess risk of HCC in liver transplant recipients has not been consistently observed and it may be an artefact of including HCC diagnosed histopathologically in the explanted organ (Vajdic et al. 2012, Koshiol et al. 2014). Furthermore, the prevalence of HBV and HCV infection is relatively high in transplant recipients and in people with HIV/AIDS compared to the general population, complicating the interpretation (Gane et al. 1996, Kotton and Fishman 2005, Alter 2006, Watt et al. 2009). The risk of HCC is increased both during dialysis and after kidney transplantation (Vajdic et al. 2006), therefore transplantation and the associated immunosuppression may not alter or promote the development of HCC in organ transplant recipients.

HCV infection is causal for NHL in immunocompetent individuals (IARC 2012), and HBV infection has been observed to increase the risk of NHL (Dalia et al. 2013). While single centre studies have reported a positive association between HCV infection and PTLD/NHL risk after liver transplantation (McLaughlin et al. 2000, Duvoux et al. 2002), the largest cohort study of US Scientific Registry of Transplant Recipients did not

observe any association (Morton et al. 2007). The association between HCV infection and risk of PTLD/NHL is more likely to be a result of chronic antigenic stimulation and clonal B-lymphocyte development (Vallat et al. 2004, Suarez et al. 2006).

Merkel cell polyomavirus (MCPyV or MCV)

A newly discovered virus, Merkel cell polyomavirus (MCPyV or MCV), is believed to be responsible for 80% of Merkel cell carcinoma (MCC), a rare neuroendocrine skin cancer (IARC 2012, Schrama et al. 2012). Both people with HIV/AIDS and solid organ transplant recipients experience a remarkably increased risk of MCC (Penn and First 1999, Engels et al. 2002, Koljonen et al. 2009). The association between immunosuppression and MCC is further supported by evidence of metastatic MCC regression after withdrawal of immunosuppressive agents (Friedlaender et al. 2002, Muirhead and Ritchie 2007, Koljonen et al. 2009). Although the mechanisms are not fully understood, the tumourigenicity of MCV may be driven by UV-induced mutations (Feng et al. 2008, Shuda et al. 2008, Schrama et al. 2012).

Type, extent and duration of immunosuppressive therapy and cancer risk

Overall, iatrogenic immunosuppression is the key risk factor for *de novo* cancer after solid organ transplantation. However, the mechanisms involved in cancer development are unclear because of the complexity of both intrinsic and extrinsic factors in the context of immunodeficiency. There has been much debate regarding whether the increased risk of cancer is due to certain types of immunosuppressive agents, the intensity of immunosuppression or both factors combined. The association between immunosuppression and the risk of cancer is strongly suggested by the

similarity of the cancer risk profile for solid organ transplant recipients and individuals with HIV/AIDS, as previously noted (Grulich et al. 2007). A causal role for immunosuppression for specific cancers, particularly but not exclusively virus-associated cancers, is also supported by the observation that their risk is reversed after the cessation of immunosuppressive agents in kidney recipients who experience graft failure (van Leeuwen et al. 2010). Similarly, a complete or partial remission of NHL after a reduction in immunosuppression has been observed in some recipients (Tsai et al. 2001, Swinnen et al. 2008, Reshef et al. 2011).

Indirect evidence of the association between cancer risk and the intensity of immunosuppression comes from observations of the higher risk of cancer in heart and lung recipients who receive higher doses of immunosuppressive agents relative to liver and kidney recipients (Euvrard et al. 1995, Ong et al. 1999, Collett et al. 2010). A dose-dependent association between immunosuppression and cancer risk has also been suggested by cohort studies. A significant increase in PTLD risk with increasing doses of muromonab-CD3 has been observed in a single-centre heart transplant study (Swinnen et al. 1990). Similarly, a high average dose of ATG was related to an 8-fold increased risk of NHL in a population-based nested case-control study (Fernberg et al. 2011). Another population-based nested case-control study demonstrated an elevated risk of cutaneous SCC with a higher accumulated dose of azathioprine (Ingvar et al. 2010). Furthermore, it has been shown in transplant recipients that a lower CD4 count, a measure of the extent of immunosuppression, was associated with an increased risk of skin cancer and of NHL (Ducloux et al. 1998, Guiguet et al. 2009).

The occurrence of cancer is believed to be the ultimate result of: impaired immune control of viral infections, DNA damage and disruption of DNA repair system, insufficient immunosurveillance of cancer cells, and the up-regulation of cytokines that potentially promote cancer progression such as TGF β-1 (Buell et al. 2005, Rama and Grinyo 2010). Corticosteroid is not IARC-classified as a human carcinogen (IARC 1987, Grosse et al. 2009). Studies of transplant recipients, predominantly kidney, have mainly focused on the association between other immunosuppressive agents and risk of the most commonly occurring cancers, PTLD/NHL and NMSC. Therefore, the evidence and potential mechanisms are mainly discussed in regard to these two malignancies.

Antibody therapy

The receipt of T-cell antibody induction, either ATG/ALG or muromonab-CD3, is strongly related to risk of PTLD/NHL in kidney transplant recipients (Opelz and Henderson 1993, Cherikh et al. 2003, Opelz et al. 2007, van Leeuwen et al. 2009, Quinlan et al. 2011, Caillard et al. 2012, Dharnidharka et al. 2012). This is likely to be due to the extent of immunosuppression, although lymphomagenesis driven by specific mechanisms of action of individual antibodies is also possible (Swinnen et al. 1990, Swinnen and Fisher 1993, Chapman et al. 2013). Muromonab-CD3 has been demonstrated to be associated with an increased risk of PTLD/NHL, particularly in higher doses (Swinnen et al. 1990, Bustami et al. 2004). Some studies have reported increased risk of PTLD/NHL with receipt of ATG/ALG (Duvoux et al. 2002, Caillard et al. 2005, Fernberg et al. 2011), but this was not supported by other studies (Cherikh et al. 2003, Bustami et al. 2004). Whilst a direct drug effect promoting lymphomagenesis has

not been established for muromonab-CD3 or ATG/ALG, it may be associated with the release of cytokines such as IL-6 and IL-10 (Swinnen and Fisher 1993, Allen et al. 2013). On the other hand, there is no evidence that basiliximab is directly carcinogenic. Several studies have observed no association between PTLD/NHL and use of basiliximab (Opelz et al. 2007, van Leeuwen et al. 2009, Caillard et al. 2012).

Evidence regarding the role of antibody therapy in other incident cancers after transplantation is limited. An Australian study of kidney transplant recipients found that the risk of cutaneous melanoma was increased with the current receipt of a T-cell depleting antibody (IRR 1.73, 95% CI 1.05-2.84) (Vajdic et al. 2009). In contrast, an Australian study of kidney transplant recipients found the current receipt of T-cell depleting antibodies conferred a lower risk of lip cancer, predominantly SCC of the lip (IRR 0.59, 95% CI 0.35-0.99); this association was observed for both ATG and muromonab-CD3 and was irrespective of the indication (that is, induction or rejection) (van Leeuwen et al. 2009). In addition, a Swedish study of transplant recipients observed that the use of ATG/ALG was not associated with the risk of cutaneous SCC in kidney recipients (Ingvar et al. 2010). However, there is no evidence regarding the association between use of antibodies and risk of cancer other than PTLD/NHL and melanoma in liver, heart and lung recipients.

Maintenance immunosuppressive therapy

Data regarding the use of specific maintenance immunosuppressive agents in cancer risk are mixed. The evidence again mainly comes from studies of PTLD/NHL and NMSC (Table 2-5, page 53). Both cyclosporine and azathioprine are classified as human

carcinogens by IARC (IARC 2012), but the clinical and biological evidence is not consistent. Recent studies examining the relative carcinogenicity of several relatively new drugs, tacrolimus, mycophenolate and mTOR inhibitors, have produced mixed results. The majority of studies only used discharge data, defined as immunosuppressive therapy at the time of separation from hospital, to assess the effect on cancer risk, however, both the type and dose of immunosuppressive agents can vary considerably over time since transplantation, thus there is considerable potential for misclassification of drug use using such data. In addition, a direct carcinogenic effect of an individual immunosuppressive agent on cancer risk in transplant recipients is difficult to assess as they are usually used in combination with at least one other agent.

CNIs and risk of PTLD/NHL

An increased risk of NHL was observed soon after the introduction of cyclosporine (Beveridge et al. 1984), and early single-centre studies investigating risk of PTLD/NHL stratified by year of transplant concluded that the introduction of cyclosporine heightened the risk of PTLD/NHL (Libertiny et al. 2001, Gao et al. 2003). These findings however must be interpreted with caution as cyclosporine combined with azathioprine was the first-line maintenance therapy. Thus, such unadjusted results are likely to be confounded by the concomitant use of other immunosuppressive agents. After adjustment for the current receipt of other agents, a study of Australian kidney transplant recipients found that the current receipt of a CNI increased the risk of early but not late NHL (van Leeuwen et al. 2009), which was concordant with an earlier study of the same population using discharge immunosuppression data (Faull et al.

2005). A Swedish study observed an elevated risk of NHL with the receipt of tacrolimus, but it was based on a very small number of incident NHLs and was not adjusted for the receipt of other agents (Fernberg et al. 2011). In this same study neither the receipt nor the dose of cyclosporine was associated with NHL risk, but again this was not adjusted for other agents (Fernberg et al. 2011).

The majority of studies have examined NHL risk in association with discharge immunosuppression and they have observed conflicting findings. Whilst neither cyclosporine (Bustami et al. 2004, Caillard et al. 2012) nor tacrolimus (Crespo-Leiro et al. 2008, Caillard et al. 2012) were associated with NHL in some population-based studies, others showed an increased risk with tacrolimus (Caillard et al. 2005, Opelz et al. 2007, Dharnidharka et al. 2012).

Carcinogenic role of CNIs in PTLD/NHL development

Evidence of a direct carcinogenic effect of CNIs is inconclusive (Grosse et al. 2009, Bugelski et al. 2010, IARC 2012). Cyclosporine and tacrolimus may contribute to cancer progression through a direct cellular effect in addition to indirectly via immunosuppression (Hojo et al. 1999, Maluccio et al. 2003). In regard to lymphomagenesis, an *in vitro* study showed that both cyclosporine and tacrolimus could enhance the survival of EBV-infected B-cells (Beatty et al. 1998) and cyclosporine could promote EBV-infected B-cell expansion by increasing IL-6 activity in peripheral blood mononuclear cells (Tanner and Menezes 1994). However, a recent study using mice models showed that cyclosporine may suppress the growth of lymphoma (Rafferty et al. 2012).

CNIs and risk of NMSC

Epidemiological studies are yet to provide convincing evidence of a role for CNIs in NMSC development. Population-based studies have shown that recipients who received a combination of corticosteroid, cyclosporine and azathioprine or sirolimus were at a higher risk of NMSC relative to those who received a combination of corticosteroid and azathioprine or sirolimus (Jensen et al. 1999, Campistol et al. 2006, Ingvar et al. 2010). But we are unable to reliably determine whether this difference is driven by cyclosporine alone or is the result of greater immunosuppression with triple compared to double therapy. A Swedish study found that transplant recipients who received a combination of corticosteroid, cyclosporine and azathioprine had a 5-fold increase in risk of cutaneous SCC compared to recipients who received corticosteroid and cyclosporine, but the ever receipt, accumulated and mean dose of cyclosporine did not confer a higher risk (Ingvar et al. 2010). In addition, cyclosporine treatment in patients with chronic skin disorders, such as atopic dermatitis, is not associated with increased risk of cutaneous SCC and BCC (Muellenhoff and Koo 2012), although the dosage of cyclosporine in this setting is lower than for transplant recipients. Evidence is limited regarding the association between tacrolimus and risk of NMSC. In a population-based study of kidney recipients, the receipt of tacrolimus at discharge decreased NMSC risk, although adjustments for the use of other agents were not made (Kasiske et al. 2004). However, the use of tacrolimus did not increase the risk of NMSC in univariate analysis in liver and heart recipients in two single-centre studies (Herrero et al. 2005, Brewer et al. 2009).

Carcinogenic role of CNIs in NMSC development

It appears that CNIs may have an indirect effect on NMSC progression but evidence for a direct carcinogenic role, rather than immunosuppression *per se*, remains elusive. An *in vivo* study showed that inhibition of calcineurin was related to the development of cutaneous SCC via the p53 pathway in immune deficient mice models (Wu et al. 2010). In addition, cyclosporine has been shown enhance the aggressiveness of cutaneous SCC through the TGF-β1 signalling pathway (Walsh et al. 2011). Higher numbers of chromosomal aberrations have been observed in both cyclosporine and tacrolimus treatment groups compared to controls in UV-induced cutaneous SCC mice models (Dworkin et al. 2009). In addition, the administration of cyclosporine or tacrolimus was found to increase the size of SCC, and progression from papilloma to SCC, in mice exposed to either agent or UVR (Yokota et al. 1989, Duncan et al. 2007, Wulff et al. 2008, Yajima et al. 2008).

Azathioprine and risk of PTLD/NHL

Epidemiological studies have not provided consistent findings with regard to the carcinogenic effect of azathioprine. A Swedish study found no association between the accumulated or average dose of azathioprine and risk of NHL; however, this was not adjusted for exposure to other immunosuppressive agents (Fernberg et al. 2011). An Australian study of kidney recipients reported that the current receipt of antiproliferatives (azathioprine or mycophenolate) was unrelated to NHL risk (van Leeuwen et al. 2009). Findings from studies using discharge immunosuppression data to examine the effect of azathioprine on NHL risk vary considerably. Some reported that azathioprine increased the risk of NHL (Kinlen et al. 1979, Cherikh et al. 2003),

while others found no association in either kidney recipients (Caillard et al. 2012) or in kidney, liver, and heart recipients (Bustami et al. 2004, Faull et al. 2005, Dharnidharka et al. 2012).

Azathioprine is IARC-classified as a human carcinogen for NHL on the basis of data from transplant recipients and patients with autoimmune disorders such as IBD (IARC 2012). A 4 to 5-fold increased risk of lymphoma has been observed in IBD patients treated with azathioprine (Beaugerie et al. 2009, Kotlyar et al. 2014). A causal relationship is indicated by the reversal of NHL risk after withdrawal of azathioprine in IBD patients (Beaugerie et al. 2009, Kotlyar et al. 2014).

Carcinogenic role of azathioprine in PTLD/NHL development

There is biological evidence that azathioprine may promote the initiation of lymphoma. Mice models show a dose-dependent effect of azathioprine on lymphoma development (Imamura et al. 1973, Ito et al. 1989, Molyneux et al. 2008). There is also evidence of that azathioprine-induced lymphomagenesis may be related to DNA mismatch repair system deficiency (Chalastanis et al. 2010), which suggests a mechanism that is independent of immunosuppression.

Azathioprine and risk of NMSC

On balance, the epidemiological evidence supports an increased risk of NMSC, particularly SCC, in association with use of azathioprine. A population-based nested case-control study found that a higher accumulated and mean dose of azathioprine was strongly related to risk of cutaneous SCC in kidney recipients adjusted for the

receipt of other immunosuppressive agents (Ingvar et al. 2010). Receipt of azathioprine at discharge was associated with an over 2-fold increased risk of cutaneous SCC regardless of other agents in a single-centre kidney transplant study (Ramsay et al. 2003), and an increased risk of NMSC in a population-based study of kidney recipients without adjustment for the other agents (Kasiske et al. 2004). An excess risk of NMSC has also been shown in IBD patients treated with azathioprine (Long et al. 2010). However, data is limited in transplant recipients other than kidney. A single-centre study found no association between the current receipt of azathioprine and risk of cutaneous SCC in heart recipients (Brewer et al. 2009), in agreement with another single-centre study of liver recipients (Herrero et al. 2005), although neither of these studies was adjusted for the receipt of other agents.

Carcinogenic role of azathioprine in NMSC development

The effect of azathioprine on NMSC risk appears to be attributable to 6-TG-induced photosensitivity to UVA exposure. The administration of azathioprine facilitates indirect DNA damage in keratinocytes by UVA-related radical oxygen species (Cadet et al. 2003, O'Donovan et al. 2005, Cadet et al. 2006, Brem and Karran 2012). Kidney recipients with cutaneous SCC have higher blood levels of 6-TG compared to those without SCC (Lennard et al. 1985). In addition, UVA skin photosensitivity and DNA damage detected in kidney transplant recipients using azathioprine was reversed when azathioprine was replaced with mycophenolate (Hofbauer et al. 2012). Furthermore, an elevated frequency of P53-mutation was detected in the skin of kidney recipients with SCC after exposure to azathioprine compared to immunocompetent patients with SCC (de Graaf et al. 2008).

There is also evidence from animal models of azathioprine induced cutaneous carcinogenesis. However, most experimental studies have assessed the association between azathioprine and NMSC initiation or progression using UVR-exposed mice, therefore the interpretation is not straightforward due to the effect of UVR-induced immunosuppression. In addition, most of the skin tumours observed in mice models were papillomas instead of SCC (Athar et al. 1991), although the progression of papillomas to SCC is possible (Kelly et al. 1987).

Mycophenolate and risk of PTLD/NHL

Although mycophenolate is also an antiproliferative agent, it may not contribute to risk of PTLD/NHL. Using discharge immunosuppression data, risk of PTLD/NHL was not associated with receipt of mycophenolate in most of the population-based studies (Dharnidharka et al. 2002, Bustami et al. 2004, Crespo-Leiro et al. 2008, Caillard et al. 2012). The risk of PTLD/NHL was even reduced with receipt of mycophenolate compared to receipt of azathioprine or those who did not receive mycophenolate in kidney recipients in some studies (Cherikh et al. 2003, Robson et al. 2005). However, these studies were mainly based on kidney recipients, they did not adjust for the receipt of other agents, and none assessed the dose-response.

Carcinogenic role of mycophenolate *in PTLD/NHL development*

Experimental studies have shown that some cancers such as leukaemia express high levels of inosine monophosphate dehydrogenase which could be inhibited by mycophenolate (Jackson et al. 1975, Weber et al. 1981, Nagai et al. 1991). Therefore,

mycophenolate could potentially inhibit cancer progression. It has been shown that mycophenolate can inhibit the growth of, and induce apoptosis of, human lymphoma cells, both EBV positive and negative (Heidt et al. 2008).

Mycophenolate and risk of NMSC

There is little data regarding mycophenolate and risk of NMSC. In a single-centre heart transplant study, the current receipt of mycophenolate was not associated with cutaneous SCC risk in univariable analysis (Brewer et al. 2009).

Carcinogenic role of mycophenolate in NMSC development

The reversal of skin photosensitivity to UVA and reduction of skin DNA damage after azathioprine was switched to mycophenolate in kidney recipients suggests mycophenolate may not be related to NMSC risk (Hofbauer et al. 2012). This is supported by experimental studies showing that mycophenolate does not enhance UV-induced skin tumour development (Duncan et al. 2007, Wulff et al. 2008, Dworkin et al. 2009). Interestingly, a combination of mycophenolate and cyclosporine can reduce the size of UV-induced skin tumours in mice, possibly due to the anti-inflammation properties of mycophenolate (Duncan et al. 2007).

mTOR inhibitors and risk of PTLD/NHL

It has been suggested that mTOR inhibitors may lead to a decreased incidence of PTLD/NHL in solid organ transplant recipients. There are case reports of a complete regression of PTLD with conversion from a CNI-based regimen to sirolimus (Pascual 2007, Boratynska and Smolska 2008). However, cohort studies have not found a

reduced NHL risk in those who received sirolimus compared with those who did not in kidney, liver, and heart recipients (Caillard et al. 2005, van Leeuwen et al. 2009, Dharnidharka et al. 2012). An increased risk of PTLD conferred by mTOR inhibitors was observed in a cohort study of 59560 kidney recipients using discharge immunosuppression data (Kirk et al. 2007), but this finding was not adjusted for the other agents or the duration of immunosuppression.

Role of mTOR inhibitors in PTLD development

Both sirolimus and everolimus have been found to inhibit B-cell proliferation in mice (Nepomuceno et al. 2003) and in cell cultures (Muthukkumar et al. 1995, Majewski et al. 2000, Majewski et al. 2003, Heidt et al. 2008).

mTOR inhibitors and NMSC

Several clinical trials have reported a decreased incidence of NMSC in recipients taking mTOR inhibitors compared with those on CNIs. A recent systematic review of randomised controlled trials showed that conversion from CNIs to mTOR inhibitors for maintenance immunosuppression was associated with a lower incidence of skin cancers including NMSC and melanoma up to 1-2 years after transplantation (Lim et al. 2014). In addition, a reduction of second skin cancers has been reported although this was in response to switching to an mTOR inhibitor together with minimising other immunosuppressive agents (Tessmer et al. 2006). Whilst this data indicates that mTOR inhibitors are a promising therapeutic option for transplant recipients with NMSC, none of the studies have evaluated NMSC risk over the long-term. Therefore, the potential beneficial effects of mTOR inhibitors require additional evaluation.

Role of mTOR inhibitors in NMSC development

The role of mTOR inhibitors has mainly been examined with respect to UVR-induced skin carcinogenesis. Sirolimus treated mice with UVB-induced SCC had fewer SCC-related chromosomal aberrations than vehicle-treated mice (Dworkin et al. 2009). In addition, although treatment of sirolimus alone or in a combination with cyclosporine in UVB-exposed mice was associated with higher cutaneous SCC numbers than the vehicle treatment group, the tumours were smaller, and less aggressive compared with cyclosporine treated mice. Further assessment suggested that sirolimus did not affect inflammation, but reduced tumour vascularity (Duncan et al. 2007). The potential anti-tumour properties of sirolimus are also indicated by a chemically induced mouse skin SCC model, showing a reduction of SCC cell growth, and a decrease in mutant P53 expression in sirolimus-treated mice compared to mice without any treatment (Amornphimoltham et al. 2008).

Rejection and risk of cancer

It is unclear whether acute rejection increases risk of cancer. The association between acute rejection and risk of cancer has been hypothesised based on the fact that higher doses of immunosuppressive agents, with or without T-cell depleting antibodies, are recommended during acute rejection. A recent study of 7153 kidney transplant recipients showed that acute rejection requiring T-cell-depleting antibody was associated with an excess risk of any cancer (HR 1.42, 95% CI 1.02-1.99). Further stratified analysis revealed that this association was only significant for genitourinary tract cancers, the most common cancer in kidney recipients (Lim et al. 2014). However,

it is uncertain whether this finding was due to the overall intensity of immunosuppression or due to certain types of agents as the dose-response relationship was not examined.

Table 2 - 4. Summary of studies examining the association between corticosteroid and cancer

Author/year	Design, period	Outcome	Cohort	Medication data	Main results
Bernatsky et al, 2007	Meta-analysis of corticosteroid and NSAIDs; 2002-2006	NHL (n=6897)	9 case-control studies and one cohort study	Categorised as 'ever-never'	OR 1.1 (95% CI 0.1–1.2)
Quinlan et al, 2010	Population based retrospective cohort study; 1999-2007	PTLD (n=762)	156,740 kidney transplant recipients	Discharge (yes, no)	Univariable model steroid user vs non-user, Early PTLD: HR 1.3 (95% CI 0.1–1.8) Late PTLD: HR 0.6 (95% CI 0.4–0.1)
Ingvar et al, 2010	Population based case-control study; 1970-1997	CSCC (n=207)	207 CSCC cases and 189 controls; 95% kidney transplant	Daily doses of corticosteroid	High vs low accumulated dose of corticosteroid: RR 3.9 (95% CI 1.2–12.3)

Abbreviations: NSAIDs: non-steroidal anti-inflammatory drugs; AID: autoimmune disease; RA: rheumatoid arthritis; CSCC: cutaneous squamous cell carcinoma. Notes: Studies included in this table are population-based cohort studies and a meta-analysis of observational studies.

Table 2 - 5. PTLD/NHL risk after solid organ transplantation in population-based cohort studies

Reference	Cohort	Analysis	Results: immunosuppressive agents HR/OR/IRR (95% CIs)	Results: other factors HR/OR/IRR (95% CIs)
Caillard, 2012, France	1998-2007 adult kidney 21,351; 327 PTLD in 181 recipients	Multivariable Cox regression; Immunosuppressive agents at discharge	Induction therapy (polyclonal vs muromonab-CD3) 1.4 (1.0–2.0) Cyclosporine (yes vs no) 0.6 (0.4–1.2) Tacrolimus (yes vs no) 0.7 (0.4–1.2) Azathioprine (yes vs no) 1.3 (0.8–2.2) Mycophenolate (yes vs no) 1.2 (0.7–2.0)	Age 33-46yrs Ref 47-60yrs 1.9 (1.2-2.9) >60yrs 2.8 (1.7-4.6) Transplant era 2006-2007 Ref 2000-2001 3.1 (1.6–6.2) 1998-1999 3.4 (1.6–6.9) EBV R-/D+ vs R+ 5.3 (3.4–8.4)
Sampaio, 2012, US	2000-2009 adult kidney 114,025; 754 PTLD	Multivariable Cox regression; Immunosuppressive agents at discharge	**EBV- PTLD:** Mycophenolate + cyclosporine vs Mycophenolate + tacrolimus: 0.5 (0.3–0.7) Sirolimus + tacrolimus vs mycophenolate +tacrolimus: 2.0 (1.3–3.1)	**EBV+PTLD :** Age >= 60 vs 41-59yrs 1.9 (1.4–2.6) Deceased donor 1.4 (1.0–1.9) **EBV-PTLD**: Ethnicity-white 2.4 (1.8–3.4) Age <18 vs 41-59yrs 3.3 (2.3–4.7) >= 60 vs 41-59yrs 1.8 (1.2–2.7)
Dharnidharka, 2012, US	2003-2010 Kidney 112,756; 580 PTLD. Heart 13,937; 140 PTLD. Liver 40,437; 383 PTLD.	Multivariable Cox regression; Immunosuppressive agents at discharge	T-cell depleting vs none 1.5 (1.2–2.0) IL-2Ra 1.1 (0.7–1.9) Heart: Cyclosporine vs Tacrolimus 0.5 (0.3–0.8) Liver: Mycophenolate yes vs no 1.3 (1.0–1.7) no CNIs vs tacrolimus 0.3 (0.1–1.0)	Age <18 vs >=18 Kidney 1.7 (1.3–2.2) Heart 3.9 (2.5–6.0) Liver 3.3 (2.4–4.4) Recipient EBV- vs + Kidney 3.6 (2.6–5.0) Heart 4.0 (2.4–6.9) Liver 1.5 (1.0–2.1) EBV mismatch Kidney 2.8 (1.3–5.7)

Table 2 – 5 (continued). PTLD/NHL risk after solid organ transplantation in population-based cohort studies

Reference	Cohort	Analysis	Results: immunosuppressive agents HR/OR (95% CIs)		Results: other factors HR/OR/IRR (95% CIs)	
Fernberg, 2011, Sweden	1970-2008 153 NHL Kidney 8177; Liver 1473; Heart 532; Lung 441; Other 458 case-control study: 37 NHL, 97 controls	Case-control study: conditional logistic regression adjusted for age, calendar year; daily doses of immunosuppressive agents	ATG ever vs never Accumulated ATG dose: No ATG <50 percentile >50 percentile muromonab-CD3 ever vs never Azathioprine ever vs never Cyclosporine ever vs never Tacrolimus yes vs no	5.6 (2.5-14.0) Ref 5.8 (1.9-18.0) 5.3 (1.6-17.0) 6.0 (0.9-67.0) 2.8 (0.8-16.0) 0.9 (0.2-5.8) 11.0 (1.2-512.0)	NA	
Quinlan, 2010, US	1999-2007 Kidney 156,740; 762 PTLD	Univariate Cox regression; Immunosuppressive agents at discharge	**Late NHL**: Antibody induction Never vs ever	1.2 (1.0–1.5)	**Early NHL** Age 20-25yrs 0-19yrs Race non-hispanic white vs other: **Late NHL** Age 20-50yrs 0-19yrs Race non-hispanic white vs other	 Ref 6.5 (5.0–8.3) 2.1 (1.7–2.7) Ref 2.9 (2.2–3.8) 1.7 (1.4–2.2)

Table 2 – 5 (continued). PTLD/NHL risk after solid organ transplantation in population-based cohort studies

Reference	Cohort	Analysis	Results: immunosuppressive agents IRR/HR (95% CIs)		Results: other factors IRR/HR (95% CIs)	
Van Leeuwen, 2009, Australia	1982-2003 Kidney 8164; 125 NHL	Poisson regression; Immunosuppressive agents at regular follow-up	**Early NHL:**		**Early onset NHL (n=27):**	
			Current receipt:		EBV- at transplant	4.7 (2.1–10.4)
			T-cell depleting antibody yes vs no	2.4 (1.1–5.3)		
			CNIs yes vs no	1.4 (0.3–6.0)		
			Antiproliferatives yes vs no	0.5 (0.2–1.2)		
			Late NHL:			
			T-cell depleting antibody yes vs no		**Late NHL (n=79):**	
			CNI		Age	1.0 (1.0–1.0)
			yes vs no	1.2 (0.4-2.3)	Time since transplant	
			Antiproliferatives		5-9.99yrs	3.6 (1.9–6.9)
			yes vs no	3.1 (1.5–6.4)	10-14.99yrs	4.6 (2.2–9.5)
					15yrs+	5.6 (1.7–18.4)
				1.1 (0.6-2.1)		
Crespo-Leiro, 2008, Spain	1984-2003 Heart 3393; Lymphoma 62	Poisson regression; Immunosuppressive agents at 3 month intervals	Univariable:		No significant results for age, sex, transplant era, smoking history	
			Induction yes vs no muromonab-CD3	1.5 (0.9-2.5)		
			yes vs no	1.2 (0.7-2.2)		
			ATG yes vs no	2.4 (1.3-4.5)		
			Antiviral prophylaxis yes vs no	0.7 (0.4–1.2)		
			Mycophenolate yes vs no	0.8 (0.4–1.8)		
			Tacrolimus yes vs no	0.8 (0.2–3.2)		

Table 2 – 5. (continued) PTLD/NHL risk after solid organ transplantation in population-based cohort studies

Reference	Cohort	Analysis	Results: immunosuppressive agents HR (95% CIs)	Results: other factors HR (95% CIs)
Faull, 2005, Australia	1970-2003 Kidney 13,516; 197 PTLD	Multivariable Cox regression; Immunosuppressive agents at discharge	Induction therapy yes vs no 1.2 (0.9–1.7) CNI vs azathioprine 3.2 (2.2–4.7)	Age 25-34 yrs Ref >65 yrs 4.5 (2.3–8.9) Recipient CMV IgG (+ vs -) 1.2 (0.8–1.8) Donor CMV IgG (+ vs -) 1.0 (0.7–1.6) EBV IgG (- vs +) 3.1 (1.8–5.4)
Caillard, 2005, US	1996-2000 25,127 kidney; 344 NHL	Multivariable Cox regression; Immunosuppressive agents at discharge	ATG/ muromonab-CD3 yes vs no 1.6 (1.2–2.0) Tacrolimus vs cyclosporine 1.6 (1.1–2.2) Mycophenolate yes vs no 0.6 (0.5–0.8) Azathioprine yes vs no 0.7 (0.5–1.0)	Malignancy as cause of transplant 4.4 (1.1–1.6) HLA mismatch 1.3 (1.1–1.6)
Bustami, 2004, US	1996-2002 kidney 38,191; PTLD 181	Multivariable Cox regression; Immunosuppressive agents at discharge	No induction Ref ATG 1.5 (0.9-2.4) rATG 3.0 (1.5-5.9) muromonab-CD3 1.7 (1.1-2.6) daclimuzab 1.8 (1.1-3.2)	Age 0.9 (0.8-1.0) Male vs female 1.4 (1.0-2.0) Race (non-hispanic vs hispanic) 1.8 (1.0-3.1)
Cherikh, 2003, US	1997-2000 38,519 kidney; PTLD number NA	Multivariable Cox regression; Immunosuppressive agents at discharge	Monoclonal induction vs none 1.7 (1.0–2.8) Mycophenolate vs azathioprine 0.6 (0.5–0.9)	Paediatric vs adult 5.3 (3.7–7.6) White vs non-white 1.4 (1.0–2.0)

Abbreviations: ATG, anti-thymocyte globulin; CNIs, calcineurin inhibitors; EBV, Epstein-Barr virus; IL-2Ra, interleukin 2 receptor antagonists; NHL, Non-Hodgkin lymphoma; PTLD, post-transplant lymphoproliferative disorder. Notes: Studies included in this table are population-based cohort studies with risk factor analysis for PTLD/NHL.

Table 2 - 6. Association between NMSC risk and immunosuppressive therapy after solid organ transplantation

First author, year, country	Period, design, PYs	Cohort, no. cases	Main results of immunosuppressive agents		Comments /notes
			All NMSC HR/RR/OR (95%CI)	SCC HR/RR/OR (95%CI)	
Ingvar, 2010, Sweden	1970-1997; Population-based case-control study PYs: NA	207 NMSC cases, 189 controls (95% kidney)		Azathioprine never Ref ever 5.2 (2.0-13.6) Cyclosporine never Ref ever 1.0 (0.5-1.8) Cyclosporine + Pred. Ref Azathioprine + Pred. 4.1 (1.4-12.2) Azathioprine + cyclosporine + Pred. 5.3 (2.0-14.4)	Adjusted for age, sex, calendar year, total accumulated dose of azathioprine, Cyclosporine, Pred.
Brewer, 2009, US	1988-2006; Single-centre retrospective study; PYs: 2097	312 heart; 306 SCC, 17 BCC		Azathioprine no Ref yes 0.9 (0.5-1.6) Mycophenolate no Ref yes 1.1 (0.6-1.9)	Time-dependent, adjusted for age, sex
Herrero, 2005, Spain	1993-1997; Retrospective Single-centre; PYs: 992	170 liver; 43 NMSC in 27 patients	Mycophenolate mofetil never Ref ever 2.5 (0.6-9.4)	NA	Adjusted for age, sex, liver function, skin type (I or II), sun burden; Tacrolimus, azathioprine not significant in univariable analysis

Table 2 - 6 (continued) Association between NMSC risk and immunosuppressive therapy after solid organ transplantation

First author, year, country	Period, design, PYs	Cohort, no. cases	Main results of immunosuppressive agents				Comments/notes
			All NMSC HR/RR/OR (95%CI)		SCC HR/RR/OR (95%CI)		
Fortina, 2004, Italy	NA; Retrospective Single-centre PYs: NA	230 adult heart; 83 SCC, 37 BCC			WLC at 3-year, percentile <50 50-75 >75	 Ref 1.6 (0.7-3.9) 4.0 (1.5-11.4)	WLC: weighted linear combination of the cumulative AZA, Cyclosporine, Pred. dose
Ramsay, 2003, Australia	Single-centre; 1999-2000; PYs: NA	361 kidney (Caucasian); 1817 SCC in 135 patients, 916 BCC in 143 patients			AZA never ever AZA + Cyclosporine + Pred AZA + Cyclosporine AZA + Pred Cyclosporine + Pred	Ref 2.4 (NA) Ref 0.5 (0.2-1.4) 0.8 (0.3-2.1) 0.4 (0.2-1.0)	Adjusted for age, sex, duration of immunosuppression
Jensen, 1999, Norway	1963-1992; population-based; PYs: NA	2235 kidney, heart; 97 SCC;			AZA + Pred Cyclosporine + Pred AZA+Cyclosporine + Pred	Ref 1.3 (0.4-3.8) 2.8 (1.4-5.3)	Adjusted for age, organ
Bouwes, 1996, Australia	1969-1994; Single-centre; PYs: NA	1158 kidney (Caucasian), 83 SCC, 52 BCC, 136 both SCC and BCC	Cyclosporine AZA (<1980) AZA (>1980) Cyclosporine to AZA Cyclosporine+AZA	Ref 1.4 (0.8-2.3) 1.1 (0.7-1.9) 0.8 (0.4-1.7) 1.1 (0.7-1.8)	Cyclosporine AZA before 1980 AZA after 1980 Cyclosporine to AZA Cyclosporine+AZA	Ref 1.3 (0.7-2.3) 1.1 (0.6-2.0) 0.8 (0.4-1.8) 1.1 (0.6-1.8)	Adjusted for age, sex

Abbreviations: AZA, azathioprine; BCC, basal cell carcinoma; NMSC, non-melanoma skin cancer; Pred, corticosteroid; SCC, squamous cell carcinoma.

Notes: Studies included in the table are cohort studies with immunosuppression data; single-centre studies were included due to lack of population-based studies.

2.6.3 Cancer-related death after solid organ transplantation

The magnitude of the overall risk of cancer-related death in transplant recipients appears comparable to the risk estimates for cancer incidence. The overall cancer SMR and SIR were 2.3-fold and 2.9-fold, respectively, in a Hong Kong cohort of kidney recipients (Cheung et al. 2012). A Spanish single-centre study of liver transplant recipients reported that the SMR for *de novo* non-cutaneous cancer was 2.9, comparable with the SIR for *de novo* non-cutaneous cancer (3.2) (Herrero et al. 2005). A recent UK study reported that increased age, receipt of a deceased-donor kidney and pre-transplant malignancy history increased the risk of cancer-related death, taking into account other causes of death as a competing risk (Warnakulasuriya 2009).

By contrast, no excess risk of overall cancer-related death was observed in a population-based study of 164,078 kidney transplant recipients registered on the United States Renal Data System (1990–2004) (Kiberd et al. 2009). In the stratified analysis, cancer-related death was only increased in those younger than 50 years at transplantation. The authors hypothesised that their finding was due to the competing risk of death from other causes in elderly transplant recipients. Although competing risk of death from other causes may have a significant impact on the analysis of cancer-specific death, this study is likely to have underestimated cancer-related deaths because cause of death was unknown for 41% of recipients and all of these deaths were classified as non-cancer-related.

Data are limited with regard to the pattern of site-specific risk of cancer-related death. In the Hong Kong study, the risk of cancer-related death was significantly elevated for

the most frequent cancers (Table 2 - 7, page 62), such as NHL (SIR 15.8, SMR 18), melanoma (SIR 9, SMR 6), and cancer of the bladder (SIR 8, SMR 4.7), kidney (SIR 12, SMR 4), stomach (SIR 2.8, SMR 3.5), colorectum (SIR 1.7, SMR 2), and lung (SIR 1.7, SMR 1.5). NHL is the most common cancer-related death after solid organ transplantation, as suggested by several studies (Thorley-Lawson and Gross 2004, Watt et al. 2009, Metcalfe et al. 2010), although the Hong Kong study is the only study to quantify the relative risk. A single study found no difference in NHL survival for transplant recipients and the Surveillance, Epidemiology, and End Results (SEER) general population (1964–2007) (Knight et al. 2009). However, central nervous system involvement in NHL after solid organ transplantation is more common than the general population (Parker et al. 2010, White et al. 2011), and this may contribute to the excess risk of NHL-related death. Recommended management of NHL after solid organ transplantation involves reducing immunosuppression, chemotherapy with or without rituximab, antiviral therapy for EBV-positive patients, surgery and radiation (Parker et al. 2010, Reshef et al. 2011, Campistol et al. 2012, Mosli et al. 2013).

In the Hong Kong study, no deaths were observed for NMSC and thyroid cancer in spite of an increased incidence of these cancers in this cohort. Some studies have suggested that skin cancers other than Merkel cell carcinoma are not as aggressive as other cancers in kidney (Briggs 2001) and liver recipients (Herrero et al. 2005), whereas a population-based Swedish study of predominantly kidney recipients found highly increased risk of death from cutaneous SCC (n = 7, SMR 52.2) (Lindelof et al. 2006).

The detailed data from the Hong Kong study indicated that the study of cancer mortality needs to be stratified by site-specific cancer, as tumour behaviour may not be the same for different cancers. Moreover, the pattern of site-specific cancer incidence and mortality may not be the same for other transplanted organs or for another geographic region. No study has quantified the risk of site-specific cancer-related death in heart and lung recipients, despite the remarkably high risk of a broad range of cancers and the higher intensity of immunosuppressive therapy used for these patients (Euvrard et al. 1995, Collett et al. 2010).

Table 2 - 7. Cancer-specific SMR and SIR in solid organ transplantation

Author, year, country	Period, PYs	Cohort, number of causes of death	Cancer-specific SMR (95%CI)		Cancer-specific SIR (95%CI)	
Cheung, 2012, Hong Kong	1972-2011; PYs: 40,246	Kidney recipients, n=4895; Total deaths N=2713; Overall cancer incidence n=299; Cancer death n=95	Overall:	2.3 (2-2.6)	Overall:	2.9 (2.6-3.3)
			NHL	18.2 (14.0-23.8)	NHL	15.8 (11.9-21.0)
			Melanoma	6.3 (1.8-21.6)	Melanoma	9.1 (2.2-36.3)
			Bladder	4.7 (2.3-9.6)	Bladder	8.2 (4.7-14.5)
			Kidney	4.4 (2.2-9.0)	Kidney	12.5 (8.5-18.4)
			Stomach	3.5 (2.3-5.4)	Stomach	2.9 (1.6-5.0)
			Leukaemia	2.8 (1.4-5.7)	Leukaemia	2.1 (0.9-5.1)
			Cervix	2.8 (1.3-6.1)	Cervix	7.2 (3.9-13.4)
			Colorectal	2.2 (1.5-3.0)	Colorectal	1.8 (1.2-2.5)
			Lung	1.5 (1.1-1.9)	Lung	1.7 (1.2-2.4)
			Breast	1.9 (1.2-2.9)	Breast	1.7 (1.0-2.8)
Kiberd, 2009, US	1990-2004, PYs: NA	Kidney recipients, n=164,078; Cancer death n=1937; Non-cancer death n=36,619 (including 15,957 missing cause of death)	Overall:	1.0 (0.9-1.0)	NA	
Lindelöf, 2006, Sweden	1970-1997, PYs: NA	All transplanted organs (kidney n=5139, liver n=397, others n=395); SCC incidence n=544 (in 201 patients); SCC death n=7	SCC:	52.2 (21.0-107.0)	NA	
Herrero, 2005, Spain*	1993-1997, PYs: NA	Liver recipients, n=187; Cutaneous cancer incidence n=35; non-cutaneous cancer incidence n=28; Cancer-related death (excluding recurrences) n=13	Overall *de novo* cancer:	2.9 (1.6-5.0)	Overall *de novo* non-cutaneous cancer: Cutaneous cancer:	3.3 (2.2-4.7) 16.0 (11.8-23.5)

Abbreviations: SMR: standardised mortality ratio; SIR: standardised incident ratio: PYs: person-years; *single centre study was included in this table due to lack of population-based studies of cancer-related death in transplanted populations.

2.6.4 Cancer screening and prevention in solid organ transplant recipients

The ability to prevent and to diagnose pre-cancerous lesions and early cancer after transplantation is reliant on targeted periodic cancer screening examinations as well as applying critical prophylactic strategies. The clinical practice guidelines provide comprehensive recommendations for cancer screening in solid organ transplant recipients (Kidney Disease: Improving Global Outcomes Transplant Work 2009, Costanzo et al. 2010, Chadban et al. 2012, Kelly et al. 2013, Martin et al. 2014). Cancer screening strategies and related issues for the most frequent cancers are briefly discussed here.

NMSC and lip cancer

Prior to transplantation, a careful evaluation is recommended for all prospective transplant recipients (Greenberg and Zwald 2011, Chadban et al. 2012). The identification of recipients at high risk of NMSC is critical for targeted surveillance. Patient education and heightened awareness is essential and recipients are encouraged to follow a monthly self-skin and lip examination. In addition, regular skin examination by a physician, preferably a dermatologist, is recommended. High risk subgroups are those of older age, a history of NMSC or actinic keratoses, fair skin type, high sun exposure history during childhood, history of sunburns, and those who live in high sun-exposure climates (Chadban et al. 2012).

PTLD/NHL

PTLD/NHL is not only the second most frequent malignancy but also a potentially life-threatening complication. A comprehensive cancer history and physical examination is

necessary before and after transplantation. Pre-transplant testing of EBV infection in both donor and recipient is an effective approach especially to identify recipients with a high risk of acquisition of EBV infection after transplantation (Parker et al. 2010). Currently, EBV viral load surveillance, particularly during the first year post-transplantation, is only recommended for paediatric or adolescent recipients since they are more likely to experience primary EBV infection at transplantation (Parker et al. 2010). Close monitoring of symptoms and signs is beneficial for adult recipients with a negative EBV serology prior to transplantation but routine surveillance for EBV infection is not recommended in adult solid organ transplant recipients (Parker et al. 2010).

Solid cancers

In terms of absolute risk, the development of solid cancers including kidney, liver, colorectal, lung, breast and prostate are of concern in solid organ transplantation. The evidence regarding the benefits and potential harms of screening for these malignancies is however limited. For kidney recipients, kidney cancer screening using cytologic or radiographic measurements is generally not recommended (Chadban et al. 2012), although ultrasonography or computed tomography scanning of native kidneys has been suggested (Kasiske et al. 2000).

Liver transplant recipients with chronic viral hepatitis, particularly those with HBV or HCV associated cirrhosis should be monitored every 6 to 12 months by serum alpha-fetoprotein and abdominal ultrasound to detect potential lesions that may lead to HCC (Lucey et al. 2013). There is no established guideline regarding the surveillance

measurements, the intervals and duration of surveillance for liver transplant recipients with HCC as the primary indication. However, close surveillance by abdominal and chest computed tomography every 6 months to 3 years after transplantation is recommended as recurrent HCC is a major cause of death (Lucey et al. 2013). Liver cancer screening for recipients of organs other than liver is not recommended (Costanzo et al. 2010, Chadban et al. 2012).

The current screening guideline recommends that liver recipients with PSC and IBD or other established risk factors for colorectal cancer (i.e. age >50 years) should undergo an annual screening colonoscopy (Lucey et al. 2013). Screening for colorectal cancer in kidney and heart transplant recipients is suggested as currently recommended for the general population (Costanzo et al. 2010, Chadban et al. 2012), although fecal occult blood testing may be less specific in transplant recipients compared with non-transplanted individuals (Collins et al. 2012).

Current guidelines recommended that female transplant recipients undergo annual cervical cancer screening with Pap smear and pelvic examination, which is more frequent than for the general population (Kasiske et al. 2000, European best practice guidelines for renal transplantation 2002). Breast cancer screening in transplant recipients should follow that for the general population.

.

Table 2 - 8. Incidence of lip cancer after solid organ transplantation in population and registry-based studies

First author, year, country	Period, PYs	Organ type, ICD code for lip cancer	No. cases	Organ type, SIR (95%CI)	
Krynitz, 2013, Sweden	1970-2008, PYs: 7450	Kidney n = 7952 Liver n = 1221 Heart and lung n = 1012 ICD code: NA	n = 64 n = 2 n = 8	Kidney Liver Heart and lung	46 (35-59) 19 (2.3-68) 84 (36-166)
Engels, 2011, US	1987-2008; NA	All organs (n = 175732), 58% kidney; ICD code: NA	n = 130	All recipients	16.8 (14.0-19.9)
Collect, 2010, UK	1980-2007; NA	All transplant recipients (n = 37617) Kidney n = 25104; Liver n = 6846 Heart n = 3609; lung n = 2058 ICD code: NA	n = 59	Kidney recipients Liver recipients Heart recipients Lung recipients	65.6 (49.9-84.6) 20.0 (5.4-51.2) 60.0 (31.0-104.8) NA
Aberg, 2008, Finland	1982-2005; 3222	All liver, n = 540; ICD code: NA	n = 1	Liver recipients	21.3 (0.54-118)
Makitie, 2008, Finland	1967-2003; 29645	Kidney n = 2884; ICD code: NA	n = 26	Kidney recipients	31.8 (20.8-46.6)
Villeneuve, 2007, Canada	1981-1998; 81273	Kidney n = 11155; ICD9 140	n = 54	Kidney recipients	23.0 (12.6-38.5)
Vajdic, 2006, Australia	1982-2003; 86898	Kidney n = 10180; ICD10 C00	n = 39	Kidney recipients	47.1 (39.1-75.1)

Table 2 – 8 (continued). Incidence of lip cancer after solid organ transplantation in population and registry-based studies

First author, year, country	Period, PYs	Organ type, ICD code	No. cases	Organ type, SIR (95%CI)	
Lindelöf, 2000, Sweden	1970-1994; NA	All recipients n = 535 (kidney n = 4712); ICD code: NA	n = 18 (male) n = 9 (female)	All recipients	Male 37.8 (22.4-59.7) Female 126 (57.8-240)
Adami, 2003, Sweden	1970-1997; 36963	All recipients n = 5931; ICD7 140	n = 40	Kidney recipients Non-kidney recipients	53.3 (38.0-72.5) 24.8 (0.6-138)
Jensen, 1999, Norway	1963-1992; PYs: 14975	2561 kidney, heart; ICD code: NA	n = 7 (male) n = 3 (female)	All recipients	Male 15 (5.9-30) Female 81 (17-237)
Birkeland, 1995, Nordic countries	1964-1986	Kidney n = 5692; ICD code: NA	n = 18	Kidney recipients	21.3 (112.6-33.6)

Abbreviations: PYs person-years; ICD International Classification of Diseases; SIR standardised incidence ratio; CI confidence interval; NA not stated.
Notes: Includes population and registry-based studies.

2.7 Summary

In this chapter, I have given an overview of the clinical practice of immunosuppression and the associated complications in solid organ transplantation. Immunosuppressive therapy is essential to improve recipient survival after transplantation, but the subsequent complications have become a principal concern. There is reliable evidence of an increased risk of cancer in solid organ recipients relative to the general population, particularly cancers with a viral cause. However, current evidence of the site-specific cancer incidence profile is based predominantly on kidney and adult recipients. In addition, data on the risk of site-specific cancer-related death are limited, and little is known for organ recipients other than kidney.

Immunosuppression is primarily responsible for the increased risk of cancer after transplantation. It is hypothesised that a higher degree of immunosuppression increases cancer risk; however, no observational study has adequately assessed the association between cancer risk and the time-dependent use and dose of individual immunosuppressive agents. Furthermore, Australia has made significant progress in solid organ transplantation since the first kidney transplant, but no clinical immunosuppression data or risk factor data is available for liver, heart and lung transplant recipients. In the following chapters, therefore, I aim to answer some of these evidence gaps in these transplanted populations.

Chapter 3 Comparison of de novo cancer incidence in Australian liver, heart and lung transplant recipients

3.1 Objectives

In this Chapter, I evaluate the pattern of cancer incidence for a population-based, national cohort of Australian liver, heart and lung transplant recipients. Specifically the aims of this Chapter are:

1. To examine the overall and site-specific *de novo* cancer incidence in liver, heart, and lung transplant recipients over two decades, and
2. To compare the age- and sex-adjusted relative risk of *de novo* cancer between recipients of different transplanted organs.

3.2 Introduction

Liver, heart and lung transplantation are established procedures for patients with end-stage organ failure. In Australia, excellent outcomes are achieved, with 1-year graft survival rates of 80–88% (ANZCOTR 2012, ANZLTR 2012). Cancer, however, has become the leading cause of death in liver and heart transplant recipients surviving for more than 5 years with a functioning graft. Immunosuppression is the primary risk factor for cancer in the transplanted population, as reinforced by the remarkably similar cancer profile in those with HIV/AIDS (Grulich et al. 2007).

Population-based cohort studies show a 2.5- to 3-fold excess risk of cancer after solid organ transplantation compared to the general population (Grulich et al. 2007, Engels et al. 2011). As it is the most common form of transplantation, kidney recipients dominate this estimate, but the spectrum of cancer risk for recipients of other organs appears broadly similar based on a few high-quality population-based studies (Aberg et al. 2008, Jiang et al. 2008, Jiang et al. 2010, Engels et al. 2011, Krynitz et al. 2013). Non-population-based estimates suggest that the risk of NHL is highest in intestinal and lung transplant recipients, followed by heart, liver and lung (Opelz and Dohler 2004), but the age- and sex-adjusted relative risk of lymphoma and solid cancer between transplanted organs has not been assessed. Furthermore, few studies have reported cancer incidence by indication for transplantation, and population-level data on cancer in paediatric recipients is scarce (Simard et al. 2011). A comparison of cancer risk in different subsets of the transplanted population will provide insights into cancer aetiology, and thus cancer prevention, in the context of immune suppression. Quantifying and understanding differences in cancer incidence between recipients of different transplanted organs will generate evidence-based clinical strategies for minimising cancer risk, and for identifying high-risk patient subsets that would benefit most from these interventions.

3.3 Methods

3.3.1 Study population

My study population comprised Australian residents who received a liver, heart and lung transplant in Australia between 1984 and 2006. The transplant recipients were registered on the Australian and New Zealand Liver Transplant Registry (ANZLTR) or the Australia and New Zealand Cardiothoracic Organ Transplant Registry (ANZCOTR). These population-based registries recorded all liver and cardiothoracic (heart, lung, and heart-lung) transplantations since 1985 and 1984, respectively. They systematically recorded the recipient's name, date of birth, sex, date of first and any subsequent transplantations, age at transplantation, and primary indication for transplantation.

I excluded non-Australian residents from the study population as they are not eligible for cancer registration in Australia. I did not exclude patients with a history of cancer prior to transplantation (n = 367), including those whose indication for transplantation was a hepatobiliary tumour (n = 93), (Oo *et al.* 2005, Vajdic *et al.* 2006, Aberg *et al.* 2008, Engels *et al.* 2011). However, these patients did not contribute person-years at risk for that cancer type. I performed a sensitivity analysis to assess the impact of excluding patients with a history of cancer. In addition, I did not include cancers or follow-up time within 30 days of transplantation (Adami *et al.* 2003, Collett *et al.* 2010, Jiang *et al.* 2010, Quinlan *et al.* 2010, Quinlan *et al.* 2011). This exclusion was necessary to avoid counting as incident those cancers that were prevalent at transplantation but not registered until they were histopathologically diagnosed in the explanted organ (Vajdic *et al.* 2006). I classified patients who received a combined liver and kidney transplant (n = 23), and those who received a combined liver, heart and lung transplant (n = 3), as liver transplant recipients;

and I classified those who received a combined heart and lung transplant (n = 137) as lung transplant recipients.

3.3.2 Data collection

Deaths and incident cancers were ascertained by record linkage with population-based administrative health datasets. Deaths were obtained from the National Death Index (NDI; 1980–2006) or the transplantation registries. Cancers were identified from the Australian Cancer Database (ACD), a register of incident primary invasive neoplasms, other than basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) of the skin which are not ascertained by the Australian population cancer registries. The date of diagnosis, topography and morphology was ascertained for each primary neoplasm diagnosed in the cohort between 1984 and 2006. The Australian cancer registries apply international rules when registering multiple primary cancers. Solid cancers were classified according to the International Classification of Diseases (ICD), 10^{th} revision, while hematopoietic neoplasms and Kaposi sarcomas were classified according to the ICD for Oncology, 3^{rd} edition.

Registrant's name, sex, date of birth, date of death, and state of residence were used during record linkage that was performed utilising an established probabilistic algorithm by taking into account a range of potential identifiers. In this process, a linkage probability or weight was computed to each potential matching record pair which had a certain threshold of probabilities to be the same identity, and a subset of paired records underwent clerical review (Jaro 1995).

I obtained cancer incidence rates for the Australian population from the ACD by five-year age group, sex, calendar year, and state, for 1982 to 2006.

I obtained ethical approval and the requirement for informed participant consent was waived because I received only de-identified data.

3.3.3 Data analysis

Crude and age- and sex-standardised incidence rate

Person-years of follow-up accrued from 30 days post transplantation until death, age 80, or 31st December 2006, whichever occurred first. I calculated crude and age- and sex-standardised cancer incidence rates (ASR), standardised to the 1996 Australian population, and 95% confidence intervals (CIs; based on the normal approximation to the binomial distribution) using annual Australian population estimates obtained from the Australian Bureau of Statistics. These rates were computed for any cancer, solid cancers, and lymphomas for paediatric recipients (0–15 years) and adults (16+ years), by transplanted organ, respectively.

Cumulative incidence of cancer

I used the competing risk extension to the Kaplan-Meier method to estimate the cumulative incidence of cancer, treating death as the competing risk. Follow-up time for

cumulative incidence of cancer was from 30 days post transplantation until the date of the first cancer diagnosis, death, age 80, or 31st December 2006, whichever occurred first.

Risk of cancer relative to the general population

I compared cancer incidence rates in transplant recipients with the Australian general population using the SIR, defined as the ratio of the observed and the expected numbers of cancers. I applied the likelihood ratio method to calculate 95% CIs for SIR for cancers with ≥10 expected cases, while exact CIs were used for cancers with <10 expected cases (Swift 2009). I computed the expected numbers of incident cancers by multiplying cohort person-years at risk by the corresponding five-year age-, sex-, state- and calendar year-specific cancer incidence rates for the Australian population. The exception was Kaposi sarcoma, where 1982 population rates were applied to avoid the impact of AIDS on the incidence of this cancer (Vajdic et al. 2006).

To understand the risk of cancer in different subgroups, I computed SIRs for the entire cohort and by transplanted organ, recipient age at transplantation (0-15 years or paediatric vs ≥16 years or adult), age at diagnosis (0–39, 40–55, ≥55 years), and primary indication for transplantation. I compared patterns of cancer risk for the different patient subgroups but importantly I did not compare SIRs statistically because of the heterogeneity in subgroup age and sex distributions (Rothman et al. 2008; p.254).

Comparison of cancer risk by transplanted organ

Within the cohort of all transplant recipients, I calculated the risk of any cancer, solid cancers and lymphomas for paediatric recipients and all recipients. As individual recipients are at risk of multiple primary cancers, I applied a marginal Cox proportional hazards model, the *Wei-Lin-Weissfeld* model, to enable inclusion of multiple cancers (Wei et al. 1989). This model is an alternative method to traditional Cox proportional hazards model for the analysis of recurrent events or the occurrence of different types of event under the assumption that all individuals are at risk for all events. I calculated hazard ratios (HR) and their 95% CI comparing the incidence of cancer by transplanted organ, age at transplant, sex, number of transplants (one, more than one; time-dependent variable), and calendar period (1984-1989, 1990-1997, 1998-2006), adjusted for one another. All variables except number of transplants satisfied the proportional hazards assumption.

I performed analyses using SAS® software v9.3 (SAS Institute Inc., Cary, NC, USA) and STATA statistical software v11.2 (StataCorp, Texas, USA).

3.4 Results

My eligible cohort comprised 4644 transplant recipients; 1926 (41%) liver, 1518 (33%) heart, and 1200 (26%) lung (Table 3 – 1, page 77). Second or higher order transplants were received by 162 (4%) patients. Approximately 9% of the cohort were paediatric (n = 415; data not shown); no paediatric patients received a lung transplant. The median age at first transplantation was 47 years (interquartile range, IQR 33–55) and it was highest for heart

(50 years, IQR 38-56) and lowest for lung recipients (45 years, IQR 30-54; data not shown). Overall, 66% of recipients were male, and the proportion of males by transplanted organ ranged from 53% (lung) to 80% (heart). The most common primary indication for transplantation was viral hepatitis (26% of liver), non-ischaemic cardiomyopathy (46% of heart) and obstructive lung disease (33% of lung) (Table 3 – 1, page 77).

I followed-up recipients for a total of 29,713 person-years. The median duration of follow-up was 5.2 years (IQR 2.0–9.9) and it was highest for heart (7.1 years) and lowest for lung recipients (3.3 years). After transplantation I observed 499 (10.7%) incident primary cancers in 463 patients; 33 patients had multiple cancers. The median age at diagnosis of first cancer was 57 years (IQR 49–63). The most frequently occurring cancers were NHL (n = 100), lip cancer (n = 58), and cutaneous melanoma (Figure 3-1, page 79). The crude overall cancer incidence rate was 1679 per 100,000 person-years and the ASR was 1693 per 100,000 (95% CI 832–2553). The 5-year cumulative incidence of cancer was 1.8% for liver, 2.2% for heart and 3% for lung recipients (Figure 3 – 2, page 80). Rates for all cancers, solid cancers, and lymphomas for paediatric and adult recipients by transplanted organ are shown in Table 3 – 2 (page 80).

Cancer risk relative to the general population: by transplanted organ

Relative to the general population, the risk of any cancer was more than 2-fold for liver (SIR = 2.20, 95% CI 1.87–2.57) and heart (SIR = 2.64, 95% CI 2.32–2.98) recipients, and more than 3-fold for lung recipients (SIR = 3.70, 95% CI 3.01–4.48) (Supplementary

Table 3 - 1, page 94, and Figure 3 - 3, page 83). The risk of lip cancer, NHL and cancer of unknown primary site were significantly elevated for recipients of all three transplanted organs (Figure 3 - 3, page 83).

Cancer risk relative to the general population: all transplant recipients

Risk of any cancer was significantly higher in transplant recipients than the matched Australian population (SIR 2.62, 95% CI 2.40–2.86). SIRs were greater than unity for 16 different cancers (Figure 3 – 1, page 79), and the relative risk was highest for Kaposi sarcoma (SIR 130), then cancer of the vulva (SIR 33), lip (SIR 24.9), NMSC (excluding BCC and SCC) (SIR 24.6) and salivary gland (SIR 18.2), followed by NHL (SIR 8.4). Most (n = 6, 75%) salivary gland cancers were squamous cell carcinomas. Risk of some epithelial cancers common in the general population, including prostate, breast, and pancreas, was not significantly elevated. Risk of all HPV-related anogenital cancers (cervix, vulva, vagina, anus and penis) was increased (SIR = 6.03, 95% CI 3.12–10.5), as was the risk of all cancers causally related to alcohol consumption (oral cavity, pharynx, oesophagus, colorectum, liver, larynx, and breast (SIR = 1.49, 95% CI 1.18–1.86).

Table 3 - 1. Characteristics of Australian liver, heart and lung transplant recipients, 1984-2006

Characteristics	Liver‡			Heart			Lung§		
	N* (%)	Person-years		N* (%)	Person-years		N* (%)	Person-years	
		Total	Median‡		Total	Median‡		Total	Median‡
Total	1926 (100)	12703	6.0 (2.2–10.2)	1518 (100)	11719	7.1 (2.9–11.9)	1200 (100)	5292	3.3 (1.5–6.5)
Age at first transplantation (years)									
0–9	248 (13.0)	1906	6.7 (2.7–12.5)	39 (2.6)	324	8.5 (3.6–12.8)	1 (0.1)	0.5	0
10–19	104 (5.4)	950	9.5 (3.8–14.0)	88 (5.8)	639	5.8 (2.0–12.0)	72 (6.0)	330	3.1 (1.3–6.5)
20–29	93(4.8)	735	7.1 (3.1–12.1)	111 (7.3)	793	5.7 (2.8–10.0)	212 (17.7)	1003	3.6 (1.5–7.5)
30–39	174 (9.0)	1450	8.2 (3.7–12.5)	168(11.0)	1457	8.2 (2.8–13.7)	189 (15.7)	852	3.1 (1.4–7.1)
40–49	540 (28.0)	3371	5.3 (2.1–9.4)	337 (22.2)	2920	8.2 (3.9–13.1)	245 (20.4)	1306	4.4 (1.8–7.7)
50–59	591 (30.7)	3467	4.8 (1.7–9.0)	587 (38.7)	4517	7.5 (3.1–11.7)	385 (32.1)	1512	3.1 (1.2–6.0)
≥60	176 (9.1)	824	3.6 (1.5–6.9)	188 (12.4)	1067	4.8 (1.7–8.7)	96 (8.0)	288	2.2 (1.2–4.3)
Sex									
Male	1191 (61.8)	7078	4.9 (1.8–8.9)	1218 (80.2)	9494	7.2 (3.1–12.0)	641 (53.4)	2827	3.3 (1.5–6.3)
Female	735 (38.2)	5624	6.9 (2.7–11.9)	300 (19.8)	2225	6.4 (2.6–11.3)	559 (46.6)	2464	3.4 (1.4–6.5)
Transplant era									
1984-1989	518 (26.9)	5949	13.4 (7.72-15.6)	679	7447	12.6 (6.67-15.2)	245	1716	6.11 (2.09-12.0)
1990-1997	288 (14.9)	2543	9.88 (8.97-10.9)	263	2094	9.52 (5.3-10.7)	224	1308	5.66 (2.39-9.33)
1998-2006	1120 (58.2)	4211	3.5 (1.51-5.96)	576	2178	3.52 (1.44-5.88)	731	2268	2.64 (1.18-4.7)
Cancer history									
No	1603 (83.2)	11371	6.32 (2.64-11.0)	1481 (97.6)	11529	7.24 (3.0-12.0)	1167 (97.3)	5171	3.41 (1.46-6.53)
Yes	323 (16.8)	1332	2.68 (1.17-5.9)	37 (2.4)	190	4.11 (1.18-8.23)	33 (2.7)	121	2.39 (1.11-4.58)
Multiple transplant (≥2)	139 (7.2)	1031	6.9 (2.2-12.5)	10 (0.7)	74	5.3 (2.5–9.4)	13 (1.1)	88	6.2 (3.1-10.7)
Primary indication for transplantation									
Non-ischemic cardiomyopathy	-			692 (45.6)	5521	7.5 (2.9–12.4)	-		
Ischaemic heart disease	-			582 (38.3)	4442	6.9 (3.2–11.7)	-		
COPD	-			-			349 (29.0)	1446	3.5 (1.6–6.0)
Obstructive lung disease	-			-			398 (33.2)	1719	3.2 (1.5–6.4)
Congenital heart disease	-			76 (5.0)	605	7.9 (2.8–12.8)	54 (4.5)	359	5.3 (2.1–11.9)
Viral hepatitis	494 (25.6)	2529	4.1 (1.7–7.9)	-			-		
Hepatobiliary tumour	93 (4.8)	350	2.3 (0.8–5.0)	-			-		
Autoimmune-related liver disease	442 (23.0)	3374	7.1 (2.6–12.1)	-			-		
Alcoholic liver disease	223 (11.6)	1406	5.8 (2.6–9.2)	-			-		
Congenital biliary disease	189 (9.8)	1571	7.7 (2.9–13.0)	-			-		
Miscellaneous	485 (25.5)	3472	6.3 (2.5–11.4)	168 (11.0)	1150	5.7 (2.5–10.6)	399 (33.2)	1767	3.0 (1.2–6.8)

Abbreviations: COPD, chronic obstructive pulmonary disease; *The counts in sub-categories may not add up to the total number due to missing data; ‡Liver transplant (n = 1900), combined liver and kidney transplant (n = 23), or combined liver, heart and lung transplant (n = 3); § Lung transplant (n = 1063) or combined lung and heart transplant (n = 137); ‡Median and interquartile range.

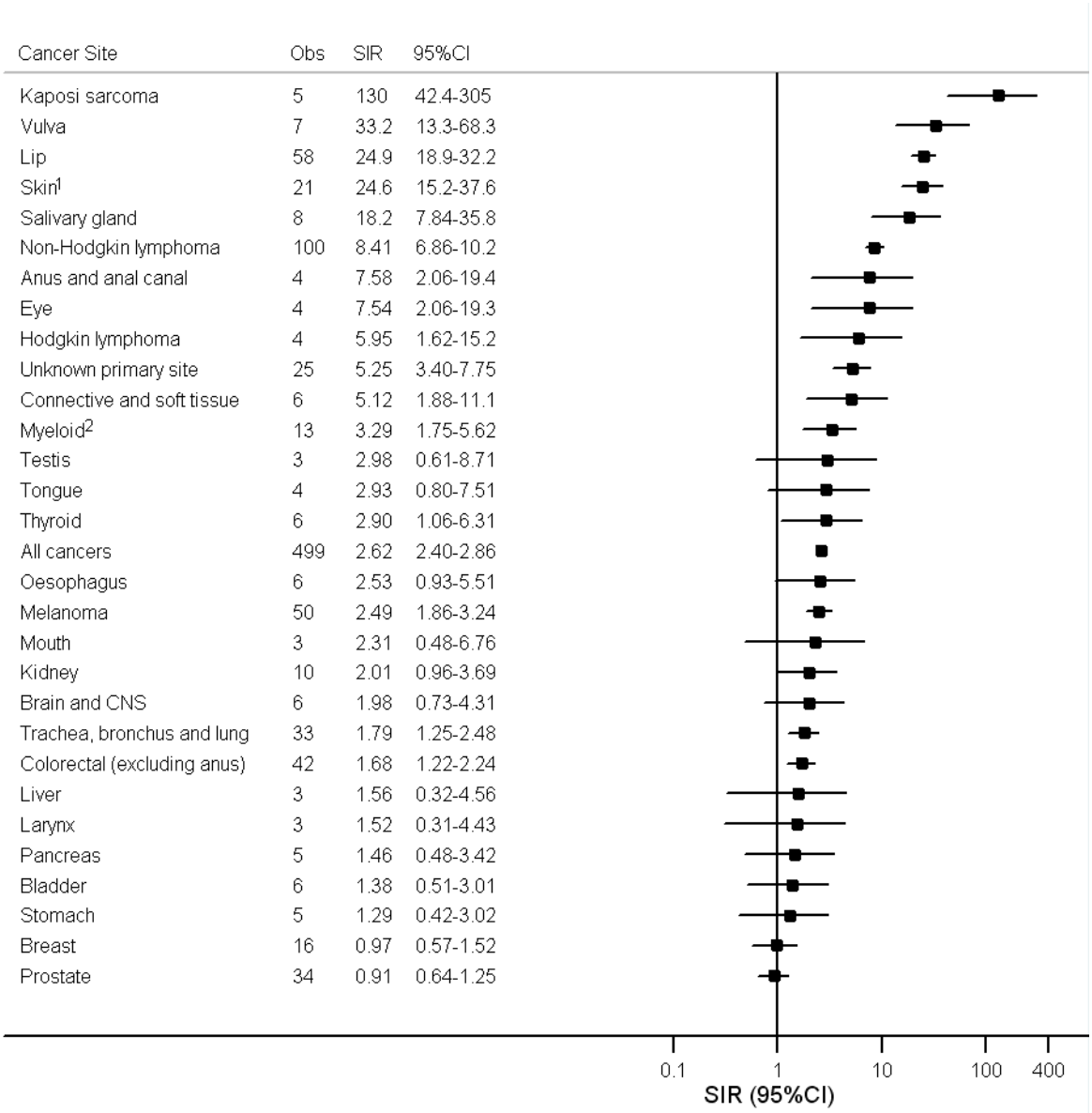


Figure 3 - 1. Site-specific cancer risk for Australian liver, heart and lung transplant recipients relative to the general population

[1]NMSC, excluding BCC and SCC; [2]Myeloid neoplasms including lymphoid/myeloid not otherwise specified.

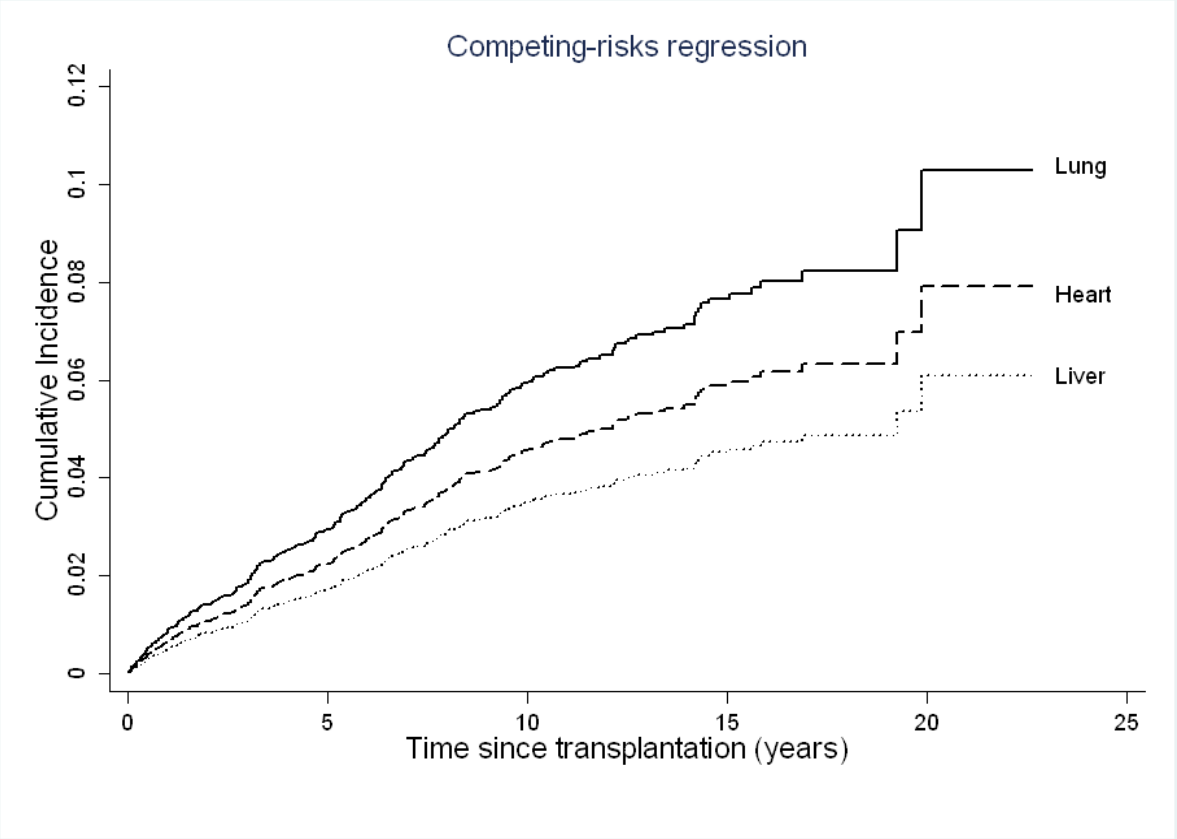


Figure 3 - 2. Cumulative incidence of cancer after transplantation by transplanted organ

Table 3 - 2. Cancer incidence in Australian paediatric and adult liver and heart and lung transplant recipients

	Paediatric recipients (0-15 years)†	Adult recipients (≥16 years)			
	Liver, heart, or lung	All adult	Liver	Heart	Lung§
Crude incidence rate per 100,000					
Any cancer‡	516	1825	1404	2195	1873
Solid cancers‡	152	1431	1140	1749	1332
Lymphoma#	364	397	273	446	541
Age- and sex-standardised incidence rate (95% CI)					
Any cancer‡	468 (233–702)	2060 (944–3176)	1149 (889–1409)	3038 (1534–4542)	2268 (1371–3165)
Solid cancers‡	156 (14–299)	1701 (588–2814)	931 (696–1166)	2508 (1022–3995)	1714 (860–2569)
Lymphoma#	311 (124–498)	361 (272–450)	222 (111–334)	530 (299–761)	553 (271–836)
Standardised incidence ratio (95% CI)					
Any cancer‡	23.8 (13.8–38.0)	2.54 (2.32–2.78)	2.29 (1.94–2.69)	2.69 (2.36–3.04)	4.28 (3.49–5.19)
Solid cancers‡	51.3 (16.6–120)	2.26 (2.03–2.49)	2.04 (1.69–2.43)	2.35 (2.04–2.70)	3.36 (2.63–4.22)
Lymphoma#	88.5 (45.7–154)	7.82 (6.33–9.53)	5.60 (3.59–8.33)	6.99 (5.02–9.48)	16.8 (11.1–24.4)

† Liver and heart transplantations only; §Lung or combined heart and lung transplantation; ‡Excluding BCC and SCC of the skin; #Includes non-Hodgkin lymphoma, Hodgkin lymphoma and lymphoma not otherwise specified.

Cancer risk relative to the general population: by recipient age

I observed 17 cases of cancer, including 10 NHL, in 415 paediatric recipients (data not shown), resulting in SIRs exceeding 20 and 80 for any cancer and lymphoma, respectively (Table 3 – 2, page 80). Cancer of the vulva, colorectum, and breast all occurred at significantly increased risk. The median age at diagnosis was 7 years (IQR 6–17) for lymphoma and 30 years (IQR 16–32) for solid cancer (data not shown).

When I considered age at diagnosis for all recipients combined, I observed a significant excess risk of any cancer, lip cancer, NHL, and melanoma for all age groups (Table 3 – 3, page 82). I found a significant excess risk for colorectal cancer in the 0–39 year age group, cancer of unknown primary site in the >40 year age group, and skin and lung cancer in the >55 year age group.

The pattern of cancer risk by transplanted organ was similar, with some noteworthy exceptions (Figure 3-3, page 83).Risk of NMSC (other than BCC and SCC) was significantly elevated in both heart (SIR = 32.8, 95% CI 17.9–55.0) and lung (SIR = 61.4, 95% CI 24.7–126) recipients, but there were no incident cases in liver recipients. Seventeen of the 21 skin cancers in cardiothoracic patients were Merkel cell carcinoma (MCC; SIR = 103, 95% CI 60.4–166). In contrast, melanoma risk was significantly increased in liver (SIR = 2.13, 95% CI 1.22–3.46) and heart (SIR = 3.04, 95% CI 2.03–4.36) but not lung (SIR = 1.64, 95% CI 0.53–3.83) recipients. Colorectal cancer risk was significantly raised in liver (SIR = 2.40,

95% CI 1.49–3.68) and lung (SIR = 2.58, 95% CI 1.12–5.09) recipients, but not in heart (SIR = 0.99, 95% CI 0.54–1.63). Only heart and lung transplant recipients exhibited significantly elevated risk of lung cancer (SIR = 2.18, 95% CI 1.39–3.22 and SIR = 3.82, 95% CI 1.65–3.53, respectively). When recipients with a history of cancer were excluded from the cohort the key findings were unchanged (data not shown).

Table 3 - 3. Cancer risk by age at diagnosis for the most frequent incident cancers in Australian liver, heart and lung transplant recipients

Cancer type	Age 0–39 years				Age 40–55 years				Age >55 years			
	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI
All cancers[1]	59	5.14	11.5	8.74–14.8	131	37	3.6	2.99–4.22	310	148	2.1	1.87–2.33
Lip	2	0.13	15.9	1.92–57.3	24	0.7	35	22.7–52.7	32	1.5	21	14.3–29.6
Skin[2]	1	0.04	23.7	0.60–132	1	0.2	5.5	0.14–30.6	19	0.6	30	18.2–47.2
Non-Hodgkin lymphoma	24	0.42	56.8	36.4–84.5	38	2.6	15	10.5–20.5	38	8.9	4.3	3.01–5.84
Unknown primary site	1	0.05	18.4	0.47–103	5	0.8	6.2	2.02–14.5	19	3.8	5.1	3.05–7.91
Melanoma	7	1.29	5.42	2.18–11.2	14	5.8	2.4	1.33–4.07	29	13	2.2	1.51–3.14
Lung	0	-	-	-	5	2.5	2	0.66–4.74	28	16	1.8	1.19–2.50
Colorectal[3]	3	0.21	14.1	2.91–41.2	9	4.1	2.2	1.00–4.16	30	21	1.5	0.99–2.03
Prostate	0	-	-	-	1	2.6	0.4	0.01–2.12	33	35	1	0.66–1.31

Abbreviations: Obs = observed number of cancers; Exp = expected number of cancers; SIR = standardised incidence ratio; [1]Excluding BCC and SCC of the skin; [2]NMSC, excluding BCC and SCC of the skin; [3]Excluding anal cancer.

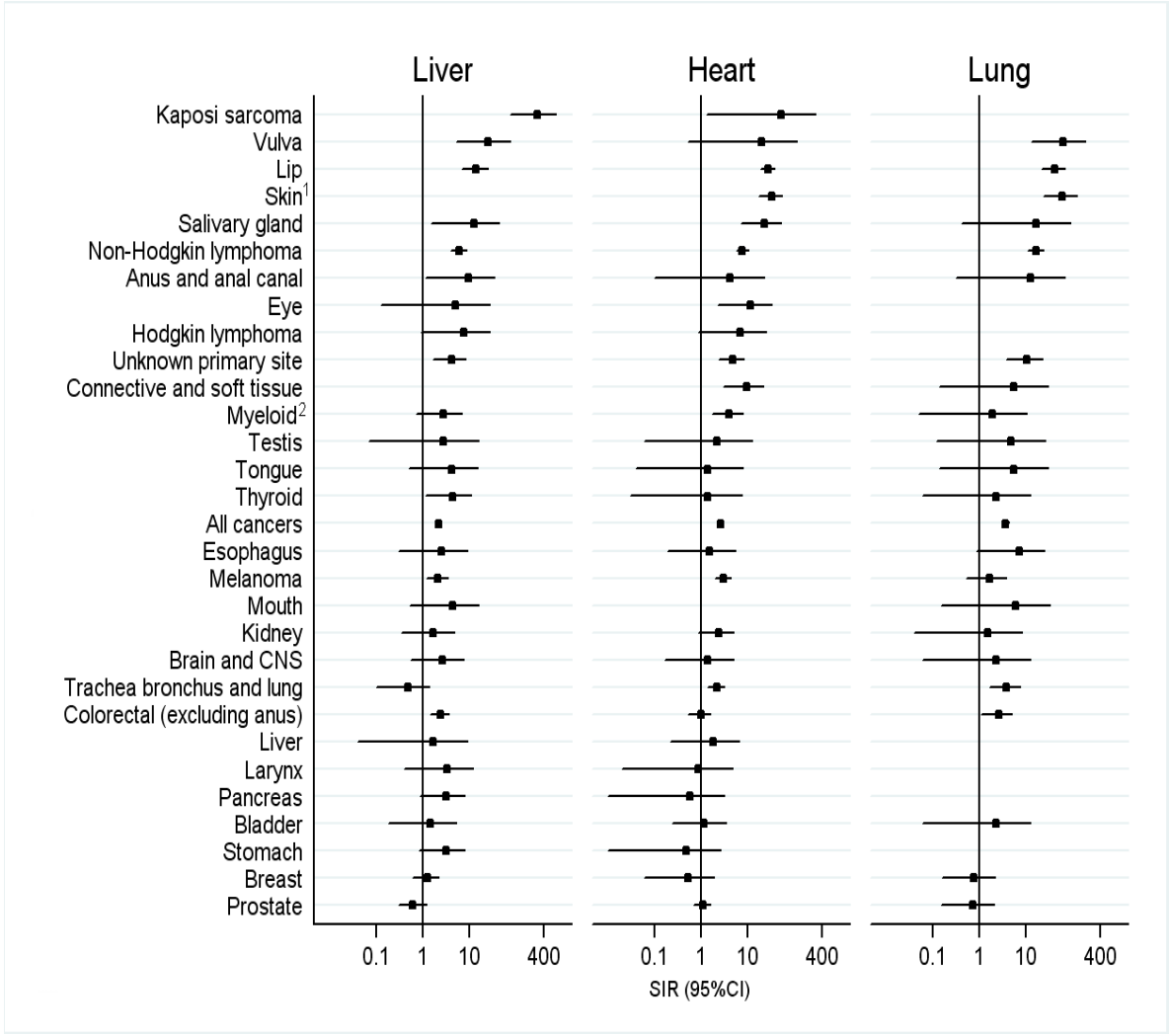


Figure 3 - 3. Site-specific cancer risk by transplanted organ relative to the general population

[1]NMSC, excluding BCC and SCC of skin; [2]Myeloid neoplasms including lymphoid/myeloid not otherwise specified.

Cancer risk relative to the general population: by indication for transplantation

Cancer risk was significantly increased regardless of the indication for transplantation, except for those receiving a liver on account of a hepatobiliary tumour (n = 93). After liver transplantation, colorectal cancer risk was increased in those with autoimmune-related liver disease (n = 12; SIR = 4.49, 95% CI 2.32–7.84) but not in those without (SIR = 1.48, 95% CI 0.68–2.82). Similarly, it was increased in those with PSC (n = 10; SIR = 9.58, 95% CI 4.59–17.6) but not in those without (SIR = 1.43, 95% CI 0.71–2.56), and in those with PSC and UC (n = 5; SIR = 12.5, 95% CI 4.06–29.1), but not in those without (SIR = 1.32, 95% CI 0.63–2.42) (data not shown).

Table 3 - 4. Cancer risk by indication for transplantation in Australian liver, heart and lung transplant recipients

Transplanted organ(s)	Primary indication	All cancers§			
		Obs	Exp	SIR	95% CI
Liver	Viral hepatitis	30	17	1.8	1.22–2.50
	Hepatobiliary tumour	2	2.1	0.9	0.11–3.39
	Autoimmune-related liver disease	60	21	2.8	2.15–3.56
	Alcoholic liver disease	24	13	1.9	1.24–2.77
	Congenital biliary disease	4	0.5	7.8	2.13–20.0
	Miscellaneous liver disease	33	16	2.1	1.44–2.85
Cardiothoracic	Non-ischemic cardiomyopathy	105	36	2.9	2.36–3.47
	Ischaemic heart disease	116	50	2.3	1.91–2.78
	Congenital heart disease	12	2.1	5.7	3.04–9.51
	Obstructive lung disease	23	3.6	6.4	4.03–9.53
	Chronic obstructive pulmonary disease	35	13	2.8	1.96–3.81
	Miscellaneous cardiothoracic disease	55	16	3.5	2.63–4.46

Abbreviations: Obs, observed number of cancers; Exp, expected number of cancers; SIR, standardised incidence ratio; CI, confidence interval; § excluding BCC and SCC of the skin;

Comparison of cancer risk by transplanted organ

After adjustment, the risk of any cancer and of lymphoma was significantly greater for heart and lung recipients compared to liver recipients (Table 3-5, page 85) The risk of solid cancer was increased in lung compared to liver recipients (HR 1.61, 95%CI 1.17–2.21) and also in lung compared to heart transplant recipients (HR 1.41, 95% CI 1.03–1.93, data not shown). Paediatric heart recipients were at higher risk of any cancer (HR 3.10, 95% CI 1.01–9.47) and lymphoma (HR 6.67, 95% CI 2.37–18.8) compared to liver recipients (data not shown). The results for adult recipients were comparable with those for the entire cohort (data not shown).

Table 3 - 5. Risk factors for cancer in Australian recipients of liver and cardiothoracic transplants

Variable	Adjusted hazard ratio (95% CI)§		
	All cancers‡	All solid cancers	All lymphomas†
Age at first transplantation (per year)	1.04 (1.03–1.05)	1.05 (1.04–1.06)	1.00 (0.98–1.01)
Sex			
Male	Ref	Ref	Ref
Female	0.80 (0.63–1.00)	0.74 (0.56–0.97)	0.99 (0.63–1.56)
Number of transplants#			
One	Ref	Ref	Ref
More than one	0.78 (0.36–1.69)	0.56 (0.21–1.50)	1.09 (0.34–3.45)
Transplanted era			
1984–1989	0.96 (0.72–1.29)	1.15 (0.83–1.59)	0.51 (0.25–1.07)
1990–1997	Ref	Ref	Ref
1998–2006	0.73 (0.57–0.94)	0.73 (0.55–0.97)	0.69 (0.42–1.12)
Transplanted organ			
Liver	Ref	Ref	Ref
Heart	1.29 (1.03–1.63)	1.15 (0.88–1.49)	1.89 (1.14–3.14)
Lung	1.66(1.27–2.17)	1.61 (1.17–2.21)	2.10 (1.25–3.54)

Abbreviations: CI, confidence interval; §Adjusted for age at transplant (single years), sex, number of transplants (as a time-dependent covariate), and calendar year at first transplantation; ‡ Excludes BCC and SCC of the skin; †Includes non-Hodgkin lymphoma, Hodgkin lymphoma and lymphoma not otherwise specified; #Time dependent.

3.5 Discussion

In this study, I confirmed that compared to the general population, the risk of cancer is increased for recipients of all studied transplanted organs, 2.2-fold for liver, 2.6-fold for heart, and 3.7-fold for lung. The pattern of site-specific risk by transplanted organ was broadly similar, confirming the critical role of immunosuppression in cancer risk after transplantation. Exceptions to this pattern suggest patient subgroups at high risk for specific cancer types. I observed a significantly higher risk of any *de novo* cancer for both heart and lung transplant recipients compared to liver transplant recipients after adjusting for age, sex, multiple transplantations, and transplant era. An increased risk was also observed for lymphomas and solid cancers, but the excess risk of solid cancers was restricted to lung recipients compared to both liver and heart recipients. My findings merit further study to understand the factors responsible for the organ-specific differences in cancer risk, particularly with respect to the degree of immunosuppression.

Population-based evidence on the relative risk of cancer in recipients of different types of solid organ is limited. A Swedish study showed an increased risk of NHL for liver, heart and lung transplant recipients relative to kidney transplant recipients, after adjustment for age, sex, year and follow-up time (Fernberg et al. 2011). My 2-fold higher risk of lymphoma in heart and in lung recipients compared to liver recipients is consistent with findings the higher risk of lymphoma in heart and lung recipients in the Collaborative Transplant Study (Opelz and Dohler 2004). My study presents the first population-based

evidence of an excess risk of any cancer in heart and lung recipients compared to liver recipients, and a significant increased risk of solid cancer in lung compared to heart and liver transplant recipients.

The hypothesised explanation for the difference in cancer risk by transplanted organ is variation in the intensity or type of immunosuppression (Euvrard et al. 1995, Ong et al. 1999, Collett et al. 2010). Several studies have found an association between post-transplantation cancer risk and receipt of induction therapy with lymphocyte depleting antibodies or maintenance immunosuppression with specific agents (Swinnen et al. 1990, Opelz and Henderson 1993, Dantal et al. 1998, Opelz and Dohler 2004, van Leeuwen et al. 2009, Neto et al. 2012). There are no published data directly comparing the dose and type of immunosuppressive agents for liver, heart and lung transplant recipients in Australia. However, Australian clinical transplantation practice has generally followed international trends, with a lower prevalence of induction therapies and lower overall immunosuppressive dose for liver transplant recipients compared to heart and lung recipients (Collett et al. 2010, Wiesner and Fung 2011).

In addition to differences in the extent and type of immunosuppression by transplanted organ, other factors may also play a role, either independent of or interacting with immunosuppression. These include patient factors, such as prevalent or acquired infection by carcinogenic agents, autoimmune disease, carcinogenic behaviours, and genetic predisposition to cancer, as well as inherent biological differences in the transplanted

tissue. A greater volume of lymphoid tissue in the lung compared to other organs, and thus greater potential for the transmission of donor lymphocytes infected with Epstein-Barr virus (EBV), has been suggested to explain the higher NHL risk in lung compared to liver transplant recipients (Cockfield 2001).

When I considered the liver and cardiothoracic transplant recipients together, my data agree with prior evidence showing a wide-ranging excess cancer risk relative to the general population (Grulich et al. 2007, Engels et al. 2011), especially cancers with a viral cause. The pattern of site-specific cancer risk by transplanted organ was broadly similar, and also largely consistent with prior population-based evidence for liver (Jain et al. 1998, Haagsma et al. 2001, Oo et al. 2005, Aberg et al. 2008, Jiang et al. 2008, Finkenstedt et al. 2009, Baccarani et al. 2010, Collett et al. 2010, Engels et al. 2011, Krynitz et al. 2013) and heart (Kellerman et al. 2009, Collett et al. 2010, Jiang et al. 2010, Engels et al. 2011, Krynitz et al. 2013), and lung transplantation (Engels et al. 2011, Krynitz et al. 2013). My study adds to existing evidence showing an increased risk of NHL, Kaposi sarcoma, colorectal cancer, lip cancer and cancer of unknown primary site after liver transplantation. My novel findings were an excess risk of cutaneous melanoma and cancer of the thyroid, vulva, anus and salivary gland. I did not confirm previously published findings of an excess risk of cancer of the liver (Engels et al. 2011), lung (Oo et al. 2005, Finkenstedt et al. 2009, Collett et al. 2010), oral cavity (Collett et al. 2010), or kidney (Haagsma et al. 2001) after liver transplantation.

My estimate for colorectal cancer risk after liver transplantation (SIR = 2.40, 95% CI 1.49–3.68) is similar to a meta-estimate for prior population-based studies (SIR = 2.6, 95% CI 1.7–4.1) (Sint Nicolaas et al. 2010). PSC is an indication for liver transplantation, and 60-80% of individuals with PSC also have inflammatory bowel disease, predominantly UC. It is established that patients with UC are at high risk of colorectal cancer (Lieberman 2009, Danese and Fiocchi 2011), and findings from a single retrospective study suggest that transplantation may not alter this inherently high risk (Hanouneh et al. 2012). In my study, an excess risk of colorectal cancer was confined to liver transplant recipients with a history of PSC and UC. My data support the continued screening of these patients to enable the early diagnosis of UC and colorectal adenomas.

Consistent with prior population-based studies (Collett et al. 2010, Jiang et al. 2010, Engels et al. 2011), I confirmed an excess risk of NHL, lip cancer and lung cancer in heart transplant recipients. However, I did not observe an increased risk of kidney (Collett et al. 2010, Jiang et al. 2010, Engels et al. 2011), oral cancer (Collett et al. 2010, Jiang et al. 2010) or multiple myeloma (Collett et al. 2010, Jiang et al. 2010). I confirmed the excess risk of cutaneous melanoma in Australian heart transplant recipients (Roithmaier et al. 2007). My novel findings for heart recipients were an elevated risk of MCC, myeloid neoplasms, and cancer of the salivary gland, eye, and unknown primary site. The increased risk of MCC, a neuroendocrine skin cancer possibly associated with infection by Merkel cell polyomavirus is in broad agreement with the only prior estimate post-transplantation, an SIR of 66 for Finnish kidney transplant recipients (Koljonen et al. 2009).

The recent report of an excess risk of Merkel cell carcinoma in US HIV/AIDS patients (SIR = 11, 95% CI 6.3–17) (Lanoy et al. 2009) supports an association between risk of this cancer and immunosuppression. However, the absence of MCCs in my cohort of liver transplant recipients suggests that MCC risk may not be related to immunosuppression *per se*, and that the intensity or types of immunosuppression or other factors are likely to be important.

In this study, I found that lung transplantation was associated with an increased risk of NHL, MCC and cancer of the vulva, lip, lung, colorectum, and unknown primary site. These findings thus confirm prior population-based evidence of an excess risk of NHL and lung cancer (Collett et al. 2010, Engels et al. 2011), but not anal cancer (Collett et al. 2010).

There are scarce data on cancer risk in paediatric liver and heart transplant recipients (Aberg et al. 2008, Simard et al. 2011). I found an increased risk for NHL, skin cancer and vulvar cancer, concurring with a Swedish paediatric cohort consisting mostly of kidney transplant recipients (Simard et al. 2011). However, the excess risk of colorectal cancer, breast cancer and Hodgkin lymphoma that I observed has not previously been reported, and requires validation in larger cohorts. Notably, most solid cancers occurred in adulthood, highlighting the need for increased clinical surveillance during this phase of life. The striking increased risk of NHL in childhood has consistently been observed (Villeneuve et al. 2007, Jiang et al. 2010) and has been associated with EBV seroconversion (Katz et al. 2007) and intensity of immunosuppression (Schubert et al. 2008). As a result,

anti-viral prophylaxis and targeted monitoring of EBV viral load in peripheral blood is recommended for high-risk paediatric patients (Lee et al. 2005).

Several potential limitations must be considered when interpreting my findings. I could not estimate the risk of BCC and SCC of the skin because these neoplasms are not recorded by all Australian cancer registries; consequently, my estimates for 'any cancer' risk exclude these cancers. In addition, it is possible that a small number of cancers were donor-derived. Moreover, I relied upon record linkage between transplant registries and routinely collected administrative data, and some false positive and negative linkages do occur. Nevertheless, the record linkage is highly sensitive and specific (Grulich et al. 1996), and linkage errors are unlikely to occur differentially by transplanted organ or cancer type. On the other hand, a potential bias would arise if there was differential participation in national cancer screening programs (breast, cervical and colorectal cancer) by transplant recipients compared to the general population, or by transplanted organ. Whilst there is some evidence that Australian kidney transplant recipients undergo more cancer screening (Wong et al. 2009), with the exception of individuals with PSC, there is no data on screening rates for liver, heart and lung transplant recipients. Furthermore, surveillance bias is unlikely to explain differences between transplanted organs in the risk of other solid cancers or of lymphomas. Whilst linkage accuracy is known, there has been no formal validation of linkage completeness, and I was unable to censor upon migration from Australia. Nevertheless, given the high quality of Australian cancer registries (Parkin et al. 2002), I expect to have identified most incident cancers. In addition, my study period

covered the years from 1984 to 2006, and, therefore, it may not be feasible to generalise the results to reflect current trends of cancer incidence in this population due to changes in clinical practice, such as immunosuppressive therapy. Whist population-based cancer incidence is not available for recent years, my findings of the pattern of overall and site-specific cancer risk in my cohort are in accordance with population-based studies, regardless of transplant eras or countries (Grulich et al. 2007, Engels et al. 2011, Krynitz et al. 2013). Finally, no adjustment for multiple statistical tests was carried out, and thus the possibility of chance finding cannot be excluded.

The key strengths of my study are the population-basis for inclusion of transplant recipients and for ascertaining deaths and cancers. The use of identical methods for transplant recipients, the general population, and for the different transplanted organs, enabled unbiased comparison of risk and also minimised the influence of selection bias and loss-to-follow-up. I included site-specific cancer risk estimates for the largest population-based series of non-kidney paediatric transplant recipients published to-date. The relatively large population size also allowed me to estimate the risk of some rare cancers, such as MCC. Finally, the systematic recording of indication for transplantation by the transplant registries allowed insight into patient subgroups at high risk of cancer.

3.6 Conclusions

I found evidence of a higher risk of cancer in heart and lung compared to liver transplant recipients in Australia. Understanding the factors responsible for these associations is

expected to lead to strategies to help reduce the cancer burden facing this high-risk patient group. Knowledge of the cancer profile by transplanted organ and patient age will facilitate early detection and improve patient outcomes.

Supplementary Tables

Supplementary Table 3 - 1 Site-specific SIRs for Australian liver, heart and lung transplant recipients by transplant type

Cancer site	Liver			Heart			Lung		
	Obs	SIR	95% CI	Obs	SIR	95% CI	Obs	SIR	95% CI
Kaposi sarcoma	4	290	79.1–743	1	51.9	1.31–289	0	–	–
Vulva	3	26	5.35–75.8	1	20.4	0.52–114	3	65	13.4–190
Lip	11	14	7.00–25.1	34	27.5	19.0–38.4	13	41.9	22.3–71.6
Skin§	0	–	–	14	32.8	17.9–55.0	7	61.4	24.7–126
Merkel cell carcinoma	0	–	–	13	93.5	49.8–160	4	160	43.6–409
Salivary gland	2	13	1.51–45.0	5	22.7	7.39–53.1	1	16.7	0.42–92.8
Non-Hodgkin lymphoma	27	6.2	4.05–8.95	46	7.8	5.71–10.41	27	16.8	11.1–24.4
Multiple myeloma	0	–	–	1	0.95	0.02–5.28	0	–	–
Anus and anal canal	2	9.7	1.18–35.2	1	4.11	0.10–22.9	1	12.6	0.32–70.1
Eye	1	5.1	0.13–28.3	3	11.5	2.37–33.5	0	–	–
Hodgkin lymphoma	2	7.7	0.93–27.8	2	7.07	0.86–25.5	0	–	–
Unknown primary site	7	4.2	1.68–8.59	12	4.76	2.46–8.31	6	10.7	3.92–23.2
Connective and soft tissue	0	–	–	5	9.53	3.10–22.2	1	5.55	0.14–30.9
Myeloid	4	2.8	0.75–7.04	8	4.03	1.74–7.94	1	1.94	0.05–10.8
Testis	1	2.8	0.07–15.8	1	2.24	0.06–12.5	1	4.81	0.12–26.8
Tongue	2	4.2	0.51–15.1	1	1.41	0.04–7.88	1	5.59	0.14–31.2
Thyroid	4	4.4	1.19–11.2	1	1.38	0.03–7.70	1	2.3	0.06–12.8
All cancers§	153	2.2	1.87–2.57	249	2.64	2.32–2.98	97	3.7	3.01–4.48
Oesophagus	2	2.5	0.31–9.10	2	1.54	0.19–5.56	2	7.19	0.87–26.0
Melanoma	16	2.1	1.22–3.46	29	3.04	2.03–4.36	5	1.64	0.53–3.83
Mouth	2	4.5	0.54–16.1	0	–	–	1	5.92	0.15–33.0
Liver	1	1.7	0.04–9.25	2	1.85	0.22–6.69	0	–	–
Kidney	3	1.7	0.35–4.94	6	2.36	0.87–5.14	1	1.51	0.04–8.40
Brain and CNS	3	2.7	0.55–7.74	2	1.37	0.17–4.96	1	2.25	0.06–12.6
Trachea bronchus and lung	3	0.5	0.10–1.42	22	2.18	1.39–3.22	8	3.82	1.65–3.53
Colorectal (excluding anus)	21	2.4	1.49–3.68	13	0.99	0.54–1.63	8	2.58	1.12–5.09
Larynx	2	3.3	0.40–12.0	1	0.86	0.02–4.80	0	–	–
Pancreas	4	3.2	0.88–8.23	1	0.57	0.01–3.18	0	–	–
Bladder	2	1.5	0.18–5.34	3	1.17	0.24–3.43	1	2.3	0.06–12.8
Stomach	4	3.1	0.85–7.99	1	0.47	0.01–2.61	0	–	–
Breast	11	1.3	0.62–2.23	2	0.53	0.06–1.91	3	0.76	0.16–2.22
Prostate	7	0.6	0.27–1.19	24	1.1	0.71–1.60	3	0.73	0.15–2.14

[1]NMSC, excluding BCC and SCC of skin; [2]Myeloid neoplasms including lymphoid/myeloid not otherwise specified.

Chapter 4 De novo cancer-related death in Australian liver, heart and lung transplant recipients

4.1 Objectives

In this Chapter, I describe pattern of death from *de novo* cancer in Australian liver, heart and lung transplant recipients. The objective of this Chapter is to examine the risk of overall and site-specific *de novo* cancer-related death in liver, heart, and lung transplant recipients.

4.2 Introduction

Immunosuppressive therapy after solid organ transplantation is widely reported to be associated with a 2- to 3-fold increased risk of cancer (Vajdic et al. 2006, Grulich et al. 2007, Collett et al. 2010), as supported by my findings reported in Chapter 3. Whilst recipients' short-term survival has been remarkably improved, cancer has become one of the leading causes of death for recipients with a functioning graft for all transplanted organs (ANZCOTR 2012, ANZLTR 2012, Christie et al. 2012, OPTN/SRTR 2012, Stehlik et al. 2012). However, comparatively few studies have quantified the risk of death from cancer in this population as summarised in Chapter 2 (Table 2 - 3, page 29) (Herrero et al. 2005, Lindelof et al. 2006, Kiberd et al. 2009, Foster et al. 2011, Cheung et al. 2012). Furthermore, previous studies did not exclude deaths from recurrent cancers (Kiberd et al. 2009, Foster et al. 2011, Cheung et al. 2012), and were limited by a large proportion of deaths of unknown cause (Kiberd et al. 2009, Foster et al. 2011). Documenting the extent and pattern of risk for *de novo* cancer-related death

in transplant recipients will guide the identification of high-risk patient subgroups, potentially leading to further improvements in long-term survival.

4.3 Methods

4.3.1 Study population

My cohort for this study was described in Chapter 3. Briefly, transplant recipients were registered on the ANZLTR or the ANZCOTR. These national population-based transplantation registries recorded all liver and cardiothoracic transplantations since 1985 and 1984, respectively, and systematically record demographic and clinical data about each recipient.

I included recipients with single (n = 4482) and second or and higher-order transplants (n = 162) in the analyses. I excluded non-Australian recipients and those who died within 30 days of transplantation (n = 287) from the study population. I did not exclude recipients with a history of cancer prior to transplantation (n = 367), including those whose indication for transplantation was a hepatobiliary tumour (n = 93), since these recipients were at risk of death from a different *de novo* cancer. However, these patients did not contribute person-years at risk for death from these cancers as I only sought to estimate the risk of death from cancers diagnosed after transplantation (i.e. *de novo* cancers). I performed a sensitivity analysis to assess the impact of excluding patients with a history of cancer (prior to 30-days post-transplantation). I classified patients who received a combined liver and kidney transplant (n = 23), and those who received a combined liver, heart and lung transplant (n = 3), as liver transplant

recipients; I classified those who received a combined heart and lung transplant (n = 137) as lung transplant recipients.

4.3.2 Data collection

I identified deaths by record linkage with the NDI, a registry of all deaths in Australia since 1980, or from the transplant registry. The underlying cause of death was available for deaths ascertained from the NDI, coded to the 9th International Classification of Diseases, 9th revision (ICD-9), between 1984 and 1997, and ICD-10 from 1998 onwards. Deaths identified from the transplant registries alone had an unknown underlying cause and thus could not be included in cause-specific analyses. I ascertained cancers by record linkage with the ACD, a register of incident primary invasive neoplasms in Australian residents. I established the date of diagnosis, topography and morphology for each cancer diagnosed between 1984 and 2006. The record linkage was performed by the Australian Institute of Health and Welfare utilising an established probabilistic record (Jaro 1995).

I obtained Australian population mortality rates for any cancer and site-specific cancers from the Australian Institute of Health and Welfare by five-year age group, sex, calendar year, and State or Territory, for 1984 to 2006.

I obtained ethical approval and the requirement for informed participant consent was waived because I received only de-identified data.

4.3.3 Data management

Classification of underlying cause of death

The underlying cause of death recorded on Australian death certificates is defined as "the disease or injury which initiated the train of morbid events leading directly to death, or the circumstances of the accident or violence which produced the fatal injury". I classified the cause of each death according to homogeneous groups defined by the Australian Bureau of Statistics and in accordance with WHO-ICD rules (Benden et al. 2011). I classified deaths as cancer-related, non-cancer related, or unknown cause. I considered cancer-related deaths as due to recurrent cancer if the cancer was diagnosed prior to transplantation or during the 30 day-post-transplantation period. Cancers diagnosed during this period were almost certainly prevalent at transplantation and some were not registered until they were histopathologically diagnosed in the explanted organ. If the cancer attributed to the death was diagnosed more than 30 days after transplantation then I considered the cause of death to be a *de novo* cancer. Some patients (n = 48) died from cancer but did not have a linked registered cancer, or they were registered with a cancer that was not attributed to their death. In these cases, if the cause of death was a liver cancer and there was a history of gallbladder cancer prior to transplantation or within 30 days of transplantation, I classified the death as attributable to a recurrent cancer (n = 2). I classified all other cancer-related deaths, including those due to non-melanocytic skin cancer (hereafter called skin cancer; C44, n = 14), which is not recorded by the Australian cancer registries, as *de novo* cancer deaths.

4.3.4 Data analysis

Mortality rates

I included all deaths in the calculation of overall mortality rates, while I excluded deaths due to an unknown cause (n = 69) from the cause-specific mortality rates. Person-years follow-up accrued from 30 days post transplantation until the date of death, age 80, or 31st December 2006, whichever occurred first. I calculated crude and age- and sex-standardised overall and cause-specific mortality rates (ASMR), standardised to the 1996 Australian population, and 95% CIs (based on the normal approximation to the binomial distribution) using annual Australian population estimates from the Australian Bureau of Statistics. I computed cause-specific mortality rates by transplanted organ for any cancer, cardiovascular disease, respiratory disease, endocrine disease, nutritional and metabolic diseases, digestive disease, and infectious disease.

Survival analyses

I applied the Kaplan-Meier method to estimate the unadjusted overall survival probabilities by transplanted organ and compared these using the log-rank test. I computed survival probabilities from 30-days after transplantation to the date of death or 31st December 2006. I also calculated the cumulative incidence of *de novo* cancer deaths treating other causes of death as a competing risk.

De novo cancer mortality risk relative to general population

I compared *de novo* cancer mortality rates in transplant recipients with those for the Australian general population using the standardised mortality ratio (SMR), defined as the ratio of the observed and the expected numbers of cancer-related deaths. I used the likelihood ratio method to calculate 95% CIs for deaths with more than 10

expected cases, and exact CIs for deaths with less than 10 expected cases (Swift 2009). I calculated the expected numbers of cancer deaths by multiplying cohort person-years at risk by the corresponding five-year age-, sex-, state- and calendar year-specific cancer mortality rates for the Australian population. I computed SMRs for the entire cohort and by transplanted organ, sex, and age at transplantation (tertiles). As for Chapter 3, I did not compare SMRs statistically because of the heterogeneity in subgroup age and sex distributions (Rothman et al. 2008; p.254).

I performed analyses using SAS® software v9.3 (SAS Institute Inc., Cary, NC, USA) and STATA statistical software v11.2 (StataCorp, Texas, USA).

4.4 Results

My eligible cohort comprised 4644 transplant recipients; 1926 (41%) liver, 1518 (33%) heart, and 1200 (26%) lung, as described in Chapter 3. The median duration of follow-up was 5.2 years (interquartile range, IQR 2.0–9.9), and 1,558 deaths were observed over a total of 29,713 person-years (Table 4-1, page 102). The median age at death was 53 years (IQR 41–60). I observed the highest number of deaths from any cause in lung transplant recipients (n = 590), followed by heart (n = 564), and liver (n = 404) recipients. There were 77 deaths in 415 paediatric liver and heart transplant recipients; the median age at death was 14 years (IQR 4–18), and most deaths occurred either during the first year after transplantation (n = 28, 36.4%) or more than five years post-transplant (n = 28, 36.4%; data not shown).

Underlying cause of death

I observed a total of 1,265 non-cancer related deaths, led by cardiovascular disease (n = 410, 32.4%), respiratory disease (n = 235, 18.6%), and endocrine, nutritional and metabolic disease (n = 168, 13.3%). The leading causes of death corresponded to the underlying cause of end-stage organ disease, that is, digestive disease for liver transplantation, cardiovascular disease for heart transplantation, and respiratory disease for lung transplantation.

A total of 224 transplant recipients died of cancer; 171 (11%) deaths were de novo cancer deaths and 53 (3.4%) were recurrent cancer deaths (Table 4-1, page 102). The median time between the first registered cancer and de novo cancer-related death was 7.1 months (IQR 1.2–24) (data not shown). Non-Hodgkin lymphoma (NHL; n = 38, 22.2%) was the most common cause of de novo cancer death, followed by cancer of unknown primary site (n = 25, 14.6%); (Figure 4-2, page 106). For those who survived more than 5 years after transplantation (n = 2395, 51.6%), de novo cancer was the 2nd leading cause of death in heart (n = 72), the 3rd leading cause of death in liver (n = 26), and the 4th leading cause of death in lung (n = 16) transplant recipients (Table 4-3, page 104). Five de novo cancer deaths occurred in paediatric transplant recipients, and the median time between transplantation and death was 6.6 years (IQR 6.4–7.6). De novo cancer was the leading (liver recipients) or the 2nd leading (heart recipients) cause of death for the 248 paediatric patients who survived more than five years after transplantation (Table 4-2, page 103).

Of the 53 recurrent cancers (23% of all cancer-related deaths), 41 were recurrent liver cancers in liver transplant recipients. The median time between the first liver

transplantation and recurrent liver cancer related death was 1.8 years (IQR 0.8–3.7) (data not shown).

Table 4 - 1. Characteristics of total deaths in Australian liver, heart and lung transplant recipients by transplanted organ

Characteristics§	Total N‡ (%)	Liver N‡ (%)	Heart N‡ (%)	Lung N‡ (%)	*p**
Total number of cohort	4644 (100)	1926 (100)	1518 (100)	1200 (100)	
Number of death	1558 (33.5)	404(21.0)	564(37.1)	590(49.2)	
Age at death, median (q1-q3)	53 (41-60)	52(43-59)	57(46-63)	51(36-58)	<0.001
Age group					<0.001
Paediatric	77 (4.9)	40 (9.9)	29 (5.1)	8 (1.4)	
Adult	1481 (95.1)	364 (90.1)	535 (94.9)	582 (98.6)	
Sex					<0.001
Male	1048 (67.3)	266(65.8)	467(82.8)	315(53.4)	
Female	510 (32.7)	138(34.2)	97(17.2)	275(46.6)	
Transplant era					<0.001
1984-1991	402 (25.8)	112 (27.7)	237 (42.0)	53 (9.0)	
1992-1996	599 (38.5)	136 (33.7)	214 (37.9)	249 (42.2)	
1997-2006	557 (35.7)	156 (38.6)	113 (20.0)	288 (48.8)	
Recipient race					<0.001
Caucasian	1376 (93.2)	315 (82.9)	491 (95.5)	570 (97.9)	
Non-Caucasian	100 (6.8)	65 (17.1)	23 (4.5)	12 (2.1)	
Recipient country of birth					<0.001
Australian or New Zealand	889 (72.0)	1329 (69.7)	842 (71.9)	546 (79.7)	
Europe	239 (19.4)	343 (18.0)	226 (19.3)	104 (15.2)	
Asia	55 (4.4)	164 (8.6)	56 (4.8)	15 (2.2)	
Other	52 (4.2)	72 (3.8)	47 (4.0)	20 (2.9)	
Multiple transplant					<0.001
No	1499 (96.2)	354 (87.6)	559 (99.1)	586 (99.3)	
Yes (≥2)	59 (3.8)	50 (12.4)	5 (0.9)	4 (0.7)	
Cancer history					<0.001
No	1438 (92.3)	314 (77.7)	555 (98.4)	569 (96.4)	
Yes	120 (7.7)	90 (22.3)	9 (1.6)	21 (3.6)	
Primary indication for transplantation					NA
Cardiomyopathy	245(15.7)	-	245(43.4)	-	
Ischaemic heart disease	246(15.8)	-	246(43.6)	-	
COPD	186(11.9)	-	-	186(31.5)	
Obstructive lung disease	170(10.9)	-	-	170(28.8)	
Congenital heart disease	44 (2.8)	-	16(2.9)	28(4.8)	
Viral hepatitis	111(7.1)	111(27.5)	-	-	
Autoimmune disease	104(6.7)	104(25.7)	-	-	
Alcoholic liver disease	44 (2.8)	44 (10.9)	-	-	

Hepatobiliary tumor	36(2.3)	36(8.9)	-	-	
Congenital biliary disease	22(1.4)	22(5.5)	-	-	
Miscellaneous	350 (22.5)	87(21.5)	57(10.1)	206(34.9)	
Underlying cause of death					<0.001
Diseases of the heart and blood vessels	410 (26.3)	40(9.9)	321(56.9)	49(8.3)	
Diseases of the respiratory system	235 (15.1)	4 (1.0)	4 (0.7)	227(38.5)	
Endocrine, nutritional and metabolic diseases	168 (10.8)	15(3.7)	10(1.8)	143(24.2)	
Diseases of the digestive system	127 (8.1)	110(27.2)	8 (1.4)	9 (1.5)	
De novo cancer	171 (11.0)	42(10.4)	97 (17.2)	32 (5.4)	
Recurrent cancer	53 (3.4)	45 (11.1)	2 (0.3)	6 (1.0)	
Infectious disease	84 (5.4)	54(13.4)	18(3.2)	12 (2.0)	
All others	310 (20.0)	94 (23.3)	104 (18.4)	112 (19.0)	

Abbreviations: COPD, Chronic obstructive pulmonary disease; § Continuous variables presented as median (interquartile range) and categorical variables presented as n (%); ‡ The counts in sub-categories may not add up to the total number due to missing data; *Non-parametric Wilcoxon rank-sum test, chi-square test or Fisher exact test as appropriate.

Table 4 - 2. Site-specific cancer mortality risk in Australian liver, heart and lung transplant recipients relative to the general population by age at transplantation

Cancer	Age at transplantation (years)								
	0-40 years			41-52 years			53-73 years		
	Obs	SMR	95%CI	Obs	SMR	95%CI	Obs	SMR	95%CI
All *de novo* cancers	27	11.1	7.30–16.1	67	3.74	2.92–4.71	77	1.92	1.52–2.38
Skin cancer§	4	237	64.5–606	10	79.4	38.1–146	9	28.1	12.8–53.3
NHL†	13	103	54.8–176	12	16.9	8.71–29.5	13	8.96	4.77–15.3
Melanoma	3	16.8	3.46–49.0	7	9.37	3.77–19.3	5	4.02	1.31–9.39
Unknown primary site	0	–		12	10.2	5.29–17.9	13	4.72	2.51–8.07
Trachea, bronchus and lung	0	–		9	2.39	1.09–4.53	15	1.52	0.85–2.51

Abbreviations: Obs: Observed number of cancer deaths; SMR: Standardised mortality ratio; CI: confidence interval; §Non-melanocytic skin cancer; †Non-Hodgkin lymphoma.

Survival analyses

Compared to all other causes of death combined, the cumulative incidence of deaths due to *de novo* cancer increased steadily over time (Figures 4-1-B, 4-1-C and 4-1-D, page 105). More than 5 years after transplantation, the rate of increase in cancer-

related deaths appeared steeper for heart compared to liver and lung transplant recipients.

Table 4 - 3. Causes of death over five years after transplantation for Australian liver, heart and lung transplant recipients

Cause of death		Transplanted organ		
	N (%)	Liver (%)	Heart (%)	Lung (%)
All deaths	628 (100)	143 (100)	312 (100)	173 (100)
Disease of the heart and blood vessels	201 (32.0)	27 (18.9)	156 (50.0)	18 (10.4)
All *de novo* cancers	114 (18.2)	26 (18.2)	72 (23.1)	16 (9.25)
All recurrent cancers	9 (1.4)	7 (4.90)	1 (0.30)	1 (0.58)
Disease of the respiratory system	68 (10.8)	2 (1.40)	4 (1.28)	62 (35.8)
Endocrine, nutritional and metabolic diseases	43 (6.80)	4 (2.80)	5 (1.60)	34 (19.7)
Diseases of the digestive system	42 (6.70)	33 (23.0)	6 (1.92)	3 (1.73)
Infectious diseases	34 (5.40)	19 (13.3)	10 (3.20)	5 (2.90)
All others	117 (18.6)	25 (17.5)	58 (18.6)	34 (19.7)

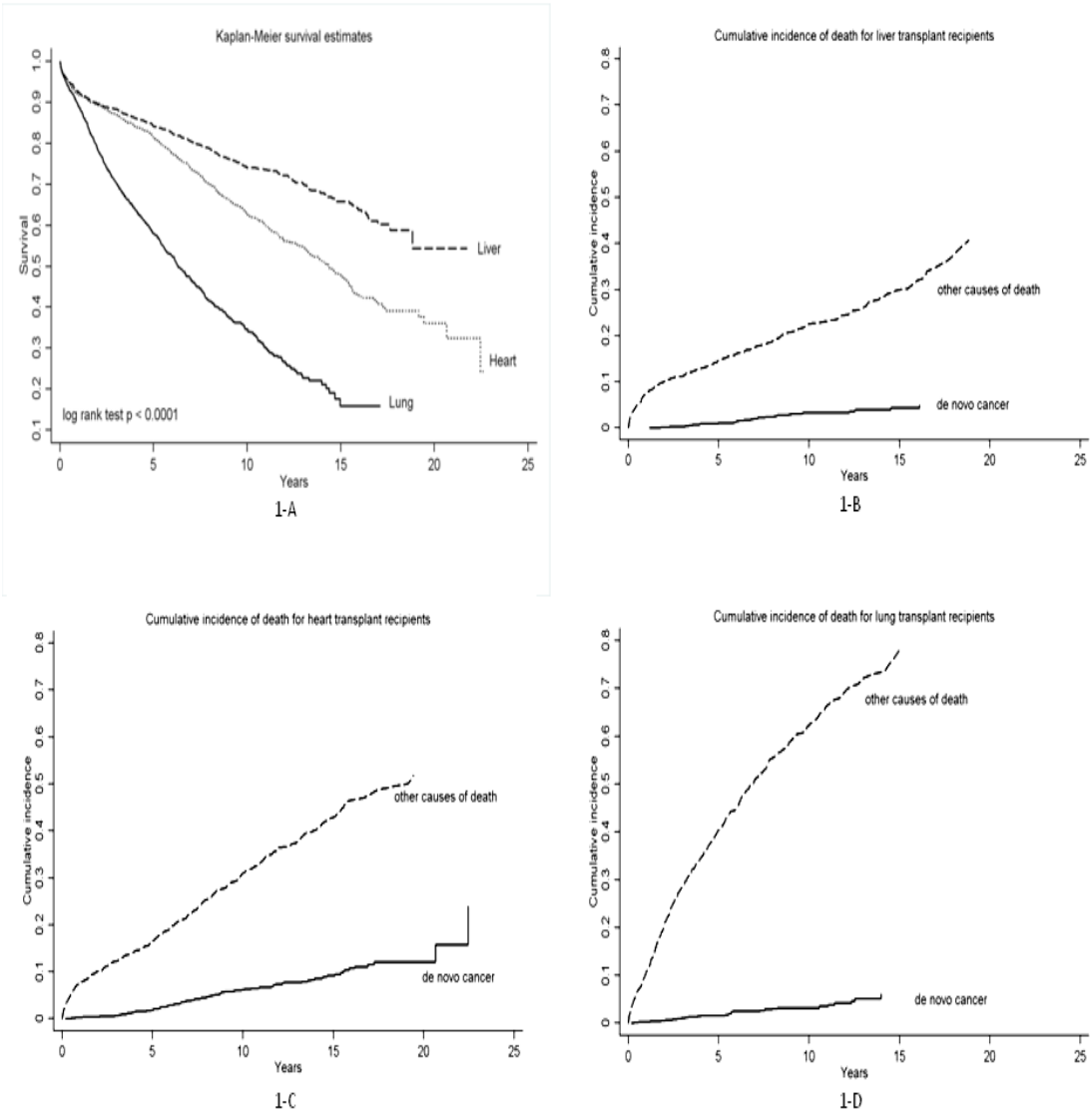


Figure 4 - 1. Survival curves by transplanted organ for Australian liver, heart, and lung transplant recipients

1-A: Kaplan-Meier curve by transplanted organ; 1-B: Cumulative incidence by causes of death for liver transplant recipients; 1-C: Cumulative incidence by causes of death for heart transplant recipients; 1-D: Cumulative incidence by causes of death for lung transplant recipients.

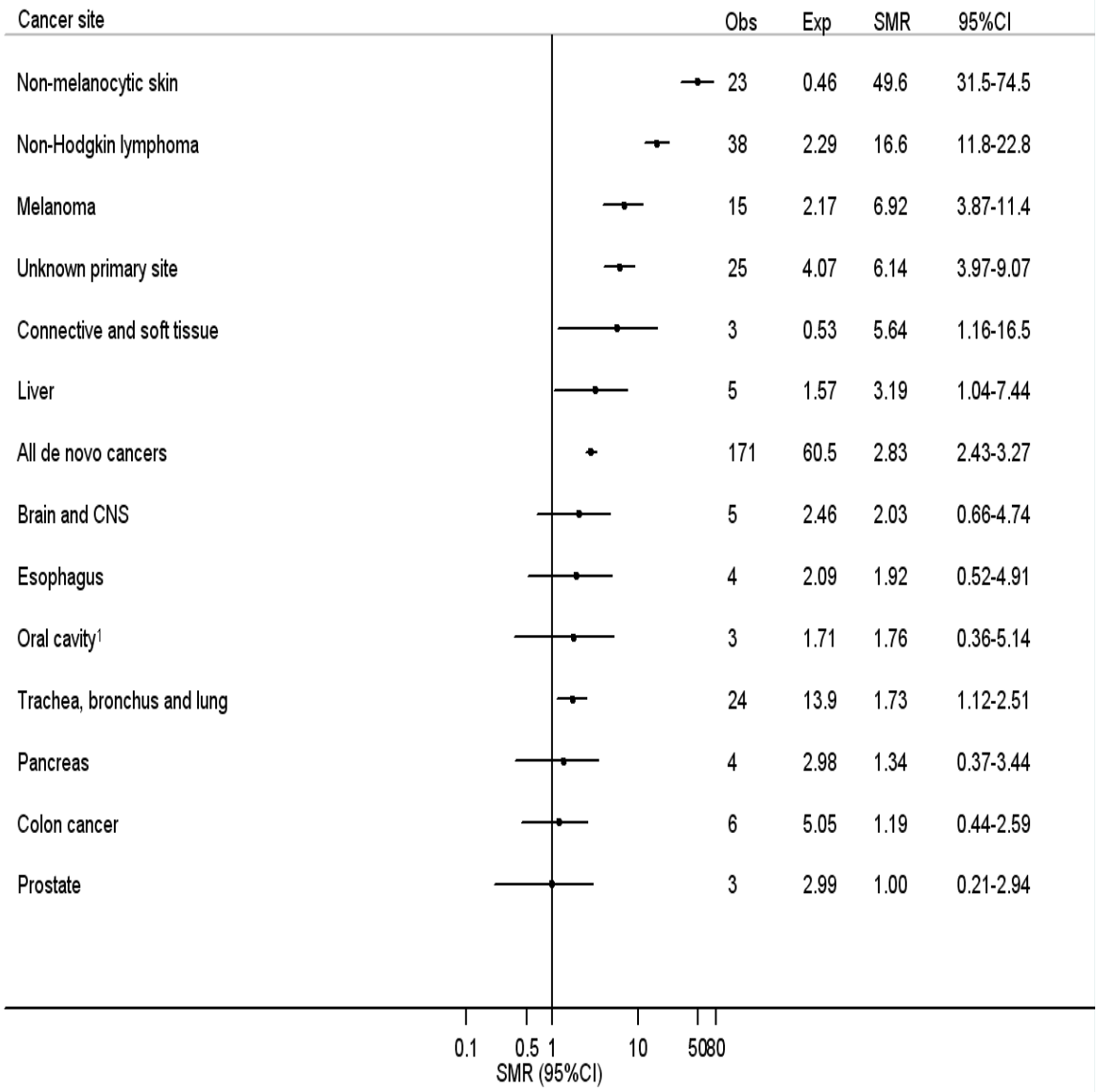


Figure 4 - 2. Site-specific cancer* mortality risk for Australian liver, heart and lung transplant recipients relative to the general population

*The risk of death by cancer site for sites with at least 3 deaths; [1]Oral cavity (C00-C14).

Crude and age- and sex-standardised mortality rates

Overall, the crude mortality rate was 5,243 per 100,000 and the age- and sex-standardised mortality rate (ASMR) was 4,948 (95% CI 4,052–5,843) per 100,000. The crude mortality rate for *de novo* cancer related death was 575 per 100,000 and the ASMR was 359 (95% CI 283–435) per 100,000. Liver transplant recipients had the lowest overall mortality rate (ASMR 2647, 95% CI 2,291–3,002) and the lowest rate of *de novo* cancer mortality (ASMR 260, 95%CI 149–370). Lung transplant recipients experienced the highest overall mortality rate (ASMR 8322, 95%CI 7374-9271), and heart transplant recipients had highest rate of *de novo* cancer mortality (ASMR 555, 95%CI 309-801) (Table 4 - 4, page 107). The crude mortality rate for recurrent liver cancer in liver transplant recipients was 322 per 100,000 and the ASMR was 268 (95% CI 162–375) per 100,000.

Table 4 - 4 The age- and sex- standardised mortality rates (ASMR) of underlying cause of death for Australian liver, heart and lung transplant recipients by transplanted organ

Underlying cause of death	ASMR (95%CI)		
	Liver	Heart	Lung
All death	2647 (2291-3003)	5597 (4304-6891)	8322 (7374-9271)
De novo cancer	260 (149-370)	555 (309-801)	327 (200-454)
Digestive disease	631 (476-786)	-	-
Infectious disease	303 (189-416)	137 (52-222)	2662 (2172-3153)
Cardiovascular disease	-	3645 (2435-4856)	-
Endocrine, Nutritional and Metabolic Diseases	-	-	2651 (1992-3311)
All other non-cancer death	1142 (885-1400)	1174 (806-1542)	2635 (2122-3149)

Abbreviations: ASMR, age and sex standardised mortality rate; CI, confidence interval.

The ASMR for *de novo* cancer deaths was 121 (95% CI 38.6–204) per 100,000 up to 2 years after transplantation, 258 (95% CI 143–373) between 2 and 5 years, and 554

(95% CI 402–707) more than 5 years after transplantation. The ASMR for NHL was 77.5 (95% CI 9.05–146) per 100,000 during the first 2 years, 53.3 (95% CI 2.94–104) between 2 and 5 years, and 172 (95% CI 70.9–273) beyond 5 years post-transplantation.

Mortality risk compared to the general population: all transplant recipients

Transplant recipients were at 10-fold risk of death from any cause compared to the general population (SMR 10.8, 95%CI 10.3–11.4). The 171 *de novo* cancer deaths corresponded to a 2.8-fold risk compared to the general population. The risk of death was significantly elevated for skin cancer, NHL, melanoma, cancer of unknown primary site, liver cancer, connective and soft tissue cancer, and lung cancer (Figure 4-2, page 106). There was no excess risk of death due to the most common epithelial cancers in the general population, specifically colon (n = 6, SMR 1.19, 95% CI 0.44–2.59), prostate (n = 3, SMR 1.00, 95% CI 0.21–2.94), and breast cancer (n = 1, SMR 0.33, 95% CI 0.01–1.82). The risk estimates did not change when those with a history of cancer prior to transplantation were excluded.

Mortality risk compared to the general population: by age and sex

Of the 77 deaths in paediatric transplant recipients, five were attributed to *de novo* cancer (SMR 41.3, 95% CI 13.4–96.5). The median age at *de novo* cancer death was 17 (IQR 8–20) years. Of the 10 paediatric recipients diagnosed with NHL after transplantation, seven died and in four cases (3 heart and 1 liver transplant recipient) the deaths were attributed to NHL.

The risk of death from any *de novo* cancer was at least 2-fold regardless of age at transplantation (Table 4 - 5, page 109). I observed a significant excess risk for skin cancer, NHL, and melanoma for the three age groups examined. The risk of death from cancer of unknown primary site was only increased in those more than 40 years of age at transplantation. I observed an excess risk of death from any *de novo* cancer for both males and females (Table 4 - 6, page 109). Both sexes also exhibited an increased risk of death due to NHL and cancer of unknown primary site. I observed that all skin cancer deaths occurred in males, and a significantly elevated risk of death due to melanoma and lung cancer was also only observed in males.

Table 4 - 5. Site-specific cancer mortality risk for Australian liver, heart and lung transplant recipients relative to the general population by age at transplantation

Cancer	Age at transplantation (years)					
	0-40 years		41-52 years		53-73 years	
	Obs	SMR (95%CI)	Obs	SMR (95%CI)	Obs	SMR (95%CI)
All cancer	27	11.1 (7.30–16.1)	67	3.74 (2.92–4.71)	77	1.92 (1.52–2.38)
Skin cancer§	4	237 (64.5–606)	10	79.4 (38.1–146)	9	28.1 (12.8–53.3)
NHL‡	13	103 (54.8–176)	12	16.9 (8.71–29.5)	13	8.96 (4.77–15.3)
Melanoma	3	16.8 (3.46–49.0)	7	9.37 (3.77–19.3)	5	4.02 (1.31–9.39)
CUP*	0	–	12	10.2 (5.29–17.9)	13	4.72 (2.51–8.07)
Lung#	0	–	9	2.39 (1.09–4.53)	15	1.52 (0.85–2.51)

Abbreviations: Obs, Observed number of cancer deaths; SMR, Standardised mortality ratio; §Non-melanocytic skin cancer (C44); ǂ Non-Hodgkin lymphoma. *cancer of unknown primary; # Trachea, bronchus and lung.

Table 4 - 6. Site-specific cancer mortality risk for Australian liver, heart and lung transplant recipients relative to the general population by sex

Cancer	Male			Female		
	Obs	SMR	95% CI	Obs	SMR	95%CI
All *de novo* cancers	143	3.07	2.59–3.60	28	2.02	1.36–2.86
Skin cancer (NMSC)	23	54.4	34.5–74.5	0	–	–
Non-Hodgkin lymphoma	30	16.8	11.3–23.9	8	16.0	6.92–31.6
Melanoma	14	7.72	4.22–13.0	1	2.81	0.07–15.7
Unknown primary site	21	6.65	4.12–10.2	4	4.38	1.19–11.2
Trachea, bronchus and lung	20	1.72	1.07–2.59	4	1.73	0.47–4.44

Abbreviations: Obs: observed number of cancer deaths; SMR: standardised mortality ratio.

Mortality risk compared to the general population: by transplanted organ

The risk of death due to any *de novo* cancer was 2-fold for liver (SMR 1.96, 95% CI 1.42–2.61), 3-fold for heart (SMR 3.05, 95% CI 2.49–3.7), and more than 4-fold for lung transplant recipients (SMR 4.41, 95% CI 3.02–6.23) (Figure 4-3, page 111). The risk of death due to NHL and cancer of unknown primary site was significantly increased for all three transplanted organs. I observed an excess risk of mortality due to skin cancer for both heart (SMR 66.1, 95% CI 39.2–104) and lung (SMR 86.4, 95% CI 23.6–221) but not liver (SMR 6.91, 95% CI 0.17–38.5) recipients. Risk of melanoma-related death was significantly increased after liver (SMR 5.26, 95% CI 1.43–13.5) and heart (SMR 8.81, 95% CI 4.22–16.2) but not lung (SMR 3.67, 95% CI 0.09–20.4) transplantation. I only observed an increased risk of death due to lung cancer in lung transplant recipients (SMR 3.98, 95% CI 1.46–8.66). There was no excess risk of *de novo* liver cancer death for liver, heart or lung transplant recipients. Lung transplant recipients exhibited a significantly increased risk of death from *de novo* cancer at all time periods post-transplantation. For liver and heart transplant recipients, I only observed a significantly increased risk of death from *de novo* cancer beyond 2 years after transplantation.

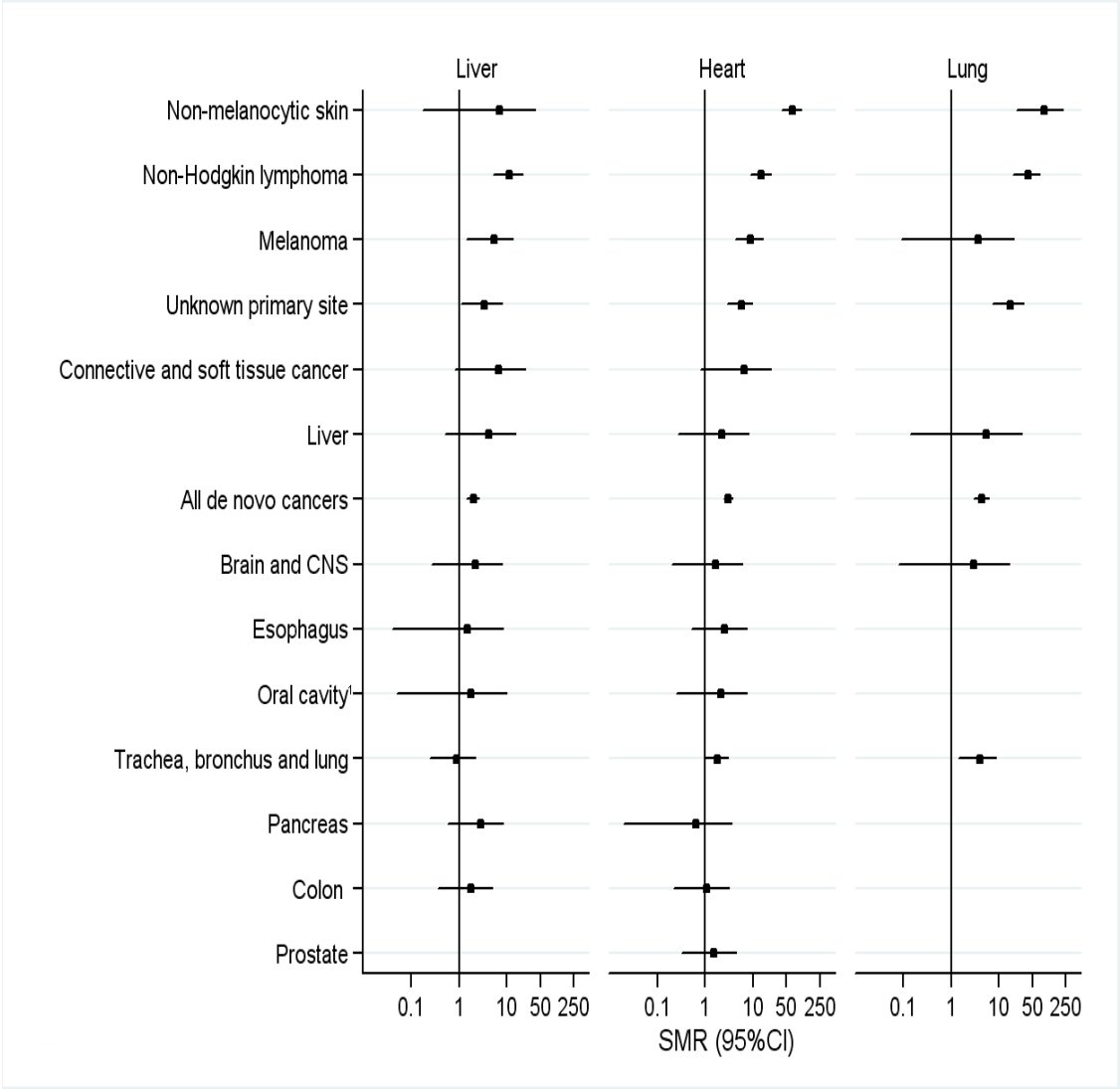


Figure 4 - 3. Site-specific *de novo* cancer mortality risk relative to the general population by transplanted organ

[1]Oral cavity (C00-C14).

Table 4 - 7. Risk of mortality due to *de novo* cancer and NHL for Australian liver, heart and lung transplant recipients by time since transplant and transplanted organ

Cancer	Years*	Liver			Heart			Lung		
		Obs	SMR	95% CI	Obs	SMR	95% CI	Obs	SMR	95% CI
All *de novo* cancers	<2	3	0.7	0.15-2.06	7	1.46	0.59-3.01	7	2.98	1.20-6.15
	2–5	12	2.1	1.08-3.64	17	2.28	1.33-3.66	9	3.68	1.68-6.98
	≥5	27	2.4	1.58-3.37	73	3.74	2.94-4.66	16	6.5	3.71-10.5
NHL†	<2	1	6	0.15-33.4	3	15.6	3.22-45.6	5	55.4	18.0-129
	2–5	2	9	1.08-32.3	3	10.3	2.13-30.1	3	31.5	6.50-92.2
	≥5	6	14	5.23-31.0	12	16.6	8.61-29.1	3	33.6	6.93-98.3

Abbreviations: SMR, Standardised mortality ratio; CI, confidence interval; *Time since transplant (years); †Non-Hodgkin lymphoma.

4.5 Discussion

In this national population-based cohort study, I found a 2.8-fold excess risk of death from *de novo* cancer in liver and cardiothoracic transplant recipients compared to the matched general population. This risk estimate is similar in magnitude to the excess risk of incident cancer in this cohort (SIR 2.62; Chapter 3), a finding that is predominantly attributed to immunosuppression. I observed that the excess risk of death from *de novo* cancer occurred regardless of recipient sex and age at transplantation, and was greatest for lung transplant recipients. NHL was the most common cause of cancer-related death. *De novo* cancer was a leading cause of late death, particularly in heart and liver transplantation, and in paediatric recipients. The excess mortality from cancer in this population reinforces the need for tailored cancer prevention strategies, evidence-based surveillance to promote early detection, and guidelines for managing immunosuppression and cancer treatment after diagnosis.

There are relatively few prior estimates of cancer-related mortality after solid organ transplantation. The estimate of a 1.96-fold risk of death from *de novo* cancer after liver transplantation is similar to that observed in a single-centre Spanish study (1990–2001) that also excluded recurrent cancers (2.93, 95% CI 1.56–5.02) (Herrero et al. 2005). A 2.3-fold risk of death from any cancer (*de novo* and recurrent) was reported in a population-based study of kidney transplant recipients in Hong Kong (1972–2011) (Cheung et al. 2012). On the other hand, no excess risk of cancer death was observed in a population-based study of 164,078 kidney transplant recipients registered on the United States Renal Data System (1990–2004) (Kiberd et al. 2009). However, the latter finding is likely to have underestimate cancer death because cause of death was unknown for 41% of recipients and all of these deaths were classified as non-cancer-related.

The site-specific pattern of cancer-related death by transplanted organ very closely mirrored that observed for incident cancers in this cohort as reported in Chapter 3 (Figure 3-3, page 83). Specifically, risk of death from NHL and cancer of unknown primary site was elevated for all three transplanted organs; risk of death from skin cancer was increased in heart and lung but not liver recipients, risk of death from melanoma was increased in liver and heart but not in lung transplant recipients, and risk of death from lung cancer was confined to heart and lung transplant recipients. Furthermore, there was no excess risk of death due to prostate or breast cancer in any transplanted group. In contrast to the cancer incidence profile, there was no excess risk of death attributed to colon cancer (n = 6) or to lip/oral cancer (n = 3).

There are no prior estimates of the risk of death from NHL in transplant recipients relative to the general population. However, in keeping with previous studies (Metcalfe et al. 2010, Watt et al. 2010), NHL was the most common cause of cancer-related death in this cohort. This may reflect the high incidence of this neoplasm in this patient group rather than a poor treatment response, as a single study found no difference in NHL survival for transplant recipients and the Surveillance, Epidemiology, and End Results (SEER) general population (1964–2007) (Knight et al. 2009). NHL in transplant recipients is managed by reducing immunosuppression, chemotherapy with or without rituximab, antiviral therapy for EBV-positive patients, surgery, and radiation (Reshef et al. 2011, Campistol et al. 2012).

Skin cancer carried the highest relative risk of cancer-related death in my cohort (SMR 50). This estimate is very similar to the risk of death from cutaneous squamous cell carcinoma (SCC) in Swedish solid organ transplant recipients (1970–1997; SMR 52.2, 95% CI 21.0–107.6), predominantly kidney (Lindelof et al. 2006). Although the skin cancer histology was not recorded, a previous Australian study of cardiothoracic transplant recipients found that 95% of non-melanocytic skin cancers were SCC (Veness et al. 1999). I found no excess risk of mortality from skin cancer in liver transplant recipients, which is consistent with prior evidence that most deaths in this patient group occur from non-cutaneous neoplasms (Herrero et al. 2005). This finding, like the different patterns in cancer incidence by transplanted organ, is likely to be largely related to the less intensive immunosuppressive therapy received by liver compared to cardiothoracic transplant recipients (Euvrard et al. 1995, Ong et al. 1999, Collett et al. 2010).

For paediatric transplant recipients, most deaths of any cause occurred during the first year and more than five years after transplantation. *De novo* cancer was the leading cause of late death, the risk relative to the general population was around 40-fold and most cancer-related deaths were attributed to NHL. In the only prior study to assess mortality in paediatric transplant recipients with NHL, heart transplant recipients had significantly lower overall survival compared to kidney or liver transplant recipients (Taj et al. 2012). Given the relatively high mortality of paediatric recipients with NHL, serial monitoring of EBV viral load has been recommended as a screening strategy for high-risk patients, and may improve outcomes (Tsai et al. 2008, Schubert et al. 2009, Kerkar et al. 2010, Taj et al. 2012).

The increased incidence and mortality from *de novo* cancer in solid organ transplant recipients justifies patient education about the need for sun protection and monthly self-skin examinations, and early reporting of new signs and symptoms. The findings indicate that males are at greatest risk of dying from non-melanoma skin cancer and melanoma. Clinically, cancer risk may be reduced by minimizing immunosuppression, viral prophylaxis, EBV viral load monitoring, and targeted cancer screening in high-risk patients (Webster et al. 2008, Reshef et al. 2011, Campistol et al. 2012). Significantly greater non-skin cancer-related survival and overall survival was reported for a period of intensive cancer surveillance (2002–2007) compared to historical surveillance (1982–2001) in Austrian liver transplant recipients (Finkenstedt et al. 2009). However, there was no adjustment for either cancer treatment or improvements in cancer outcomes over time. The intensive surveillance was an annual chest and abdominal CT

scan, urological evaluation (including prostate specific antigen (Zazgornik et al.) test), gynaecological evaluation (including Papanicolau smear and mammography), dermatological screening, and 3-yearly colonoscopy except in patients with a history of adenoma or inflammatory bowel disease (1-yearly). Nevertheless, as incidence rates of prostate and breast cancer are not increased in organ transplant recipients, there is no evidence to suggest screening practices for these malignancies that would differ from those for the general population.

The strengths of my study include the population-based design and the relatively large size, allowing stratification by organ and cancer type. A limitation is that some of the cancer-related deaths did not have a matching linked cancer registry record; however, most of these were skin cancers, the majority of which are not notifiable neoplasms in Australia. Also, there is the potential for differential misclassification of the underlying cause of death in individuals with multiple morbidities (such as those with end-stage organ disease and cancer), and this may have led to an under-estimation of the true risk of cancer-related death in this patient group (Sarfati et al. 2010). Furthermore, 4% of deaths were due to an unknown cause, and these cases were not included in the cause-specific analyses. Despite these limitations, the rank order of cause-specific mortality was in accordance with previous reports (Watt et al. 2010, Christie et al. 2011, Stehlik et al. 2011).

4.6 Conclusions

In this Chapter, I quantified the risk of *de novo* cancer-related mortality in Australian liver and cardiothoracic transplant recipients, overall and in relation to organ type,

time since transplantation, and recipient age and sex. This population-based data provides additional momentum to calls for a review of guidelines for cancer prevention and screening for solid organ transplant recipients, and the generation of robust evidence in these areas (Webster et al. 2008).

Chapter 5 Immunosuppressive therapies in a national cohort of Australian liver, heart and lung transplant recipients, 1984 to 2006

5.1 Objectives

In this Chapter I describe the immunosuppressive therapy of a population-based, national cohort of liver, heart and lung transplant recipients in Australia. Specifically, the objectives are:

1. To describe, quantify and compare the immunosuppressive drugs and dosages prescribed to recipients of different organs
2. To describe and quantify the immunosuppression profiles over two decades

5.2 Introduction

The use of immunosuppressive therapy is critical in patient survival in solid organ transplantation, but the associated risk of cancer is of concern. A 3-to-4-fold excess risk of cancer relative to the general population has been observed for recipients of all organ types (Adami et al. 2003, Vajdic et al. 2006, Jiang et al. 2008, Engels et al. 2011). Observational research indicates that heart and lung transplant recipients have a higher risk of any cancer, and a higher risk of specific cancers such as NHL, compared to liver recipients (Adami et al. 2003, Opelz et al. 2007, Fernberg et al. 2011, Na et al. 2013). It has been hypothesised that this difference in cancer risk profile may be explained by

differences in the degree of immunosuppression by organ type (Euvrard et al. 1995, Ong et al. 1999, Collett et al. 2010).

Patient characteristics and risks determine the initial type and dose of immunosuppressive agents and any changes over time following transplantation. Furthermore, the clinical availability and pattern of use of classes of drugs and individual agents has changed over time. Clinical practice guidelines and OPTN/SRTR data indicate key differences in immunosuppression practice by organ type. For example, the use of induction antibody is less common in liver compared to heart and lung transplantation (Meier-Kriesche et al. 2006). Further, CNI monotherapy with corticosteroid withdrawal may only be considered in highly selected low-risk heart transplant recipients, whereas it is recommended for a substantial proportion of liver recipients (Meier-Kriesche et al. 2006, Matin et al. 2008, Collett et al. 2010, Costanzo et al. 2010, Singh and Watt 2012). However, there has been no direct comparison of immunosuppressive drug type and dose by organ type.

5.3 Methods

5.3.1 Study population

I performed a retrospective population-based cohort study of Australian liver (n = 1926, 41%), heart (n = 1518, 33%), and lung (n = 1200, 26%) transplant recipients 1984-2006 based on data from the population-based liver (ANZLTR) and cardiothoracic (ANZCOTR) transplant registries and 18 transplantation units at 12 Australian hospitals. For this

analysis, I excluded recipients with no retrievable transplantation medical records and those with some clinical but no immunosuppression therapy data (n = 470) (Figure 5 - 1, page 120). This excluded subset consisted of 31 (1.6%) liver, 298 (19.6%) heart, and 141 (11.8%) lung transplant recipients. Of the transplant recipients excluded from this study, 178 (3.8%) were transplanted 1984-1994, 34 (0.7%) during 1994-1997, and 258 (6.1%) during 1998-2006.

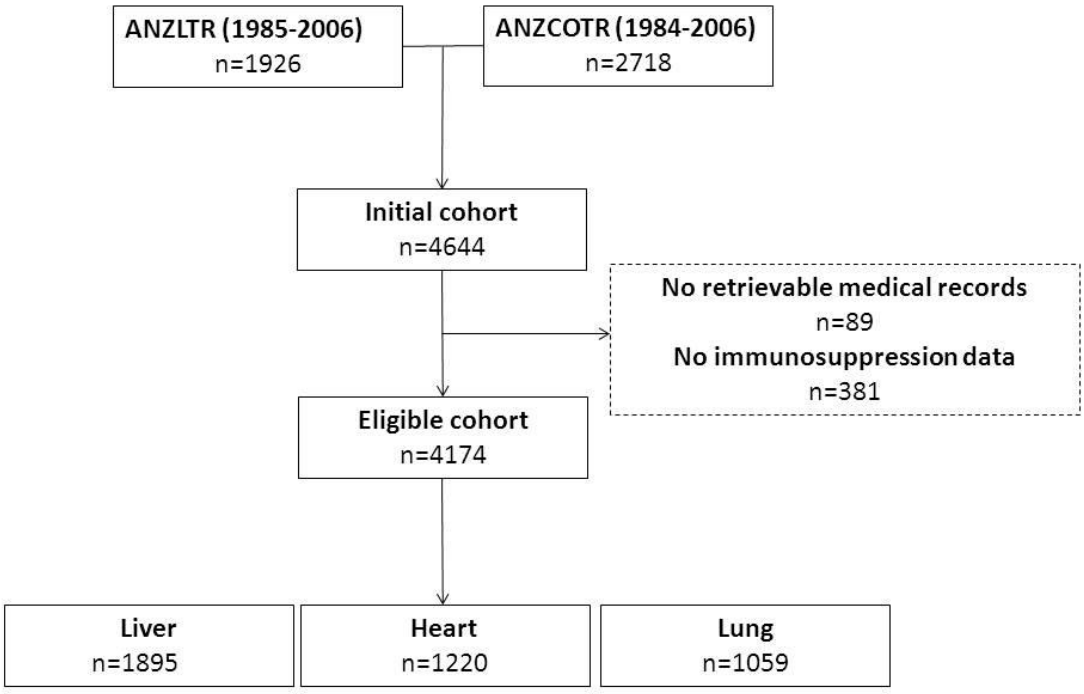


Figure 5 - 1. Flowchart of the cohort for immunosuppression study

Abbreviations: ANZLTR: Australian and New Zealand Liver Transplant Registry; ANZCOTR: Australia and New Zealand Cardiothoracic Organ Transplant Registry.

Ethical approval was obtained and the requirement for informed consent was waived as only de-identified data were received.

5.3.2 Data collection

The transplant registries prospectively collected demographic and some clinical data including organ type, primary indication, transplant date, age at transplant, sex, and date of death. I supplemented these records with data abstracted from medical records at all Australian transplantation unit (18 units at 12 hospitals) including comorbidities and immunosuppressive agents. I ascertained recipient's weight and prescribed immunosuppressive and corticosteroid agents at induction (i.e. peri-operative therapy), at regular intervals thereafter (i.e. maintenance therapy), and during episodes of rejection requiring treatment.

I recorded the receipt of antibody agents, including IL-2Ra(basiliximab, daclizumab) and T-cell depletive antibodies, both muromonab-CD3 and polyclonal (anti-thymocyte/anti-lymphocyte globulins, ATG/ALG), during induction therapy and rejection episodes. I documented the receipt of individual immunosuppressive agents and their doses (mg/day or mg/kg/day) at transplant, 3 months, 6 months, and 1, 2, 5, 10, 15 and 20 years after transplantation. As this data was missing for a substantial proportion of recipients at

transplantation and 2 years (15% and 43% respectively) I excluded these time points from this analysis.

Immunosuppression data included the use of cyclosporine, tacrolimus, azathioprine, mycophenolate (mycophenolate mofetil or enteric-coated mycophenolate sodium), sirolimus and everolimus. I did not distinguish the different formulations of ATG/ALG, cyclosporine or tacrolimus. I collected the use of oral corticosteroids and intravenous corticosteroid pulse therapy at induction and rejection episodes, but I did not differentiate the type of acute rejection (cellular or humoral).

5.3.3 Data preparation

I followed-up organ recipients from the date of first transplant until re-transplantation, 80 years of age, death, or the end of follow-up (31 December 2006), whichever occurred first. In Australia, tacrolimus and mycophenolate were approved by the Therapeutic Goods Administration in 1997, and sirolimus and everolimus in 2002 and 2005, respectively. However, tacrolimus and mycophenolate were used from 1995, and sirolimus from 1998, in clinical trials in Australia. I therefore categorised transplant era according to the broad availability of immunosuppressive agents for this cohort; 1984-1994, 1995-1997, and 1998-2006. I included all drug use, whether it was approved use, approved only under a special access scheme (Section 100 of the Australian Pharmaceutical Benefits Scheme), or in clinical trial.

I used recipient weight to standardise the dose of individual agents to mg/kg/day. Weight was not recorded at 27% of observation times. Assuming the data were missing at random, I assessed three different methods to impute the missing weight values (Allison 2001, Engels and Diehr 2003, Donders et al. 2006). First, I replaced the missing weight value with the recipient's last known weight, i.e. the last observation carried forward (LOCF) method. Second, I used the mean value of the weight before and after the missing value, if available, to replace the missing value (Engels and Diehr 2003). Third, I replaced the missing weight values with values from cases similar to the case with the missing values using a linear mixed model including age at transplantation, sex, and weight at other observation times for the same individual (Rubin 1978, European Medicines Agency 2010). The results before and after imputation using each of the above methods are shown in Table 5 - 1, page 141. The second method could not be applied at 5- and 10-years after transplantation due to the larger proportion of missing values. I found no notable differences in the median recipient weight at each observation time before and after imputation using either the LOCF or mixed model methods, however, the percentage of missing values using the mixed model was reduced to 8-15% compared with 16-28% using the LOCF method, so I chose the mixed model approach.

I converted all dosages of mycophenolate acid to equivalent dosages of mycophenolate (Staatz and Tett 2007). I reviewed the immunosuppressive therapy data and identified

potential outlying dose values (i.e. >1.5-times the interquartile range (IQR) from the lower or upper quartile dose). I changed these values to missing, unless there was a clear and logical pattern, in which case I retained them. The type and dose of individual immunosuppressive agents was missing at 18% and 31% of observation times, respectively. Assuming the data were missing at random, I imputed the missing values, where possible, using the LOCF method. This is the conventional method for imputing longitudinal medication data, especially a combination of binary (drug type) and continuous (drug dose) data where most recipients received more than one immunosuppressive agent, with the dose of one agent likely to be related to the dose of the other (European Medicines Agency 2009).

The receipt and median dosage of immunosuppressive agents at 3-months and 5-years post-transplantation before and after imputation are shown in Table 5 - 2 and Table 5 - 3 (page 142-144). The results based on the imputed data were consistent with the results based on the original data. This was also the case for the other time points (data not shown).

5.3.4 Data analysis

I compared the receipt of induction antibody, antibody rejection, corticosteroid therapy, and each immunosuppressive agent by organ type, and various other recipient subgroups (e.g. age, sex) using Pearson chi-square tests (categorical variables with all expected cell

frequencies over five), or Fisher exact tests as appropriate (categorical variables with any of the expected cell frequencies five or less). Since the doses of immunosuppressive agents were not normally distributed, I used the Kruskal-Wallis non-parametric test to compare the median dose of each immunosuppressive agent for recipient subgroups; if the test result was significant, I applied a post-hoc multiple comparison procedure for non-parametric pairwise differences across three or more groups in order to identify in which groups the median doses differed statistically significantly from one another (Dunn 1964, Elliott and Hynan 2011). I illustrated the change in dose by time since transplantation by plotting box-and-whisker plots, and quantified the trend in change in dose over time using the Mann-Kendall trend test.

I modelled the receipt of induction antibody, antibody rejection, each immunosuppressive agent and the number of agents (monotherapy vs. combination therapy) by organ type, adjusting for age at transplantation, sex, race, transplant year, dialysis prior to transplantation, primary indications, and immunosuppressive agents using logistic regression. For corticosteroids the maximum likelihood estimation did not converge due to quasi-complete separation of data (Albert and Anderson 1984), thus adjusted estimates were not possible. I modelled the median dose of each immunosuppressive agent by organ type, adjusting for age at transplantation, sex, transplant year, antibody induction therapy (yes/no), and the dose of other immunosuppressive agents given simultaneously, using quantile regression (McGreevy et al. 2009). Antibody rejection, sirolimus and

everolimus were not included in the adjustment due to their low prevalence. The adjustment could not be performed beyond 10 years after transplantation due to insufficient numbers of patients across each of the strata.

I performed analyses using SAS 9.3 (SAS Institute, Inc., Cary, USA) and STATA statistical software 11.2 (StataCorp, Texas, USA), or R statistical software v3.1.3 (R Development Core Team, 2014).

5.4 Results

5.4.1 Cohort characteristics

The eligible cohort consisted of 4174 transplant recipients, 1895 (46%) liver, 1220 (29%) heart, and 1059 (25%) lung, of whom 405 were paediatric (302 liver, 83 heart, and 20 lung). The median follow-up time was 5.6 years (IQR 2.4-10.2). There were significant differences in the distribution of transplant year and recipient and donor characteristics between organ types (Table 5 - 4, page 146).

Seventy-two precents of the cohort (n = 3019) had complete data on immunosuppressive agents for the entire follow-up time. The percentage of liver recipients with missing drug dose data at 1, 5, 10, and 15 years after transplantation was 15%, 16%, 20%, and 34%. The corresponding figures for heart recipients were 19%, 15%, 19%, and 34%, and for lung 21%, 29%, 24%, and 75% (9 of 12 recipients surviving 15 years) (Figure 5 - 2, page 127).

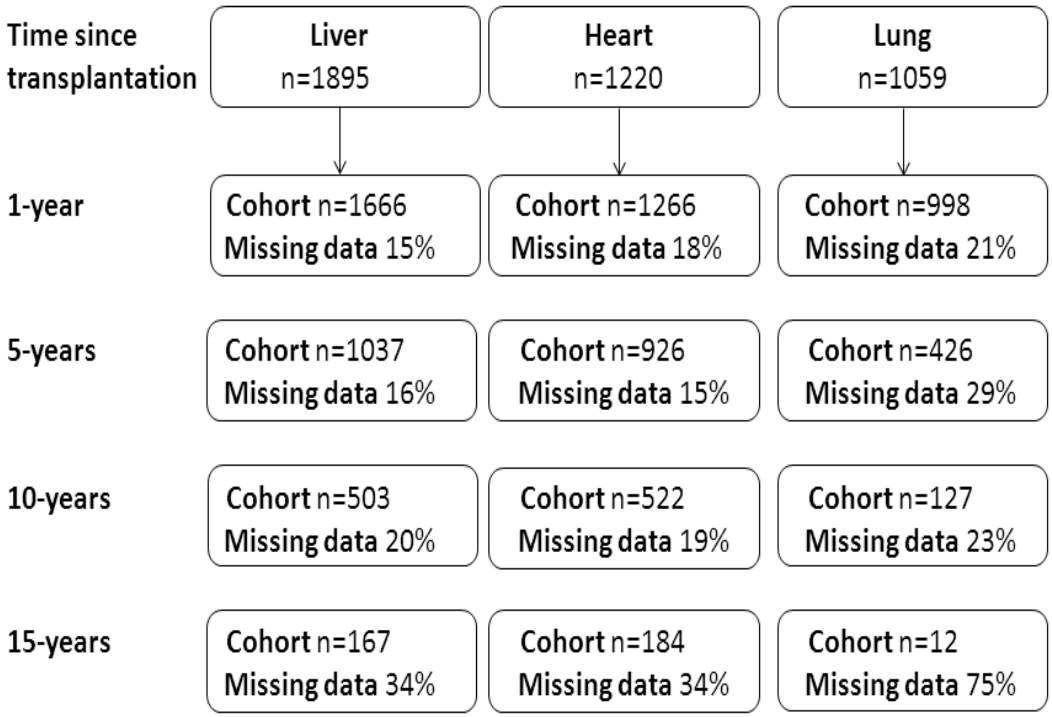


Figure 5 - 2. Flowchart of the cohort and extent of missing immunosuppression data by organ type

5.4.2 Induction immunosuppression

Around 22% of all transplant recipients received induction antibody and 25% received intravenous corticosteroids (Table 5-5, page 148). Induction antibody was most common for heart recipients (42%), adults (23%), and those transplanted in the earliest era (1984-1994; 37%). There was also significant variation in induction antibody use by primary indication, dialysis history and intravenous corticosteroids (data not shown). As expected, use of ATG/ALG and muromonab-CD3 decreased substantially after 1994 and IL-2Ra was

the most common induction antibody therapy in the latest period (1998-2006; 11%). In the most recent era (1998-2006), 198 (4.7%) heart, 75 (1.8%) lung and 68 (1.6%) liver recipients received induction antibody. After adjustment for confounding, induction antibody therapy was significantly more common in heart (odds ratio, OR 8.22, 95% CI 5.18-13.2) and lung (OR 5.87, 95% CI 3.78-9.21) compared to liver recipients.

5.4.3 Therapy for acute rejection

Approximately 36% of the cohort was treated for acute rejection (Table 5-6, page 149); most commonly liver recipients (44%), paediatric patients (49%), females (41%), and those transplanted in the earliest era (1984-1994; 50%). Intravenous corticosteroid pulse was the most frequent therapy (86% of those treated and 29% of all transplant recipients); only 8% received antibody therapy, most commonly liver recipients (10%). As expected, the use of antibodies decreased significantly over time, from 17% in 1984-1994, to 9% in 1995-1997, and 4% in 1998-2006 ($p < 0.001$). There was also significant variation in antibody rejection therapy by organ type, age, sex (Table 5-6, page 149), dialysis history, intravenous corticosteroids, antibody induction therapy, donor age, and recipient CMV serostatus at transplantation (data not shown). After adjustment, antibody rejection therapy was significantly more common in liver (OR 1.84, 95% CI 1.27-2.70) and lung (OR 2.19, 95% CI 1.29-3.68) compared to heart recipients.

5.4.4 Corticosteroids

At least 85% of the cohort received oral corticosteroids 3 months after transplantation. Compared to heart and lung, liver recipients were significantly less likely to use corticosteroids at all follow-up times ($p < 0.001$; Figure 5-3, page 150). Receipt of corticosteroids decreased over time from transplantation for liver recipients only; 5 years after transplantation, 34% of liver recipients were corticosteroid-free, compared to 28% of heart and 1% of lung recipients.

5.4.5 Maintenance immunosuppressive therapies

There were marked differences in maintenance immunosuppressive regimen by organ type, year of transplant, and time since transplantation (Figure 5 – 4 to Figure 5 - 6; page 151-153; Table 5 - 7, page 159). Overall, the use of cyclosporine declined over time after the introduction of tacrolimus. Similarly, azathioprine use decreased and mycophenolate increased over time, but the extent of these changes was greater than for the CNIs.

Across all eras, a greater proportion of liver recipients received CNI monotherapy than heart and lung recipients at all follow-up times ($p < 0.001$, data not shown). During 1998-2006, 35-50% liver, 3-4% heart and 10% lung recipients received monotherapy and there was also significant variation by age, sex, race, transplant year, primary indication, dialysis history, diabetes history, antibody induction therapy, and recipient CMV serostatus at transplantation (data not shown). After adjustment, liver recipients were more likely to

receive monotherapy compared to heart and lung recipients at 3 months, 1 year and 5 years post-transplantation ($p < 0.001$).

Maintenance immunosuppression by transplant era

As expected, for those transplanted 1984-1994, the combination of cyclosporine and azathioprine was the cornerstone for heart and lung transplantation, it was used by at least 90% of recipients at 3 months and 60% at 10 years (Figure 5-4, page 151). This combination was less common for liver transplantation, particularly with increasing time since transplantation (40% at 5 years, 25% at 10 years).

For patients transplanted 1995-1997, the combination of cyclosporine and azathioprine at 3 months and 1 year declined to 80% of heart and lung and 40% of liver recipients (Figure 5-5, page 152; Table 5 - 7, page 159). At 10 years this further declined to 30% of heart and lung, and 10% of liver recipients. In heart transplantation the combination of cyclosporine and mycophenolate increased from 14% at 5 years to 27% at 10 years whereas the combination of tacrolimus and mycophenolate was used by less than 5% of recipients at 5 and 10 years. Five and 10 years after transplantation, cyclosporine monotherapy was used by 10% of heart and lung and 30% of liver transplant recipients.

Compared to prior eras, patients transplanted in 1998-2006 were less likely to start immunosuppressive therapy with the combination cyclosporine and azathioprine, and as

expected there were generally fewer changes in immunosuppressive agents over time since transplantation (Figure 5-6, page 153; Table 5-7, page 159). Mycophenolate, with cyclosporine or tacrolimus, was used by around 50% of heart recipients. The greatest changes over time were observed for lung recipients, with use of cyclosporine and azathioprine declining from 50% to 20% in concert with increases in the use of tacrolimus as monotherapy or in combination with mycophenolate or azathioprine. Tacrolimus-based therapies were the mainstay for liver recipients, and CNI monotherapy increased from 35% at 3 months to 50% at 1 and 5 years after transplantation. mTOR inhibitors were used by a small proportion of transplant recipients; 3% at 3 months and 4% at 5 years. CNI-free regimens were used by 1.6% liver, 6.0% heart and 1.7% lung recipients at 1 year, and 6.6% liver, 8.0% heart and 8.0% lung recipients at 5 years.

In adjusted analyses for patients transplanted 1998-2006, lung and heart recipients were more likely to receive cyclosporine at 3 months (OR (95% CI): 17.0 (8.67-35.2) and 11.2 (5.57-23.6), respectively), 1 year (9.45 (4.84-19.1) and 9.08 (4.49-18.9)) and 5 years (3.34 (1.21-9.62) and 7.17 (2.58-21.4)) compared to liver recipients. Heart recipients were also more likely to receive mycophenolate (6.04 (3.01-12.2), 6.76 (3.27-14.2) and 9.13 (3.26-26.9) at 3 months, 1 year and 5 years, respectively) and lung recipients azathioprine (2.52 (1.43-4.54), 3.30 (1.78-6.17) and 4.48 (1.57-13.4) at 3 months, 1 year and 5 years, respectively) than liver recipients. Liver recipients, on the other hand, were more likely to receive tacrolimus compared to heart and lung recipients at 3 months, 1 year and 5 years

($p < 0.001$). There were no differences between heart and lung transplant recipients. All of these differences were maintained when only adult recipients were considered.

Dose of immunosuppressive agents by time since transplantation

As anticipated, the median dose of several immunosuppressive drugs decreased with increasing time since transplantation, particularly the first 5 years (Figure 5-4 to Figure 5-6, Table 5-8 to Table 5 - 10, page 159-162). For the entire cohort the reduction in median dose over 5 years was around 50% for CNIs and 20-30% for antiproliferative agents. The median dose of cyclosporine was 5.00 mg/kg/day at 3 months and 2.63 mg/kg/day at 5 years. The corresponding values were 0.12 and 0.05 mg/kg/day for tacrolimus, 34.1 and 23.5 mg/kg/day for mycophenolate, 1.36 and 1.06 mg/kg/day for azathioprine, 0.04 and 0.03 mg/kg/day for sirolimus, and 0.02 and 0.01 mg/kg/day for everolimus. Dosages were stable between 5 and 10 years, except for cyclosporine, which continued to decline ($p < 0.05$). Between 3 months and 10 years after transplantation the median dose of cyclosporine and mycophenolate significantly decreased for all transplanted organs ($p < 0.05$), the median dose of azathioprine significantly declined for heart and lung transplant recipients ($p < 0.05$) but not liver, and the median dose of tacrolimus significantly declined for liver and lung recipients ($p < 0.05$) but not heart (Figure 5 - 7, Figure 5 - 8, page 154-155).

Dose of immunosuppressive agents by recipient subgroup

The median dose of several individual immunosuppressive agents differed significantly by recipient age, sex, year of transplant, and primary indication (Table 5-8 to Table 5-10, page 159-162). Overall, higher dosages (per kg) were received by paediatric compared to adult recipients (and their primary indications), females compared to males, and those transplanted during the early compared to the latest era.

The median dose of immunosuppressive agents by transplanted organ before and after adjustment for potential confounders at 3 months, and 1 and 5 years are shown in Figure 5 – 9 to Figure 5 - 11 (page 156-158) and Table 5 - 11 (page 163) . Before adjustment, compared to heart transplant recipients, liver recipients received a higher dose of cyclosporine ($p < 0.001$), no difference in tacrolimus dose, a lower dose of azathioprine ($p < 0.001$), and a lower dose of mycophenolate at 3 months and 1 year after transplantation ($p < 0.001$). After adjustment, the results for tacrolimus, azathioprine, and mycophenolate were unchanged, but there was no difference in cyclosporine dose until 5 years after transplantation. The significance was retained when comparisons were restricted to adults.

Compared to lung transplant recipients, liver recipients received a similar dose of cyclosporine and tacrolimus, a lower dose of azathioprine ($p < 0.001$), and a lower dose of mycophenolate at 3 months ($p < 0.001$). After adjustment, liver recipients received a higher dose of cyclosporine at 5 years ($p < 0.05$), a lower dose of tacrolimus at 1 year ($p <$

0.05), a lower dose of azathioprine ($p < 0.05$) at all follow-up times, and no difference in mycophenolate dose.

Compared to lung transplant recipients, heart recipients received a lower dose of cyclosporine at 3 months and 1 year ($p < 0.001$), no difference in tacrolimus dose, a higher dose of azathioprine at all follow-up times ($p < 0.001$), and a higher dose of mycophenolate at 1 year ($p < 0.001$). After adjustment, compared to lung recipients, heart recipients received a lower dose of cyclosporine at 1 year ($p < 0.05$), no difference in tacrolimus, a higher dose of azathioprine at 1 and 5 years ($p < 0.05$), and no difference in mycophenolate.

5.5 Discussion

I have described and compared the immunosuppression of a national population-based cohort of liver, heart and lung transplant recipients over 20 years. Induction, maintenance and rejection immunosuppression varied significantly by organ type, but also recipient age, sex, year of transplant, time since transplantation and other recipient and donor characteristics. Over the 20 year period there were decreases in the use of muromonab-CD3, cyclosporine, and azathioprine, and increases in the use of IL-2Ra, tacrolimus, and mycophenolate. As expected, overall, with increasing time since transplantation, the median dose of individual agents declined, and regimens were more likely to change. Liver recipients were less likely to use corticosteroids than heart and lung recipients. After

adjustment for potential confounders, induction antibody was significantly less common in liver compared to heart and lung recipients. Antibody rejection therapy was significantly more common in liver and lung compared to heart recipients. Liver recipients were more likely to receive CNI monotherapy, with or without corticosteroids, compared to heart and lung recipients. Liver recipients also consistently received lower doses of azathioprine compared to heart and lung recipients.

This is the first population-based study to describe and compare the type and degree of iatrogenic immunosuppression for national cohorts of liver, heart and lung transplant recipients. My finding of higher rates of antibody induction in heart and lung compared to liver transplant recipients is consistent with international and United States transplant registry data (Meier-Kriesche et al. 2006, Taylor et al. 2006, Matin et al. 2008, Klipa et al. 2010). However, the proportion of these transplant recipients who receive antibody induction is lower in Australia compared to the United States, mirroring the situation for kidney transplantation (Adu et al. 2003, Webster et al. 2010). In general, antibody induction is reserved for recipients at high risk of early acute rejection, to avoid or delay the use, or to enable lower initial dose of CNIs in those with significant renal or hepatic dysfunction, or to avoid or reduce early high-dose corticosteroid use. Consistent with Australian kidney transplantation and international experience in recent years, I observed an increase in use of IL-2Ra and a decrease in use of muromonab-CD3 (Adu et al. 2003, Taylor et al. 2006, Matin et al. 2008, Klipa et al. 2010). This finding is in line with a reduced

incidence of acute rejection with IL-2Ra (LaCasce 2006, Pascual 2007, Christie et al. 2012) and safety concerns about muromonab-CD3 induction therapy (Gao et al. 2003, Chang et al. 2008), including increased risk of lymphoma (Swinnen et al. 1990, Webster et al. 2004, O'Neill et al. 2006). Muromonab-CD3 is no longer indicated for transplant recipients.

In agreement with international reports (Meier-Kriesche et al. 2006, Matin et al. 2008, Stehlik et al. 2010), I observed a reduction in the incidence of acute rejection over time, a trend that has been attributed to advances in immunosuppressive therapy. Overall, I found that 33% of recipients experienced at least one treated episode of acute rejection, more commonly in liver compared to heart and lung recipients, and treatment was mostly intravenous corticosteroid pulse therapy. My observations are comparable with international data for rejection during the first year after heart (30%; 2003-2008) (Stehlik et al. 2010) and lung transplantation (34%; 2004-2011) (Christie et al. 2012), as well as liver transplantation in the United States (43%; 1998-2003) (OPTN/SRTR 2012). I also found a higher rate of rejection in paediatric and female recipients, as previously reported in heart recipients (Stehlik et al. 2010) and paediatric liver recipients (Christie et al. 2012). In my data the timing of the acute rejection was not known, but most rejections will have occurred within 1 year of transplantation (Matin et al. 2008, Polesel et al. 2008, Stehlik et al. 2010, Martinu et al. 2011, Yusen et al. 2013).

As anticipated, I found that prior to 1998 the combination of cyclosporine and azathioprine dominated immunosuppressive therapy for heart transplantation. I showed a decline in the use of this combination since 1998, and an increase in tacrolimus- and mycophenolate-based regimens, consistent with international clinical practice trends (Meier-Kriesche et al. 2006, Stehlik et al. 2010). During the latest era (1998-2006), the most common maintenance regimen for heart transplant recipients was cyclosporine and mycophenolate. Furthermore, in agreement with United States practice (Meier-Kriesche et al. 2006), I showed that changes in regimen during this period occurred throughout follow-up but predominantly during the first year. Unlike kidney transplantation in Australia (Adu et al. 2003), I did not observe a marked increasing use of CNI-free regimens over the study period.

I found that immunosuppressive therapy was very similar for lung and heart transplant recipients prior to 1998. Since 1998, use of the combination cyclosporine and azathioprine declined, and tacrolimus-based therapies increased, more so in lung compared to heart recipients, in line with international trends (Meier-Kriesche et al. 2006, Christie et al. 2012). During the latest era (1998-2006), the most common regimen for lung transplant recipients at 1 year was cyclosporine-based, in contrast to international data during 2002-2011 showing majority use of tacrolimus-based regimens (Christie et al. 2012). However, by year 5, there was approximately equivalent use of cyclosporine- and tacrolimus-based regimens. In agreement with United States practice (Meier-Kriesche et al. 2006), during

the latest era I observed the greatest variation over time since transplantation for regimen choice among lung transplant recipients, believed to reflect their higher incidence of late infections and chronic rejection.

I found that maintenance immunosuppression regimens used in liver transplantation were consistently different to those used in heart and lung transplantation. CNI monotherapy, first cyclosporine and increasingly tacrolimus, was used by around 40% of the liver cohort at any point in time. CNI-free regimens were rarely observed. Furthermore, prior to 1998 the uptake of tacrolimus in liver transplantation exceeded that in heart and lung transplantation, and after 1998 tacrolimus-based regimens were used by the majority of liver recipients. Corticosteroid withdrawal was also more common in liver transplantation. These patterns and trends are in complete alignment with data from OPTN/SRTR in the US (Meier-Kriesche et al. 2006, Matin et al. 2008), however, there was no decline in azathioprine in favour of mycophenolate.

Minimising the degree of immunosuppression is the goal in long-term maintenance therapy, and this is achieved by reducing the number and/or the dosage of immunosuppressive agents. As noted above, I found that monotherapy was more commonly achieved in liver compared to heart and lung transplant recipients. In contrast, I observed a reduction in the median dosages of azathioprine with increasing time post-transplantation for heart and lung but not liver recipients. The dose of azathioprine was,

however, lower in liver than in heart and lung transplant recipients at every time point I examined. There was no consistent pattern for CNIs, but liver recipients used a higher dose of cyclosporine compared to heart and lung transplant recipients at 5 years. I contend that on the balance of this evidence, liver recipients receive a lower degree of immunosuppression than heart and lung transplant recipients. There are no prior published comparisons of immunosuppressive drug type and dosages by organ type.

The key strengths of my study are the population-base and the adjusted comparison of immunosuppressive drugs and drug dose by organ type. The transplant registries ascertained all recipients, thereby avoiding selection bias. As I had data on recipient and donor characteristics that may influence the choice of immunosuppressive agents and their dose, I was able to compare organ groups taking into account these potential confounding factors.

On the other hand, several limitations must also be considered. Being a retrospective study, I was reliant on the availability and quality of medical records. There were missing data, to a greater extent for heart and lung compared to liver transplant recipients. As I used the LOCF approach to impute the missing immunosuppression data, I am likely to have underestimated changes in immunosuppressive regimen over time, and overestimated the dose with increasing time since transplantation. Reassuringly however, I observed no notable differences between the analyses carried out based on the original

and imputed data. I could not identify clinical trial participants or collect immunosuppression trough levels, thus the doses do not represent actual drug concentrations. In addition, I am unable to exclude residual confounding as an explanation for the differences by organ type because I did not have information on every characteristic that may influence the choice and dose of immunosuppression after transplantation, such as infection and renal dysfunction.

5.6 Conclusions

I have used population-based data over 20 years to address a question of long-standing interest in transplantation research. My data demonstrates clear differences in immunosuppression therapies for Australian liver, heart and lung transplant recipients and these differences mirror international transplantation clinical practice. My novel finding of a statistically significantly lower degree of immune suppression in liver compared to heart and lung transplant recipients may explain the lower cancer risk in liver compared to cardiothoracic transplant recipients. I also demonstrate marked changes in immune suppression over time since transplantation, changes that must be taken into account in observational studies examining the relationship between iatrogenic immune suppression and post-transplantation outcomes.

Table 5 - 1 Median recipient weight before and after imputation at 3 months, and 1, 5, and 10 years after transplantation using three different imputation methods

Weight	Before imputation		After imputation					
			Mean weight method		LOCF method		Mixed model	
	N	%	N	%	N	%	N	%
3-months								
Number of patients*	4382	100	4382	100	4382	100	4382	100
Median (IQR) (kg)	68.0 (56.0-78.0)		68.0 (56.0-79.0)		68.0 (57.0-79.0)		68.6 (57.0-79.0)	
Non-missing	3101	70.8	3242	84.6	3675	83.9	3708	84.6
Missing	1281	29.2	1140	15.4	707	16.1	674	15.4
1-year								
Number of patients*	3930	100	3930	100	3930	100	3930	100
Median (IQR) (kg)	74.0 (62.0-85.0)		74.0 (62.0-85.0)		73.0 (61.0-85.0)		73.0 (60.2-84.1)	
Non-missing	2678	68.1	2767	86.3	2888	73.5	3392	86.3
Missing	1252	31.9	1163	13.7	1042	26.5	538	13.7
5-years								
Number of patients*	2380	100	2380	100	2380	100	2380	100
Median (IQR) (kg)	77.0 (64.0-88.0)		77.0 (63.0-88.0)		77.0 (63.0-88.0)		76.0 (63.0-87.2)	
Non-missing	1597	67.1	1597	67.1	1764	74.2	2151	90.4
Missing	783	32.9	783	32.9	616	25.8	229	9.60
10-years								
Number of patients*	1149	100	1149	100	1149	100	1149	100
Median (IQR) (kg)	76.0 (63.0-88.0)		76.0 (63.0-88.0)		75.0 (63.0-87.0)		75.0 (63.0-87.0)	
Non-missing	702	61.0	702	61.0	825	71.8	1056	91.9
Missing	447	38.9	447	38.9	324	28.2	93	8.10

*The total number of transplant recipients who were still alive at each follow-up time.

Table 5 - 2 Receipt and dosage (mg/kg/day) of immunosuppressive agents at 3 months post-transplantation, before and after imputation of missing data for weight and agent type and dose

Immunusupressive agent	Transplanted organ (original data)						Transplanted organ (imputed data)					
	Liver		Heart		Lung		Liver		Heart		Lung	
	N	%	N	%	N	%	N	%	N	%	N	%
All	1851	100	1384	100	1147	100	1851	100	1384	100	1147	100
CNIs (cyclosporine/tacrolimus)												
Yes	1707	92.2	1143	82.6	973	84.8	1792	96.8	1156	83.5	1016	88.5
No	12	0.70	24	1.70	9	0.80	33	1.80	28	2.00	11	1.00
Missing	132	7.10	217	15.7	165	14.4	26	1.40	200	14.5	120	10.5
Cyclosporine												
Yes	814	44.0	80	5.80	144	12.6	871	47.1	84	6.10	147	12.8
Median dose* (IQR)	716	5.69 (4.17-7.69)	968	4.39 (3.25-6.08)	600	5.00 (3.00-7.42)	863	5.51 (4.11-7.53)	1098	4.38 (3.17-6.03)	872	5.32 (3.63-8.00)
No	905	48.9	1087	78.5	837	73.0	952	51.4	1100	79.5	879	76.6
Missing	132	7.10	217	15.7	166	14.5	28	1.51	200	14.5	121	10.6
Tacrolimus												
Yes	801	43.3	54	3.90	135	11.8	837	45.2	54	3.90	136	11.9
Median dose* (IQR)	612	0.12 (0.08-0.18)	43	0.13 (0.08-0.19)	89	0.14 (0.08-0.19)	668	0.12 (0.08-0.18)	54	0.13 (0.08-0.18)	119	0.14 (0.08-0.20)
No	918	49.6	1113	80.4	846	73.8	986	53.3	1130	81.7	890	77.6
Missing	132	7.1	217	15.7	166	14.4	28	1.50	200	14.5	121	10.5

*median dose, mg/kg/day

Table 5 – 2 (continued) Receipt and dosage (mg/kg/day) of immunosuppressive agents at 3 months post-transplantation, before and after imputation of missing data for weight and agent type and dose

Immunusupressive agent	Transplanted organ (original data)						Transplanted organ (imputed data)					
	Liver		Heart		Lung		Liver		Heart		Lung	
	N	%	N	%	N	%	N	%	N	%	N	%
Antiproliferative (azathioprine/mycophenolate)												
Yes	1075	58.1	1030	74.4	878	76.5	1157	62.5	1050	75.9	922	80.4
No	644	34.8	137	9.90	104	9.10	668	36.1	134	9.70	105	9.10
Missing	132	7.10	217	15.7	165	14.4	26	1.40	200	14.5	120	10.5
Azathioprine												
Yes	936	50.6	735	53.1	690	60.2	1014	54.7	748	54.0	732	63.8
Median dose* (IQR)	747	1.09 (0.85-1.39)	669	1.64 (1.32-1.90)	495	1.67 (1.00-2.00)	904	1.07 (0.84-1.41)	741	1.67 (1.30-1.92)	711	1.52 (1.00-2.00)
No	782	42.2	397	28.7	292	25.5	810	43.8	401	29.0	295	25.7
Missing	133	7.20	252	18.2	165	14.3	27	1.50	235	17.0	120	10.5
Mycophenolate												
Yes	138	7.50	260	18.8	188	16.4	142	7.60	267	19.3	190	16.5
Median dose *(IQR)	93	25.6 (20.8-30.3)	208	36.1 (29.4-41.4)	152	36.2 (28.0-45.6)	102	25.6 (18.9-30.8)	265	36.4 (29.4-41.7)	189	36.0 (27.2-44.8)
No	1580	85.3	872	63.0	794	69.2	1682	90.9	882	63.7	837	73.0
Missing	133	7.20	252	18.2	165	14.4	27	1.50	235	17.0	120	10.5
mTORs (sirolimus/everolimus)												
Yes	56	3.00	54	3.90	90	7.80	11	0.60	49	3.50	82	7.10
No	1790	96.7	1214	87.7	1031	89.9	1835	99.1	1218	88.0	1039	90.6
Missing	5	0.30	116	8.40	26	2.30	5	0.30	117	9	26	2.30
Sirolimus												
Yes	5	0.30	94	6.80	7	0.60	5	0.30	95	6.90	7	0.60
Median dose* (IQR)	3	0.10 (0.05-0.24)	71	0.04 (0.03-0.07)	3	0.07 (0.02-0.18)	3	0.1 (0.05-0.24)	76	0.04 (0.03-0.07)	5	0.05 (0.02-0.07)
No	1790	96.7	1236	89.3	1050	91.5	1835	99.1	1240	89.6	1058	92.2
Missing	56	3.00	54	3.90	90	7.90	11	0.60	49	3.5	82	7.20
Everolimus												
Yes	0	0	22	1.60	19	1.70	0	0	22	1.6	19	1.70
Median dose* (IQR)	0	-	20	0.02 (0.01-0.03)	17	0.02 (0.01-0.03)			20	0.02 (0.01-0.03)	17	0.02 (0.01-0.03)
No	1817	98.2	1318	95.2	1073	93.5	1845	99.7	1320	95.4	1076	93.8
Missing	34	1.80	44	3.20	55	4.80	6	0.30	42	3.00	52	4.50

*median dose, mg/kg/day

Table 5 - 3 Receipt and dosage of immunosuppressive agents at 5 years post-transplantation before and after imputation of missing data for weight and agent type and dose

Immunusupressive agent	Transplanted organ (before imputation)						Transplanted organ (after imputation)					
	Liver		Heart		Lung		Liver		Heart		Lung	
	N	%	N	%	N	%	N	%	N	%	N	%
All	1036	100	923	100	425	100	1036	100	923	100	425	100
CNIs (cyclosporine/tacrolimus)												
Yes	838	80.9	743	80.5	315	74.1	885	85.4	783	84.8	345	81.2
No	42	4.05	21	2.30	16	3.80	42	4.10	23	2.50	17	4.00
Missing	156	15.1	159	17.2	94	22.1	109	10.5	117	12.7	63	14.8
Cyclosporine												
Yes	542	52.3	698	75.6	238	56.0	575	55.5	736	79.7	261	61.4
Median dose* (IQR)	362	2.74 (2.08-3.57)	557	2.41 (1.77-3.41)	184	2.53 (1.69-3.55)	540	2.83 (2.1-3.73)	730	2.44 (1.79-3.41)	254	2.90 (2.00-4.00)
No	338	32.6	66	7.2	93	21.9	352	34.0	70	7.60	101	23.8
Missing	156	15.1	159	17.2	94	22.1	109	10.5	117	12.7	63	14.8
Tacrolimus												
Yes	295	28.4	45	4.9	77	18.1	309	29.8	47	5.10	84	19.8
Median dose* (IQR)	235	0.05 (0.03-0.08)	36	0.06 (0.03-0.11)	37	0.06 (0.03-0.12)	292	0.05 (0.03-0.08)	47	0.05 (0.03-0.10)	59	0.07 (0.03-0.12)
No	585	56.5	719	77.9	254	59.8	618	59.7	759	82.2	278	65.4
Missing	156	15.1	159	17.2	94	22.1	109	10.5	117	12.7	63	14.8

*median dose, mg/kg/day

Table 5 - 3 (continued) Receipt and dosage of immunosuppressive agents at 5-years post-transplantation before and after imputation of missing data for weight and agent type and dose

Immunusupressive agent	Transplanted organ (before imputation)						Transplanted organ (after imputation)					
	Liver		Heart		Lung		Liver		Heart		Lung	
	N	%	N	%	N	%	N	%	N	%	N	%
Antiproliferative (azathioprine)												
Yes	374	36.1	690	74.8	274	64.5	402	38.8	731	79.2	301	70.8
No	506	48.8	74	8.00	57	13.4	526	50.8	75	8.10	58	13.7
Missing	156	15.1	159	17.2	94	22.1	108	10.4	117	12.7	66	15.5
Azathioprine												
Yes	296	28.6	541	58.6	208	48.9	322	31.1	576	62.4	229	53.9
Median dose* (IQR)	185	0.85 (0.67-1.15)	448	1.24 (0.85-1.54)	154	1.00 (1.00-1.60)	298	0.85 (0.66-1.15)	570	1.26 (0.85-1.56)	214	1.00 (0.77-1.53)
No	583	56.3	223	24.2	122	28.7	605	58.4	230	24.9	129	30.4
Missing	157	15.1	159	17.2	95	22.4	109	10.5	117	12.7	67	15.8
Mycophenolate												
Yes	77	7.4	149	16.1	65	15.3	79	7.60	155	16.8	71	16.7
Median dose *(IQR)	71	22.0 (17.4-27.8)	108	24.5 (17.9-33.8)	52	26.5 (16.0-33.9)	78	21.4 (17.2-27.8)	155	24.4 (16.7-34.2)	70	23.4 (15.0-31.0)
No	802	77.4	615	66.7	265	62.4	848	81.9	651	70.5	287	67.5
Missing	157	15.1	159	17.2	95	22.3	109	10.5	117	12.7	67	15.8
mTORs (sirolimus/everolimus)												
Yes	7	0.7	51	5.5	26	6.10	7	0.7	53	5.70	27	6.40
No	966	93.2	844	91.4	343	80.7	1008	97.3	865	93.7	380	89.4
Missing	63	6.1	28	3.00	56	13.2	21	2.00	5	0.60	18	4.20
Sirolimus												
Yes	7	0.7	49	5.3	23	5.40	7	0.70	51	5.50	24	5.70
Median dose* (IQR)	5	0.04 (0.03-0.05)	37	0.02 (0.01-0.03)	8	0.03 (0.02-0.05)	7	0.03 (0.02-0.05)	43	0.02 (0.01-0.03)	13	0.04 (0.02-0.05)
No	966	93.2	846	91.7	346	81.4	1008	97.3	867	93.9	383	90.1
Missing	63	6.1	28	3.00	56	13.2	21	2.0	5	0.60	18	4.20
Everolimus												
Yes	0	0	2	0.2	3	0.70	0	0	2	0.20	3	0.7
Median dose* (IQR)			2	0.01 (0.01-0.01)	1	0.03	0	-	2	0.01 (0.01-0.01)	1	0.03
No	1012	97.7	910	98.6	397	93.4	1015	98.0	916	99.2	404	95.1
Missing	24	2.30	11	1.2	25	5.90	21	2.00	5	0.60	18	4.20

*median dose, mg/kg/day

Table 5 - 4 Characteristics of Australian liver, heart, and lung transplant recipients, 1984-2006

Characteristics at transplant	Liver		Heart		Lung		*p#*
	n	%	n	%	n	%	
Total	1895	100	1220	100	1059	100	
Age							<0.001
Paediatric (≤15 years)	302	15.9	83	6.80	20	1.90	
Adult (>15 years)	1593	84.1	1137	93.2	1039	98.1	
Sex							<0.001
Male	1170	61.7	983	80.6	567	53.5	
Female	725	38.3	237	19.4	492	46.5	
Race							<0.001
Caucasian	1540	82.5	1141	94.1	1030	97.7	
Non-Caucasian	326	17.5	71	5.90	24	2.30	
Unknown	29		8		5		
Transplant year (era)							<0.001
1984-1994	510	26.9	530	43.4	224	21.1	
1995-1997	285	15.0	240	19.7	216	20.4	
1998-2006	1100	58.1	450	36.9	619	58.5	
Primary indication							<0.001
Viral hepatitis	486	25.7					
Autoimmune disease	436	23.0					
Alcoholic liver disease	223	11.8					
Congenital biliary disease	188	9.90					
Hepatobiliary tumour	88	4.60					
Non-ischaemic cardiomyopathy			537	44.0			
Ischaemic heart disease			473	38.8			
Congenital heart disease			69	5.70	52	4.90	
Obstructive lung disease					352	33.2	
COPD					313	29.6	
Miscellaneous	474	25.0	141	11.5	342	32.3	
Alcohol history							<0.001
Yes	953	65.3	877	78.7	557	75.8	
No	507	34.7	237	21.3	178	24.2	
Unknown	435		106		324		
Smoking history							<0.001
Yes	663	44.8	743	63.3	558	75.8	
No	818	55.2	431	36.7	400	24.2	
Unknown	414		46		101		
Cancer history*							<0.001
Yes	312	16.5	31	2.50	31	2.90	
No	1583	83.5	1189	97.5	1028	97.1	

Table 5 – 4 (continued) Characteristics of Australian liver, heart, and lung transplant recipients, 1984-2006

Characteristics at transplant	Liver		Heart		Lung		*p*
	n	%	n	%	n	%	
Dialysis history							<0.001
Yes	69	4.10	28	2.60	4	0.60	
No	1662	95.9	1030	97.4	617	99.4	
Unknown	164		162		438		
Diabetes history							<0.01
Yes	265	14.4	131	12.5	102	19.2	
No	1571	85.6	917	97.5	429	80.8	
Unknown	59		172		528		
Hypertension history							<0.001
Yes	162	13.9	336	34.9	110	21.5	
No	1003	86.1	627	65.1	401	78.5	
Unknown	730		257		548		
Recipient CMV IgG status							<0.001
Positive	1297	71.5	809	66.4	665	63.7	
Negative	517	28.5	409	33.6	379	36.3	
Unknown	81		2		15		
Recipient EBV IgG status							<0.001
Positive	1065	79.5	513	70.9	566	83.8	
Negative	275	20.5	210	29.1	109	16.2	
Unknown	555		497		384		
Recipient anti-HCV antibody							<0.001
Positive	419	25.7	6	0.80	3	0.50	
Negative	1214	74.3	748	99.2	549	99.5	
Unknown	262		466		507		
Other transplanted organ							NA
No	1895	100	1199	98.3	1054	99.5	
Yes, kidney	0	0	21	1.70	2	0.20	
Yes, heart	0	0	0	0	3	0.30	
Donor sex							<0.001
Male	1088	57.4	866	71.0	619	58.7	
Female	763	40.3	353	29.0	435	41.3	
Donor age, median (IQR)	35	(20-49)	30	(20-43)	34	(22-46)	<0.001

*Excluding non-melanoma skin cancer and cancers diagnosed before 1982; COPD Chronic obstructive pulmonary disease; CMV Cytomegalovirus; EBV Epstein Barr virus; HCV hepatitis C virus ; #Non-parametric Wilcoxon rank-sum test, chi-square test or Fisher exact test as appropriate.

Table 5 - 5 Receipt of antibody induction therapy by age group, sex and year of transplant in Australian liver, heart and lung transplant recipients

	Total	Age group			Transplant era				Transplanted organ			
		Adult	Paediatric		1984-1994	1995-1997	1998-2006		Liver	Heart	Lung	
	N (%)	N (%)	N (%)	*p* #	N (%)	N (%)	N (%)	*p* #	N (%)	N (%)	N (%)	*p* #
Total	4174 (100)	3769 (100)	405 (100)		1264 (100)	741 (100)	2169 (100)		1895 (100)	1220 (100)	1059 (100)	
Intravenous corticosteroids				<0.001				<0.001				<0.001
Yes	1036 (24.8)	981 (26.0)	55 (13.6)		398 (31.5)	239 (32.3)	399 (18.4)		514 (27.1)	386 (31.6)	136 (12.8)	
No	3138 (75.2)	2788 (74.0)	350 (86.4)		866 (68.5)	502 (67.8)	1770 (81.6)		1381 (72.9)	834 (68.4)	923 (87.2)	
Antibody induction				<0.001				<0.001				<0.001
Yes	846 (22.3)	791 (23.3)	55 (13.9)		444 (37.3)	52 (7.60)	350 (18.3)		152 (8.20)	491 (41.6)	203 (27.0)	
No	2947 (77.7)	2606 (76.7)	341 (86.1)		747 (62.7)	633 (92.4)	1567 (81.7)		1707 (91.8)	690 (58.4)	550 (73.0)	
Missing	381	372	9		73	56	252		36	39	306	
ATG/ALG				<0.001				<0.001				<0.001
Yes	512 (13.6)	485 (14.3)	27 (6.80)		350 (29.6)	36 (5.20)	126 (6.60)		9 (0.50)	364 (31.0)	139 (18.6)	
No	3263 (86.4)	2894 (85.7)	369 (93.2)		832 (70.4)	649 (94.7)	1782 (93.4)		1845 (99.5)	809 (69.0)	609 (81.4)	
Missing	399	390	9		82	56	261		41	47	311	
OKT3				0.02				<0.001				<0.001
Yes	141 (3.70)	118 (3.10)	23 (5.80)		111 (9.40)	17 (2.50)	13 (0.70)		84 (4.50)	53 (4.50)	4 (0.50)	
No	3636 (96.3)	3263 (86.6)	373 (94.2)		1073 (90.6)	668 (97.5)	1895 (99.3)		1773 (95.5)	1123 (95.5)	740 (99.5)	
Missing	397	388	9		80	56	261		38	44	315	
IL-2Ra				<0.001				<0.001				<0.001
Yes	218 (5.50)	210 (5.90)	8 (2.00)		0	0	218 (11.2)		69 (3.70)	87 (7.20)	62 (7.20)	
No	3728 (94.5)	3336 (94.1)	392 (98.0)		1264 (100)	741 (100)	1723 (88.8)		1808 (96.3)	1125 (92.8)	795 (92.8)	
Missing	228	223	5		0	0	228		18	8	202	

Abbreviations: IL-2Ra, Interleukin 2 receptor antagonists; #P value shown: Chi-square test or Fisher exact test as appropriate.

Table 5 - 6 Receipt of therapy for acute rejection by age group, sex and year of transplant in Australian liver, heart and lung transplant recipients

	Total	Age group			Transplant era				Transplanted organ			
		Adult	Paediatric		1984-1994	1995-1997	1998-2006		Liver	Heart	Lung	
	N (%)	N (%)	N (%)	*p#*	N (%)	N (%)	N (%)	*p#*	N (%)	N (%)	N (%)	*p#*
Total	4174 (100)	3769 (100)	405 (100)		1264 (100)	741 (100)	2169 (100)		1895 (100)	1220 (100)	1059 (100)	
Treated rejection				<0.001				<0.001				<0.001
Yes	1512 (36.2)	1314 (34.9)	198 (48.9)		629 (49.8)	290 (39.1)	593 (27.3)		824 (43.5)	398 (32.6)	290 (27.4)	
No	2662 (63.8)	2455 (65.1)	207 (51.1)		635 (50.2)	451 (60.9)	1576 (72.7)		1071 (56.5)	822 (67.4)	769 (72.6)	
Intravenous corticosteroids				<0.001				<0.001				<0.001
Yes	1294 (31.0)	1105 (29.3)	189 (46.7)		538 (42.6)	247 (33.3)	509 (23.5)		765 (40.4)	300 (24.6)	229 (21.6)	
No	2880 (69.0)	2664 (70.7)	216 (53.3)		726 (57.4)	494 (66.7)	1660 (76.5)		1130 (59.6)	920 (75.4)	830 (78.4)	
Any antibody				<0.001				<0.001				0.007
Yes	362 (8.7)	306 (8.1)	56 (13.8)		212 (16.8)	66 (8.9)	84 (3.9)		190 (10.0)	83 (6.8)	89 (8.4)	
No	3812 (91.3)	3463 (91.9)	349 (86.2)		1052 (83.2)	675 (91.1)	2085 (96.1)		1705 (89.0)	1137 (93.2)	970 (91.6)	
ATG/ALG				0.22				<0.001				<0.001
Yes	184 (4.4)	171 (4.5)	13 (3.2)		108 (8.5)	40 (5.4)	36 (1.7)		32 (1.7)	68 (5.6)	84 (7.9)	
No	3990 (95.6)	3598 (95.5)	392 (96.8)		1156 (91.5)	701 (94.6)	2133 (98.3)		1863 (98.3)	1152 (94.4)	975 (92.1)	
OKT3				<0.001				<0.001				<0.001
Yes	203 (4.9)	157 (4.2)	46 (11.4)		133 (10.5)	28 (3.8)	42 (1.9)		180 (9.5)	20 (1.6)	3 (0.3)	
No	3971 (95.1)	3612 (95.8)	359 (88.6)		1131 (89.5)	713 (96.2)	2127 (98.1)		1715 (90.5)	1200 (98.4)	1056 (99.7)	

#*P* value shown: Chi-square test or Fisher exact test as appropriate.

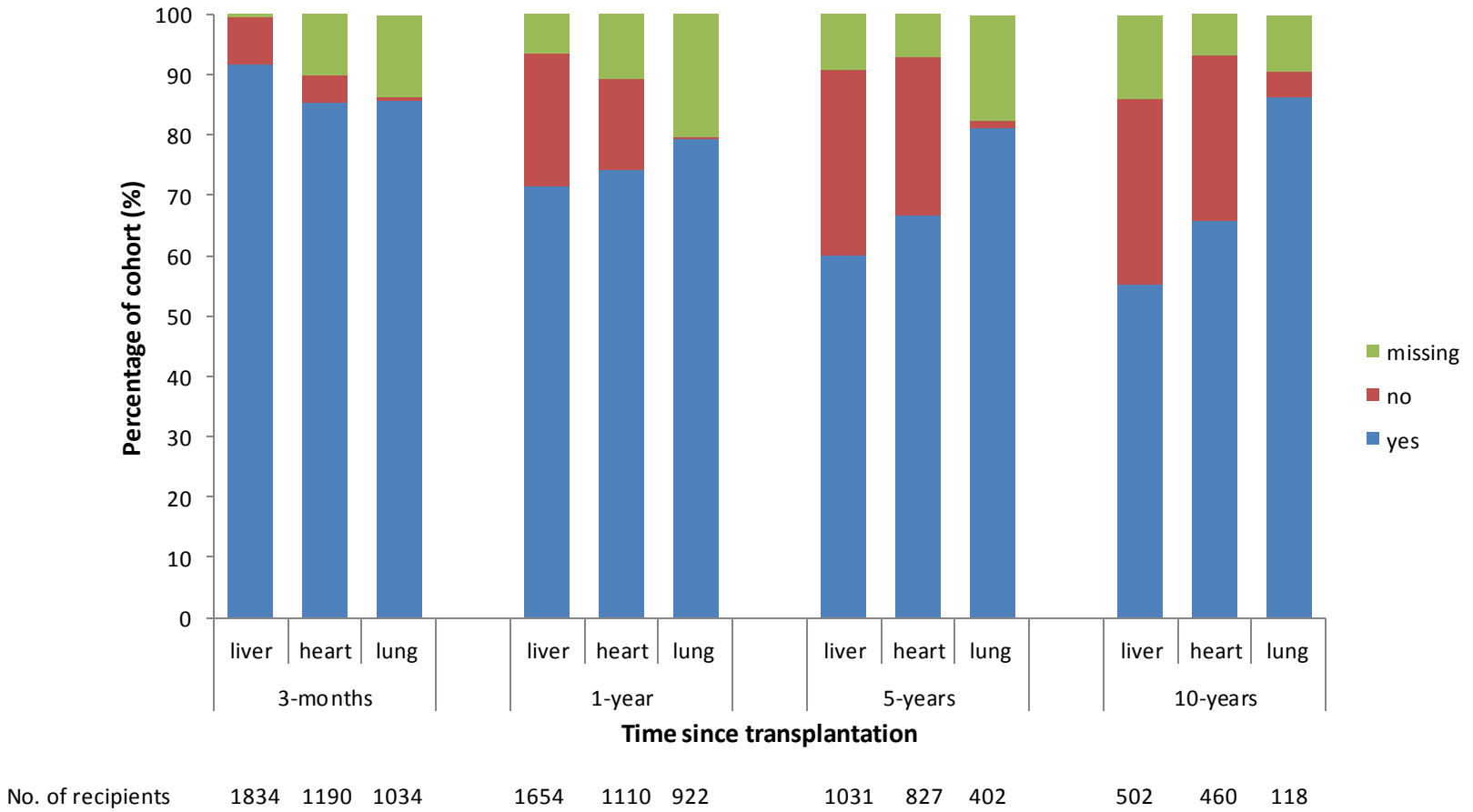


Figure 5 - 3. The receipt of corticosteroids by transplant organ type at 3 months, and 1, 5, and 10 years after transplantation

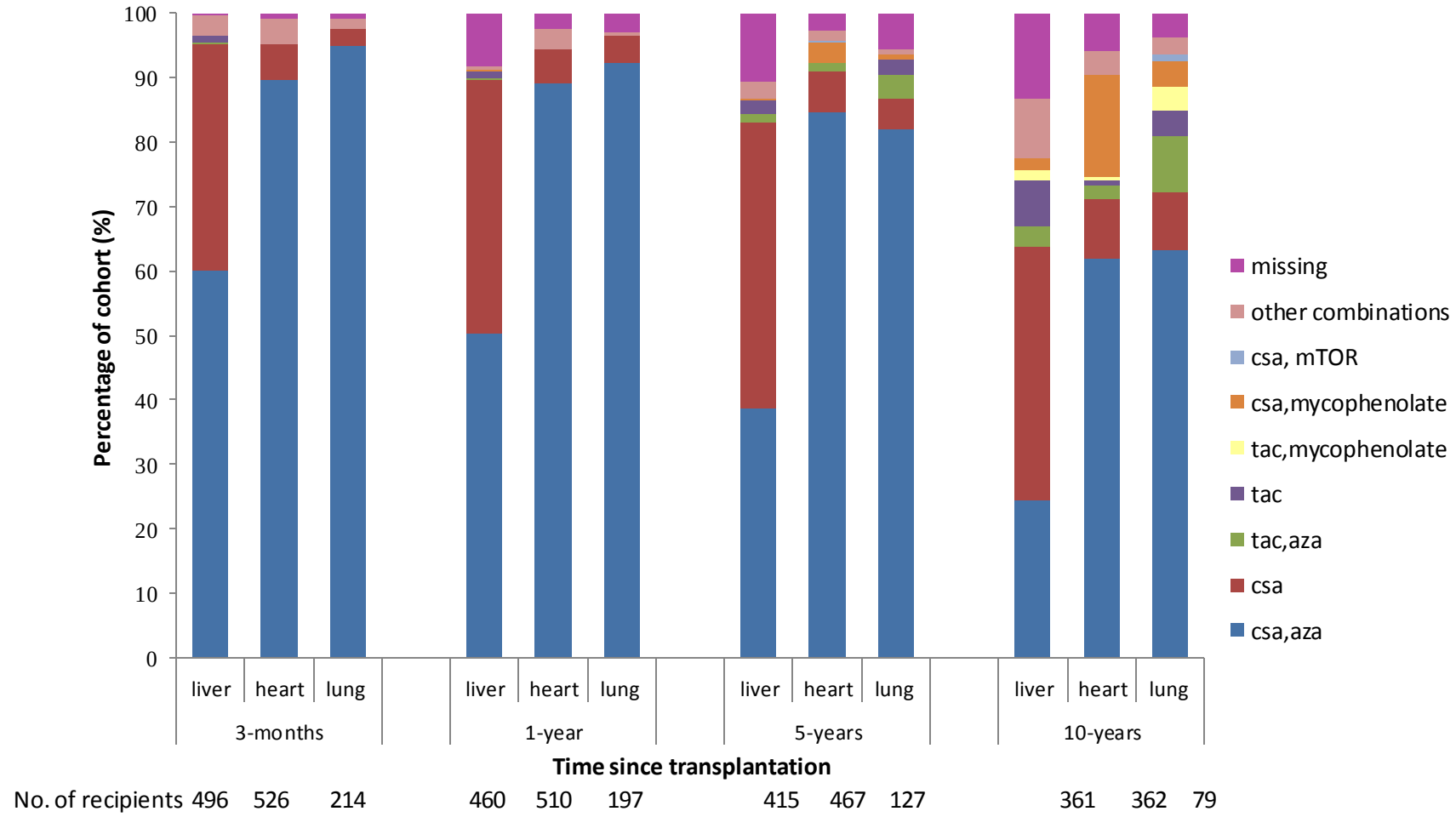


Figure 5 - 4. Immunosuppressive drug combinations by time since transplantation for Australians who received a liver, heart or lung transplant between 1984 and 1994

Notes: "other combinations" include mTOR with azathioprine/mycophenolate with or without cyclosporine/tacrolimus. Figure shows the regimens used by individuals still alive at a given point in time.

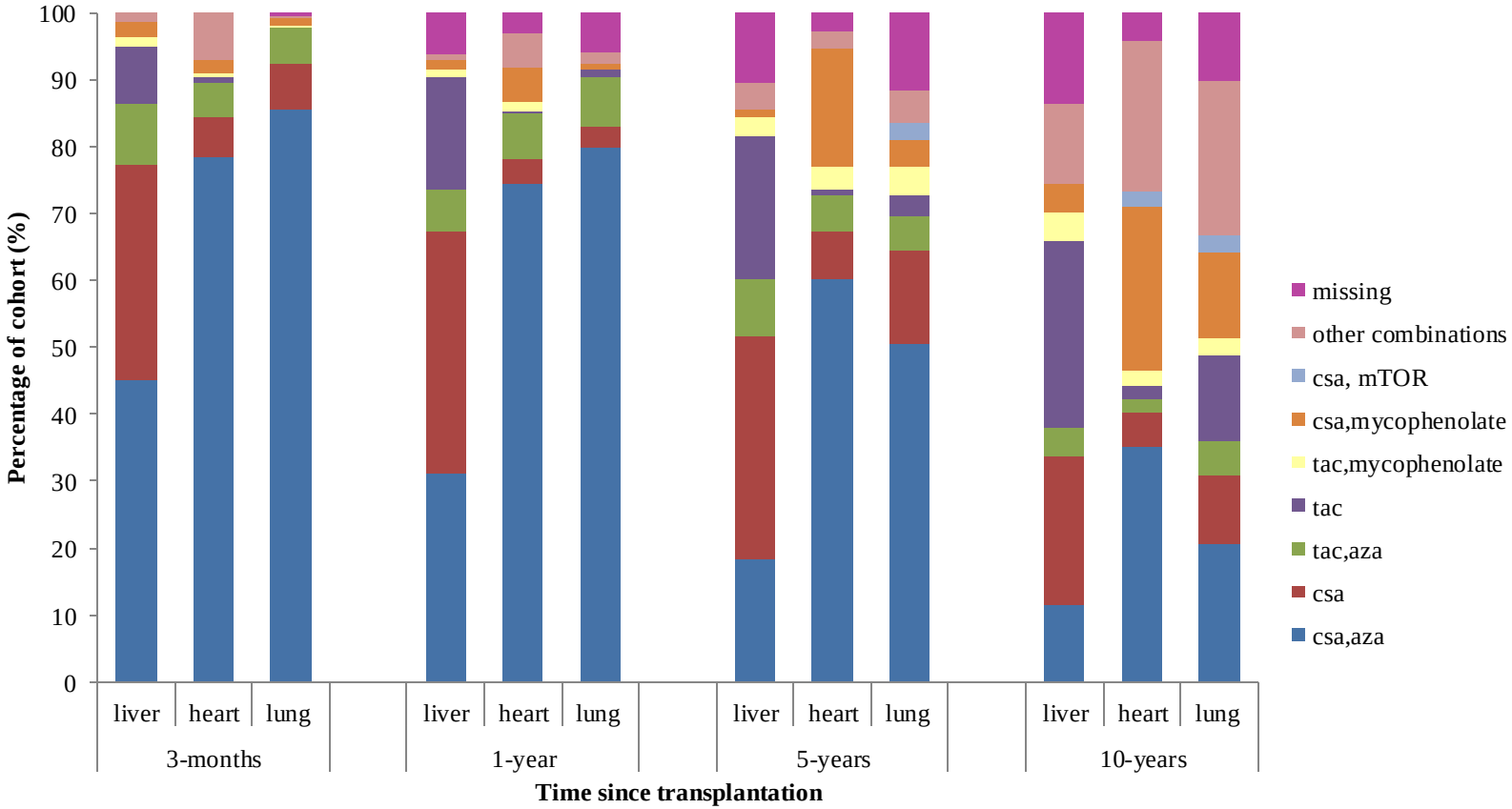


Figure 5 - 5. Immunosuppressive drug combinations by time since transplantation for Australians who received a liver, heart or lung transplant between 1995 and 1997

Notes: "other combinations" is mTOR with azathioprine/mycophenolate with or without cyclosporine A/tacrolimus. It is important to note that the figure shows the regimens used by individuals at a given point in time and different individuals are alive at each follow-up time post-transplantation.

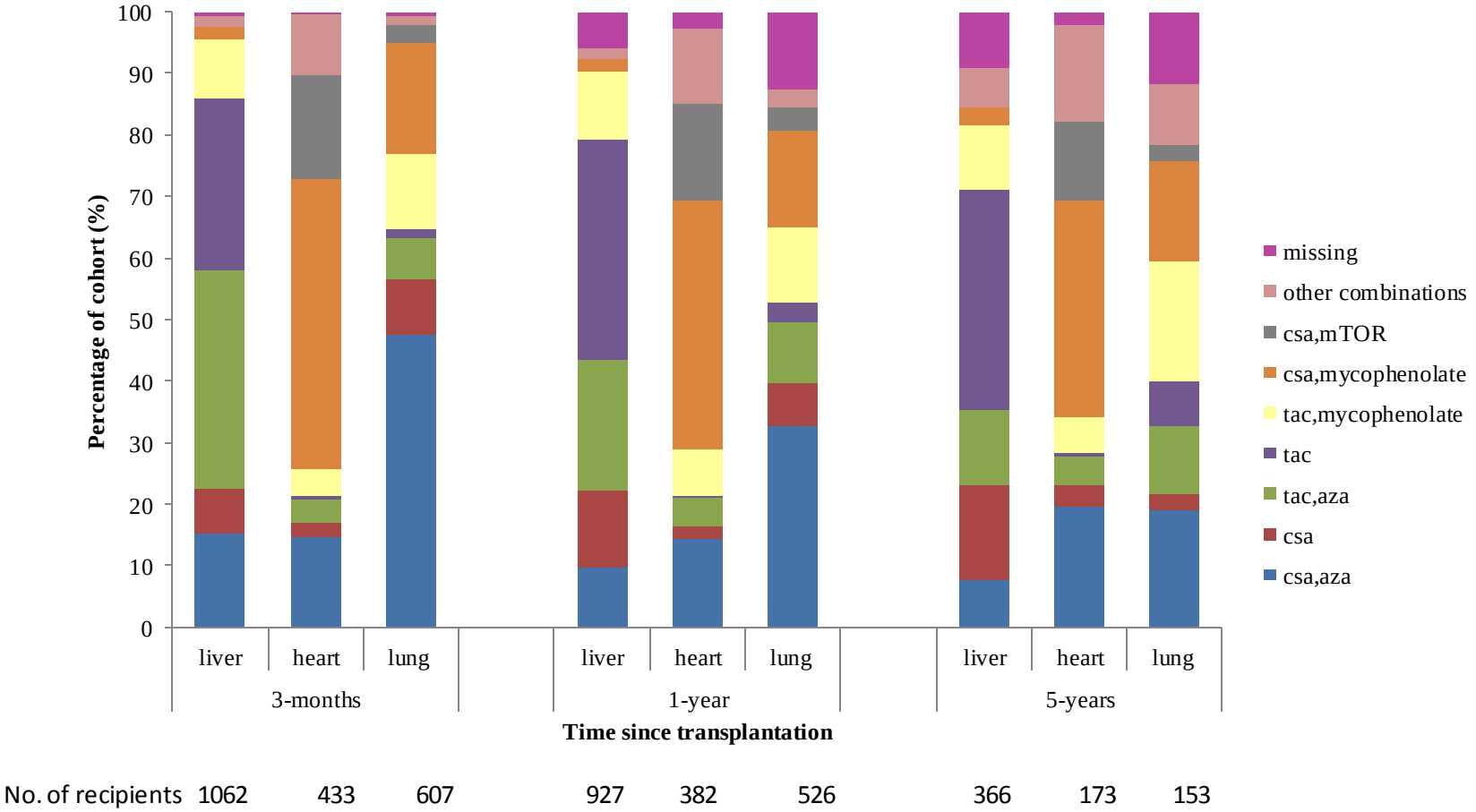


Figure 5 - 6. Immunosuppressive drug combinations by time since transplantation for Australians who received a liver, heart or lung transplant between 1998 and 2006

Notes: "other combinations" is mTOR with azathioprine/mycophenolate with or without cyclosporine A/tacrolimus. It is important to note that the figure shows the regimens used by individuals at a given point in time and different individuals are alive at each f ollow-up time post-transplantation.

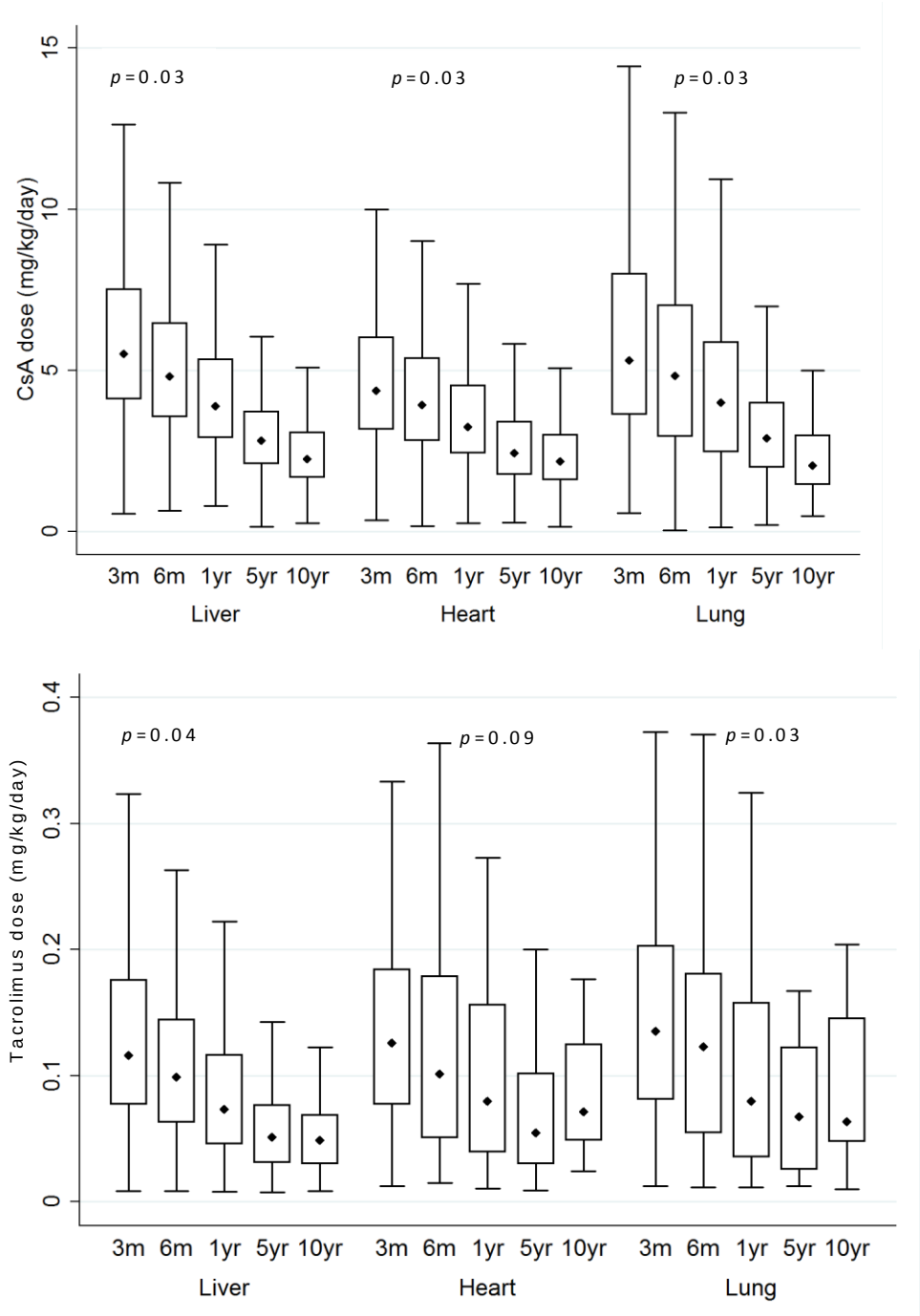
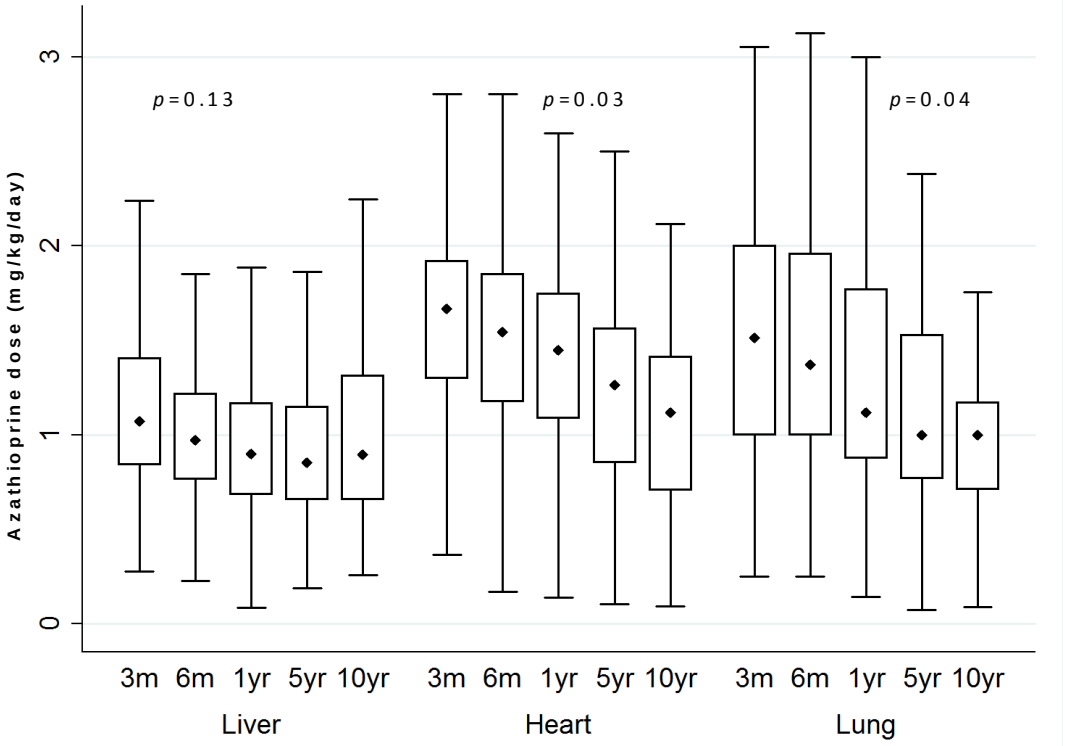


Figure 5 - 7. Change in median (IQR) cyclosporine and tacrolimus dose (mg/kg/day) by time since transplantation and organ type

P value shown: Mann-Kendall trend test.



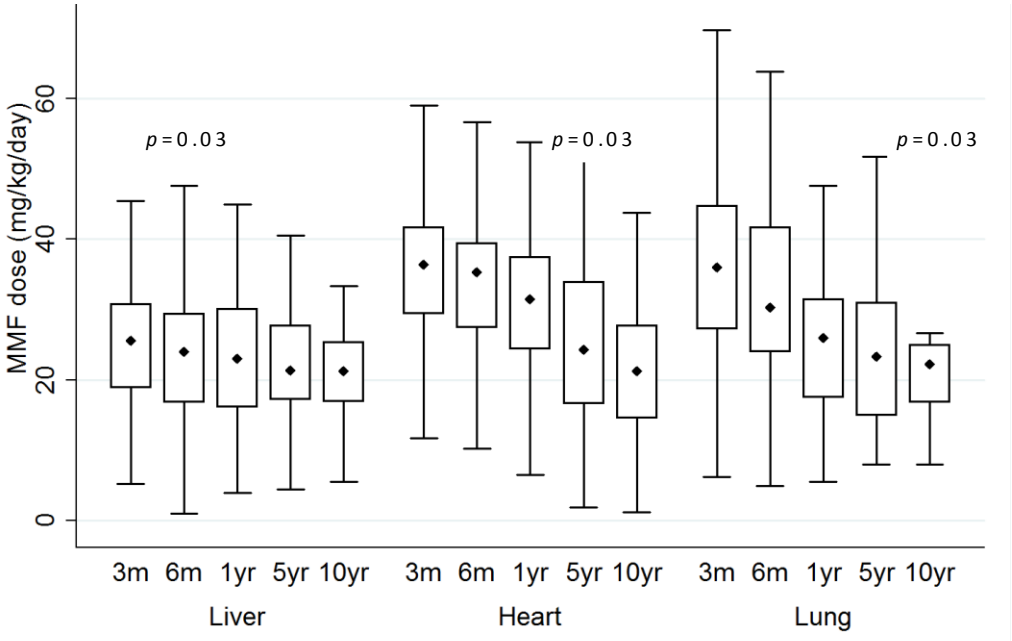


Figure 5 - 8. Change in median (IQR) mycophenolate and azathioprine dose (mg/kg/day) by time since transplantation and organ type.
P value shown: Mann-Kendall trend test.

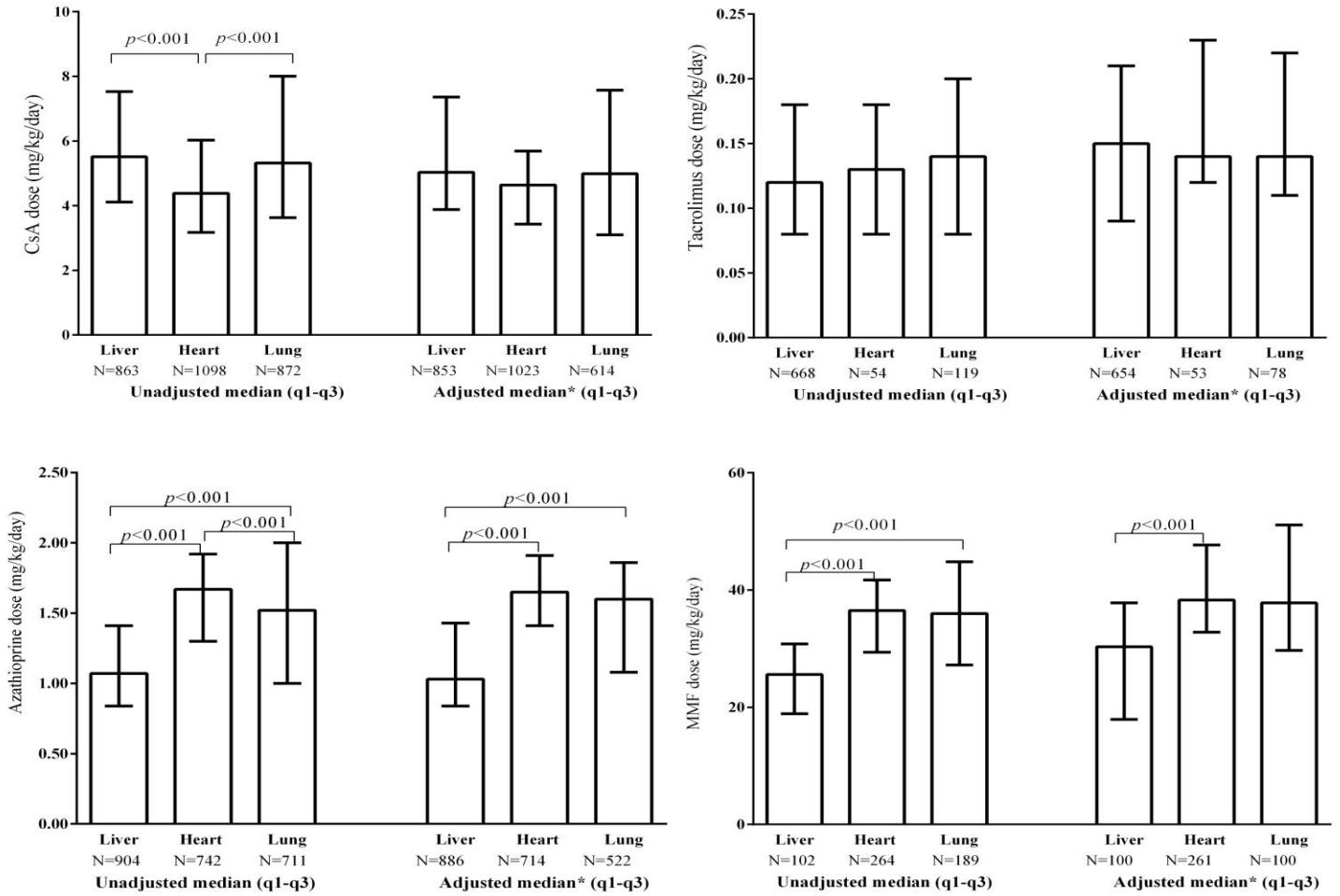


Figure 5 - 9. Unadjusted and adjusted median dose of immunosuppressive agent by organ type 3 months after transplantation

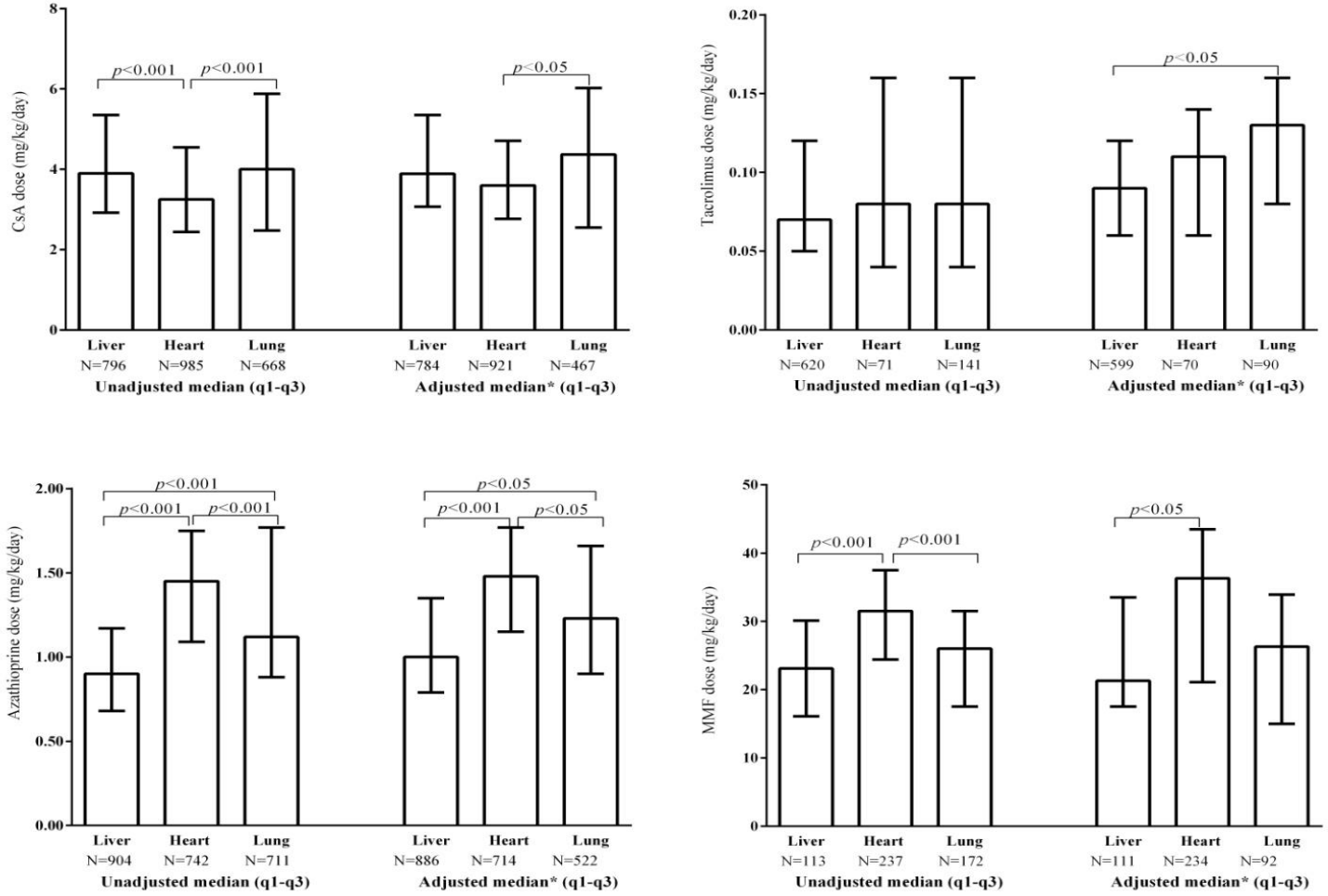


Figure 5 - 10. Unadjusted and adjusted median (IQR) dose (mg/kg/day) of immunosuppressive agent by organ type 1 year after transplantation

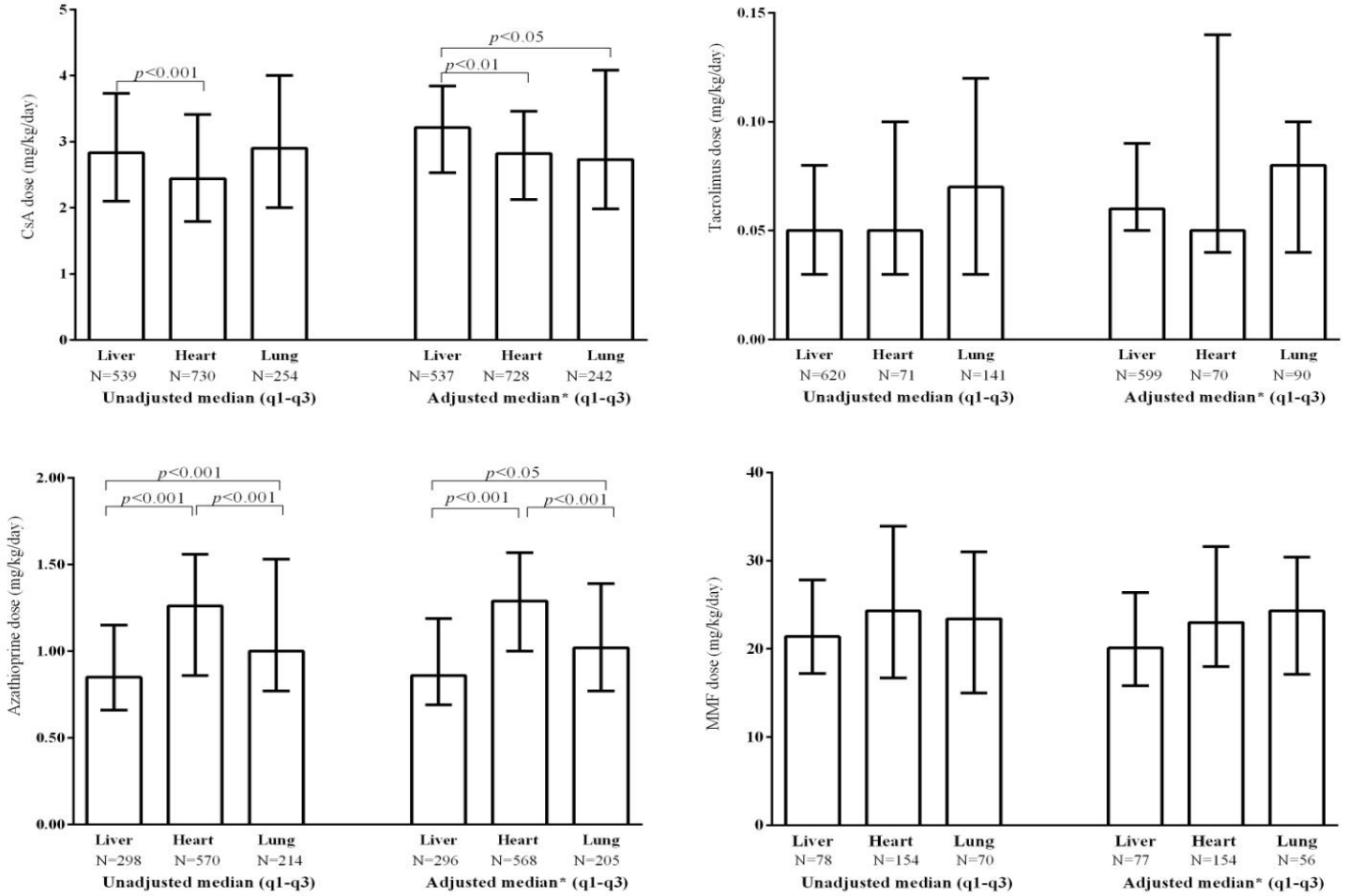


Figure 5 - 11. Unadjusted and adjusted median (IQR) dose (mg/kg/day) of immunosuppressive agent by organ type 5 years after transplantation

Table 5 - 7. Immunosuppressive drug combinations at 3 months, and 1, 5 and 10 years after transplantation by organ type, 1984-2006*

Drug combinations	3 months post-transplant				1 year post-transplant			
	Liver, N (%)	Heart, N (%)	Lung, N (%)	Total, N (%)	Liver, N (%)	Heart, N (%)	Lung, N (%)	Total, N (%)
Total	1834 (100)	1190 (100)	1034 (100)	4058 (100)	1654 (100)	1110 (100)	922 (100)	3686 (100)
CsA, AZA	585 (31.9)	716 (60.2)	674 (65.2)	1975 (48.7)	403 (24.4)	672 (60.5)	513 (55.6)	1588 (43.1)
CsA	339 (18.5)	53 (4.45)	75 (7.25)	467 (11.5)	394 (23.8)	43 (3.87)	51 (5.53)	488 (13.2)
TAC, AZA	404 (21.8)	28 (2.35)	51 (4.93)	483 (11.9)	216 (13.1)	32 (2.88)	66 (7.16)	314 (8.52)
CsA,mycophenolate	28 (1.50)	210 (17.6)	111 (10.7)	349 (8.60)	26 (1.57)	166 (14.9)	85 (9.22)	277 (7.51)
TAC	325 (17.6)	5 (0.42)	10 (0.97)	340 (8.38)	380 (23.0)	3 (0.27)	20 (2.17)	403 (10.9)
TAC,mycophenolate	107 (5.80)	19 (1.60)	75 (7.25)	201 (4.95)	105 (6.35)	31 (2.79)	82 (8.89)	218 (5.91)
CsA, AZA, mTORi	0	73 (6.13)	18 (1.74)	91 (2.24)	0 (0)	60 (5.41)	20 (2.17)	80 (2.17)
Others	37 (2.00)	80 (6.72)	13 (1.26)	130 (3.20)	19 (1.15)	73 (6.58)	19 (2.06)	111 (3.01)
Missing	9 (0.49)	6 (0.50)	7 (0.68)	22 (0.54)	111 (6.71)	30 (2.70)	66 (7.16)	207 (5.62)
	5 years post-transplant				10 years post-transplant			
	Liver, N (%)	Heart, N (%)	Lung, N (%)	Total, N (%)	Liver, N (%)	Heart, N (%)	Lung, N (%)	Total, N (%)
Total*	1029 (100)	826 (100)	401 (100)	2256 (100)	501 (100)	459 (100)	118 (100)	1078 (100)
CsA, AZA	233 (22.6)	541 (65.5)	194 (48.4)	968 (42.9)	104 (20.8)	258 (56.2)	58 (49.2)	420 (39.0)
CsA	325 (31.6)	49 (5.93)	27 (6.73)	401 (17.8)	173 (34.5)	39 (8.50)	11 (9.32)	223 (20.7)
TAC	70 (6.80)	24 (2.91)	28 (6.98)	122 (5.41)	18 (3.59)	9 (1.96)	9 (7.63)	36 (3.34)
CsA,mycophenolate	15 (1.46)	109 (13.2)	31 (7.73)	155 (6.87)	13 (2.59)	81 (17.6)	8 (6.78)	102 (9.46)
TAC, AZA	193 (18.7)	3 (0.36)	18 (4.49)	214 (9.49)	64 (12.8)	5 (1.09)	8 (6.78)	77 (7.14)
TAC,mycophenolate	45 (4.37)	16 (1.94)	35 (8.73)	96 (4.25)	12 (2.39)	4 (0.87)	4 (3.39)	20 (1.86)
CsA, AZA, mTORi	0 (0)	23 (2.78)	7 (1.75)	30 (1.33)	0 (0)	2 (0.44)	2 (1.69)	4 (0.37)
Others	45 (4.37)	40 (4.84)	22 (5.49)	107 (4.74)	50 (9.98)	36 (7.84)	11 (9.32)	97 (9.00)
Missing	103 (10.0)	21 (2.54)	39 (9.73)	163 (7.23)	67 (13.4)	25 (5.45)	7 (5.93)	99 (9.18)

* Table shows the regimens used by individuals still alive at a given point in time. Abbreviations: CsA, cyclosporine; AZA, azathioprine; TAC, tacrolimus; mTORi, mammalian target of rapamycin inhibitor.

Table 5 - 8. Median dose (mg/kg/day) of individual immunosuppressive agents 3 months after transplantation in Australian heart, lung and liver transplant recipients

	CNIs						Antiproliferative						mTORi					
	Cyclosporine			Tacrolimus			Mycophenolate			Azathioprine			Sirolimus			Everolimus		
	N	Median (q1-q3)	*P#*	N	Median (q1-q3)	*P#*	N	Median (q1-q3)	*P#*	N	Median (q1-q3)	*P#*	N	Median (q1-q3)	*P#*	N	Median (q1-q3)	*P#*
Total	2833	5.00 (3.57–7.02)		841	0.12 (0.08–0.18)		555	34.1 (26.7–41.6)		2357	1.36 (0.97–1.80)		84	0.04 (0.03–0.07)		37	0.02 (0.01–0.03)	
Age			<0.001			<0.001			0.78			0.96			0.55			-
Adult	2629	4.89 (3.47–6.67)		671	0.11 (0.07–0.15)		539	34.1 (26.7–41.6)		2123	1.36 (0.96–1.82)		82	0.04 (0.03–0.07)		37	0.02 (0.01–0.03)	
Paediatric	204	8.33 (6.00–12.1)		170	0.20 (0.13–0.30)		16	31.0 (25.8–42.3)		234	1.36 (1.04–1.67)		2	0.06 (0.03–0.09)		0	-	
Sex			<0.001			<0.001			<0.001			0.50			0.05			0.04
Male	1873	4.79 (3.45–6.60)		524	0.11 (0.07–0.16)		382	32.3 (25.6–39.0)		1523	1.36 (0.97–1.79)		69	0.04 (0.02–0.06)		24	0.01 (0.01–0.03)	
Female	960	5.56 (3.88–8.00)		317	0.13 (0.09–0.20)		173	37.0 (29.0–46.9)		834	1.37 (0.96–1.82)		15	0.05 (0.04–0.09)		13	0.03 (0.02–0.03)	
Transplant era			<0.001			0.12			0.20			<0.001			-			-
1984-1992	722	6.25 (4.65–8.05)		1	0.27		0	-		595	1.35 (1.00–1.72)		0	-		0	-	
1993-1997	1038	5.00 (3.49–7.15)		80	0.13 (0.10–0.18)		16	30.6 (23.9–44.9)		868	1.64 (1.06–1.99)		0	-		0	-	
1998-2006	1073	4.22 (3.13–5.96)		760	0.12 (0.08–0.18)		539	34.1 (26.7–41.1)		894	1.19 (0.89–1.56)		84	0.04 (0.03–0.07)		37	0.02 (0.01–0.03)	
Primary indication			<0.001			<0.001			<0.001			<0.001			0.15			0.21
Nonischemic cardiomyopathy	489	4.62 (3.29-6.25)		21	0.13 (0.08-0.19)		121	36.8 (29.4-43.5)		332	1.65 (1.30-1.92)		29	0.04 (0.03-0.07)		5	0.01 (0.01-0.03)	
Ischaemic heart disease	429	4.05 (2.99-5.38)		14	0.09 (0.05-0.14)		93	36.6 (30.0-40.5)		288	1.68 (1.31-1.92)		33	0.03 (0.02-0.06)		8	0.02 (0.01-0.03)	
COPD	264	4.42 (3.00-6.06)		34	0.11 (0.06-0.13)		59	33.3 (24.4-42.0)		212	1.35 (0.98-1.94)		0	-		5	0.02 (0.01-0.02)	
Obstructive lung disease	276	8.00 (5.04-11.0)		56	0.16 (0.13-0.25)		79	39.3 (30.8-49.2)		226	1.63 (1.00-2.00)		3	0.07 (0.02-0.18)		6	0.04 (0.02-0.06)	
Congenital heart disease	107	5.21 (3.73-7.76)		6	0.18 (0.16-0.25)		17	33.3 (28.6-43.5)		85	1.75 (1.12-2.00)		3	0.04 (0.03-0.04)		0		
Viral hepatitis	218	4.93 (3.72-6.40)		165	0.10 (0.07-0.13)		24	25.0 (21.2-29.6)		227	1.01 (0.80-1.33)		0	-		0		
Hepatobiliary tumour	28	6.04 (4.16-7.51)		28	0.09 (0.07-0.15)		4	27.6 (22.1-32.8)		39	0.96 (0.78-1.54)		1	0.24		0		
Autoimmune-related liver diseas	222	5.98 (4.38-7.50)		134	0.11 (0.08-0.15)		27	27.8 (21.4-33.3)		214	1.02 (0.82-1.39)		0	-		0		
Alcoholic liver disease	113	4.76 (3.72-6.00)		76	0.10 (0.07-0.15)		13	23.5 (18.2-29.4)		110	1.02 (0.90-1.32)		0	-		0		
Congenital biliary disease	75	11.5 (7.00-17.8)		90	0.22 (0.13-0.30)		3	30.0 (5.00-32.0)		90	1.24 (0.96-1.47)		0	-		0		
Miscellaneous	612	5.01 (3.61-7.00)		217	0.13 (0.08-0.19)		115	31.3 (24.2-39.5)		534	1.35 (1.00-1.79)		15	0.05 (0.04-0.09)		13	0.02 (0.01-0.02)	

Abbreviations: COPD, Chronic obstructive pulmonary disease; # The Kruskal-Wallis nonparametric test was used to compare median doses of specific immunosuppressive agents between recipient subgroups.

Table 5 - 9. Median dose (mg/kg/day) of individual immunosuppressive agents 1 year after transplantation in Australian heart, lung and liver transplant recipients

	CNIs						Antiproliferatives						mTORi					
	Cyclosporine			Tacrolimus			Mycophenolate			Azathioprine			Sirolimus			Everolimus		
	N	Median (q1-q3)	*P#*	N	Median (q1-q3)	*P#*	N	Median (q1-q3)	*P#*	N	Median (q1-q3)	*P#*	N	Median (q1-q3)	*P#*	N	Median (q1-q3)	*P#*
Total	2449	3.66 (2.60-5.15)		832	0.07 (0.04-0.12)		522	27.6 (20.6-35.1)		1857	1.15 (0.82-1.61)		86	0.04 (0.02-0.05)		35	0.02 (0.01-0.03)	
Age			<0.001			<0.001			0.48			0.85			0.13			-
Adult	2279	3.48 (2.53-4.88)		698	0.07 (0.04-0.11)		496	27.8 (20.8-34.9)		1718	1.16 (0.82-1.61)		85	0.04 (0.02-0.05)		35	0.02 (0.01-0.03)	
Paediatric	170	6.91 (5.00-10.0)		134	0.15 (0.08-0.22)		26	23.2 (15.4-41.7)		139	1.14 (0.83-1.67)		1	0.09		0	-	
Sex			<0.001			<0.001			0.43			0.42			0.005			0.16
Male	1623	3.38 (2.50-4.74)		512	0.06 (0.04-0.10)		364	27.1 (21.4-34.5)		1210	1.19 (0.83-1.61)		68	0.03 (0.02-0.05)		23	0.02 (0.01-0.02)	
Female	826	4.23 (2.86-6.00)		320	0.09 (0.06-0.15)		158	28.9 (18.0-37.0)		647	1.09 (0.81-1.6)		18	0.05 (0.04-0.08)		12	0.03 (0.01-0.04)	
Transplant era			<0.001			0.17			0.06			<0.001			-			-
1984-1994	1088	4.35 (3.09-5.95)		6	0.12 (0.09-0.16)		2	52.8 (45.0-60.6)		859	1.27 (0.88-1.69)		0	-		0	-	
1995-1997	532	3.63 (2.59-5.00)		95	0.08 (0.05-0.14)		20	30.4 (22.7-37.9)		439	1.22 (0.80-1.69)		0	-		0	-	
1998-2006	829	2.97 (2.20-3.95)		731	0.07 (0.04-0.12)		500	27.4 (20.5-34.5)		559	1.00 (0.75-1.39)		86	0.04 (0.02-0.05)		35	0.02 (0.01-0.03)	
Primary indication			<0.001			<0.001			<0.001			<0.001			0.06			0.22
Nonischemic cardiomyopathy	438	3.37 (2.45-4.64)		28	0.07 (0.04-0.18)		97	31.6 (25.0-37.5)		318	1.43 (1.10-1.76)		24	0.04 (0.03-0.05)		4	0.01 (0.01-0.02)	
Ischaemic heart disease	387	3.03 (2.33-4.05)		22	0.05 (0.03-0.09)		91	29.7 (23.5-36.5)		271	1.45 (1.12-1.72)		33	0.03 (0.02-0.04)		9	0.02 (0.01-0.02)	
COPD	205	3.12 (1.87-4.51)		38	0.05 (0.03-0.09)		58	25.2 (17.2-29.1)		167	1.00 (0.70-1.50)		5	0.02 (0.02-0.03)		5	0.02 (0.01-0.03)	
Obstructive lung disease	199	5.86 (3.76-8.64)		67	0.12 (0.05-0.20)		69	29.0 (18.2-37.7)		182	1.35 (0.91-1.98)		3	0.08 (0.06-0.12)		6	0.03 (0.02-0.04)	
Congenital heart disease	91	4.90 (3.00-6.60)		9	0.18 (0.09-0.21)		15	28.6 (23.8-42.9)		81	1.52 (1.00-1.90)		3	0.04 (0.01-0.06)		0	-	
Viral hepatitis	204	3.22 (2.43-3.94)		151	0.06 (0.03-0.08)		25	27.4 (21.3-30.8)		140	0.90 (0.66-1.18)		0	-		0	-	
Hepatobiliary tumour	27	3.67 (2.37-5.19)		26	0.05 (0.02-0.08)		3	28.2 (17.2-32.3)		28	0.88 (0.73-1.01)		1	0.11		0	-	
Autoimmune related liver diseas	207	4.35 (3.23-5.56)		132	0.08 (0.06-0.11)		27	25.3 (16.1-30.3)		165	0.88 (0.69-1.15)		1	0.06		0	-	
Alcoholic liver disease	103	3.57 (2.86-4.26)		75	0.06 (0.04-0.09)		16	20.1 (13.8-25.9)		68	0.89 (0.65-1.07)		1	0.03		0	-	
Congenital biliary disease	63	7.22 (5.96-10.7)		73	0.15 (0.08-0.20)		10	19.9 (15.4-26.9)		44	1.04 (0.80-1.19)		0	-		0	-	
Miscellaneous	525	3.82 (2.60-5.13)		211	0.09 (0.05-0.14)		111	27.4 (19.4-35.7)		393	1.07 (0.83-1.56)		15	0.03 (0.02-0.05)		11	0.01 (0.01-0.03)	

Abbreviations: COPD, Chronic obstructive pulmonary disease; # The Kruskal-Wallis nonparametric test was used to compare median doses of specific immunosuppressive agents between recipient subgroups.

Table 5 - 10. Median dose (mg/kg/day) of individual immunosuppressive agents 5 years after transplantation in Australian heart, lung and liver transplant recipients

	CNIs						Antiproliferatives						mTORi					
	Cyclosporine			Tacrolimus			Mycophenolate			Azathioprine			Sirolimus			Everolimus		
	N	Median (q1-q3)	P #	N	Median (q1-q3)	P #	N	Median (q1-q3)	P #	N	Median (q1-q3)	P #	N	Median (q1-q3)	P #	N	Median (q1-q3)	P #
Total	1523	2.63 (1.95–3.62)		397	0.05 (0.03–0.08)		302	23.5 (16.4–31.2)		1082	1.06 (0.75–1.49)		62	0.03 (0.02–0.04)		3	0.01 (0.01–0.03)	
Age			<0.001			<0.001			0.29			0.14			-			-
Adult	1394	2.53 (1.85–3.44)		335	0.05 (0.03–0.08)		290	23.5 (16.4–31.7)		1004	1.06 (0.74–1.47)		62	0.03 (0.02–0.04)		3	0.01 (0.01–0.03)	
Paediatric	129	4.48 (3.33–5.71)		62	0.09 (0.06–0.13)		12	21.5 (16.9–26.1)		78	1.08 (0.81–1.63)		0	-		0	-	
Sex			<0.001			<0.001			0.28			0.30			0.02			-
Male	1034	2.50 (1.81–3.44)		225	0.05 (0.03–0.08)		211	23.8 (16.7–32.0)		726	1.10 (0.74–1.51)		52	0.02 (0.02–0.03)		3	0.01 (0.01–0.03)	
Female	489	2.99 (2.14–4.00)		172	0.06 (0.04–0.09)		91	21.7 (14.7–30.6)		356	1.00 (0.76–1.45)		10	0.04 (0.03–0.06)		0	-	
Transplant era			<0.001			0.004			0.08			0.006			0.24			-
1984-1994	1008	2.96 (2.10–4.00)		54	0.07 (0.06–0.11)		38	28.3 (18.9–34.5)		754	1.13 (0.75–1.53)		4	0.05 (0.03–0.06)		0		
1995-1997	385	2.30 (1.69–2.98)		181	0.05 (0.04–0.08)		125	21.1 (14.0–29.4)		263	1.00 (0.76–1.36)		32	0.03 (0.02–0.04)		0		
1998-2006	130	2.04 (1.58–2.60)		162	0.05 (0.03–0.08)		139	23.5 (18.4–32.3)		65	0.96 (0.76–1.32)		26	0.02 (0.01–0.03)		3	0.01 (0.01–0.03)	
Primary indication			<0.001			<0.001			0.02			<0.001			0.08			-
Nonischemic cardiomyopathy	331	2.54 (1.89-3.45)		25	0.06 (0.03-0.10)		69	25.0 (16.5-32.7)		266	1.27 (0.89-1.54)		19	0.02 (0.02-0.03)		1	0.01	
Ischaemic heart disease	290	2.19 (1.69-3.00)		11	0.04 (0.02-0.06)		58	23.6 (17.9-33.0)		220	1.24 (0.81-1.57)		19	0.02 (0.01-0.03)		1	0.01	
COPD	70	2.05 (1.40-3.03)		13	0.05 (0.03-0.07)		21	15.0 (12.8-24.0)		53	1.00 (0.48-1.45)		4	0.03 (0.02-0.05)		0	-	
Obstructive lung disease	68	4.00 (2.64-6.01)		33	0.08 (0.05-0.13)		31	25.9 (16.7-37.5)		66	1.00 (0.82-1.67)		8	0.04 (0.03-0.05)		0	-	
Congenital heart disease	60	3.20 (2.49-4.06)		6	0.12 (0.04-0.20)		4	18.9 (14.8-30.3)		53	1.20 (0.89-1.56)		0	-		0	-	
Viral hepatitis	117	2.42 (1.77-2.86)		58	0.04 (0.03-0.06)		21	22.7 (14.9-27.8)		50	0.80 (0.61-0.96)		2	0.04 (0.03-0.06)		0	-	
Hepatobiliary tumour	12	3.41 (2.33-4.57)		9	0.04 (0.01-0.05)		2	20.2 (13.7-26.7)		9	0.87 (0.79-0.93)		0	-		0	-	
Autoimmune related liver diseas	149	3.08 (2.35-3.72)		87	0.06 (0.04-0.08)		16	16.9 (12.1-20.4)		107	0.85 (0.71-1.19)		0	-		0	-	
Alcoholic liver disease	66	2.12 (1.64-2.68)		29	0.03 (0.02-0.04)		17	22.7 (21.1-27.0)		36	0.85 (0.66-1.43)		2	0.02 (0.01-0.02)		0	-	
Congenital biliary disease	49	5.26 (3.44-6.55)		40	0.08 (0.05-0.10)		1	20		23	0.82 (0.68-0.93)		0	-		0	-	
Miscellaneous	311	2.81 (2.00-3.90)		86	0.05 (0.03-0.08)		62	26.6 (18.5-34.5)		199	1.00 (0.67-1.47)		8	0.03 (0.03-0.05)		1	0.03	

Abbreviations: COPD, Chronic obstructive pulmonary disease; * The Kruskal-Wallis nonparametric test was used to compare median doses of specific immunosuppressive agents between recipient subgroups.

Table 5 - 11. Unadjusted and adjusted median doses (mg/kg/day) of individual immunosuppressive agents at 3 months, 1 year and 5 years after transplantation in Australian heart, lung and liver transplant recipients

	Unadjusted median						Adjusted median*					
	Liver		Heart		Lung		Liver		Heart		Lung	
	N	median (q1-q3)	N	median (q1-q3)	N	median (q1-q3)	N	median* (q1-q3)	N	median* (q1-q3)	N	median* (q1-q3)
3-months												
CNIs												
Cyclosporine	863	5.51 (4.11–7.53)	1098	4.38 (3.17–6.03)	872	5.32 (3.63–8.00)	853	5.03 (3.88-7.36)	1023	4.64 (3.43-5.69)	614	4.99 (3.10-7.57)
Tacrolimus	668	0.12 (0.08–0.18)	54	0.13 (0.08–0.18)	119	0.14 (0.08–0.20)	654	0.15 (0.09-0.21)	53	0.14 (0.12-0.23)	78	0.14 (0.11-0.22)
Antiproferatives												
Mycophenolate	102	25.6 (18.9–30.8)	264	36.5 (29.4–41.7)	189	36.0 (27.2–44.8)	100	30.3 (17.9-37.8)	261	38.3 (32.8-47.7)	100	37.8 (29.7-51.1)
Azathioprine	904	1.07 (0.84–1.41)	742	1.67 (1.30–1.92)	711	1.52 (1.00–2.00)	886	1.03 (0.84-1.43)	714	1.65 (1.41-1.91)	522	1.60 (1.08-1.86)
1-year												
CNIs												
Cyclosporine	796	3.90 (2.92-5.35)	985	3.25 (2.44-4.55)	668	4.00 (2.48-5.88)	784	3.89 (3.07-5.35)	921	3.60 (2.77-4.71)	467	4.37 (2.55-6.02)
Tacrolimus	620	0.07 (0.05-0.12)	71	0.08 (0.04-0.16)	141	0.08 (0.04-0.16)	599	0.09 (0.06-0.12)	70	0.11 (0.06-0.14)	90	0.13 (0.08-0.16)
Antiproferatives												
Mycophenolate	113	23.1 (16.1-30.1)	237	31.5 (24.4-37.5)	172	26.0 (17.5-31.5)	111	21.3 (17.5-33.5)	234	36.3 (21.1-43.5)	92	26.3 (15.0-33.9)
Azathioprine	588	0.90 (0.68-1.17)	703	1.45 (1.09-1.75)	566	1.12 (0.88-1.77)	573	1.00 (0.79-1.35)	673	1.48 (1.15-1.77)	411	1.23 (0.90-1.66)
5-years												
CNIs												
Cyclosporine	539	2.83 (2.10–3.73)	730	2.44 (1.79–3.41)	254	2.90 (2.00–4.00)	537	3.21 (2.53-3.84)	728	2.82 (2.12-3.46)	242	2.73 (1.98-4.08)
Tacrolimus	291	0.05 (0.03–0.08)	47	0.05 (0.03–0.10)	59	0.07 (0.03–0.12)	291	0.06 (0.05-0.09)	47	0.05 (0.04-0.14)	58	0.08 (0.04-0.10)
Antiproferatives												
Mycophenolate	78	21.4 (17.2–27.8)	154	24.3 (16.7–33.9)	70	23.4 (15.0–31.0)	77	20.1 (15.8-26.4)	154	23.0 (18.0-31.6)	56	24.3 (17.1-30.4)
Azathioprine	298	0.85 (0.66–1.15)	570	1.26 (0.85–1.56)	214	1.00 (0.77–1.53)	296	0.86 (0.69-1.19)	568	1.29 (1.00-1.57)	205	1.02 (0.77-1.39)

*Adjusted median doses were adjusted for age at transplantation, sex, transplant year, antibody induction therapy (yes/no), all the other drug doses that were used at the same time. Antibody induction therapy was not included in the adjustment at 5-years after transplantation.

Chapter 6 Role of immunosuppression in risk of non-Hodgkin lymphoma in Australian liver, heart and lung transplant recipients

6.1 Objectives

In this Chapter I examine the role of immunosuppressive therapy in risk of NHL in a population-based cohort of liver and cardiothoracic transplant recipients in Australia between 1984 and 2006. Specifically the objective of this Chapter is to explore the association between the type, dose and duration of immunosuppressive therapy and risk of early and late NHL after transplantation.

6.2 Introduction

Non-Hodgkin lymphoma (NHL) is the most life-threatening manifestation of post-transplant lymphoproliferative disorder (PTLD), the term for a spectrum of lymphomas that develop as a result of uncontrolled lymphocyte proliferation after solid organ transplantation (Loren et al. 2003, Swerdlow et al. 2008, Kinch et al. 2014). After skin cancer, NHL is the second most common cancer in adult and the most common cancer in paediatric transplant recipients (Engels et al. 2011, Schober et al. 2013).

There are two types of transplant-related NHL with distinctly different clinical, histological and epidemiological characteristics. Early NHL, occurring within 1 or 2 years of transplantation, is of B-cell origin and closely associated with Epstein-Barr virus (EBV) infection or reactivation due to potent immunosuppression (Ghobrial et al.

2005, van Leeuwen et al. 2009, Quinlan et al. 2011, Caillard et al. 2012). Late NHL, on the other hand, includes a greater proportion of T-cell origin lymphomas (Dotti et al. 2000, Ghobrial et al. 2005, Quinlan et al. 2011) and appears less likely to be directly related to EBV infection (Pintilie 2007, van Leeuwen et al. 2009, Kinch et al. 2014).

An association between the intensity of iatrogenic immunosuppression and NHL risk is hypothesised on the basis of the strong association between low CD4+ lymphocyte count and NHL risk in HIV-immunosuppression (Guiguet et al. 2009). It is also predicted by the higher risk of NHL in heart and lung compared to liver transplant recipients (Fernberg et al. 2011, Na et al. 2013), and their relative levels of immunosuppression (Collett et al. 2010, Wiesner and Fung 2011). Establishing the role of individual agents in lymphomagenesis, including distinguishing a direct drug effect from an indirect effect due to the intensity of immunosuppression is challenging because recipients typically receive multiple immunosuppressive agents, and drugs and drug dosages usually vary over time as observed in Chapter 5. Many studies have identified an increased risk associated with receipt of T-cell depleting antibodies (Swinnen et al. 1990, Opelz et al. 2006, van Leeuwen et al. 2009, Caillard et al. 2012, Dharnidharka et al. 2012), but no consistent findings have been observed for maintenance immunosuppressive agents, typically measured at discharge from hospital (Melosky et al. 1992, Birkeland and Hamilton-Dutoit 2003, Bustami et al. 2004, Caillard et al. 2005, Faull et al. 2005, Quinlan et al. 2011, Caillard et al. 2012). A population-based nested case-control study found no clear evidence of an association between NHL risk and the average or cumulative dose of azathioprine or cyclosporine after adjustment for age and calendar year (Fernberg et al. 2011). However, no population-based study has

assessed the independent effect of individual immunosuppressive agents on NHL risk with longitudinal data on the type and dose of individual agents.

6.3 Materials and methods

6.3.1 Study population

I performed a retrospective population-based cohort study of all Australian liver (n = 1926, 41%), heart (n = 1518, 33%), and lung (n = 1200, 26%) transplant recipients, 1984-2006. The transplant registries, ANZLTR and ANZCOTR, were described in full in Chapter 3. For this analysis, I excluded recipients with no retrievable transplantation medical records (n = 89, 2%); this included two liver and 87 heart transplant recipients. I also excluded 13 patients with a diagnosis of NHL prior to transplantation; I retained 379 patients with history of another cancer (liver n = 317, heart n = 32, lung n = 30) as they were at risk of NHL.

I obtained ethical approval and the requirement for informed participant consent was waived because I received de-identified data.

6.3.2 Data collection

I ascertained incident NHL diagnoses by record linkage between the transplant registers and the ACD, a register of incident primary invasive neoplasms, between 1 January 1984 and 31 December 2006. The registrant's name, sex, date of birth, date of death, and state of residence were used to link records using an established probabilistic algorithm. I identified NHL diagnoses on the basis of International Classification of Diseases for Oncology (ICD) codes (Turner et al. 2010). Polymorphic

PTLD (ICDO-3 9970/1, lymphoproliferative disorder) is not routinely registered by the ACD, and was not included in this study. I identified deaths from the transplant registers or by record linkage with the NDI, a registry of all deaths in Australia since 1980.

The demographic characteristics of recipients (age, sex, race) and donors (age, sex) and some clinical information (date of transplantation, organ type, primary indication, subsequent transplantation) were systematically and prospectively collected by the registers. From transplant unit medical records I retrospectively collected recipient country of birth, smoking and alcohol consumption, weight, height, and blood type, history of cancer, diabetes mellitus, cardiovascular disease, hypertension, autoimmune disease, and dialysis prior to transplantation. I abstracted CMV IgG serostatus for recipients and donors at transplantation, recipient EBV IgG serostatus at transplantation and during follow-up and recipient infection with HBV and HCV at transplantation. I also collected the use of prophylactic anti-CMV immunoglobulin and antiviral agents at transplantation.

Information on receipt of antibody included T-cell-depleting antibody (ATG/ALG, muromonab-CD3) and IL-2Ra (basiliximab, daclizumab) at induction or rejection. I recorded immunosuppressive agents at transplantation, and 3 months, 6 months, and 1, 5, 10, 15 and 20 years after transplantation, specifically the use and dosage of cyclosporine, tacrolimus, azathioprine, mycophenolate, sirolimus and everolimus.

6.3.3 Data management

Data on EBV serostatus at transplantation was missing for 1667 (37%) of the cohort; 569 (13%) liver, 658 (14%) heart and 440 (10%) lung recipients. I replaced missing values by using multiple imputation by chained equations (White et al. 2011). The variables I used in the imputation were age, sex, organ type, transplant era, primary indication, CMV IgG serostatus, antibody induction therapy, and the outcome variable NHL (Allison 2000). As the timing of EBV infection after transplantation was not available, I classified EBV infection as positive at transplantation, positive only after transplantation, or negative until the end of follow-up.

My management of the immunosuppressive therapy data is described in detail in Chapter 5. Briefly, I imputed missing recipient weight values, standardised the dose of individual agents to mg/kg/day, and imputed missing data on the type and dose of individual immunosuppressive agents, for a maximum of one consecutive follow-up time point. As the date of antibody rejection therapy was not available, I used antibody induction therapy (yes/no) in the analysis for early NHL and antibody induction or rejection therapy (ever/never) in the analysis for late NHL, assuming acute rejection preceded late NHL.

6.3.4 Data analysis

Person-years of follow-up accrued from the date of transplantation until the date of NHL diagnosis, age 80, death, or 31 December 2006, whichever occurred first. I observed 100 cases of NHL (90 in adults, 10 in paediatric) and 1538 deaths (1461 in adults, 77 in paediatric) during 26046 person-years of follow-up; the median follow-up time was 5.15 (IQR 2.02–9.74) years. I examined risk factors for early and late NHL

separately; given the sharp decrease in NHL incidence during the second year, I classified NHL diagnosed within the first year as early NHL, and all other NHL diagnoses as late NHL (Figure 6-1, page 170).

As recipient age (paediatric vs adult) significantly modified the incidence of NHL by organ type, I restricted the subsequent analyses to adults (n = 4131); the paediatric group was too small (n = 411 with 10 cases of NHL) to be analysed separately. Characteristics of recipients with and without NHL and with low versus high initial immunosuppressive dose were compared using Student t tests (normally distributed continuous variables), Wilcoxon rank-sum tests (non-normally distributed continuous variables), and Pearson chi-square tests (categorical variables with all expected cell frequencies over five), or Fisher exact tests (categorical variables with any of the expected cell frequencies five or less).

I examined three measures of immunosuppression. I first tested a time-dependent binary variable for the current receipt of each immunosuppressive agent, irrespective of dose. Second, I tested a time-dependent continuous variable for the current daily dose of each immunosuppressive agent. I further categorised the current dose of each agent as low or high relative to the median dose at each follow-up time. Third, to capture the overall dose of immunosuppressive therapy received, I modelled the mean dose (mg/kg/day) of each immunosuppressive agent during follow-up (first year for early NHL and the entire follow-up for late NHL), calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point, weighted by the length of each

follow-up time interval relative to the total duration of use. I categorised the mean dose of each agent as low or high relative to the median value.

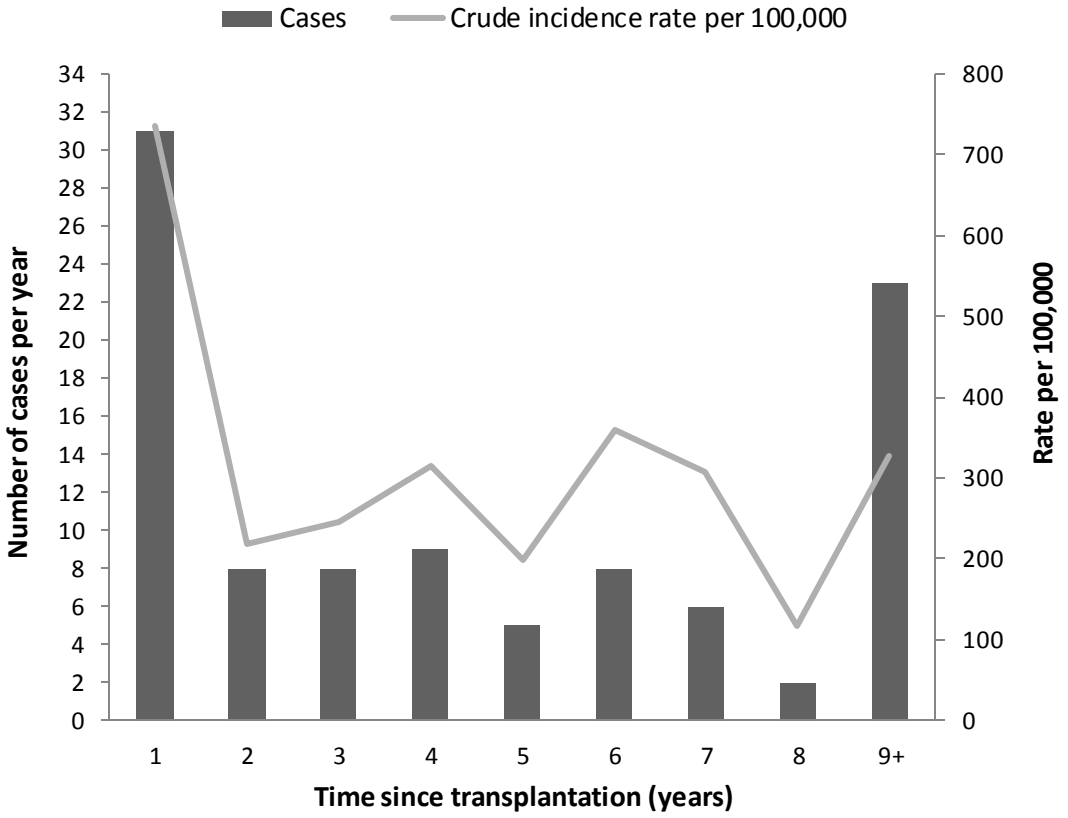


Figure 6 - 1. Crude NHL incidence rates (per 100,000) and number of NHL cases per year by time since transplantation in Australian liver, heart and lung transplant recipients, 1984-2006

I restricted analyses for tacrolimus and mycophenolate to the years from 1997 when these agents were used by my cohort. As sirolimus and everolimus were not used until 2002, and 2005, respectively, I was unable to model these agents individually due to low usage and limited follow-up time. I did not examine corticosteroid use as a

potential risk factor for NHL because it is not considered carcinogenic to humans (Bernatsky et al. 2007, Swift 2009).

I applied the Fine and Gray (Fine and Gray 1999) proportional subdistribution hazard model to estimate hazard ratios (HRs) of early and late NHL, accounting for death as a competing risk. For late NHL, I excluded recipients with NHL within the first year; however, I counted deaths within the first year as they contributed to the competing risk analysis. I used age as the underlying time-scale in the modelling, and evaluated time since transplantation as a potential risk factor in the late NHL model.

I used two models to examine the three measures of immunosuppression (current receipt, current dose, and mean dose of each agent). In the basic model, I adjusted each measure of immunosuppressive agent or other risk factor of interest for age at transplantation, sex, transplant year, and transplanted organ. In the complete model, I further adjusted each immunosuppressive agent for the other immunosuppressive agents (except those within the same class and thus not given simultaneously when considering the time-dependent measures current receipt and current dose), and other variables that fulfilled the criteria for additional confounding factors (i.e. were associated with immunosuppressive agents and NHL and/or death after adjustment for the variables in the basic model). I also applied cause-specific hazard models to estimate HRs of early and late NHL to allow comparison with prior risk estimates. I analysed the risk factors for late NHL including all follow-up time (maximum 23 years) and censoring the follow-up at 10 years, after which the population at risk of NHL reduced significantly (Figure 6-1 page 170, Figure 6 - 3, page 174).

I carried out analyses based on both original and imputed data. Although imputation attenuated the results obtained based on the original data, I observed no notable differences (Supplementary Table 6 – 1, page 197). The modelling results presented are based on imputed data.

I performed analyses using Stata statistical software v13.0 (StataCorp, Texas, USA).

6.4 Results

6.4.1 Cohort characteristics

The cohort comprised 4131 adult transplant recipients, 1615 (39%) liver, 1342 (32%) heart and 1174 (28%) lung. Early NHL developed in 29 recipients (6 liver, 5 heart, and 18 lung), and late NHL in 61 recipients (17 liver, 35 heart, and 9 lung). Diffuse large B-cell lymphoma was the most common subtype, comprising about 45% of both early and late NHL. No early NHLs but 6 late NHLs were T-cell lymphomas.

Recipients who developed early or late NHL and those who did not differed by era of transplantation, organ type, EBV serostatus, and induction with ATG/ALG (Table 6 - 1, page 176). Specifically, early and late NHL occurred more often in the earlier transplant era, lung recipients were more likely to develop early NHL, heart recipients were more likely to develop late NHL, EBV seronegativity was associated with both early and late NHL, those who received ATG/ALG were less likely to develop early and late NHL, and receipt of either cyclosporine or azathioprine was associated with early and late NHL. In terms of the characteristics of recipients receiving low compared to high dose

immunosuppressive agents at transplantation, younger recipients received a higher dose of one or more agents, as did females, those transplanted in the earliest era, heart and lung transplant recipients, recipients seronegative for EBV or CMV, recipients receiving ATG/ALG induction therapy, recipients not receiving basiliximab induction therapy, and recipients on a low dose of another agent (Table 6-2, page 177). Several of these associations, including for EBV, were no longer significant after adjustment for age, sex, transplant year and organ type. Figure 6 - 3 (page 178) shows the extent of missing immunosuppression data at 1-year, 5-years, 10-years and 15-years post-transplantation.

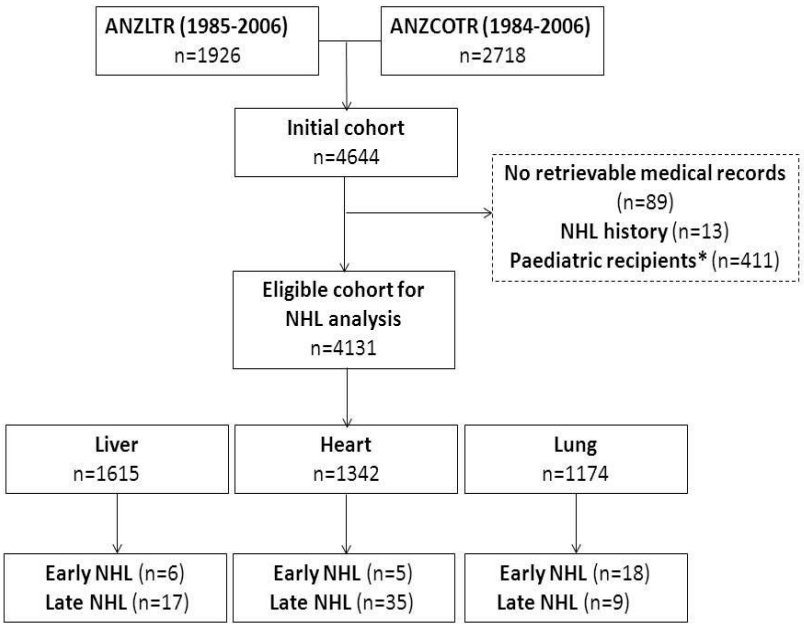


Figure 6 - 2. Flowchart of the cohort and outcome by organ type

***** As recipient age (paediatric vs adult) significantly modified the incidence of NHL by organ type, I restricted the subsequent analyses to adults; the paediatric group was too small (n = 411 with 10 cases of NHL) to be analysed separately.

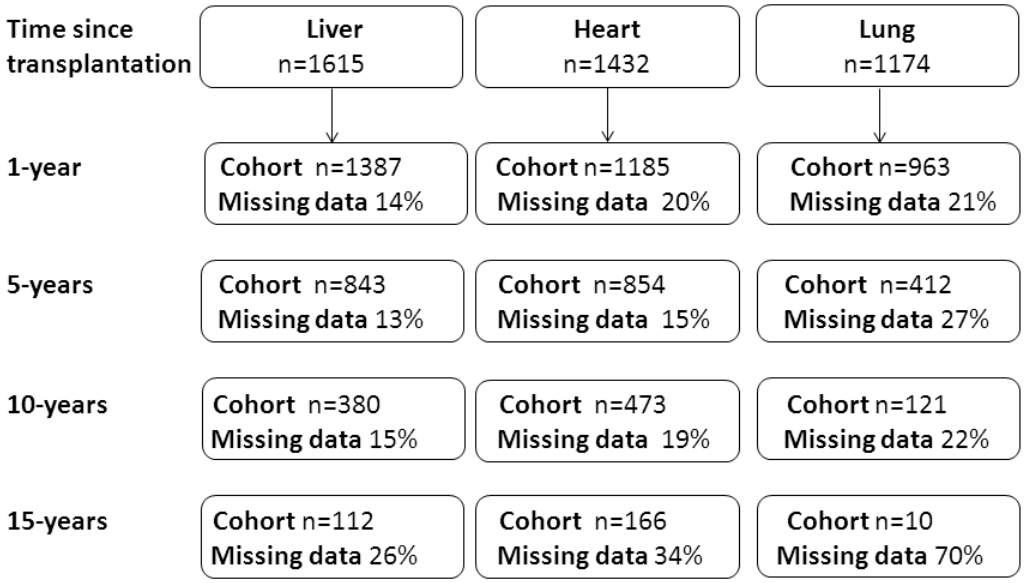


Figure 6 - 3. Flowchart of the cohort and extent of missing immunosuppression data by organ type

6.4.2 Risk factors for early NHL

In the basic models, increased risk of early NHL was associated with lung transplantation, older age, earlier transplant era, negative recipient EBV serology at transplantation, the current receipt of tacrolimus and mycophenolate, and high current and mean dose of mycophenolate (Table 6-3, page 180). A history of alcohol drinking and the current receipt of cyclosporine were associated with a decreased risk of early NHL in basic models (Table 6 - 3, page 180).

In the complete (final) model, use of muromonab-CD3 induction therapy was associated with an increased risk of early NHL (HR 5.66, 95 % CI 1.13-28.3), and a unit increase in either the current or mean dose of azathioprine conferred a significant 2-fold excess risk of early NHL (Table 6 - 3, page 180). Furthermore, those receiving a

high mean daily dose of azathioprine (>1.30 mg/kg/day) were at a 6-fold risk of early NHL compared to those receiving a low dose.

6.4.3 Risk factors for late NHL

In the basic model, increased risk of late NHL was associated with heart transplantation, increasing time since transplantation, acquired EBV infection after transplantation, recipient blood type O, and high mean dose of azathioprine (Table 6-4, page 183).

In the complete (final) model, the significant positive association with time since transplantation was strengthened (HR 6.20, 95 % CI 1.99-19.3), as was the association with azathioprine; both higher current dose (HR 1.54, 95 % CI 1.02-2.31) and higher mean dose (HR 1.78, 95 % CI 1.12-2.84) were associated with elevated risk of late NHL (Table 6 - 4**,** page 183). Those receiving a high mean daily dose of azathioprine (>1.14 mg/kg/day) were at a 2-fold risk of late NHL compared to those receiving a low dose. Late NHL risk was also associated with increasing age (HR 1.12, 95 % CI 1.04-1.22 per year of age).

All of the significant findings for late NHL were retained when the follow-up time was restricted to 10 years post-transplantation (Supplementary Table 6 - 1, page 197). The cause-specific hazard ratio estimates for early NHL were comparable with the subdistribution hazard model estimates, but age and time since transplantation were significantly associated with risk of late NHL only in the subdistribution hazard model (Supplementary Table 6 - 2, page 200).

Table 6 - 1. Baseline characteristics of adult liver, heart and lung transplant recipients with and without NHL, 1984-2006†

Variable§	N†	Early NHL	Late NHL	No NHL	*P*‡
	(*n* 4131)	(*n* = 29)	(*n* = 61)	(*n* = 4041)	
Recipient age	49 (39-57)	49 (39-57)	48 (39-54)	49 (39-55)	0.69
Recipient sex					0.44
Male	2771 (67.1)	22 (75.9)	38 (62.3)	2711 (67.1)	
Female	1360 (32.9)	7 (24.1)	23 (37.7)	1330 (32.9)	
Transplant era					<0.001
1984-1994	1279 (21.0)	11 (37.9)	30 (49.2)	1238 (30.6)	
1995-1997	704 (17.0)	7 (24.2)	18 (29.5)	679 (16.8)	
1998-2006	2148 (52.0)	11 (37.9)	13 (21.3)	2124 (52.6)	
Recipient race					0.17
Caucasian	3649 (90.6)	26 (96.3)	57 (96.6)	3566 (90.5)	
Non-Caucasian	377 (9.40)	1 (0.70)	2 (0.40)	374 (9.50)	
First transplanted organ					<0.001
Liver	1615 (39.1)	6 (20.7)	17 (27.9)	1592 (39.4)	
Heart	1342 (32.5)	5 (17.2)	35 (57.4)	1302 (37.2)	
Lung	1174 (28.4)	18 (62.1)	9 (14.7)	1147 (28.4)	
Recipient serological status					
EBV					<0.001
Positive	2102 (83.9)	10 (52.6)	18 (56.3)	2074 (84.5)	
Negative	404 (16.1)	9 (47.4)	14 (43.7)	381 (15.5)	
CMV					0.73
Positive	2819 (70.0)	19 (67.9)	40 (65.5)	2760 (70.0)	
Negative	1211 (30.0)	9 (32.1)	21 (34.5)	1181 (30.0)	
Induction antibody					
ATG/ALG					0.04
Yes	487 (14.3)	1 (5.00)	13 (25.5)	473 (14.2)	
No	2910 (85.7)	19 (95.0)	38 (74.5)	2853 (85.8)	
Muromonab-CD3					0.30
Yes	122 (3.60)	2 (10.0)	2 (3.90)	118 (3.60)	
No	3275 (96.4)	18 (90.0)	49 (96.1)	3208 (96.4)	
Basiliximab					0.45
Yes	199 (5.90)	0	2 (3.90)	197 (5.90)	
No	3198 (94.1)	20 (100)	49 (96.1)	3129 (94.1)	

Table 6 – 1. (continued) Baseline characteristics of liver, heart and lung transplant recipients with and without NHL, 1984-2006†

Variable§	N†	Early NHL	Late NHL	No NHL	*P*‡
	(*n* 4131)	(*n* 29)	(*n* 61)	(*n* 4041)	
Immunosuppressive agent					
Cyclosporine					0.002
Yes	2777 (74.4)	17 (65.4)	51 (94.4)	2709 (74.2)	
No	956 (25.6)	9 (34.6)	3 (5.6)	944 (25.8)	
Tacrolimus*					0.02
Yes	838 (37.5)	7 (58.3)	2 (10.0)	836 (62.2)	
No	1396 (62.5)	19 (41.7)	18 (90.0)	1378 (37.8)	
Mycophenolate*					0.09
Yes	592 (26.5)	5 (41.7)	2 (10.0)	590 (26.6)	
No	1643 (73.5)	21 (58.3)	18 (90.0)	1625 (73.4)	
Azathioprine					0.008
Yes	2316 (62.6)	19 (73.1)	44 (81.5)	2253 (62.3)	
No	1382 (37.4)	7 (26.9)	10 (18.5)	1366 (37.7)	
Dose					
Cyclosporine	4.89 (3.46-6.68)	5.00 (2.91-8.00)	4.82 (3.42-6.10)	4.89 (3.47-6.72)	0.99
Tacrolimus*	0.11 (0.07-0.15)	0.10 (0.03-0.16)	0.11 (0.07-0.15)	0.11 (0.07-0.15)	0.90
Mycophenolate *	33.9 (25.6-41.1)	32.3 (29.4-44.8)	28.4 (24.6-32.1)	33.9 (25.6-41.1)	0.62
Azathioprine	1.37 (0.96-1.83)	1.64 (1.16-1.89)	1.43 (1.06-1.82)	1.36 (0.95-1.83)	0.08

Notes: § Continuous variables presented as median (interquartile range) and categorical variables presented as n (%); † The counts in sub-categories may not add up to the total number due to missing data; ‡ Non-parametric Wilcoxon rank-sum test, chi-square test or Fisher exact test as appropriate; *Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents.

Table 6 - 2. Baseline characteristics of adult transplant recipients who received low and high dose of immunosuppressive agents at transplantation

Variable†‡	Cyclosporine			Tacrolimus*			Mycophenolate*			Azathioprine		
	Low	High	*P*	Low	High	*P*	Low	High	*P*	Low	High	*P*
N	1340	1337		333	332		279	274		1098	1078	
Recipient age												
Median (q1-q3)	51 (43-56)	46 (33-53)	<0.001	50 (43-57)	48 (37-55)	0.002	49 (40-56)	48 (31-56)	0.10	49 (41-55)	48 (35-54)	<0.001
Sex			<0.001			<0.001			0.008			0.63
Male	967 (72.2)	846 (63.3)		244 (73.3)	189 (56.9)		208 (74.5)	176 (64.2)		732 (66.7)	708 (65.7)	
Female	373 (27.8)	491 (36.7)		89 (26.7)	143 (43.1)		71 (25.5)	98 (35.8)		366 (33.3)	370 (34.3)	
Transplant era			<0.001			0.14			0.34			<0.001
1984-1994	400 (29.8)	639 (47.8)		0	0		0	0		391 (35.6)	484 (44.9)	
1995-1997	280 (20.9)	285 (21.3)		15 (4.50)	24 (7.20)		7 (2.50)	3 (1.10)		196 (17.9)	304 (28.2)	
1998-2006	660 (49.3)	413 (30.9)		318 (95.5)	308 (92.8)		272 (97.5)	271 (98.9)		511 (46.5)	290 (26.9)	
Recipient race			0.28			0.46			0.32			0.04
Caucasian	1220 (91.5)	1224 (92.6)		278 (85.0)	288 (87.0)		252 (90.3)	254 (92.7)		981 (90.2)	989 (92.7)	
Noncaucasian	114 (8.50)	98 (7.40)		49 (15.0)	43 (13.0)		27 (9.70)	20 (7.30)		107 (9.80)	78 (7.30)	
First transplanted organ			<0.001			0.001			<0.001			<0.001
Liver	321 (24.0)	427 (31.9)		268 (80.5)	242 (72.9)		90 (32.2)	10 (3.60)		578 (52.6)	195 (18.1)	
Heart	652 (48.6)	402 (30.1)		22 (6.60)	13 (3.90)		104 (37.3)	157 (57.3)		208 (18.9)	488 (45.3)	
Lung	367 (27.4)	508 (38.0)		43 (12.9)	77 (23.2)		85 (30.5)	107 (39.1)		312 (28.5)	395 (36.6)	

Notes: †The counts in sub-categories may not add up to the total number due to missing data. ǂ The dose of each agent at transplantation was categorised as low or high relative to the median value of the dose. * Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents. ‡ Non-parametric Wilcoxon rank-sum test, chi-square test or Fisher exact test as appropriate.

Table 6 - 2. (continued) Baseline characteristics of adult transplant recipients who received low and high dose of immunosuppressive agents at transplantation

Variable†ǂ	Cyclosporine			Tacrolimus*			Mycophenolate*			Azathioprine		
	Low	High	*P* ‡	Low	High	*P* ‡	Low	High	*P* ‡	Low	High	*P* ‡
Recipient serological status												
EBV			<0.001			0.04			0.75			0.27
Positive	688 (84.6)	617 (75.6)		242 (92.0)	233 (86.6)		202 (87.8)	190 (88.8)		569 (80.8)	475 (78.4)	
Negative	125 (15.4)	199 (24.4)		21 (8.00)	36 (13.4)		28 (12.2)	24 (11.2)		135 (19.2)	131 (21.6)	
CMV			<0.001			0.28			0.69			0.06
Positive	968 (73.3)	870 (66.2)		232 (71.4)	222 (67.5)		179 (64.6)	172 (63.0)		780 (72.4)	731 (68.7)	
Negative	353 (26.7)	444 (33.8)		93 (28.6)	107 (32.5)		98 (35.4)	101 (37.0)		298 (27.6)	333 (31.3)	
Antibody induction												
ATG/ALG			0.11			0.73			0.003			<0.001
Yes	251 (20.8)	217 (18.2)		5 (1.60)	3 (1.00)		27 (11.1)	47 (21.2)		125 (12.5)	228 (23.7)	
No	956 (79.2)	973 (81.8)		313 (98.4)	295 (99.0)		216 (88.9)	175 (78.8)		875 (87.5)	734 (76.3)	
Muromonab-CD3			0.94			0.94			0.37			0.30
Yes	47 (3.90)	47 (4.00)		3 (0.90)	3 (1.00)		4 (1.60)	1 (0.50)		48 (4.80)	37 (3.80)	
No	1160 (96.1)	1143 (96.0)		315 (99.1)	295 (99.0)		239 (98.4)	221 (99.5)		952 (95.2)	925 (96.2)	
Basiliximab			<0.001			0.97			0.32			0.63
Yes	69 (5.70)	33 (2.80)		36 (11.3)	34 (11.4)		58 (23.9)	62 (27.9)		24 (2.40)	20 (2.10)	
No	1138 (94.3)	1157 (97.2)		282 (88.7)	264 (88.6)		185 (76.1)	160 (72.1)		976 (97.6)	942 (97.9)	
Immunosuppressive agent												
Mycophenolate*			0.84			0.01						
Low	106 (45.3)	53 (44.2)		58 (71.6)	42 (52.5)							
High	128 (54.7)	67 (55.8)		23 (28.4)	38 (47.5)							
Azathioprine			0.99			0.001						
Low	390 (48.0)	469 (48.0)		120 (77.4)	96 (60.4)							
High	422 (52.0)	508 (52.0)		35 (22.6)	63 (39.6)							

Notes: †The counts in sub-categories may not add up to the total number due to missing data. ǂThe dose of each agent at transplantation was categorised as low or high relative to the median value of the dose. * Tacrolimus and Mycophenolate restricted to the years from 1997 based on the availability of these agents. ‡ Non-parametric Wilcoxon rank-sum test, chi-square test or Fisher exact test as appropriate.

Table 6 - 3. Risk factors for early NHL after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model

Risk factor	N	NHL	Death	Basic model‡		N	NHL	Death	Complete model†	
				NHL§	Death§				NHL§	Death§
				HR (95%CI)	HR (95%CI)				HR (95%CI)	HR (95%CI)
Age at transplantation (per single year)	4131	29	324	**2.44 (1.28–4.63)**	**2.09 (1.46–3.00)**	2933	16	196	2.12 (0.78–5.89)	**1.58 (1.00–2.51)**
Sex										
Female	1360	7	93	Ref	Ref	922	4	52	Ref	Ref
Male	2771	22	231	2.13 (0.90–5.04)	**1.29 (1.01–1.67)**	2011	12	144	1.98 (0.62–6.27)	**1.40 (1.00–1.95)**
Race										
Non-Caucasian	377	1	35	Ref	Ref					
Caucasian	3649	26	271	1.68 (0.24–11.8)	**0.64 (0.44–0.93)**					
Transplant era										
1998-2006	2148	11	143	Ref	Ref	1464	6	92	Ref	Ref
1995-1997	704	7	56	2.32 (0.89–6.05)	1.22 (0.89–1.68)	534	5	45	1.67 (0.49–5.65)	1.47 (0.97–2.22)
1984-1994	1279	11	125	**2.66 (1.12–6.35)**	**1.64 (1.25–2.14)**	935	5	59	1.14 (0.31–4.28)	1.12 (0.71–1.76)
First transplanted organ										
Liver	1615	6	112	Ref	Ref	1238	4	78	Ref	Ref
Heart	1342	5	102	0.62 (0.19–2.04)	0.87 (0.65–1.17)	1038	4	60	0.61 (0.13–2.95)	0.80 (0.51–1.26)
Lung	1174	18	110	**4.70 (1.84–12.0)**	**1.53 (1.17–1.99)**	657	8	58	3.05 (0.67–13.9)	**1.53 (1.02–2.28)**
Recipient EBV status at transplantation										
Positive	3068	15	205	Ref	Ref					
Negative	638	10	35	**2.70 (1.03–7.07)**	0.78 (0.52–1.19)					
Overall recipient EBV status										
Positive at transplantation	2102	10	113	Ref	Ref					
Positive only after transplantation	48	3	2	**12.7 (2.67–60.9)**	0.78 (0.17–3.57)					
Negative	148	3	2	**4.99 (1.03–24.1)**	**0.23 (0.06–0.89)**					
ATG/ALG induction therapy										
No	2891	19	195	Ref	Ref					
Yes	487	1	30	0.18 (0.02–1.39)	0.89 (0.58–1.38)					
Muromonab CD-3 induction therapy										
No	3259	18	210	Ref	Ref	2832	14	185	Ref	Ref
Yes	122	2	17	3.23 (0.77–13.7)	**2.47 (1.45–4.21)**	101	2	11	**5.66 (1.13–28.3)**	1.87 (0.97–3.58)
Basiliximab induction therapy										
No	3189	22	214		Ref					
Yes	199	0	9	NA	0.66 (0.32–1.33)					
CMV immunoglobulin										
No	1435	7	73	Ref	Ref					
Yes	392	6	37	2.17 (0.49–9.53)	**2.02 (1.33–3.05)**					
CMV antiviral therapy										
No	1903	11	172	Ref	Ref					
Yes	2228	18	152	1.55 (0.74–3.27)	**0.78 (0.62–0.97)**					
Alcohol drinking										
Never	580	7	40	Ref	Ref					
Ever	2398	11	149	**0.30 (0.12–0.75)**	0.80 (0.55–1.17)					
Cancer history										
No	3763	26	282	Ref	Ref					
Yes	368	3	42	2.15 (0.63–7.30)	**1.81 (1.27–2.57)**					
Subsequent transplantation										
No	4012	29	311		Ref					
Yes (>=2)	119	0	13	NA	1.41 (0.77–2.59)					
Donor age (per single year)	4082	28	316	1.02 (0.99–1.04)	**1.02 (1.01–1.02)**					

Table 6 – 3. (continued) Risk factors for early NHL after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model

Immunosuppressive agent	N	NHL	Death	Basic model‡		N	NHL	Death	Complete model†	
				NHL§	Death§				NHL§	Death§
				HR (95%CI)	HR (95%CI)				HR (95%CI)	HR (95%CI)
Current receipt										
Cyclosporine	3738	26	247			3335	20	214		
No				Ref	Ref				Ref	Ref
Yes				**0.26 (0.08–0.86)**	**0.60 (0.41–0.87)**				0.45 (0.09–2.30)	**0.64 (0.42–0.96)**
Tacrolimus*	2403	12	139			2105	8	120		
No				Ref	Ref					Ref
Yes				**6.60 (1.23–35.3)**	0.79 (0.50–1.24)				NA	0.68 (0.42–1.09)
Mycophenolate *	2403	12	139			2105	8	120		
No				Ref	Ref					Ref
Yes				**4.58 (1.54–13.6)**	1.20 (0.77–1.86)				NA	1.24 (0.76–2.03)
Azathioprine	3705	26	245			3335	20	214		
No				Ref	Ref				Ref	Ref
Yes				1.18 (0.51–2.71)	**0.74 (0.56–0.98)**				2.62 (0.73–9.37)	0.74 (0.54–1.02)
Number of drugs	3703	26	245							
1				Ref	Ref					
>1				1.67 (0.50–5.64)	**0.44 (0.32–0.60)**					
Current dose (mg/kg/day)										
Cyclosporine	3678	26	236	0.84 (0.70–1.01)	0.96 (0.91–1.02)	3180	16	200	1.00 (0.86–1.16)	0.98 (0.92–1.04)
Tacrolimus*	2251	11	135	NA	0.07 (0.002–2.71)	1927	8	113	NA	0.03 (0.0003–2.48)
Mycophenolate*	2366	11	137	**1.04 (1.00–1.07)**	1.00 (0.99–1.01)	1926	8	112	NA	1.00 (0.96–1.01)
Azathioprine	3604	22	232	1.14 (0.68–1.91)	0.98 (0.79–1.21)	3105	16	198	**1.79 (1.02–3.14)**	0.93 (0.73–1.18)
Categorised current dose¶										
Cyclosporine	3678	26	236			3180	16	200		
No				3.24 (0.89–11.8)	**1.59 (1.08–2.36)**				2.95 (0.39–22.5)	**1.61 (1.05–2.48)**
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				0.78 (0.28–2.14)	0.80 (0.58–1.12)				2.57 (0.69–9.55)	0.94 (0.65–1.36)
Tacrolimus*	2251	11	135			1927	8	113		
No				0.16 (0.03–1.16)	0.77 (0.46–1.30)				NA	0.86 (0.49–1.49)
Low (≤median)				Ref	Ref				NA	Ref
High (>median)				0.73 (0.19–2.82)	**0.41 (0.19–0.86)**				NA	**0.33 (0.14–0.78)**
Mycophenolate*	2366	11	137			1926	8	112		
No				0.43 (0.10–1.78)	**0.57 (0.36–0.92)**				NA	**0.54 (0.33–0.90)**
Low (≤median)				Ref	Ref				NA	Ref
High (>median)				2.82 (0.52–15.3)	**0.37 (0.18–0.76)**				NA	**0.31 (0.13–0.72)**
Azathioprine	3604	22	232			3105	16	198		
No				1.19 (0.43–3.28)	**1.53 (1.08–2.18)**				0.69 (0.14–3.40)	**1.51 (1.01–2.24)**
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				1.57 (0.49–5.07)	1.25 (0.87–1.82)				2.47 (0.74–8.22)	1.10 (0.74–1.64)
Mean dose# (mg/kg/day)										
Cyclosporine	3514	26	235	0.86 (0.71–1.05)	0.98 (0.93–1.04)	2933	16	196	1.02 (0.86–1.22)	1.00 (0.94–1.06)
Tacrolimus*	2162	11	135	NA	0.11 (0.003–4.61)	1819	8	112	NA	0.01 (0.0004–2.69)
Mycophenolate*	2292	11	137	**1.03 (1.00–1.06)**	1.00 (0.99–1.01)	1819	8	112	1.02 (0.97–1.08)	1.00 (0.98–1.02)
Azathioprine	3446	22	231	1.18 (0.68–2.05)	1.03 (0.83–1.29)	2933	16	196	**2.20 (1.21–4.01)**	1.00 (0.75–1.34)
Categorised mean dose¶										
Cyclosporine										
No	890	9	65	**3.78 (1.04–13.8)**	**1.65 (1.10–2.49)**	744	4	56	1.24 (0.31–4.97)	**9.13 (4.90–17.0)**
Low (≤median)	1312	7	83	Ref	Ref	1118	3	65	Ref	Ref
High	1312	10	87	0.96 (0.33–2.79)	1.01 (0.72–1.40)	1160	9	78	2.80 (0.67–11.6)	1.11 (0.76–1.61)
Tacrolimus*										
No	1460	5	100	0.17 (0.02–1.19)	0.80 (0.48–1.33)	1192	4	82	NA	**2.63 (1.18–5.88)**
Low (≤median)	355	3	25	Ref	Ref	332	3	24	NA	Ref
High (>median)	347	3	10	0.70 (0.18–2.64)	0.39 (0.19–0.83)	311	1	6	NA	**0.27 (0.11–0.66)**
Mycophenolate*										
No	1705	6	98	0.33 (0.09–1.22)	0.73 (0.44–1.20)	1336	6	77	NA	0.94 (0.49–1.78)
Low (≤median)	294	3	23	Ref	Ref	250	2	22	NA	Ref
High (>median)	293	2	16	0.96 (0.17–5.43)	0.64 (0.33–1.22)	233	0	13	NA	0.65 (0.32–1.31)
Azathioprine										
No	1291	7	92	1.95 (0.63–6.02)	1.39 (0.99–1.95)	1007	3	78	1.39 (0.20–9.38)	**1.55 (1.03–2.34)**
Low (≤median)	1078	4	63	Ref	Ref	980	2	57	Ref	Ref
High (>median)	1078	11	76	2.80 (0.74–6.02)	1.12 (0.78–1.61)	946	11	61	**6.35 (1.38–29.3)**	1.02 (0.69–1.50)

Notes: NA, not available due to insufficient number;
§The counts in sub-categories may not add up to the total number due to missing data.
‡Basic model: Immunosuppressive agent or other variable of interest, adjusted for age at transplantation, sex, transplant year, and transplanted organ (if not the variable of interest).
† Complete model: Basic model plus the variables muromonab-CD3 induction and other immunosuppressive agents in the respective category (i.e. current receipt, current dose, categorised current dose, mean dose, categorised mean dose). For time-dependent variables (i.e. current receipt, current dose, categorised current dose), immunosuppressive agents from the same class cannot be given simultaneously and thus are not adjusted for. For variables other than immunosuppressive agents, results adjusted for the mean dose of immunosuppressive agents are presented.
*Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents.
#The mean dose of each agent was calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point (weighted by the length of each follow-up time interval). The median mean dose for cyclosporine was 4.55 (IQR 3.32-6.25) mg/kg/day, for tacrolimus 0.10 (IQR 0.06-0.14), for mycophenolate 32.3 (IQR 24.7-39.2), and for azathioprine 1.30 (IQR 0.93-1.74).
¶The dose of individual agents was categorised as low or high relative the median value of dose.

Table 6 - 4. Risk factors for late NHL after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model

Risk factor	N	NHL	Death	Basic model §,‡		N	NHL	Death	Complete model†	
				NHL	Death				NHL	Death
				HR (95%CI)	HR (95%CI)				HR (95%CI)	HR (95%CI)
Age at transplantation (per single years)	4102	61	1405	1.02 (0.98–1.06)	**1.05 (1.03–1.07)**	2939	48	995	**1.12 (1.04–1.22)**	1.01 (0.99–1.04)
Sex										
Female	1353	23	442	Ref	Ref	953	18	311	Ref	Ref
Male	2749	38	963	0.58 (0.33–1.03)	**1.19 (1.06–1.34)**	1986	30	684	0.65 (0.35–1.21)	**1.23 (1.06–1.42)**
Race										
Non-Caucasian	376	2	89	Ref	Ref					
Caucasian	3623	57	1260	2.50 (0.59–10.6)	0.90 (0.71–1.14)					
Transplant era										
1998-2006	2137	13	403	Ref	Ref	1551	11	292	Ref	Ref
1995-1997	697	18	314	1.88 (0.92–3.84)	**1.25 (1.08–1.45)**	515	14	229	0.97 (0.44–2.13)	0.98 (0.81–1.19)
1984-1994	1268	30	688	1.15 (0.59–2.26)	**1.41 (1.22–1.62)**	873	23	474	0.59 (0.27–1.27)	0.97 (0.81–1.17)
Time since transplantation (years)										
0-0.99	4102	0	324	NA	**1.56 (1.29-1.88)**	2939	0	224	NA	**1.76 (1.40–2.21)**
1-1.99	3535	8	180	Ref	Ref	2566	4	113	Ref	Ref
2-4.99	3114	18	329	1.28 (0.55–3.01)	0.82 (0.68–1.00)	2285	16	252	2.30 (0.75–7.02)	0.98 (0.78–1.24)
5+	2109	35	572	**3.68 (1.52–8.92)**	1.10 (0.88–1.40)	1518	28	406	**6.20 (1.99–19.3)**	1.14 (0.87–1.48)
First transplanted organ										
Liver	1609	17	353	Ref	Ref	1136	14	249	Ref	Ref
Heart	1337	35	489	**2.41 (1.30–4.48)**	1.13 (0.97–1.31)	1010	27	349	1.79 (0.86–3.72)	1.12 (0.93–1.36)
Lung	1156	9	563	0.70 (0.30–1.63)	**3.45 (3.00–3.96)**	793	7	397	0.56 (0.20–1.55)	**2.92 (2.43–3.50)**
Recipient EBV status at transplantation										
Positive	3053	35	894	Ref	Ref					
Negative	628	17	261	1.97 (0.98–3.98)	1.01 (0.80–1.27)					
Recipient EBV status after transplantation										
Positive	2209	30	477	Ref	Ref					
Negative	145	1	51	0.18 (0.02–1.35)	0.96 (0.72–1.29)					
Overall recipient EBV status										
Positive at transplantation	2092	18	441	Ref	Ref					
Positive only after transplantation	45	8	13	**16.1 (5.76–45.1)**	0.99 (0.58–1.69)					
Negative	145	1	51	0.41 (0.05–3.15)	0.97 (0.72–1.32)					
CMV concordance										
Recipient –/ Donor –	482	5	169	Ref	Ref					
Recipient –/ Donor +	694	16	209	2.52 (0.92–6.90)	1.00 (0.82–1.22)					
Recipient +/ Donor + or Donor –	1740	40	938	1.41 (0.55–3.60)	1.10 (0.93–1.29)					

Table 6-4. (continued) Risk factors for late NHL after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model

Risk factor	N	NHL	Death	Basic model§*	
				NHL	Death
				HR (95%CI)	HR (95%CI)
Donor age	4054	61	1376	1.0 (0.98–1.02)	**1.01 (1.01–1.02)**
Recipient blood type					
A	1779	19	624	Ref	Ref
AB	187	4	63	2.1 (0.67–6.04)	0.98 (0.76–1.27)
B	468	6	141	1.16 (0.46–2.91)	0.91 (0.75–1.09)
O	1662	32	571	**1.81 (1.03–3.19)**	0.99 (0.88–1.11)
Smoking history					
Never	1306	16	367	Ref	Ref
Ever	1959	34	765	1.51 (0.83–2.76)	**1.43 (1.24–1.64)**
Cancer history					
No	3737	58	1294	Ref	Ref
Yes	365	3	111	0.96 (0.28–3.25)	**1.98 (1.58–2.49)**
Diabetes					
No diabetes	1954	35	512	Ref	Ref
Preexisting diabetes	493	3	158	0.46 (0.14–1.8)	**1.77 (1.47–2.13)**
Post-transplant diabetes	559	7	157	0.8 (0.35–1.8)	1.16 (0.98–1.38)
Dialysis					
Never	2504	40	604	Ref	Ref
Ever	277	3	139	0.79 (0.28–2.22)	**2.12 (1.76–2.56)**
Multiple transplantation					
No	3983	60	1359	Ref	Ref
Yes (>=2)	119	1	46	0.66 (0.09–4.98)	**1.42 (1.0–2.01)**
CMV immunoglobulin					
No	1428	20	338	Ref	Ref
Yes	386	5	144	0.95 (0.34–2.63)	**1.41 (1.14–1.74)**
ATG/ALG (induction/rejection)					
Never	3060	37	917	Ref	Ref
Ever	652	17	333	1.33 (0.69–2.54)	0.99 (0.85–1.14)
Muronomab CD-3 (induction/rejection)					
Never	3440	46	1132	Ref	Ref
Ever	274	8	120	1.54 (0.69–3.41)	**1.38 (1.11–1.7)**
Basiliximab (induction/rejection)					
Never	3496	51	1219	Ref	Ref
Ever	214	2	30	1.32 (0.29–5.97)	0.93 (0.63–1.38)

Table 6-4. (continued) Risk factors for late NHL after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model

Immunosuppressive agent	N	NHL	Death	Basic model §,‡		N	NHL	Death	Complete model†	
				NHL	Death				NHL	Death
				HR (95%CI)	HR (95%CI)				HR (95%CI)	HR (95%CI)
Current receipt										
Cyclosporine	3725	49	1149			3722	49	1147		
No				Ref	Ref				Ref	Ref
Yes				0.78 (0.36–1.71)	0.89 (0.75–1.05)				0.82 (0.38–1.78)	**0.83 (0.70–0.99)**
Tacrolimus*	3421	42	909			3420	42	909		
No				Ref	Ref				Ref	Ref
Yes				0.78 (0.32–1.95)	0.99 (0.81–1.20)				0.76 (0.30–1.94)	1.01 (0.83–1.22)
Mycophenolate*	3421	42	909			3420	42	909		
No				Ref	Ref				Ref	Ref
Yes				0.67 (0.24–1.84)	**0.80 (0.66–0.98)**				0.54 (0.19–1.52)	**0.78 (0.64–0.95)**
Azathioprine	3723	49	1147			3722	49	1147		
No				Ref	Ref				Ref	Ref
Yes				1.59 (0.82–3.13)	0.94 (0.82–1.07)				1.71 (0.87–3.36)	0.89 (0.78–1.02)
Number of drugs	3722	49	1147							
1				Ref	Ref					
>1				0.99 (0.42–2.33)	**0.67 (0.57-0.78)**					
Current dose (mg/kg/day)										
Cyclosporine	3697	49	1121	0.85 (0.73–1.00)	**1.02 (1.00–1.04)**	3646	49	1098	0.93 (0.77–1.12)	1.01 (0.98–1.04)
Tacrolimus*	3341	42	878	NA	0.32 (0.04–2.65)	3308	42	853	NA	0.28 (0.03–2.34)
Mycophenolate*	3401	42	904	0.99 (0.95–1.02)	0.99 (0.98–1.02)	3311	42	857	0.99 (0.95–1.02)	0.99 (0.98–1.00)
Azathioprine	3680	49	1113	1.31 (0.90–1.89)	1.01 (0.91–1.11)	3599	49	1074	**1.54 (1.02–2.31)**	0.97 (0.88–1.08)
Categorised current dose¶										
Cyclosporine	3697	49	1121			3646	49	1098		
No				1.14 (0.50–2.61)	1.12 (0.94–1.34)				1.12 (0.48–2.61)	1.20 (0.99–1.44)
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				0.86 (0.43–1.70)	1.00 (0.87–1.14)				0.92 (0.47–1.79)	1.00 (0.87–1.15)
Tacrolimus*	3341	42	878			3308	42	853		
No				1.28 (0.38–4.28)	0.89 (0.69–1.15)				1.33 (0.40–4.42)	0.86 (0.66–1.12)
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				1.24 (0.28–5.60)	**0.69 (0.49–0.98)**				1.09 (0.24–4.95)	**0.68 (0.48–0.97)**
Mycophenolate*	3401	42	904			3311	42	857		
No				1.46 (0.43–5.00)	1.16 (0.92–1.48)				1.74 (0.47–6.40)	1.22 (0.95–1.57)
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				1.03 (0.20–5.26)	0.85 (0.61–1.19)				0.97 (0.18–5.08)	0.87 (0.62–1.23)
Azathioprine	3680	49	1113			3599	49	1074		
No				0.68 (0.31–1.49)	1.05 (0.90–1.22)				0.63 (0.29–1.37)	1.04 (0.89–1.22)
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				1.32 (0.64–2.72)	0.98 (0.84–1.13)				1.30 (0.64–2.65)	0.94 (0.81–1.22)
Mean dose# (mg/kg/day)										
Cyclosporine	3260	48	1073	1.02 (0.94–1.10)	**1.09 (1.06–1.12)**	2939	48	995	1.04 (0.94–1.15)	**1.09 (1.07–1.12)**
Tacrolimus*	2954	41	857	NA	0.17 (0.02–1.41)	2718	41	781	NA	0.30 (0.04–2.21)
Mycophenolate*	2954	41	857	0.98 (0.95–1.01)	**0.98 (0.97–0.99)**	2718	41	781	0.99 (0.96–1.02)	**0.98 (0.97–0.99)**
Azathioprine	3186	48	1045	**1.76 (1.23–2.53)**	**1.20 (1.07–1.35)**	2939	48	995	**1.78 (1.12–2.84)**	**1.14 (1.00–1.29)**
Categorised mean dose¶										
Cyclosporine										
No	835	3	131	0.40 (0.11–1.48)	**1.60 (1.26–2.02)**	638	3	111	0.61 (0.10–3.80)	**4.46 (2.84–7.02)**
Low (≤median)	1213	22	356	Ref	Ref	1140	23	326	Ref	Ref
High	1212	23	586	1.16 (0.63–2.15)	**1.91 (1.67–2.20)**	1161	23	558	1.44 (0.76–2.72)	**2.14 (1.84–2.48)**
Tacrolimus*										
No	2064	34	717	1.64 (0.51–5.31)	**1.46 (1.12–1.90)**	1859	34	646	1.60 (0.38–6.63)	**3.44 (2.22–5.33)**
Low (≤median)	446	3	68	Ref	Ref	426	3	66	Ref	Ref
High (>median)	444	4	72	1.45 (0.33–6.39)	1.19 (0.84–1.69)	433	4	69	1.42 (0.31–6.54)	1.17 (0.82–1.67)
Mycophenolate*										
No	2211	34	741	2.51 (0.77–8.12)	**2.35 (1.86–2.98)**	1875	34	652	2.33 (0.73–7.41)	**2.71 (2.11–3.49)**
Low (≤median)	456	3	74	Ref	Ref	421	3	67	Ref	Ref
High (>median)	456	4	70	1.66 (0.35–7.95)	1.36 (0.98–1.90)	422	4	62	1.90 (0.39–9.38)	1.15 (0.80–1.65)
Azathioprine										
No	1105	5	213	0.44 (0.15–1.36)	1.18 (0.98–1.42)	933	5	189	0.73 (0.24–2.23)	**1.58 (1.29–1.94)**
Low (≤median)	1042	14	379	Ref	Ref	998	14	365	Ref	Ref
High (>median)	1039	29	453	1.80 (0.95–3.40)	**1.16 (1.01–1.33)**	1008	29	441	**1.93 (1.01–3.68)**	**1.19 (1.03–1.37)**

Notes: NA, not available due to insufficient number;
§ The counts in sub-categories may not add up to the total number due to missing data.
‡ Basic model: Immunosuppressive agent or other variable of interest, adjusted for age at transplantation, sex, transplant year and transplanted organ (if not the variable of interest).
† Complete model: Basic model plus the variables muromonab-CD3 induction and other immunosuppressive agents in the respective category (i.e. current receipt, current dose, categorised current dose, mean dose, categorised mean dose). For time-dependent variables (i.e. current receipt, current dose, categorised current dose), immunosuppressive agents from the same class (CNI: cyclosporine, tacrolimus, antiproliferative: mycophenolate, azathioprine), cannot be given simultaneously and thus are not adjusted for. For variables other than immunosuppressive agents, results adjusted for the mean dose of immunosuppressive agents are presented.
* Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents.
# The mean dose of each agent was calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point (weighted by the length of each follow-up time interval). The median dose for cyclosporine was 3.18 (IQR 2.36-4.38) mg/kg/day, for tacrolimus 0.07 (IQR 0.04-0.11), for mycophenolate 26.8 (IQR 19.7-34.1), and for azathioprine 1.14 (IQR 0.82-1.50).
¶ The dose of individual agents was categorised as low or high relative to the median value of dose.

6.5 Discussion

In this population-based study of Australian adult liver, heart and lung transplant recipients, I found that a high dose of azathioprine was independently associated with a moderate dose-related risk of both early and late NHL. After adjustment for the dose of individual immunosuppressive agents, the risk of NHL did not differ between transplanted organs. I also showed that muromonab-CD3 induction therapy was independently associated with a substantial increased risk of early NHL, and increasing age and increasing duration of immunosuppression are independent risk factors for late NHL.

In this study the rate of NHL was highest during the first year post-transplantation, decreased sharply during the second year, and peaked again after the fifth year. This pattern accords with previous population-based studies (Adami et al. 2003, Villeneuve et al. 2007, Caillard et al. 2012), but differs from Australian kidney transplant recipients where the highest risk was observed within two years of transplantation (Faull et al. 2005, van Leeuwen et al. 2009). In line with previous studies (Knight et al. 2009, van Leeuwen et al. 2009), the majority of early NHL in this cohort were diffuse large B-cell lymphomas while some late NHLs were T-cell lymphomas. The bimodal incidence of NHL with different pathologic characteristics supports two mechanisms of lymphomagenesis (Ghobrial et al. 2005, van Leeuwen et al. 2009, Schober et al. 2013).

Prior to statistical adjustment for immunosuppression, risk of early NHL was increased for lung relative to liver transplantation, and risk of late NHL was increased for heart relative to liver transplantation, as previously observed (Ghobrial et al. 2005, Fernberg

et al. 2011). After adjustment for immunosuppression, the risk of both early and late NHL did not differ by organ type. This finding suggests that differences in NHL risk between transplanted organs are mainly attributable to organ-specific differences in the type and extent of immunosuppression (Penn 1990, Swerdlow et al. 2008, Fernberg et al. 2011).

EBV is an established and strong risk factor for early NHL after transplantation. Immunosuppressed recipients who acquire primary EBV infection after transplantation are less able to develop an adequate level of EBV-specific cytotoxic CD8+ T-cells (EBV-CTL), which can lead to unrestrained B-cell proliferation (Shpilberg et al. 1999, Cohen 2000). The receipt of T-cell depleting antibodies may further hamper the production of EBV-CTLs (Yajima et al. 2009). In this study, EBV seronegativity at transplant was related to early NHL but did not confound the relationship between immunosuppression and early NHL risk.

I confirmed the increased risk of early NHL with muromonab-CD3 induction therapy observed in prior studies (Swinnen et al. 1990, Bustami et al. 2004). The significance was only apparent after adjustment for dose of immunosuppressive agents, indicating the confounding role of intensity of immunosuppression driven by the concomitant use of the other immunosuppressive agents. Muromonab-CD3 is a murine monoclonal antibody against the human CD3-receptor T-cell complex, which activates and then eliminates circulating T-cells (Swinnen and Fisher 1993), but the precise biology underlying the heightened risk of NHL is not known. Due to significant side-effects,

including the high incidence of PTLD/NHL (Swinnen et al. 1990, Cherikh et al. 2003), muromonab-CD3 is no longer indicated for transplant recipients.

The finding of a non-significant association between ATG/ALG and NHL risk is consistent with a study of US kidney transplant recipients (Bustami et al. 2004), but in contrast with a Swedish nested case-control study (Fernberg et al. 2011). In the Swedish study ATG/ALG was however received by 60% of the cohort, whereas in this study and the US study exposure to this agent was much less prevalent (12% and 10% respectively). IL-2Ra, targeting only activated T-lymphocytes as opposed to resting T-cells (Henry and Rajab 2002), was also not associated with NHL risk in my cohort, in agreement with prior studies (Caillard et al. 2005, van Leeuwen et al. 2009, Fernberg et al. 2011).

As previously observed (van Leeuwen et al. 2009, Fernberg et al. 2011, Caillard et al. 2012), I found that older age increased the risk of late NHL. This relationship is thought to reflect age-related increases in immune senescence and associated impaired immune surveillance (Cockfield 2001). The positive association between increasing time since transplantation and late NHL may be due to chronic antigenic stimulation from the transplanted organ, leading to T-cell exhaustion (Bucks et al. 2009), which may explain NHL preferentially localising in the allograft region (Opelz and Henderson 1993, Kew et al. 2000, Opelz and Dohler 2004). Another hypothesis is chronic immune dysregulation and perhaps chronic infection by a carcinogenic agent (Cockfield 2001). Univariable analysis of cross-sectional data from adult kidney transplant recipients has shown an increase in detectable EBV DNA with increasing time since transplantation

and with use of azathioprine (OR 1.5, 95 % CI 0.9-2.3), a decrease in detectable EBV DNA with use of mycophenolate (OR 0.3, 95 % CI 0.2-0.4), and an increased risk of PTLD with persistent EBV DNAemia (Morton et al. 2014). The precise role of EBV in the development of late NHL remains uncertain.

I found that a high dose of azathioprine independently increased the risk of NHL. I observed this association for early and late NHL and for the current and mean dose of azathioprine. Except for the association between mean azathioprine dose and late NHL risk, these associations only became significant after complete adjustment. This supports confounding by the concurrent use of other immunosuppressive agents or possibly by the overall intensity of immunosuppression. No previous transplantation study has shown that azathioprine dose increases risk of NHL independent of other immunosuppressive agents. In a nested case-control study, neither the receipt nor the accumulated or average categorised dose of azathioprine was associated with increased risk of NHL, but risk estimates were not adjusted for the dose of other agents or the duration of immunosuppression (Fernberg et al. 2011). The finding of no association with the current receipt of azathioprine (i.e. irrespective of dose) is consistent with a previous study of Australian kidney transplant recipients (van Leeuwen et al. 2009). Except for one study of US kidney recipients, where both azathioprine and mycophenolate were associated with reduced risk of PTLD (Caillard et al. 2005), studies examining the relationship with discharge immunosuppressive agents have found no association between azathioprine and NHL risk in kidney (Bustami et al. 2004, Faull et al. 2005, Caillard et al. 2012), heart (Dharnidharka et al. 2012) or liver (Dharnidharka et al. 2012) recipients. Indirect evidence of the promotion

of lymphomagenesis by azathioprine is however indicated by a poorer survival rate after NHL diagnosis in kidney transplant recipients using azathioprine (Caillard et al. 2006).

Although azathioprine use has decreased over the past two decades, it has not been completely replaced by other agents (Na et al. 2014); in 2012 at least 10% of adult lung recipients received azathioprine at discharge and 1-year post-transplantation (OPTN/SRTR 2012). Azathioprine is classified by IARC as a human carcinogen causing NHL, and skin cancer (Grosse et al. 2009, IARC 2012). The current use of azathioprine and its metabolite, 6-mercaptopurine, is associated with a 4 to 5-fold risk of lymphoma in patients with inflammatory bowel disease (Beaugerie et al. 2009, Kotlyar et al. 2014). Importantly, in terms of evidence of a causal relationship, the excess risk of lymphoma is reversible after cessation of azathioprine in this patient group (Beaugerie et al. 2009, Kotlyar et al. 2014). Biological evidence in animals and humans showing azathioprine promotes lymphomagenesis via genotoxicity and immunosuppression is also convincing. An increased frequency of somatic mutations has been observed in IBD patients treated with thiopurine (Nguyen et al. 2009), the direct mutagenic effect has also been evidenced in some *in vitro* studies (Swann et al. 1996, Smith et al. 1999, Yuan and Wang 2008). In addition, it appears that the lymphomagenic effect of azathioprine is possibly related to DNA mismatch repair system deficiency (Chalastanis et al. 2010).

In agreement with prior studies I found that receipt of mycophenolate was not related to risk of late NHL (Caillard et al. 2005, Robson et al. 2005, Crespo-Leiro et al. 2008,

Caillard et al. 2012). In contrast, I found the current receipt, increasing current and mean dose of mycophenolate increased risk of early NHL before adjustment for other agents, but I could not examine the independent association due to insufficient numbers. Although this finding warrants further study, a positive association is not predicted on the basis of evidence that mycophenolate inhibits B-cell stimulation and B-cell lymphoma cells and exerts anti-inflammatory effects (Heidt et al. 2008, Bugelski et al. 2010).

I found that risk of early NHL was decreased with current receipt of cyclosporine and increased with current receipt of tacrolimus before adjustment for other agents. After adjustment, the association with cyclosporine was no longer significant, and I could not assess the independent association with tacrolimus due to insufficient numbers. I found no association between these agents and risk of late NHL. Prior evidence of an association between NHL risk and these agents is mixed. Current receipt of any CNIs was associated with increased risk of late but not early NHL in Australian kidney transplant recipients (van Leeuwen et al. 2009), similar to findings from the same population based on discharge immunosuppression (Faull et al. 2005). In the Swedish nested case-control study, there was no clear association between use or dose of cyclosporine and NHL risk, and a suggested increased risk with use of tacrolimus based on very small numbers (Fernberg et al. 2011). Finally, studies using discharge immunosuppression have been inconsistent, some showing no association between NHL risk and cyclosporine (Bustami et al. 2004, Caillard et al. 2012) or tacrolimus (Crespo-Leiro et al. 2008, Caillard et al. 2012), and others showing an increased risk with tacrolimus (Opelz and Dohler 2004, Caillard et al. 2012, Dharnidharka et al. 2012).

Cyclosporine is IARC-classified as a human carcinogen for NHL, skin cancer, and cancer at multiple other sites (IARC 2012). However, as noted above, most observational studies of transplant recipients did not assess the independent role of individual agents, and cyclosporine was traditionally prescribed with azathioprine. There is no conclusive evidence that cyclosporine is directly mutagenic (Grosse et al. 2009, Bugelski et al. 2010, IARC 2012). Although both cyclosporine and tacrolimus have been shown to promote EBV infected B-cell proliferation (Beatty et al. 1998), a recent study suggested that cyclosporine may suppress the growth of B-cell lymphoma in mice (Rafferty et al. 2012). While my findings must be replicated, they challenge the IARC classification for cyclosporine as being carcinogenic for NHL. Further study is also needed to examine the independent association between tacrolimus and NHL risk, but biological evidence of carcinogenicity is limited. An increased incidence of lymphomas has been observed in herpes virus infected mice treated with tacrolimus (Mistrikova et al. 1999), and a mutagenic effect has been suggested in an *in vitro* study (Oliveira et al. 2004). On the other hand, no genotoxicity was found in kidney transplant recipients treated with tacrolimus (Ozturk et al. 2008).

I found no independent association between NHL risk and lifestyle factors, comorbidities, donor and recipient CMV serostatus, or prophylactic anti-CMV immunoglobulin and antiviral agents, in accordance with prior population-based studies (Faull et al. 2005, Opelz et al. 2009), although immunoglobulin has been reported to prevent the development of early NHL in kidney recipients (Opelz et al. 2007).

Key strengths of my study are the population base for the transplant recipients, NHL diagnoses and deaths, minimising bias, and the comprehensive data on dose of immunosuppressive agents and clinical risk factors over 23-years. As the immunosuppression data for my cohort is comparable with international transplantation practice (Chapter 5), my findings are likely to be generalizable. Moreover, I examined two approaches to modelling the dose of each immunosuppressive agent. This is the first population-based study to examine risk of NHL in relation to dose of individual immunosuppressive agents, adjusted for other agents, and accounting for competing risk of death. I observed significant confounding by dose of immunosuppressive agents, thereby demonstrating the need for adjusted analyses. Competing risk of death is also important because more than a third of participants died during follow-up, and immunosuppression and other factors were associated with death. Both age and duration of immunosuppression are significant risk factors for death, therefore, the association between these factors and risk of NHL was not observed when I censored at the event of first event (death) in the cause specific model.

The main limitation is my reliance on the accuracy, completeness and availability of medical records. EBV viral load, EBV genome in the tumour, and HLA mismatches were not routinely tested or recorded in recipient medical records. The timing of rejection, acquired viral infections, and acquired comorbidities were not collected, thus I examined them as fixed variables assuming their occurrence preceded NHL. In addition, some potentially relevant confounders, such as primary indication, HBV/HCV infection,

and mTOR inhibitors, were collected but could not be evaluated due to low prevalence and need to be assessed in larger studies. Therefore, I am unable to exclude residual confounding. My findings are also limited by the scope of the study period from 1984 to 2006, which may not be representative of contemporary clinical practice, particularly, immunosuppressive therapy. Muromonab-CD3 is no longer used and azathioprine is currently used infrequently in transplant recipients. Despite the long follow-up, my statistical power was limited by a relatively small number of incident NHLs. Statistical power was further reduced in the complete models restricted to patients without any missing data, and thus significant associations may not have been detected. Missing data was imputed to the extent possible and there were no notable differences in results based on original and imputed data, supporting the assumption of ignorable drop-outs. Like previous studies (Allen et al. 2013), I was unable to reliably model the overall intensity of immunosuppression, however, the results for each agent were adjusted for the dose of the other agent. As this was an observational study, I am unable to exclude the effect of propensity to receive different immunosuppressive agents between different recipient subgroups. Finally, as I performed multiple comparisons, the possibility of chance findings cannot be excluded. However, the consistency of the results for individual immunosuppressive agents is reassuring.

6.6 Conclusions

I found consistent evidence of a moderate increased risk of both early and late NHL associated with higher doses of azathioprine. I confirmed the prior evidence of different risk profiles for early and late post-transplant NHL. Whilst NHL is rare, my

findings support the avoidance of azathioprine where possible, and careful monitoring of patient subgroups at high risk.

Supplementary Tables

Supplementary Table 6 - 1. Risk factors for late NHL after adult liver, heart and lung transplantation, censored at ten years follow-up

Risk factor	N	NHL	Death	Complete model §,‡	
				NHL	Death
				HR (95%CI)	HR (95%CI)
Age at transplantation (per single year)	3010	43	853	**1.12 (1.02–1.22)**	**1.06 (1.01–1.11)**
Sex					
Female	979	17	282	Ref	Ref
Male	2031	26	571	0.59 (0.31–1.10)	**1.17 (1.01–1.36)**
Transplant era					
1998-2006	1551	11	292	Ref	Ref
1995-1997	522	14	218	1.10 (0.48–2.49)	1.04 (0.85–1.27)
1984-1994	937	18	343	0.58 (0.23–1.46)	0.96 (0.78–1.18)
Time since transplantation (years)					
0-0.99	3010	0	224	NA	**1.68 (1.33–2.11)**
1-1.99	2637	4	113	Ref	Ref
2-4.99	2356	16	252	2.31 (0.75–7.07)	1.07 (0.84–1.35)
5-9.99	1589	23	264	**5.91 (1.91–18.3)**	1.31 (0.94–1.82)
First transplanted organ					
Liver	1174	13	227	Ref	Ref
Heart	1032	24	258	1.76 (0.83–3.75)	0.97 (0.79–1.19)
Lung	804	6	368	0.50 (0.16–1.51)	**2.68 (2.22–3.25)**
Current receipt of immunosuppressive agent					
Cyclosporine	3722	44	982		
No				Ref	Ref
Yes				0.76 (0.34–1.70)	0.86 (0.71–1.04)
Tacrolimus*	3383	37	745		
No				Ref	Ref
Yes				0.77 (0.29–2.01)	0.96 (0.78–1.18)
Mycophenolate*	3383	37	745		
No				Ref	Ref
Yes				0.45 (0.14–1.41)	**0.71 (0.58–0.89)**
Azathioprine	3722	44	982		
No				Ref	Ref
Yes				1.95 (0.97–3.95)	0.99 (0.86–1.15)
Current dose (mg/kg/day)					
Cyclosporine	3644	44	945	0.87 (0.68–1.10)	1.02 (0.99–1.04)
Tacrolimus*	3260	37	700	NA	0.20 (0.02–1.93)
Mycophenolate*	3262	37	705	0.98 (0.94–1.02)	0.99 (0.98–1.00)
Azathioprine	3595	44	923	**1.71 (1.13–2.58)**	1.04 (0.93–1.15)

Supplementary Table 6-1. (continued) Risk factors for late NHL after adult liver, heart and lung transplantation, censored at ten years follow-up

Immunosuppressive agent	N	NHL	Death	Complete model §,‡	
				NHL	Death
				HR (95%CI)	HR (95%CI)
Categorised current dose¶					
Cyclosporine	3644	44	945		
No				1.26 (0.52–3.03)	1.20 (0.99–1.47)
Low (≤median)				Ref	Ref
High (>median)				1.01 (0.49–2.04)	1.08 (0.92–1.26)
Tacrolimus*	3260	37	700		
No				1.31 (0.39–4.40)	0.92 (0.70–1.21)
Low (≤median)				Ref	Ref
High (>median)				1.07 (0.24–4.83)	**0.69 (0.49–0.99)**
Mycophenolate*	3262	37	705		
No				1.71 (0.44–6.70)	**1.48 (1.11–1.96)**
Low (≤median)				Ref	Ref
High (>median)				0.62 (0.10–4.01)	1.01 (0.69–1.49)
Azathioprine	3595	44	923		
No				0.59 (0.26–1.35)	0.99 (0.84–1.17)
Low (≤median)				Ref	Ref
High (>median)				1.43 (0.67–3.05)	0.98 (0.84–1.16)
Mean dose# (mg/kg/day)					
Cyclosporine	3010	43	853	1.02 (0.91–1.13)	**1.09 (1.06–1.12)**
Tacrolimus*	2758	36	640	NA	0.48 (0.05–4.63)
Mycophenolate*	2758	36	640	1.00 (0.97–1.03)	0.99 (0.98–1.00)
Azathioprine	3010	43	853	**1.90 (1.22–2.96)**	**1.19 (1.04–1.36)**
Categorised mean dose¶					
Cyclosporine					
No	638	3	110	0.47 (0.07–3.06)	**3.43 (2.04–5.76)**
Low (≤median)	1175	21	283	Ref	Ref
High	1197	19	460	1.23 (0.61–2.47)	**1.88 (1.60–2.22)**
Tacrolimus*					
No	1939	29	513	1.33 (0.30–5.95)	**1.88 (1.21–2.91)**
Low (≤median)	409	3	62	Ref	Ref
High (>median)	410	4	65	1.37 (0.31–6.09)	1.14 (0.80–1.64)
Mycophenolate*					
No	2048	30	536	1.19 (0.32–4.43)	**2.00 (1.46–2.74)**
Low (≤median)	355	3	54	Ref	Ref
High (>median)	355	3	50	1.17 (0.21–6.57)	1.01 (0.67–1.52)
Azathioprine					
No	952	5	180	0.67 (0.20–2.20)	**1.45 (1.17–1.81)**
Low (≤median)	1026	12	302	Ref	Ref
High (>median)	1032	26	371	**2.12 (1.05–4.29)**	**1.24 (1.06–1.46)**

Notes: NA, not available due to insufficient number;

§ The counts in sub-categories may not add up to the total number due to missing data.

‡Complete model: Basic model plus the variables muromonab-CD3 induction and other immunosuppressive agents in the respective category (i.e. current receipt, current dose, categorised current dose, mean dose, categorised mean dose). For time-dependent variables (i.e. current receipt, current dose, categorised current dose), immunosuppressive agents from the same class (CNI: cyclosporine, tacrolimus, Antiproliferative: mycophenolate, azathioprine), cannot be given simultaneously and thus are not adjusted for. For variables other than immunosuppressive agents, results adjusted for the mean dose of immunosuppressive agents are presented.

* Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents.

The mean dose of each agent was calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point (weighted by the length of each follow-up time interval). For early NHL model, the mean dose of each agent was calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point (weighted by the length of each follow-up time interval). The median mean dose for cyclosporine was 4.55 (IQR 3.32-6.25) mg/kg/day, for tacrolimus 0.10 (IQR 0.06-0.14), for mycophenolate 32.3 (IQR 24.7-39.2), and for azathioprine 1.30 (IQR 0.93-1.74). For late NHL model, the median dose for cyclosporine was 3.18 (IQR 2.36-4.38) mg/kg/day, for tacrolimus 0.07 (IQR 0.04-0.11), for mycophenolate 26.8 (IQR 19.7-34.1), and for azathioprine 1.14 (IQR 0.82-1.50).

¶ The dose of individual agents was categorised as low or high relative to the median value of dose.

Supplementary Table 6 - 2. Complete cause-specific hazard models for risk of early and late NHL and death

Risk factor	Early NHL‡					Late NHL‡				
	N	NHL	Death	NHL	Death	N	NHL	Death	NHL	Death
				HR (95%CI)	HR (95%CI)				HR (95%CI)	HR (95%CI)
Age at transplantation	2933	16	196	1.53 (0.27–8.80)	1.53 (0.93–2.51)	2939	48	995	0.97 (0.85–1.11)	0.97 (0.94–1.00)
Sex										
Female	922	4	52	Ref	Ref	953	18	311	Ref	Ref
Male	2011	12	144	2.09 (0.62–7.04)	**1.40 (1.01–1.95)**	1986	30	684	0.67 (0.36–1.23)	**1.22 (1.06–1.41)**
Transplant era										
1998-2006	1464	6	92	Ref	Ref	1551	11	292	Ref	Ref
1995-1997	534	5	45	1.75 (0.44–6.98)	1.47 (0.98–2.20)	515	14	229	0.91 (0.39–2.13)	0.99 (0.82–1.19)
1984-1994	935	5	59	1.12 (0.24–5.09)	1.12 (0.74–1.70)	873	23	474	0.49 (0.21–1.16)	0.92 (0.77–1.10)
Time since transplantation (years)										
0-0.99						2939	0	224	NA	**1.80 (1.43–2.27)**
1-1.99						2566	4	113	Ref	Ref
2-4.99						2285	16	252	1.79 (0.58–5.53)	0.92 (0.73–1.16)
5+						1518	28	406	2.56 (0.64–10.3)	0.84 (0.61–1.16)
First transplanted organ										
Liver	1238	4	78	Ref	Ref	1136	14	249	Ref	Ref
Heart	1038	4	60	0.56 (0.11–2.93)	0.80 (0.53–1.20)	1010	27	349	1.87 (0.89–3.90)	1.14 (0.95–1.36)
Lung	657	8	58	2.98 (0.75–11.9)	**1.54 (1.04–2.27)**	793	7	397	0.72 (0.27–1.94)	**2.93 (2.45–3.49)**
OKT3 induction therapy										
No	2832	14	185	Ref	Ref					
Yes	101	2	11	**6.88 (1.24–38.3)**	**1.90 (1.01–3.59)**					
Current receipt of immunosuppressive agent										
Cyclosporine	3335	20	214			3722	49	1147		
No				Ref	Ref				Ref	Ref
Yes				0.40 (0.10–1.54)	**0.63 (0.43–0.92)**				0.84 (0.38–1.85)	0.85 (0.72–1.00)
Tacrolimus*	2105	8	120			3420	42	909		
No					Ref				Ref	Ref
Yes				NA	0.68 (0.42–1.11)				0.72 (0.28–1.84)	1.00 (0.83–1.21)
Mycophenolate*	2105	8	120			3420	42	909		
No					Ref				Ref	Ref
Yes				NA	1.24 (0.77–2.00)				0.50 (0.19–1.32)	**0.76 (0.63–0.93)**
Azathioprine	3335	20	214			3722	49	1147		
No				Ref	Ref				Ref	Ref
Yes				2.57 (0.67–9.82)	0.75 (0.55–1.02)				1.85 (0.94–3.63)	0.95 (0.83–1.08)
Current dose (mg/kg/day)										
Cyclosporine	3180	16	200	1.00 (0.86–1.17)	0.98 (0.93–1.03)	3646	49	1098	0.95 (0.78–1.15)	1.01 (0.98–1.03)
Tacrolimus*	1927	8	113	NA	NA	3308	42	853	NA	0.26 (0.03–1.95)
Mycophenolate*	1926	8	112	NA	1.00 (0.99–1.01)	3311	42	857	0.98 (0.95–1.02)	0.99 (0.98–1.00)
Azathioprine	3105	16	198	1.84 (0.91–3.75)	0.93 (0.76–1.15)	3599	49	1074	**1.59 (1.04–2.43)**	1.00 (0.90–1.10)

Supplementary Table 6 - 2. (continued) Complete cause-specific models for risk of early and late NHL and death

Immunosuppression	Early NHL‡					Late NHL‡				
	N	NHL	Death	NHL	Death	N	NHL	Death	NHL	Death
				HR (95%CI)	HR (95%CI)				HR (95%CI)	HR (95%CI)
Categorised current dose¶										
Cyclosporine	3697	49	200			3646	49	1098		
No				3.06 (0.53–17.7)	**1.62 (1.07–2.46)**				1.09 (0.47–2.54)	1.18 (0.99–1.41)
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				2.58 (0.65–10.3)	0.95 (0.66–1.35)				0.93 (0.47–1.82)	1.00 (0.87–1.15)
Tacrolimus*	3341	42	113			3308	42	853		
No				NA	0.85 (0.49–1.49)				1.40 (0.39–4.95)	0.88 (0.68–1.14)
Low (≤median)				NA	Ref				Ref	Ref
High (>median)				NA	**0.33 (0.14–0.79)**				1.09 (0.24–5.01)	**0.69 (0.49–0.97)**
Mycophenolate*	3401	42	112			3311	42	857		
No				NA	**0.54 (0.32–0.90)**				1.93 (0.55–6.78)	1.27 (0.99–1.63)
Low (≤median)				NA	Ref				Ref	Ref
High (>median)				NA	**0.31 (0.14–0.70)**				0.97 (0.19–4.86)	0.87 (0.61–1.22)
Azathioprine	3680	49	198			3599	49	1074		
No				0.78 (0.15–4.04)	**1.50 (1.03–2.20)**				0.61 (0.28–1.35)	1.02 (0.88–1.20)
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				3.10 (0.87–11.1)	1.11 (0.74–1.66)				1.31 (0.64–2.67)	0.95 (0.81–1.11)
Mean dose# (mg/kg/day)										
Cyclosporine	3260	48	196	1.04 (0.86–1.24)	1.00 (0.95–1.05)	2939	48	995	1.07 (0.92–1.24)	**1.10 (1.07–1.12)**
Tacrolimus*	2954	41	112	NA	NA	2718	41	781	NA	0.26 (0.04–1.81)
Mycophenolate*	2954	41	112	1.02 (0.96–1.08)	1.00 (0.98–1.01)	2718	41	781	0.98 (0.95–1.01)	**0.98 (0.97–0.99)**
Azathioprine	3186	48	196	**2.30 (1.04–5.10)**	1.01 (0.79–1.28)	2939	48	995	**1.83 (1.08–3.09)**	**1.16 (1.03–1.30)**
Categorised mean dose¶										
Cyclosporine										
No	744	4	56	1.44 (0.06–36.6)	**9.16 (5.28–15.9)**	638	3	111	0.66 (0.14–3.19)	**4.56 (3.18–6.52)**
Low (≤median)	1118	3	65	Ref	Ref	1140	23	326	Ref	Ref
High	1160	9	78	2.87 (0.69–12.0)	1.11 (0.78–1.59)	1161	23	558	1.62 (0.86–3.04)	**2.15 (1.86–2.49)**
Tacrolimus*										
No	1192	4	82	NA	**2.61 (1.19–5.75)**	1859	34	646	1.67 (0.43–6.52)	**2.24 (1.59–3.16)**
Low (≤median)	332	3	24	NA	Ref	426	3	66	Ref	Ref
High (>median)	311	1	6	NA	**0.27 (0.11–0.67)**	433	4	69	1.39 (0.30–6.47)	1.14 (0.81–1.61)
Mycophenolate*										
No	1336	6	77	NA	0.68 (0.39–1.18)	1875	34	652	2.61 (0.78–8.74)	**2.53 (1.94–3.29)**
Low (≤median)	250	2	22	NA	Ref	421	3	67	Ref	Ref
High (>median)	233	0	13	NA	0.65 (0.32–1.34)	422	4	62	1.90 (0.41–8.79)	1.14 (0.80–1.62)
Azathioprine										
No	1007	3	78	1.63 (0.18–14.8)	**1.55 (1.03–2.33)**	933	5	189	0.70 (0.23–2.16)	**1.58 (1.29–1.93)**
Low (≤median)	980	2	57	Ref	Ref	998	14	365	Ref	Ref
High (>median)	946	11	61	**7.41 (1.51–36.4)**	1.03 (0.69–1.52)	1008	29	441	1.92 (0.99–3.71)	**1.22 (1.06–1.41)**

‡ Complete model: Immunosuppressive agent of interest adjusted for age at transplantation, sex, transplant year, transplanted organ, muromonab-CD3 induction, and other immunosuppressive agents in respective category (i.e. current receipt, current dose, categorised current dose, mean dose, categorised mean dose). For time-dependent variables (i.e. current receipt, current dose, categorised current dose), immunosuppressive agent from

the same class (CNI: cyclosporine, tacrolimus; antiproliferative: mycophenolate, azathioprine), not given simultaneously, not adjusted for. For variables other than immunosuppressive agents, the results adjusted for the mean dose of immunosuppressive agents are presented.
* Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents.
# The mean dose of each agent was calculated as the weighted sum of dosages (mg/kg) at each follow-up time point (weighted by the length of each follow-up time interval). The median dose for cyclosporine was 3.29 (IQR 2.41-4.54) mg/kg/day, for tacrolimus 0.07 (IQR 0.04-0.11), for mycophenolate 27.9 (IQR 20.8-35.4), and for azathioprine 1.14 (IQR 0.82-1.53).
¶ The dose of individual agents was categorised as low or high relative to the median value of dose.

Supplementary Table 6 - 3. Basic cause-specific and subdistribution models for risk of early NHL (n = 29) and death within the first year (n = 324), other putative risk factors that were not included in the complete models

Risk factor	N	NHL	Death	Cause-specific model		Subdistribution model	
				NHL	Death	NHL	Death
				HR (95%CI)	HR (95%CI)	SHR (95%CI)	SHR (95%CI)
Donor sex (first transplantation)							
Female	1507	10	116	Ref	Ref	Ref	Ref
Male	2586	19	201	0.99 (0.43–2.27)	0.89 (0.70–1.13)	0.99 (0.43–2.26)	0.89 (0.70–1.14)
Blood type							
A	1793	14	147	Ref	Ref	Ref	Ref
AB	189	2	17	1.36 (0.30–6.15)	1.12 (0.67–1.86)	1.38 (0.31–6.03)	1.11 (0.67–1.85)
B	473	5	37	1.60 (0.56–4.61)	1.01 (0.70–1.45)	1.60 (0.55–4.66)	1.00 (0.70–1.44)
O	1669	7	121	0.53 (0.21–1.33)	0.88 (0.69–1.13)	0.53 (0.21–1.30)	0.89 (0.70–1.13)
Smoking history							
Never	1317	11	82	Ref	Ref	Ref	Ref
Ever	1972	13	150	0.71 (0.28–1.77)	1.16 (0.86–1.56)	0.72 (0.27–1.95)	1.16 (0.86–1.55)
Diabetes at first transplantation							
No	2733	18	146	Ref	Ref	Ref	Ref
Yes	495	2	43	0.62 (0.14–2.79)	**1.54 (1.08–2.19)**	0.59 (0.12–2.90)	**1.54 (1.09–2.19)**
Dialysis history							
Never	2952	18	158	Ref	Ref	Ref	Ref
Ever	88	1	11	2.62 (0.31–22.0)	**2.51 (1.34–4.72)**	2.48 (0.30–20.3)	**2.46 (1.34–4.52)**
Autoimmune disease history							
No	3871	26	260	Ref	Ref	Ref	Ref
Yes	260	3	18	2.27 (0.61–8.40)	0.93 (0.57–1.53)	2.30 (0.65–8.05)	0.93 (0.56–1.52)
Cardiovascular disease (first transplantation)							
No	1784	14	85	Ref	Ref	Ref	Ref
Yes	546	4	47	0.74 (0.15–3.61)	1.75 (1.12–2.74)	0.71 (0.09–5.53)	1.75 (1.14–2.67)
Hypertension (first transplantation)							
No	1574	13	78	Ref	Ref	Ref	Ref
Yes	788	3	66	1.05 (0.32–3.42)	**1.97 (1.34–2.90)**	1.05 (0.31–3.58)	**1.97 (1.36–2.85)**
Recipient CMV IgG (first transplantation)							
Positive	2819	19	220	Ref	Ref	Ref	Ref
Negative	1211	9	92	1.13 (0.44–2.39)	0.98 (0.76–1.27)	1.02 (0.44–2.34)	0.98 (0.77–1.26)
Donor CMV IgG (first transplantation)							
Negative	1461	9	106	Ref	Ref	Ref	Ref
Positive	2573	20	205	1.33 (0.60–2.94)	1.11 (0.88–1.41)	1.34 (0.60–2.99)	1.11 (0.88–1.40)
CMV concordance (first transplantation)							
Recipient -/Donor -	485	3	37	Ref	Ref	Ref	Ref
Recipient -/Donor +	700	6	49	1.75 (0.43–7.09)	0.97 (0.63–1.49)	1.76 (0.44–7.04)	0.97 (0.63–1.49)
Recipient +/(Donor + or Donor –)	2759	19	218	1.36 (0.39–4.74)	1.04 (0.73–1.49)	1.38 (0.42–4.55)	1.04 (0.74–1.47)
Immunosuppressive therapy history							
Never	3727	26	297	Ref	Ref	Ref	Ref
Ever	404	3	27	0.69 (0.20–2.35)	0.73 (0.49–1.10)	0.73 (0.21–2.48)	0.74 (0.49–1.11)

Supplementary Table 6 - 3. (continued) Basic cause-specific and subdistribution models for risk of late NHL (n = 61) and death (n = 1405), other putative risk factors that were not included in the complete models

Risk factor	N	NHL	Death	Cause-specific models		Subdistribution models	
				NHL	Death	NHL	Death
				HR (95%CI)	HR (95%CI)	SHR (95%CI)	SHR (95%CI)
Transplanted organ sub-type							
Liver	1609	17	353	Ref	Ref	Ref	Ref
Heart	1337	35	489	**2.58 (1.40–4.77)**	1.15 (0.99–1.33)	**2.44 (1.32–4.53)**	1.13 (0.97–1.31)
Bilateral lung	689	3	274	0.68 (0.19–2.40)	**3.34 (2.82–3.97)**	0.42 (0.12–1.52)	**3.34 (2.82–3.96)**
Single lung	264	3	174	1.38 (0.40–4.76)	**3.70 (3.08–4.57)**	0.86 (0.24–2.99)	**3.66 (3.05–4.38)**
Recipient BMI at transplantation	3318	54	1129	1.00 (0.97–1.03)	1.00 (0.98–1.01)	1.00 (0.98–1.02)	1.00 (0.98–1.02)
Rejection ever							
Never	2803	41	892	Ref	Ref	Ref	Ref
Ever	1299	20	513	0.96 (0.55–1.67)	1.10 (0.98–1.23)	0.92 (0.53–1.58)	1.10 (0.98–1.23)
Donor sex (first transplantation)							
Female	1497	23	479	Ref	Ref	Ref	Ref
Male	2567	37	900	0.86 (0.49–1.50)	0.97 (0.86–1.09)	0.88 (0.52–1.48)	0.97 (0.86–1.09)
Alcohol drinking history							
Never	573	12	166	Ref	Ref	Ref	Ref
Ever	2387	36	781	0.74 (0.37–1.51)	1.07 (0.90–1.28)	0.72 (0.36–1.45)	1.08 (0.90–1.29)
Autoimmune disease history							
No	3845	57	1339	Ref	Ref	Ref	Ref
Yes	257	4	66	1.44 (0.49–4.24)	0.93 (0.72–1.20)	1.46 (0.52–4.14)	0.93 (0.71–1.21)
Recipient CMV IgG (first transplantation)							
Positive	2800	40	963	Ref	Ref	Ref	Ref
Negative	1202	21	396	1.28 (0.74–2.21)	0.93 (0.83–1.05)	1.29 (0.77–2.17)	0.93 (0.83–1.05)
Donor CMV IgG							
Negative	1452	17	480	Ref	Ref	Ref	Ref
Positive	2553	44	874	1.59 (0.90–2.78)	1.09 (0.98–1.22)	1.54 (0.88–2.71)	1.08 (0.97–1.21)
Immunosuppressive therapy before transplantation							
No	3701	55	1249	Ref	Ref	Ref	Ref
Yes	401	6	156	1.24 (0.52–2.95)	0.93 (0.78–1.10)	1.22 (0.51–2.91)	0.93 (0.78–1.10)
CMV antiviral therapy							
No	1892	27	692	Ref	Ref	Ref	Ref
Yes	2210	34	713	1.21 (0.72–2.02)	0.93 (0.84–1.04)	1.22 (0.72–2.07)	0.93 (0.84–1.04)

Supplementary Table 6 - 4. Basic subdistribution models of immunosuppressive agent for risk of early NHL (n = 29), late NHL (n = 61) and death (n = 1405) based on the original data

Immunosuppressive agent	Early NHL‡					Late NHL‡				
	N	NHL	Death	NHL	Death	N	NHL	Death	NHL	Death
				HR (95%CI)	HR (95%CI)				HR (95%CI)	HR (95%CI)
Current receipt										
Cyclosporine	3726	25	198			3713	45	1035		
No				Ref	Ref				Ref	Ref
Yes				**0.28 (0.09–0.88)**	0.76 (0.51–1.12)				0.77 (0.34–1.73)	**0.74 (0.62–0.89)**
Tacrolimus*	2373	12	107			3413	38	820		
No				Ref	Ref				Ref	Ref
Yes				**7.21 (1.28–40.8)**	0.91 (0.55–1.52)				0.90 (0.33–2.41)	1.18 (0.95–1.46)
Mycophenolate*	2375	12	107			3414	38	820		
No				Ref	Ref				Ref	Ref
Yes				**6.43 (2.09–19.8)**	1.64 (0.99–2.71)				0.57 (0.17–1.88)	1.01 (0.82–1.25)
Azathioprine	3705	25	196			3714	45	1033		
No				Ref	Ref				Ref	Ref
Yes				0.82 (0.35–1.94)	**0.58 (0.42–0.78)**				**2.17 (1.02–4.61)**	**0.78 (0.67–0.90)**
Number of drugs	3702	25	196			3713	45	1033		
1				Ref	Ref				Ref	Ref
>1				1.57 (0.46–5.38)	**0.49 (0.36–0.68)**				1.56 (0.66–3.69)	**0.60 (0.50-0.71)**
Current dose (mg/kg/day)										
Cyclosporine	3488	23	163	0.86 (0.73–1.01)	0.95 (0.89–1.01)	3553	35	767	0.83 (0.68–1.01)	**0.92 (0.87–0.96)**
Tacrolimus*	2191	11	101	NA	0.38 (0.004–31.7)	3305	37	761	NA	0.18 (0.01–2.34)
Mycophenolate*	2286	11	104	**1.04 (1.00–1.09)**	1.01 (0.99–1.02)	3341	37	794	0.98 (0.94–1.02)	1.00 (0.99–1.01)
Azathioprine	3539	21	166	0.92 (0.60–1.41)	**0.74 (0.61–0.90)**	3617	37	829	1.27 (0.86–1.89)	**0.81 (0.72–0.91**
Categorised current dose¶										
Cyclosporine	3488	23	163			3553	35	767		
No				3.07 (0.81–11.6)	1.07 (0.68–1.68)				1.30 (0.55–3.07)	**1.37 (1.11–1.69)**
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				1.03 (0.37–2.89)	**0.61 (0.41–0.92)**				0.66 (0.27–1.61)	**0.68 (0.57–0.82)**
Tacrolimus*	2191	11	101			3305	37	761		
No				**0.09 (0.01–0.62)**	0.58 (0.33–1.03)				1.16 (0.26–5.09)	**0.70 (0.52–0.93)**
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				0.52 (0.16–1.72)	**0.32 (0.13–0.81)**				1.35 (0.22–8.22)	**0.47 (0.30–0.73)**
Mycophenolate*	2286	11	104			3341	37	794		
No				0.53 (0.07–4.28)	**0.34 (0.20–0.60)**				2.72 (0.37–19.8)	0.83 (0.63–1.09)
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				13.3 (1.28–137)	**0.27 (0.11–0.69)**				1.90 (0.18–20.6)	0.69 (0.46–1.04)
Azathioprine	3539	21	166			3617	37	829		
No				1.48 (0.54–4.04)	**2.01 (1.34–3.00)**				0.44 (0.19–1.04)	**1.36 (1.13–1.63)**
Low (≤median)				Ref	Ref				Ref	Ref
High (>median)				1.63 (0.54–4.96)	1.22 (0.78–1.90)				0.92 (0.19–2.06)	0.86 (0.71–1.04)

‡ Basic model: Immunosuppressive agent or other variable of interest, adjusted for age at transplantation, sex, transplant year and transplanted organ (if not the variable of interest).

* Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents.
# The mean dose of each agent was calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point (weighted by the length of each follow-up time interval). ¶ The dose of individual agents was categorised as low or high relative to the median value of dose.

Supplementary Table 6 - 4 (continued). Basic subdistribution models of immunosuppressive agent for risk of early NHL (n = 29), late NHL (n = 61) and death (n = 1405) based on the original data

Immunosuppressive agent	Early NHL‡					Late NHL‡				
	N	NHL	Death	NHL	Death	N	NHL	Death	NHL	Death
				HR (95%CI)	HR (95%CI)				HR (95%CI)	HR (95%CI)
Mean dose# (mg/kg/day)										
Cyclosporine	2173	15	126	1.07 (0.91–1.27)	0.97 (0.89–1.06)	1883	26	491	1.09 (0.97–1.23)	**1.05 (1.01–1.09)**
Tacrolimus*	1346	8	81	NA	0.09 (0.005–17.7)	2089	32	604	NA	0.21 (0.01–4.00)
Mycophenolate*	1561	8	85	1.00 (0.94–1.07)	1.02 (1.00–1.04)	2303	32	621	0.96 (0.90–1.02)	**0.98 (0.97–0.99)**
Azathioprine	2126	14	127	1.89 (0.93–3.86)	1.01 (0.77–1.34)	1867	26	496	**2.12 (1.48–3.02)**	**1.29 (1.00–1.51)**
Categorised mean dose¶										
Cyclosporine										
No	620	2	41	1.08 (0.12–9.62)	1.82 (0.89–3.72)	601	1	74	0.21 (0.03–1.68)	**1.76 (1.21–2.57)**
Low (≤median)	777	4	42	Ref	Ref	641	14	186	Ref	Ref
High	776	9	43	2.04 (0.44–9.39)	1.00 (0.64–1.57)	641	11	231	0.93 (0.38–2.32)	**1.41 (1.16–1.72)**
Tacrolimus*										
No	920	4	60	**0.10 (0.01–0.89)**	0.96 (0.49–1.92)	1578	28	529	1.24 (0.30–5.16)	**1.07 (0.77–1.48)**
Low (≤median)	216	4	15	Ref	Ref	257	2	45	Ref	Ref
High (>median)	210	0	6	NA	0.44 (0.17–1.14)	254	2	30	1.32 (0.20–8.75)	0.78 (0.48–1.29)
Mycophenolate*										
No	1259	6	59	0.50 (0.09–2.54)	**0.29 (0.16–0.54)**	1782	30	545	NA	**1.94 (1.43–2.64)**
Low (≤median)	152	2	19	Ref	Ref	267	0	44	Ref	Ref
High (>median)	150	0	7	NA	**0.39 (0.15–0.96)**	254	2	32	NA	1.04 (0.65–1.66)
Azathioprine										
No	516	2	36	1.69 (0.19–14.8)	**1.97 (1.23–3.16)**	493	0	78	NA	**1.71 (1.26–2.32)**
Low (≤median)	814	2	35	Ref	Ref	687	9	177	Ref	Ref
High (>median)	796	10	56	**4.46 (1.11–17.8)**	**1.82 (1.19–2.78)**	687	17	241	1.90 (0.88–4.16)	**1.61 (1.33–1.95)**

‡ Basic model: Immunosuppressive agent or other variable of interest, adjusted for age at transplantation, sex, transplant year and transplanted organ (if not the variable of interest).
* Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents.
# The mean dose of each agent was calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point (weighted by the length of each follow-up time interval).
¶ The dose of individual agents was categorised as low or high relative to the median value of dose.

Chapter 7 Role of immunosuppression in risk of lip cancer in Australian adult liver, heart and lung transplant recipients

7.1 Objectives

In this Chapter I examine the role of immunosuppressive therapy in the risk of lip cancer in a population-based cohort of liver and cardiothoracic transplant recipients in Australia between 1984 and 2006. Specifically the objective of this Chapter is to explore the association between the type, dose and duration of immunosuppressive therapy and risk of lip cancer after transplantation.

7.2 Introduction

Lip cancer is one of the most common cancers in solid organ transplant recipients, occurring at a relative risk of 20 to 80 compared to the general population in population-based studies (Grulich et al. 2007, Engels et al. 2011, Krynitz et al. 2013), and in my cohort. Another immune deficient population, individuals with HIV/AIDS, experience a 2.8-fold increased risk of lip cancer (Grulich et al. 2007). This approximately 10-fold difference in risk indicates that iatrogenic immunosuppressive therapy may contribute both directly and indirectly, via immunosuppression, to the development of lip cancer. While the current use of cyclosporine or azathioprine has been shown to increase lip cancer risk in Australian kidney transplant recipients (van Leeuwen et al. 2009), there is no published evidence of a dose-related association between an individual immunosuppressive agent and lip cancer risk. On the other

hand, there is evidence of a relationship between the currency, extent, and duration of immunosuppression and lip cancer risk in transplant recipients. Strikingly, in kidney transplant recipients whose grafts fail and immunosuppressive therapy is ceased, lip cancer risk is fully reversed (van Leeuwen et al. 2010). Lip cancer risk also increases with increasing duration of immunosuppressive therapy in kidney transplant recipients (van Leeuwen et al. 2009). In addition, lip cancer is more common in heart and lung than liver transplant recipients (Chapter 3), a pattern that parallels the relative dose of immunosuppression by organ type as shown in Chapter 5.

In both the general and transplant population, almost 90% of lip cancers are squamous cell carcinomas (SCCs) and localized on the vermillion or external lip (King et al. 1995, de Visscher et al. 1997, de Visscher et al. 1998, Perea-Milla Lopez et al. 2003, van Leeuwen et al. 2009, Czerninski et al. 2010, Ariyawardana and Johnson 2013). Established risk factors in the general population are older age, male sex, and fair skin type (de Visscher et al. 1998, de Visscher and van der Waal 1998, Perea-Milla Lopez et al. 2003, Czerninski et al. 2010). Solar ultraviolet radiation (UVR) is classified by the International Agency for Research on Cancer (IARC) as having limited evidence of carcinogenicity for lip cancer (IARC 2012). The role of other factors such as smoking, alcohol consumption (Secretan et al. 2009), and human papillomavirus (HPV) infection (de Visscher and van der Waal 1998) in lip cancer aetiology is uncertain. In kidney transplant recipients increased lip cancer risk is associated with older age, male sex, fair skin, smoking, and possibly greater ambient UVR exposure (King et al. 1995, van Leeuwen et al. 2009, López-Pintor et al. 2011).

7.3 Materials and methods

7.3.1 Study population

I performed a retrospective population-based cohort study of all Australian liver (n = 1926, 41%), heart (n = 1518, 33%), and lung (n = 1200, 26%) transplant recipients, 1984-2006. The transplant registries, ANZLTR and ANZCOTR, were described in full in Chapter 3. I excluded recipients (n = 89, 2%) with no retrievable transplantation medical records (liver n = 2, heart = 87). I also excluded two recipients with a diagnosis of lip cancer prior to transplantation; I retained 390 patients with history of another cancer (liver n = 322, heart n = 35, lung n = 33) as they were at risk of lip cancer.

I obtained ethical approval and the requirement for informed participant consent was waived because I received de-identified data.

7.3.2 Data collection

I ascertained incident lip cancer diagnoses by record linkage between the transplant registers and the ACD, a register of incident primary invasive neoplasms, between 1 January 1984 and 31 December 2006. I used registrant's name, sex, date of birth, date of death, and state of residence to link records using an established probabilistic algorithm (Jaro 1995). I identified lip cancer diagnoses on the basis of International Classification of Diseases for Oncology codes (ICD10 C000-C009) and I excluded cancers arising in the cutaneous part of the lip (ICD10 C430) and melanomas. I categorised the neoplasm location as upper vermillion (C000), lower vermillion (C001), inner lip and commissures (C003-C006), or unspecified (C009). I identified deaths from

the transplant registers or by record linkage with the National Death Index, a registry of all deaths in Australia since 1980.

The demographic characteristics of recipients (age, sex, race) and donors (age, sex) and some clinical information (date of transplantation, organ type, primary indication, subsequent transplantation) were systematically and prospectively collected by the transplant registers. I retrospectively collected data on recipient demographic and lifestyle characteristics, comorbidities, immunosuppressive agents and antiviral prophylaxis from transplantation unit medical records. I collected recipient country of birth, smoking and alcohol consumption, weight, height, blood type, history of cancer, diabetes mellitus, cardiovascular disease, hypertension, autoimmune disease, and dialysis prior to transplantation. I abstracted CMV IgG serostatus for recipients and donors at transplantation, recipient EBV IgG serostatus at transplantation and during follow-up and recipient infection with HBV and HCV at transplantation. I also collected the use of prophylactic anti-CMV immunoglobulin and antiviral agents at transplantation.

I collected information on receipt of T-cell-depleting antibody (ATG/ALG, muromonab-CD3) and IL-2Ra (basiliximab, daclizumab) at induction or rejection. I recorded immunosuppressive agents at transplantation, and 3 months, 6 months, and 1, 5, 10, 15 and 20 years after transplantation, including the use and dosage of cyclosporine, tacrolimus, azathioprine, mycophenolate, sirolimus and everolimus.

7.3.3 Data management

My management of the immunosuppressive therapy data is described in detail in Chapter 5. Briefly, I imputed missing recipient weight values, standardised the dose of individual agents to mg/kg/day, and imputed missing data on the type and dose of individual immunosuppressive agents, for a maximum of one consecutive follow-up time point. As the date of antibody rejection therapy was not available, I used antibody induction therapy (yes/no) and antibody induction or rejection therapy (ever/never) in the analysis, assuming acute rejection preceded lip cancer.

7.3.4 Data analysis

Person-years of follow-up accrued from the date of transplantation until the date of lip cancer diagnosis, age 80, death, or 31 December 2006, whichever occurred first. As no paediatric recipients (n = 412) developed lip cancer, I restricted the analysis to adults only (n = 4141). I compared characteristics of recipients with and without lip cancer and with low versus high initial immunosuppressive dose using Student t-tests, Wilcoxon rank-sum tests, Pearson chi-square tests, or Fisher exact tests as appropriate.

I examined three measures of immunosuppression. I first tested a time-dependent binary variable for the current receipt of each immunosuppressive agent, irrespective of dose. Second, I tested a time-dependent continuous variable for the current daily dose of each immunosuppressive agent. Third, to capture the overall dose of immunosuppressive therapy received, I modelled the mean dose (mg/kg/day) of each immunosuppressive agent during follow-up, calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point, weighted by the length of each

follow-up time interval relative to the total duration of use. I dichotomized the current and mean dose of each agent as low or high relative to the median value.

I restricted analyses for tacrolimus and mycophenolate to the years from 1997 when these agents were used by my cohort. As sirolimus and everolimus were not used until 2002 and 2005 respectively, I was unable to model them due to low usage and limited follow-up time. I did not examine corticosteroid use as a potential risk factor for lip cancer because it is not considered carcinogenic (IARC 1987).

I applied the Fine and Gray (Fine and Gray 1999) proportional subdistribution hazard model to estimate hazard ratios (HRs) of lip cancer, accounting for death as a competing risk. I used age as the underlying time-scale in the modelling, and evaluated time since transplantation as a potential risk factor. I used two models to examine the three measures of immunosuppression (current receipt, current dose, and mean dose of each agent). In the basic model, I adjusted each measure of immunosuppressive agent or other risk factor of interest for age at transplantation, sex, transplant year and organ type. In the complete model, I further adjusted each immunosuppressive agent for the other immunosuppressive agents (except those within the same class and thus not given simultaneously when considering the time-dependent measures current receipt and current dose), and additional confounding factors (i.e. factors that were associated with immunosuppressive agents and lip cancer and/or death after adjustment for the variables in the basic model). I also applied cause-specific hazard models to estimate HRs of lip cancer to allow comparison with prior risk estimates. I analysed the risk factors for lip cancer including all follow-up time (maximum 23 years)

and censoring the follow-up at 10 years, after which the population at risk of lip cancer reduced significantly (Figure 7-1, page 214).

I carried out analyses based on both original and imputed data and did not observe notable differences (Supplementary Table 7 - 1, page 237). The results presented are based on imputed data.

I performed analyses using Stata statistical software v13.0 (StataCorp, Texas, USA).

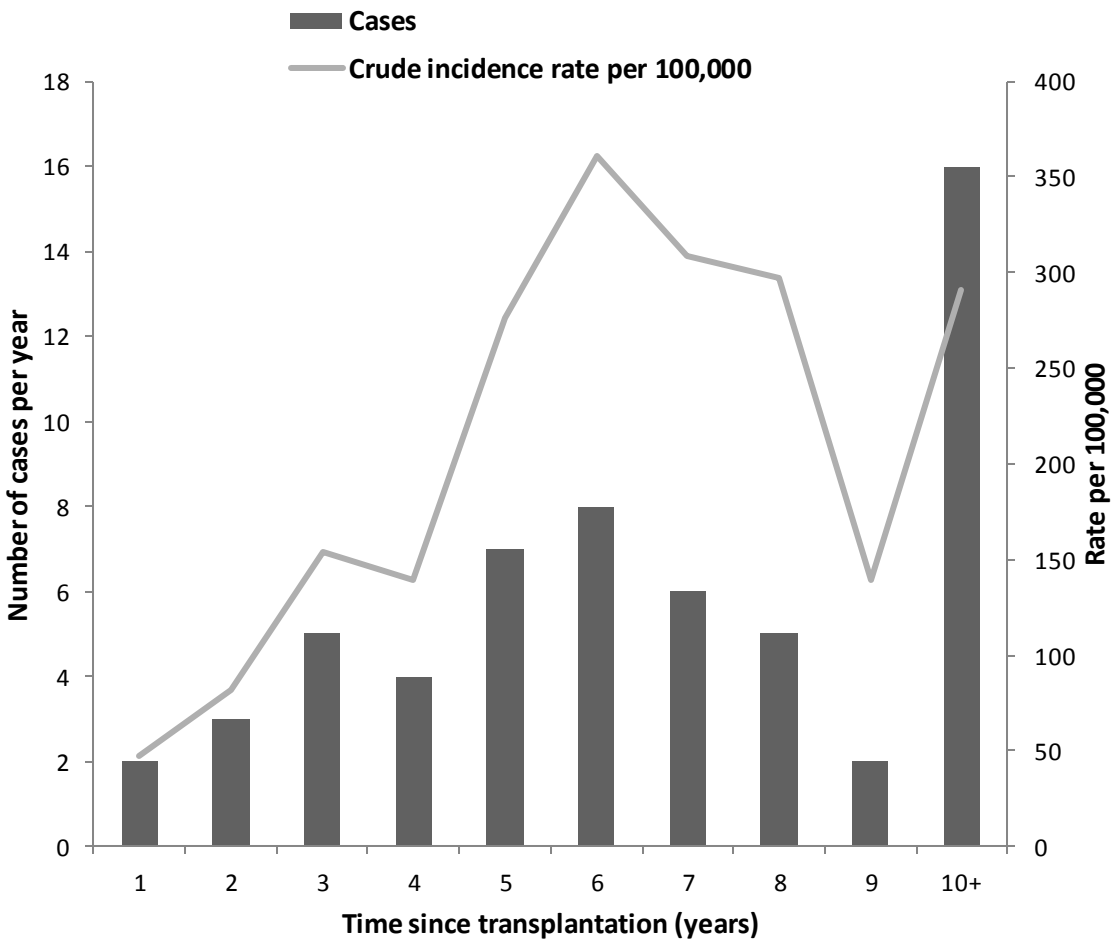


Figure 7-1. Crude lip cancer incidence rates (per 100,000) and number of lip cancer cases per year by time since transplantation in Australian liver, heart and lung transplant recipients, 1984-2006

7.4 Results

7.4.1 Cohort characteristics

My cohort comprised 4141 transplant recipients, 1620 (39%) liver, 1344 (33%) heart and 1177 (28%) lung. The median age at transplantation was 49 years (interquartile range, IQR 39-55) (Table 7 - 1, page 216). I observed 58 cases of lip cancer and 1434 deaths during 29353 person-years of follow-up; the median follow-up time was 5.28 (IQR 2.12-9.91) years. All except one case was SCC. The median age at lip cancer diagnosis was 57 years (IQR 50-61). Forty-three (74%) lip cancers were located on the lower vermillion, 7 (12%) on the upper vermillion, 3 (5%) on the inner lip and commissures, and for 5 the location was unspecified (9%). The crude incidence of lip cancer increased by time since transplantation up to 5 years post-transplantation (Figure 7-1, page 214).

Compared to those without lip cancer, recipients who developed lip cancer were more likely to be Caucasian, transplanted during the earlier era (1984-1994), receive a heart transplant, have a history of smoking, and receive cyclosporine and azathioprine at transplantation (Table 7 - 1, page 216). I found no differences between these patient groups in drug dose at transplantation (Table 7 - 2, page 218). Younger recipients received a higher dose of one or more agents, as did females, those transplanted in the earliest era, lung transplant recipients, and non-smokers (Table 7 - 2, page 218).

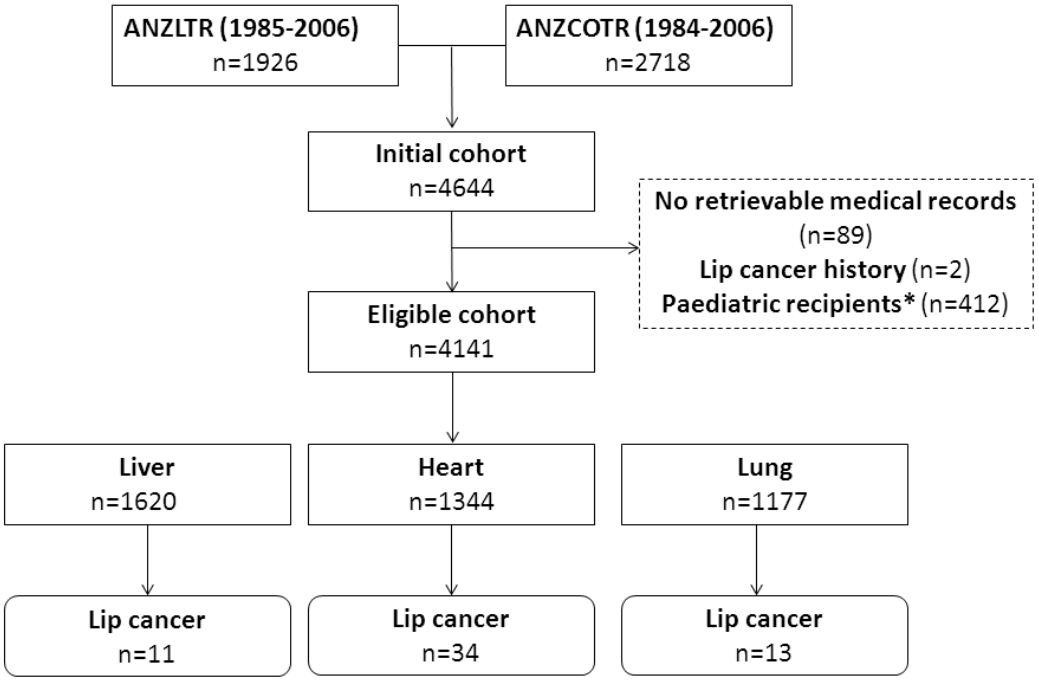


Figure 7 - 2. Flowchart of the cohort and outcome by organ type

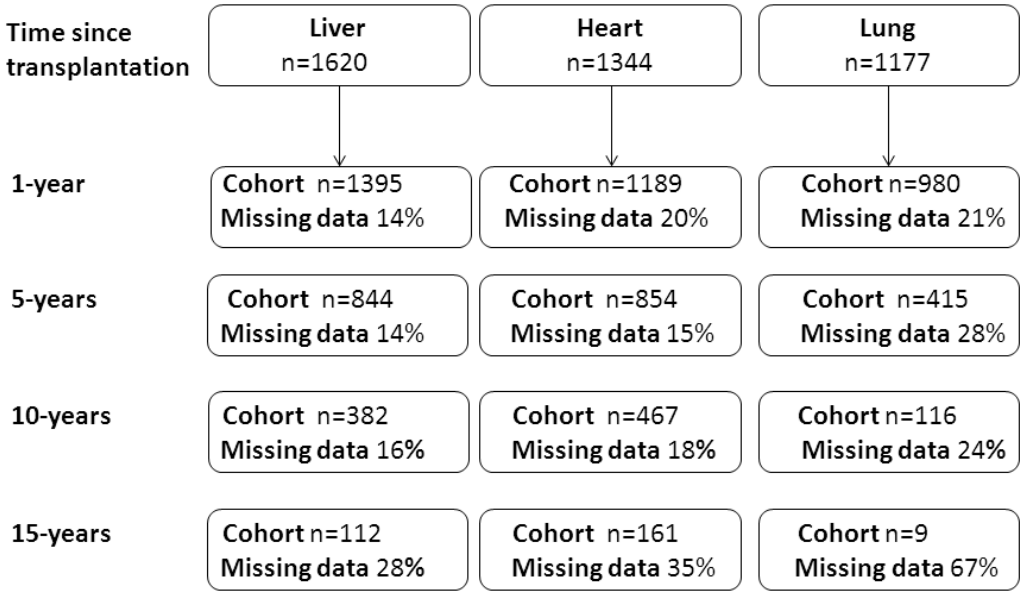


Figure 7 - 3. Flowchart of the cohort and extent of missing immunosuppression data by organ type

Table 7 - 1. Baseline characteristics of adult liver, heart and lung transplant recipients with and without lip cancer, 1984-2006

Characteristic§	All†	Lip cancer†	No lip cancer†	*P*‡
	(*n* 4141)	(*n* 58)	(*n* 4083)	
Recipient age	49 (39-55)	49 (44-53)	49 (39-55)	0.44
Sex				0.09
Male	2779 (67.1)	45 (77.6)	2734 (67.0)	
Female	1362 (32.9)	13 (22.4)	1349 (33.0)	
Transplant era				<0.001
1984-1994	1283 (31.0)	49 (84.5)	1234 (30.2)	
1995-1997	704 (17.0)	6 (10.3)	698 (17.1)	
1998-2006	2154 (52.0)	3 (5.20)	2151 (52.7)	
Recipient race				0.02
Caucasian	3658 (90.6)	54 (100)	3604 (90.5)	
Non-Caucasian	378 (9.40)	0	378 (9.50)	
Transplanted organ				<0.001
Liver	1620 (39.1)	11 (19.0)	1609 (39.4)	
Heart	1344 (32.5)	34 (58.6)	1310 (32.1)	
Lung	1177 (28.4)	13 (22.4)	1164 (28.5)	
Smoking history				0.003
Ever	1979 (60.0)	38 (80.9)	1941 (59.7)	
Never	1319 (40.0)	9 (19.1)	1310 (40.3)	
Immunosuppressive agent				
Cyclosporine				<0.001
Yes	2784 (74.4)	49 (96.1)	2735 (74.1)	
No	959 (25.6)	2 (3.90)	957 (25.9)	
Tacrolimus*				0.60
Yes	847 (37.6)	1 (25.0)	846 (37.6)	
No	1405 (62.4)	3 (75.0)	1402 (62.4)	
Mycophenolate*				0.23
Yes	599 (26.6)	0	599 (26.6)	
No	1654 (73.4)	4 (100)	1650 (73.4)	
Azathioprine				<0.001
Yes	2322 (62.6)	44 (86.3)	2278 (62.3)	
No	1387 (37.4)	7 (13.7)	1380 (37.7)	
Dose (mg/kg/day)				
Cyclosporine	4.89 (3.46-6.70)	5.15 (3.50-6.89)	4.89 (3.46-6.68)	0.95
Tacrolimus*	0.11 (0.07-0.15)	0.02	0.11 (0.07-0.15)	NA
Mycophenolate*	33.7 (25.6-41.1)	0	33.7 (25.6-41.1)	NA
Azathioprine	1.37 (0.96-1.83)	1.61 (1.03-1.89)	1.37 (0.96-1.83)	0.11

NA, not available due to insufficient number ; § Continuous variables presented as median (interquartile range) and categorical variables presented as n (%); † The counts in sub-categories may not add up to the total number due to missing data; ‡ Non-parametric Wilcoxon rank-sum test, chi-square test or Fisher exact test as appropriate; * Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents.

Table 7 - 2. Baseline characteristics of adult transplant recipients who received low and high dose immunosuppressive agents at transplantation

Varible†‡	Cyclosporine			Tacrolimus*			Mycophenolate*			Azathioprine		
	Low	High	*P* §	Low	High	*P* §	Low	High	*P* §	Low	High	*P* §
N	1343	1342		333	332		278	277		1100	1082	
Recipient age			<0.001			0.002			0.10			<0.001
Median	51	46		50	48		50	48		49	48	
IQR	43-56	33-53		43-57	37-55		40-56	31-56		41-55	35-54	
Sex			<0.001			<0.001			0.008			0.63
Male	971 (72.3)	848 (63.2)		244 (73.3)	189 (56.9)		209 (75.2)	177 (63.9)		733 (66.7)	711 (65.7)	
Female	372 (27.7)	494 (36.8)		89 (26.7)	143 (43.1)		69 (24.8)	100 (36.1)		367 (33.3)	371 (34.3)	
Transplant era			<0.001			0.14			0.34			<0.001
1984-1994	400 (29.8)	643 (47.9)		0	0		0	0		392 (35.6)	487 (45.0)	
1995-1997	279 (20.8)	286 (21.3)		15 (4.50)	24 (7.20)		7 (2.50)	3 (1.10)		196 (17.8)	304 (28.1)	
1998-2006	664 (49.4)	413 (30.8)		318 (95.5)	308 (92.8)		271 (97.5)	274 (98.9)		512 (46.6)	291 (26.9)	
Recipient race			0.28			0.46			0.32			0.04
Caucasian	1224 (91.5)	1228 (92.6)		278 (85.0)	288 (87.0)		251 (90.3)	256 (92.4)		983 (90.2)	993 (92.7)	
Noncaucasian	113 (8.50)	99 (7.40)		49 (15.0)	43 (13.0)		27 (9.70)	21 (7.60)		107 (9.80)	78 (7.30)	
First transplanted organ			<0.001			0.001			<0.001			<0.001
Liver	321 (23.9)	431 (32.1)		268 (80.5)	242 (72.9)		88 (31.7)	11 (4.00)		579 (52.6)	198 (18.3)	
Heart	654 (48.7)	402 (30.0)		22 (6.60)	13 (3.90)		105 (37.8)	157 (56.7)		208 (18.9)	489 (45.2)	
Lung	368 (27.4)	509 (37.9)		43 (12.9)	77 (23.2)		85 (30.5)	109 (39.1)		313 (28.5)	395 (36.6)	
Smoking history			<0.001			0.27			0.09			0.58
Ever	856 (68.3)	663 (55.1)		155 (58.9)	164 (54.3)		166 (65.1)	150 (57.9)		594 (61.5)	613 (60.3)	
Never	397 (31.7)	541 (44.9)		108 (41.1)	138 (45.7)		89 (34.9)	109 (42.1)		372 (38.5)	404 (39.7)	
Immunosuppressive agent												
Mycophenolate*			0.66			0.006						
Low	108 (45.8)	52 (43.3)		57 (71.2)	40 (50.0)							
High	128 (54.2)	68 (56.7)		23 (28.8)	40 (40.0)							
Azathioprine			0.99			0.001						
Low	390 (48.0)	471 (48.0)		120 (77.4)	96 (60.4)							
High	423 (52.0)	511 (52.0)		35 (22.6)	63 (39.6)							

† Continuous variables presented as median (interquartile range) and categorical variables presented as n (%); ‡ The counts in sub-categories may not add up to the total number due to missing data; § Non-parametric Wilcoxon rank-sum test, chi-square test or Fisher exact test as appropriate; * Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents

7.4.2 Risk factors for lip cancer

In the basic models, I showed increased risk of lip cancer was associated with lung transplantation, older age at transplantation, earlier transplant era, increasing time since transplantation (> 2 years), CMV antiviral therapy, blood types B and O, antibody induction therapy, and higher current and mean dose of azathioprine (Table 7 - 3, page 220).

In the complete model, I found that risk of lip cancer was associated with older age at transplantation (HR 1.14, 95% CI 1.04-1.25 per year of age), earlier transplant era (HR 8.73, 95% CI 1.11-68.7 for 1984-1994 vs 1998-2006), greater time since transplantation (HR 9.86, 95% CI 2.10-46.3 for >5 vs <1 years), and a history of smoking (HR 2.71, 95% CI 1.09-6.70; Table 7 - 3, page 220). The significant positive association with azathioprine was strengthened for both higher current daily dose (HR 1.79, 95% CI 1.21-2.66) and higher mean dose (HR 2.28, 95% CI 1.18-4.38). I observed no significant findings for the dichotomized current or mean dose for any agent (data not shown).

All of the significant findings for risk of lip cancer were retained when the follow-up time was restricted to 10 years post-transplantation (Table 7 - 5, page 224). The cause-specific hazard ratio estimates were comparable with the subdistribution hazard ratio estimates, but age, transplant era and time since transplantation were significantly associated with lip cancer risk only in the subdistribution hazard model (Supplementary Table 7 - 1, page 237).

Table 7 - 3. Risk factors for lip cancer after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model

Risk factor	N	Lip cancer	Death	Basic model §,‡		N	Lip cancer	Death	Complete model†	
				Lip cancer	Death				Lip cancer	Death
				HR (95%CI)	HR (95%CI)				HR (95%CI)	HR (95%CI)
Age at transplantation	4141	58	1434	**1.06 (1.02–1.10)**	**1.05 (1.04–1.07)**	2701	44	911	**1.14 (1.04–1.25)**	1.02 (0.99–1.05)
Sex										
Female	1362	13	457	Ref	Ref	851	7	282	Ref	Ref
Male	2779	45	977	1.26 (0.65–2.44)	**1.16 (1.03–1.30)**	1850	37	629	1.78 (0.76–4.21)	1.12 (0.97–1.31)
Race										
Non-Caucasian	378	0	89	Ref	Ref					
Caucasian	3658	54	1288	NA	0.93 (0.73–1.18)					
Transplant era										
1984-1994	1283	49	690	**11.4 (3.52–36.8)**	**1.38 (1.20–1.58)**	794	37	420	**8.73 (1.11–68.7)**	0.85 (0.70–1.04)
1995-1997	704	6	328	2.98 (0.75–11.9)	**1.29 (1.11–1.49)**	480	6	219	3.55 (0.40–31.6)	0.96 (0.79–1.17)
1998-2006	2154	3	416	Ref	Ref	1427	1	272	Ref	Ref
Time since transplantation (years)										
0-0.99	4141	2	332	Ref	Ref	2701	2	196	Ref	Ref
1-1.99	3564	3	188	2.16 (0.36–13.0)	**0.67 (0.56–0.81)**	2370	2	98	1.41 (0.20–10.1)	**0.58 (0.45–0.73)**
2-4.99	3135	16	345	**6.13 (1.39–27.1)**	**0.57 (0.48–0.67)**	2112	10	244	3.32 (0.74–14.9)	**0.65 (0.52–0.80)**
5+	2113	37	569	**15.2 (3.18–72.5)**	**0.77 (0.61–0.97)**	1402	30	373	**9.86 (2.10–46.3)**	0.76 (0.58–1.00)
First transplanted organ										
Liver	1620	11	362	Ref	Ref	993	7	231	Ref	Ref
Heart	1344	34	497	1.82 (0.93–3.56)	1.15 (0.99–1.33)	983	28	340	1.16 (0.50–2.72)	1.04 (0.85–1.27)
Lung	1177	13	575	**2.43 (1.06–5.52)**	**3.37 (2.93–3.86)**	725	9	340	1.39 (0.45–4.26)	**2.27 (1.87–2.76)**
Smoking history										
Never	1319	9	375	Ref	Ref	1043	6	287	Ref	Ref
Ever	1979	38	777	2.05 (0.96–4.39)	**1.45 (1.27–1.67)**	1658	38	624	**2.71 (1.09–6.70)**	**1.46 (1.25–1.71)**
CMV antiviral therapy										
No	1908	22	709	Ref	Ref					
Yes	2223	36	725	**2.32 (1.31–4.12)**	0.91 (0.82–1.01)					
Recipient blood type										
A	1798	16	635	Ref	Ref					
AB	189	2	66	1.38 (0.32–5.98)	1.03 (0.79–1.34)					
B	474	10	144	**2.47 (1.12–5.44)**	0.91 (0.76–1.09)					
O	1673	30	582	**2.10 (1.14–3.86)**	1.00 (0.90–1.12)					
Any antibody induction										
No	3343	31	1119	Ref	Ref					
Yes	798	27	315	**2.04 (1.14–3.67)**	**0.86 (0.75–0.99)**					
Any antibody										
Never	3071	30	990	Ref	Ref					
Ever	1070	28	444	1.52 (0.89–2.62)	0.90 (0.80–1.01)					

Table 7 - 3 (continued). Risk factors for lip cancer after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model

Immunosuppression	N	Lip cancer	Death	Basic model §,‡		N	Lip cancer	Death	Complete model†	
				Lip cancer	Death				Lip cancer	Death
				HR (95%CI)	HR (95%CI)				HR (95%CI)	HR (95%CI)
Current receipt										
Cyclosporine	3761	50	1169			3254	46	1032		
No				Ref	Ref				Ref	Ref
Yes				1.59 (0.48–5.29)	**0.71 (0.60–0.85)**				4.33 (0.53–35.8)	**0.71 (0.59–0.86)**
Tacrolimus*	3460	38	921			3007	35	833		
No				Ref	Ref				Ref	Ref
Yes				1.15 (0.32–4.15)	1.09 (0.89–1.33)				0.50 (0.06–4.12)	1.09 (0.88–1.35)
Mycophenolate*	3460	38	921			3007	35	833		
No				Ref	Ref				Ref	Ref
Yes				1.25 (0.41–3.79)	0.96 (0.79–1.17)				2.06 (0.35–12.0)	0.88 (0.71–1.08)
Azathioprine	3759	50	1167			3254	46	1032		
No				Ref	Ref				Ref	Ref
Yes				1.66 (0.77–3.55)	**0.80 (0.70–0.92)**				2.30 (0.85–6.24)	**0.85 (0.73–0.99)**
Number of drugs	3510	49	1169							
1				Ref	Ref					
>1				2.55 (0.91–7.15)	**0.58 (0.49-0.68)**					
Current dose (mg/kg/day)										
Cyclosporine	3733	50	1140	0.92 (0.81–1.05)	**0.96 (0.93–0.99)**	3208	45	993	1.07 (0.92–1.23)	**0.94 (0.91–0.98)**
Tacrolimus*	3378	38	888	NA	0.20 (0.03–1.50)	2952	34	791	NA	0.12 (0.01–1.06)
Mycophenolate*	3441	37	915	0.99 (0.95–1.04)	1.00 (0.99–1.02)	2959	34	797	0.99 (0.95–1.04)	0.99 (0.98–1.00)
Azathioprine	3716	50	1133	**1.56 (1.11–2.19)**	**0.87 (0.79–0.97)**	3187	46	975	**1.79 (1.21–2.66)**	**0.84 (0.75–0.94)**
Mean dose# (mg/kg/day)										
Cyclosporine	3291	48	1090	1.00 (0.87–1.15)	**1.10 (1.07–1.12)**	2701	44	911	1.08 (0.90–1.29)	**1.10 (1.07–1.13)**
Tacrolimus*	2951	38	857	NA	**0.06 (0.01–0.51)**	2511	34	731	NA	0.10 (0.01–0.92)
Mycophenolate*	3105	37	879	0.95 (0.90–1.01)	**0.98 (0.97–0.99)**	2511	34	731	0.95 (0.89–1.02)	**0.98 (0.97–0.99)**
Azathioprine	3214	48	1063	**1.84 (1.13–2.99)**	**1.19 (1.07–1.33)**	2701	44	911	**2.28 (1.18–4.38)**	1.12 (0.98–1.28)

§ The counts in sub-categories may not add up to the total number due to missing data.
‡ Basic model: immunosuppressive agent or other variable of interest, adjusted for age at transplantation, sex, transplant year, and transplanted organ (if not the variable of interest).
† Complete model: basic model plus time since transplantation, smoking history and other immunosuppressive agents in the respective category (i.e. current receipt, current dose, categorized current dose, mean dose, categorized mean dose). For time-dependent variables (i.e. current receipt, current dose, categorized current dose), immunosuppressive agents from the same class cannot be given simultaneously and thus are not adjusted for. For variables other than immunosuppressive agents, results adjusted for the mean dose of immunosuppressive agents are presented.
* Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents; # The mean dose of each agent was calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point (weighted by the length of each follow-up time interval); The median mean dose for cyclosporine was 3.40 (IQR 2.56-4.65) mg/kg/day, for tacrolimus 0.08 (IQR 0.05-0.11), for mycophenolate 28.0 (IQR 20.8-35.7), and for azathioprine 1.16 (IQR 0.84-1.53).

Table 7 - 4. Other risk factors for lip cancer after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model

Risk factor	Number of transplant recipients			Basic model§,‡	
				Lip cancer	Death
	Cohort	Lip	Death	HR (95%CI)	HR (95%CI)
Country of birth					
Australia/New Zealand	2335	41	797	Ref	Ref
Europe	672	9	237	0.69 (0.34–1.41)	1.03 (0.89–1.19)
Asia	225	0	53	NA	1.03 (0.76–1.39)
Other/unknown	909	8	347	**0.41 (0.19–0.88)**	**1.22 (1.06–1.39)**
Transplanted organ sub-type					
Liver	1620	11	362	Ref	Ref
Heart	1344	34	497	1.81 (0.93–3.53)	**1.15 (1.00–1.34)**
Bilateral lung	699	6	278	**3.57 (1.24–10.3)**	**3.17 (2.68–3.75)**
Single lung	270	4	176	1.97 (0.59–6.51)	**3.54 (2.96–4.23)**
Donor age	4092	57	1405	1.00 (0.98–1.02)	**1.01 (1.01–1.02)**
Cancer history					
No	3763	58	1318	Ref	Ref
Yes	378	0	116	NA	**1.89 (1.52–2.36)**
Diabetes					
No diabetes	1972	33	524	Ref	Ref
Pre-existing diabetes	565	8	157	0.92 (0.43–1.98)	1.13 (0.95–1.35)
Post-transplant diabetes	496	5	159	1.05 (0.39–2.82)	**1.74 (1.45–2.09)**
Dialysis					
Never	2400	37	600	Ref	Ref
Ever	325	6	141	1.15 (0.47–2.78)	**2.01 (1.66–2.43)**
Rejection ever					
Never	2455	34	757	Ref	Ref
Ever	1312	17	525	0.91 (0.49–1.68)	**1.18 (1.05–1.32)**
Multiple transplantation					
No	4035	58	1388	Ref	Ref
Yes (>=2)	106	0	46	NA	**2.45 (1.74–3.45)**
CMV immunoglobulin					
No	1437	15	346	Ref	Ref
Yes	394	5	147	0.81 (0.25–2.56)	**1.39 (1.13–1.72)**

Table 7 - 4 (continued). Other risk factors for lip cancer after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model

Risk factor	Number of transplant recipients			Basic model§,‡	
				Lip cancer	Death
	Cohort	Lip	Death	HR (95%CI)	HR (95%CI)
Donor sex (first transplantation)					
Female	1509	14	494	Ref	Ref
Male	2594	44	913	1.21 (0.64–2.30)	0.96 (0.86–1.09)
Alcohol drinking history					
Never	582	5	173	Ref	Ref
Ever	2402	38	788	1.04 (0.38–2.86)	1.07 (0.89–1.28)
Autoimmune disease history					
No	3879	57	1365	Ref	Ref
Yes	262	1	69	0.39 (0.05–2.91)	0.93 (0.71–1.21)
Recipient EBV status at transplantation					
Positive	2107	18	447	Ref	Ref
Negative	406	13	139	1.01 (0.35–2.92)	1.12 (0.90–1.39)
Recipient EBV status after transplantation					
Positive	2228	20	491	Ref	Ref
Negative	149	2	50	0.21 (0.03–1.24)	0.91 (0.68–1.23)
Overall recipient EBV status					
Positive at transplantation	2107	18	447	Ref	Ref
Positive only after transplantation	48	2	18	1.79 (0.40–7.93)	1.29 (0.79–2.12)
Negative	149	2	50	0.19 (0.03–1.17)	0.95 (0.70–1.29)
Recipient CMV IgG (first transplantation)					
Positive	2825	40	981	Ref	Ref
Negative	1214	17	406	1.15 (0.64–2.05)	0.93 (0.83–1.05)
Donor CMV IgG					
Negative	1463	21	484	Ref	Ref
Positive	2581	35	901	1.15 (0.67–1.96)	1.10 (0.99–1.23)
CMV concordance					
Recipient –/ Donor –	486	8	169	Ref	Ref
Recipient –/ Donor +	702	8	220	1.02 (0.38–2.74)	1.05 (0.86–1.28)
Recipient +/ Donor + or Donor –	2765	39	957	0.90 (0.42–1.94)	1.12 (0.96–1.32)
Immunosuppressive therapy before transplantation					
No	3736	54	1275	Ref	Ref
Yes	405	4	159	0.83 (0.27–2.54)	0.94 (0.79–1.11)

§ The counts in sub-categories may not add up to the total number due to missing data.
‡ Basic model: immunosuppressive agent or other variable of interest, adjusted for age at transplantation, sex, transplant year, and transplanted organ (if not the variable of interest).

Table 7 - 5. Risk factors for lip cancer after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model, censored at ten years after transplantation

Risk factor	Number of transplant recipients			Complete model†	
				Lip cancer	Death
	Cohort	Lip cancer	Death	HR (95%CI)	HR (95%CI)
Age at transplantation (per single year)	2763	35	786	**1.23 (1.08–1.40)**	**1.06 (1.01–1.11)**
Sex					
Female	873	6	254	Ref	Ref
Male	1890	29	532	1.62 (0.62–4.23)	1.07 (0.91–1.26)
Transplant era					
1984-1994	851	28	305	8.89 (0.96–86.7)	0.81 (0.65–1.01)
1995-1997	485	6	209	4.16 (0.41–42.3)	1.09 (0.88–1.34)
1998-2006	1427	1	272	Ref	Ref
Time since transplantation (years)					
0-0.99	2763	2	196	Ref	Ref
1-1.99	2432	2	98	1.44 (0.20–10.3)	**0.60 (0.47–0.77)**
2-4.99	2174	10	244	4.12 (0.92–18.3)	**0.73 (0.57–0.92)**
5-9.99	1464	21	248	**13.9 (2.71–70.8)**	0.90 (0.63–1.30)
First transplanted organ					
Liver	1026	7	210	Ref	Ref
Heart	1001	20	260	0.83 (0.33–2.09)	0.93 (0.74–1.17)
Lung	736	8	316	1.13 (0.34–3.75)	**2.18 (1.77–2.68)**
Smoking history					
Never	1074	4	249	Ref	Ref
Ever	1689	31	537	**3.44 (1.11–10.6)**	**1.50 (1.27–1.78)**
Current receipt of immunosuppressive agent					
Cyclosporine	3254	37	886		
No				Ref	Ref
Yes				3.88 (0.45–33.5)	**0.70 (0.57–0.86)**
Tacrolimus*	2978	27	688		
No				Ref	Ref
Yes				0.47 (0.05–4.28)	1.06 (0.85–1.33)
Mycophenolate*	2978	27	688		
No				Ref	Ref
Yes				1.11 (0.26–4.74)	0.81 (0.64–1.03)
Azathioprine	3254	37	886		
No				Ref	Ref
Yes				3.16 (0.97–10.3)	0.90 (0.76–1.06)
Current dose (mg/kg/day)					
Cyclosporine	3207	36	858	1.05 (0.89–1.24)	**0.95 (0.92–0.99)**
Tacrolimus*	2917	26	655	NA	**0.09 (0.01–0.94)**
Mycophenolate*	2925	26	662	0.96 (0.89–1.03)	1.00 (0.99–1.00)
Azathioprine	3185	37	841	**2.10 (1.43–3.08)**	0.91 (0.81–1.02)

Table 7 - 5 (continued). Risk factors for lip cancer after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model, censored at ten years after transplantation

Risk factor				Complete model†	
	Number of transplant recipients			Lip cancer	Death
	Cohort	Lip cancer	Death	HR (95%CI)	HR (95%CI)
Mean dose (mg/kg/day)					
Cyclosporine	2763	35	786	1.08 (0.89–1.30)	**1.09 (1.05–1.12)**
Tacrolimus*	2548	26	607	NA	0.15 (0.01–1.53)
Mycophenolate*	2548	26	607	0.96 (0.89–1.04)	0.99 (0.98–1.00)
Azathioprine	2763	35	786	**2.59 (1.34–4.99)**	**1.16 (1.00–1.34)**

§ The counts in sub-categories may not add up to the total number due to missing data.
‡ Basic model: immunosuppressive agent or other variable of interest, adjusted for age at transplantation, sex, transplant year, and transplanted organ (if not the variable of interest).
† Complete model: basic model plus time since transplantation, smoking history and other immunosuppressive agents in the respective category (i.e. current receipt, current dose, categorized current dose, mean dose, categorized mean dose). For time-dependent variables (i.e. current receipt, current dose, categorized current dose), immunosuppressive agents from the same class cannot be given simultaneously and thus are not adjusted for. For variables other than immunosuppressive agents, results adjusted for the mean dose of immunosuppressive agents are presented.
* Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents.
# The mean dose of each agent was calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point (weighted by the length of each follow-up time interval). The median mean dose for cyclosporine was 3.48 (IQR 2.61-4.72) mg/kg/day, for tacrolimus 0.07 (IQR 0.05-0.11), for mycophenolate 27.8 (IQR 20.5-34.8), and for azathioprine 1.16 (IQR 0.83-1.53).

7.5 Discussion

In this population-based cohort of Australian adult liver, heart and lung transplant recipients, I found that lip cancer risk was independently associated with higher doses of azathioprine, both before and after adjusting for the use of other immunosuppressive agents and the duration of immunosuppression. Moreover, the increased lip cancer risk in lung compared to liver recipients was apparently explained by differences in immunosuppressive therapy between these two groups. Another novel result was the increased lip cancer risk in those transplanted in the earlier (prior to 1998) compared to the later era (1998-2006). I confirmed that increased duration of

immunosuppressive therapy, older age, and smoking history are independent risk factors for lip cancer in solid organ transplant recipients.

In my cohort, almost all lip cancers cases were SCC and most originated in the lower vermillion. This distribution is consistent with previous studies of transplant recipients (van Leeuwen et al. 2009) and the general population (Vukadinovic et al. 2007, Czerninski et al. 2010). There are epidemiological similarities between SCC originating in the lip and the skin, including the age and sex profile, high risk skin phenotype, and relationship with UVR exposure (de Visscher and van der Waal 1998, Euvrard et al. 2003). They also have similar molecular characteristics including aberrant expression of p53 and epithelial cyclooxygenase-2 overexpression (Berner et al. 1993, Crosthwaite et al. 1996, McGregor et al. 1997, Tripp et al. 2003, Rojas et al. 2009). It is highly likely therefore that lip SCC and cutaneous SCC will also share risk factors in transplant recipients, and thus knowledge of risk factors for lip cancer will inform strategies to reduce the significant burden from cutaneous SCC in solid organ transplantation (O'Reilly Zwald and Brown 2011).

I observed that risk of lip cancer was higher in lung compared to liver transplant recipients, but this difference was no longer significant after adjusting for immunosuppressive therapy. My findings suggest that the organ-specific variations in relation to lip cancer risk, and possibly also cutaneous SCC incidence (Krynitz et al. 2013), are largely attributable to differences in the degree of immunosuppression.

I found that increasing age was significantly associated with elevated lip cancer risk, as observed in both kidney recipients (van Leeuwen et al. 2009) and the general population (Czerninski et al. 2010). This may reflect age-related reductions in immune surveillance (Heinbokel et al. 2013) or increasing cumulative UVR exposure. Similar to findings in the general population (de Visscher et al. 1998, Moore et al. 1999), and a study of lip cancer in Australian kidney transplant recipients that assessed risk in relation to country of birth (van Leeuwen et al. 2009), I observed no lip cancers in non-Caucasian recipients. As melanin provides protection against solar UVR exposure (Wenczl et al. 1998, Nordlund 2007, Brenner and Hearing 2008), this association has been attributed to higher levels of melanin within the vermillion of dark-skinned compared to light-skinned individuals (Thibodeau and D'Ambrosio 1997). However, unlike the general population (Czerninski et al. 2010) and Australian kidney transplant recipients (van Leeuwen et al. 2009), I found comparable lip cancer risk for male and female recipients. The sex-specific differences in the general population are believed to be driven by the relative amounts of outdoor work and therefore higher cumulative UVR exposure in males compared to females (Moore et al. 1999, Perea-Milla Lopez et al. 2003, Czerninski et al. 2010). It is possible although unverifiable that liver, heart and lung transplant recipients are less likely to perform outdoor work than kidney transplant recipients due to poorer general health. Another explanation is that my results would be different if I was able to adjust for a measure of sun exposure, as was available in the kidney transplant study. Finally, cigarette smoking was positively associated with lip cancer risk in my cohort, a finding consistent with Australian kidney transplant recipients (van Leeuwen et al. 2009) and the general population (Perea-Milla Lopez et al. 2003).

As observed for lip cancer in Australian kidney transplant recipients (van Leeuwen et al. 2009) and non-melanoma skin cancer (NMSC) in transplant recipients (Euvrard et al. 2003, Ramsay et al. 2003, Herrero et al. 2005, Moloney et al. 2006, Casabonne et al. 2009, Ingvar et al. 2010, Krynitz et al. 2013), I found lip cancer risk increased with increasing time since transplantation or duration of immunosuppression. Increasing duration of immunosuppression may also be a measure of increasing duration of infection with an oncogenic virus, such as HPV infection. However, the prevalence of HPV is comparable in immune deficient and immunocompetent individuals with or without lip SCC and cutaneous SCC (Shimizu et al. 2004, Casabonne et al. 2009), and rates of infection with most types of HPV do not appear to increase with increasing time since transplantation (Casabonne et al. 2009).

In my study, a higher current or mean dose of azathioprine was associated with a moderate dose-related lip cancer risk. This association was strengthened after adjusting for the dose of other agents, smoking history, and duration of immunosuppressive therapy, at least partly reflecting the confounding role of the dose of other agents or the overall extent of immunosuppression. Unlike a prior study of lip cancer in Australian kidney recipients (van Leeuwen et al. 2009), but in agreement with a single-centre study of cutaneous SCC in heart recipients (Brewer et al. 2009), the current receipt of azathioprine (i.e. irrespective of dose) was not significantly associated with lip cancer risk. No previous study has examined the dose-related effect of azathioprine on lip cancer risk, and the evidence for cutaneous SCC risk is contradictory. A single-centre study found risk of cutaneous SCC in heart transplant

recipients was associated with the overall level of immunosuppression but not the cumulative dose of individual agents. In this study, the median dosages of cyclosporine, azathioprine and corticosteroid were converted into a single score to represent the overall level of immunosuppression assuming the biological effect of each agent is equivalent (Fortina et al. 2004). In contrast, a population-based, nested case-control study found that higher cumulative and higher mean dose of azathioprine were both strongly associated with an increased risk of cutaneous SCC (Ingvar et al. 2010). Two studies (Ramsay et al. 2003, Kasiske et al. 2004) reported an increased risk of NMSC in association with azathioprine at discharge, but did not adjust for the receipt of other agents. A higher risk of NMSC has also been observed in patients with inflammatory bowel disease treated with azathioprine (Long et al. 2010). The contradictory findings are thus not unexpected given the varied measures of drug exposure and the lack of adjustment for other immunosuppressive agents and other confounding factors.

Azathioprine is IARC-classified as a human carcinogen for NHL and NMSC (IARC 2012). Whilst azathioprine-induced mismatch repair system deficiency is thought to be responsible for lymphomagenesis, this deficiency has not been observed in post-transplantation cutaneous SCC (Wisgerhof et al. 2009, Perrett et al. 2010). A different pathway of carcinogenesis has been identified involving increased photosensitization via the incorporation of 6-thioguanine (6-TG), the active metabolite of azathioprine, into DNA (O'Donovan et al. 2005). DNA 6-TG is a photosensitiser with a maximum absorbance in the UVA region (340nm) (Karran and Attard 2008). In cells cultured with DNA 6-TG, UVA can indirectly damage DNA by inducing reactive oxygen species (Cadet

et al. 2003, O'Donovan et al. 2005, Cadet et al. 2006, Brem and Karran 2012). In support of this mechanism of SCC development, significantly higher levels of 6-TG have been detected in the blood of kidney recipients who developed cutaneous SCC than those who did not in a single-centre observational study. These two patient groups did not differ in age, sex, doss or duration of immunosuppression, history of acute rejection, serum creatinine levels or number of warts (Lennard et al. 1985). More direct evidence of carcinogenesis is indicated by a recent study showing a reversal in UVA skin photosensitivity and DNA damage in kidney transplant recipients when switched from azathioprine to mycophenolate (Hofbauer et al. 2012).

In agreement with the study of Australian kidney transplant recipients (van Leeuwen et al. 2009), I found no association between lip cancer risk and the use of mycophenolate. There is no other epidemiological evidence linking mycophenolate to lip cancer or cutaneous SCC although data are limited. In a single-centre heart transplant study, the current receipt of mycophenolate was not associated with cutaneous SCC risk in univariable analysis (Brewer et al. 2009). In addition, without adjustment for other agents, mycophenolate did not increase NMSC risk in a US study using discharge data (Kasiske et al. 2004). Although mycophenolate is an antiproliferative agent, it is not a photosensitiser, and my findings are consistent with *in vivo* studies showing that mycophenolate does not enhance UV-induced NMSC development (Duncan et al. 2007, Wulff et al. 2008, Dworkin et al. 2009).

I found no association between the current receipt of cyclosporine and lip cancer risk, in contrast to prior evidence for Australian kidney transplant recipients showing a

modest increased risk (van Leeuwen et al. 2009), but in agreement with a single-centre study of cutaneous SCC in heart recipients (Brewer et al. 2009). I also found no association between the dose of cyclosporine and lip cancer risk. While no prior study has examined this relationship, my finding is in accordance with a nested case-control study of kidney transplant recipients where cutaneous SCC risk was unrelated to cyclosporine dose without adjustment for the dose of other agents (Ingvar et al. 2010). Use of cyclosporine at discharge did not confer a higher risk of NMSC in a US study, although again, this is was without adjustment for other agents or the duration of immunosuppression (Kasiske et al. 2004). Two studies have reported that the combination of cyclosporine, azathioprine, and corticosteroid confers a higher risk of NMSC compared to the use of cyclosporine alone or azathioprine and corticosteroid (Glover et al. 1997, Jensen et al. 1999), but this most likely reflects the relative intensity of immunosuppression rather than a direct effect of cyclosporine. In addition, cyclosporine treatment in patients with chronic skin disorders, such as atopic dermatitis, is not associated with increased risk of cutaneous SCC and BCC (Muellenhoff and Koo 2012), although the dose of cyclosporine in this setting is lower than for transplant recipients.

Regarding biological evidence that cyclosporine promotes SCC carcinogenesis, cyclosporine has been shown to inhibit DNA repair and apoptosis in UVB-irritated human keratinocytes (Yarosh et al. 2005, Canning et al. 2006) and to enhance tumour size and progression from papilloma to skin cancer in UVR-exposed mice (Wulff et al. 2008). Cyclosporine is believed to promote cutaneous SCC in mice through the transforming growth factor β signalling pathway (Wulff et al. 2008); however, the

direct carcinogenic role of cyclosporine is uncertain. Most of the experimental studies used UVR-exposed mice models, where the effect of cyclosporine is complicated by UVR-induced immunosuppression (Gensler and Chen 1991, Murphy 2009).

I was unable to assess the dose-related effect of tacrolimus on lip cancer risk due to the low variation in the dosage of this drug. No prior study has examined the association between lip cancer risk and tacrolimus, and the balance of evidence with respect to cutaneous SCC risk suggests no association. In a population-based study of kidney recipients, use of tacrolimus at discharge decreased NMSC risk, although this included both cutaneous SCC and BCC and was unadjusted for use of other agents or duration of immunosuppression (Kasiske et al. 2004). Other prior studies only examined the effect of tacrolimus in univariable analyses. The current receipt of tacrolimus was not associated with risk of cutaneous SCC in a single-centre heart transplant study (Brewer et al. 2009). Similarly, receipt of tacrolimus at discharge did not increase risk of NMSC in liver recipients (Herrero et al. 2005). In mice models, tacrolimus was observed to cause higher numbers of chromosomal aberrations and increase the size of UV-induced cutaneous SCC compared to controls (Duncan et al. 2007, Dworkin et al. 2009) but this may be an effect of immunosuppression *per se* rather than a direct effect of this agent.

The receipt of antibody induction therapy increased lip cancer risk in my basic model, but this association was not robust to complete adjustment, especially adjustment by the dose of individual maintenance immunosuppression. The current receipt of T-cell–depleting antibodies decreased lip cancer risk in Australian kidney transplant recipients

(van Leeuwen et al. 2009), however I was unable to examine antibody therapy time-dependently. Regarding the relationship between antibody use and skin cancer, a single-centre study reported that muromonab-CD3, a potent T-cell depleting antibody, increased the cumulative risk of skin cancer in heart recipients (Lampros et al. 1998), but other studies have found no association between the receipt of antibodies and NMSC risk (Jensen et al. 1999, Ingvar et al. 2010). A mouse model study found that CD4-depleting antibodies increased UV-induced cutaneous inflammation and subsequent cutaneous SCC (Hatton et al. 2007), suggesting that CD4+ T-cells play an important role in the acute inflammatory and carcinogenic response of the skin to UVR.

In my study, I found no association between lip cancer risk and alcohol consumption history, comorbidities such as autoimmune disease, CMV or EBV infection, or prophylactic anti-CMV immunoglobulin. Lip cancer risk was increased in association with the receipt of CMV antiviral therapy in the basic model, but this was not significant after adjusting for the administration of immunosuppressive therapy.

The key strengths of my study include the population-base for the transplant recipients, lip cancer diagnoses and deaths, thereby minimising bias. The accuracy of incident lip cancer diagnoses was assured by Australian cancer registration which is highly reliable (Grulich et al. 1996) and the linkage technique is highly sensitive and specific (Grulich et al. 1996). My analyses utilised comprehensive data on the longitudinal dose of immunosuppressive agents and extensive clinical risk factors for three organ types over a 23 year period. To the best of my knowledge, this is the first

study to examine the relationship between lip cancer risk and the duration and dose of individual immunosuppressive agents accounting for the competing risk of death. A competing risk analysis was necessary because of the high rate of death in this population. In addition, I examined two approaches to modelling the dose of each immunosuppressive agent; my findings were consistent, implying that both the level of current and overall immunosuppression is relevant to lip cancer risk. I also analysed the median current and mean dose as a potential threshold for azathioprine-induced risk of lip cancer but, unlike with respect to NHL in Chapter 6, it did not significantly separate the two groups. Further larger studies are thus needed to detect whether a potential dose threshold exists for lip cancer risk.

My study also has several limitations. I was reliant on the accuracy, completeness, and availability of medical records and am thus unable to exclude an effect due to residual confounding. In particular, variables representing personal or ambient solar UVR exposure, sun sensitivity, and pigmentation were not available. My finding of an association between transplant era and lip cancer risk may be confounded by UVR exposure, but in support of a true association, the same time trend exists for lip cancer in the general Australian population (AIHW and AACR 2012, Ariyawardana and Johnson 2013). Data on past alcohol consumption and smoking were missing for a proportion of the cohort, and the measurement of these exposures was crude (ever/never). Information on immunosuppressive agents and their dose during follow-up was also missing for some recipients. Reassuringly however, there were no notable differences in the results based on the original and imputed data, supporting the assumption of ignorable drop-outs.

My findings are also limited by the scope of the study period from 1984 to 2006, which may not be representative of contemporary clinical practice, particularly, immunosuppressive therapy. Azathioprine is currently used infrequently in transplant recipients. Additionally, due to the low prevalence of mammalian target of rapamycin (mTOR) inhibitors during my study period, I was unable to assess the relationship between lip cancer risk and use of these agents. mTOR inhibitors may have antitumor properties; conversion from calcineurin inhibitors to mTOR inhibitors has been shown to lower the risk of skin cancer up to 1 to 2 years post-transplantation, although the longer-term benefit is uncertain (Lim et al. 2014). Furthermore, similar to previous studies (Allen et al. 2013), I was unable to model the overall intensity of iatrogenic immunosuppression; nevertheless, the results for each agent were adjusted for the dose of the other agents. Despite the long follow-up time, I observed a relatively small number of lip cancer cases, which limited my statistical power. My statistical power was further weakened in the complete models restricted to patients without any missing data, potentially precluding the detection of a significant association. As this was an observational study, I am unable to exclude the effect of propensity to receive different immunosuppressive agents between the different recipient subgroups. Finally, the possibility of chance findings cannot be excluded but encouragingly my immunosuppression findings were comparable among my different approaches.

7.6 Conclusions

My study provides the first evidence of a causal association between lip cancer risk and higher doses of azathioprine after solid organ transplantation. With over two decades

of follow-up time, my study also helped clarify the independent effects of ageing, smoking history and duration of immunosuppressive therapy on lip cancer risk. My findings support the avoidance of azathioprine in high-risk patient subgroups, together with sun protection in transplant recipients who are not candidates for alternative immunosuppressive agents, and regular monitoring of those at high risk of both lip cancer and cutaneous SCC.

Supplementary Tables

Supplementary Table 7 - 1. Immunosuppressive therapy and risk of lip cancer after adult liver, heart and lung transplantation in the presence of competing risk of death based on proportional subdistribution hazards model: original data

Immunosuppressive agent	Number of transplant recipients			Lip cancer[§‡]	Death[§‡]
	Cohort	Lip cancer	Death	HR (95%CI)	HR (95%CI)
Current receipt					
Cyclosporine	3749	48	1043		
No				Ref	Ref
Yes				1.63 (0.49–5.40)	**0.73 (0.61–0.88)**
Tacrolimus*	3430	36	823		
No				Ref	Ref
Yes				1.13 (0.31–4.14)	1.13 (0.91–1.39)
Mycophenolate*	3431	36	823		
No				Ref	Ref
Yes				1.36 (0.45–4.10)	0.99 (0.80–1.22)
Azathioprine	3750	48	1041		
No				Ref	Ref
Yes				1.59 (0.74–3.43)	**0.79 (0.69–0.92)**
Current dose (mg/kg/day)					
Cyclosporine	3588	40	774	0.97 (0.85–1.11)	**0.91 (0.87–0.98)**
Tacrolimus*	3320	36	762	NA	0.11 (0.01–1.43)
Mycophenolate*	3358	35	796	1.00 (0.96–1.04)	1.00 (0.99–1.01)
Azathioprine	3653	42	836	**1.65 (1.17–2.32)**	**0.82 (0.73–0.92)**
Mean dose# (mg/kg/day)					
Cyclosporine	1889	36	493	1.08 (0.95–1.23)	**1.05 (1.01–1.09)**
Tacrolimus*	2085	34	601	NA	0.12 (0.01–2.58)
Mycophenolate*	2299	34	619	0.97 (0.91–1.02)	**0.98 (0.97–0.99)**
Azathioprine	1873	35	499	1.62 (0.99–2.66)	**1.33 (1.14–1.55)**

§ The counts in sub-categories may not add up to the total number due to missing data; ‡ Basic model: adjusted for age at transplantation, sex, transplant year, and transplanted organ;
* Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents; # The mean dose of each agent was calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point (weighted by the length of each follow-up time interval); The median mean dose for cyclosporine was 3.25 (IQR 2.43-4.45) mg/kg/day, for tacrolimus 0.08 (IQR 0.05-0.11), for mycophenolate 26.3 (IQR 19.7-32.5), and for azathioprine 1.36 (IQR 1.01-1.66).

Supplementary Table 7 - 2. Immunosuppressive therapy and risk of lip cancer after adult liver, heart and lung transplantation in the presence of competing risk of death based on cause-specific hazards model

Risk factor	Number of transplant recipients			Complete model†	
				Lip cancer	Death
	Cohort	Lip cancer	Death	HR (95%CI)	HR (95%CI)
Age at transplantation (per single year)	2701	44	911	0.98 (0.87–1.10)	0.97 (0.93–1.00)
Sex					
Female	851	7	282	Ref	Ref
Male	1850	37	629	1.84 (0.78–4.36)	1.14 (0.98–1.32)
Transplant era					
1984-1994	794	37	420	6.84 (0.87–53.6)	0.83 (0.69–1.01)
1995-1997	480	6	219	3.28 (0.38–28.1)	0.96 (0.79–1.16)
1998-2006	1427	1	272	Ref	Ref
Time since transplantation (years)					
0-0.99	2701	2	196	Ref	Ref
1-1.99	2370	2	98	1.03 (0.14–7.37)	**0.55 (0.43–0.70)**
2-4.99	2112	10	244	1.91 (0.40–9.08)	**0.56 (0.46–0.70)**
5+	1402	30	373	2.93 (0.51–16.8)	**0.52 (0.37–0.72)**
First transplanted organ					
Liver	993	7	231	Ref	Ref
Heart	983	28	340	1.17 (0.47–2.94)	1.02 (0.85–1.24)
Lung	725	9	340	1.78 (0.62–5.11)	**2.31 (1.91–2.79)**
Smoking history					
Never	1043	6	287	Ref	Ref
Ever	1658	38	624	**3.06 (1.27–7.40)**	**1.52 (1.30–1.78)**
Current receipt of immunosuppressive agent					
Cyclosporine	3254	46	1032		
No				Ref	Ref
Yes				4.13 (0.54–31.5)	**0.73 (0.61–0.86)**
Tacrolimus*	3007	35	833		
No				Ref	Ref
Yes				0.48 (0.06–3.84)	1.08 (0.88–1.32)
Mycophenolate*	3007	35	833		
No				Ref	Ref
Yes				0.86 (0.24–3.06)	0.85 (0.69–1.04)
Azathioprine	3254	46	1032		
No				Ref	Ref
Yes				2.47 (0.98–6.22)	0.87 (0.76–1.01)
Current dose (mg/kg/day)					
Cyclosporine	3208	45	993	1.08 (0.91–1.28)	**0.94 (0.92–0.97)**
Tacrolimus*	2952	34	791	NA	**0.11 (0.01–0.98)**
Mycophenolate*	2959	34	797	0.98 (0.93–1.04)	1.00 (0.99–1.00)
Azathioprine	3187	46	975	**1.84 (1.18–2.87)**	**0.90 (0.81–0.99)**

Supplementary Table 7 - 2 (continued). Immunosuppressive therapy and risk of lip cancer after adult liver, heart and lung transplantation in the presence of competing risk of death based on cause-specific hazards model

Risk factor	Number of transplant recipients			Complete model†	
				Lip cancer	Death
	Cohort	Lip cancer	Death	HR (95%CI)	HR (95%CI)
Mean dose (mg/kg/day)[5]					
Cyclosporine	2701	44	911	1.18 (0.97–1.43)	**1.10 (1.07–1.13)**
Tacrolimus*	2511	34	731	NA	**0.09 (0.01–0.74)**
Mycophenolate*	2511	34	731	0.94 (0.88–1.00)	**0.98 (0.97–0.99)**
Azathioprine	2701	44	911	**2.42 (1.24–4.74)**	**1.15 (1.01–1.30)**

§ The counts in sub-categories may not add up to the total number due to missing data.
‡ Basic model: immunosuppressive agent or other variable of interest, adjusted for age at transplantation, sex, transplant year, and transplanted organ (if not the variable of interest).
† Complete model: basic model plus time since transplantation, smoking history and other immunosuppressive agents in the respective category (i.e. current receipt, current dose, categorized current dose, mean dose, categorized mean dose). For time-dependent variables (i.e. current receipt, current dose, categorized current dose), immunosuppressive agents from the same class cannot be given simultaneously and thus are not adjusted for. For variables other than immunosuppressive agents, results adjusted for the mean dose of immunosuppressive agents are presented.
* Tacrolimus and mycophenolate restricted to the years from 1997 based on the availability of these agents.
# The mean dose of each agent was calculated as the weighted sum of dosages (mg/kg/day) at each follow-up time point (weighted by the length of each follow-up time interval). The median mean dose for cyclosporine was 3.40 (IQR 2.56-4.65) mg/kg/day, for tacrolimus 0.08 (IQR 0.05-0.11), for mycophenolate 28.0 (IQR 20.8-35.7), and for azathioprine 1.16 (IQR 0.84-1.53).

Chapter 8 Conclusions

In this thesis I have provided a detailed comparison of cancer incidence, cancer-related mortality, immunosuppressive therapy, and cancer risk by organ type for a cohort of Australian liver, heart and lung transplant recipients followed for over two decades. I have addressed key gaps in evidence and understanding in each of these areas. Immunosuppression therapy is the key to improving recipient outcomes and the pivotal potentially modifiable risk factor for complications after transplantation. I compared overall and site-specific profiles of cancer incidence and cancer-related death with the general population and among different transplanted organs. In addition, I have presented the most comprehensive data on the immunosuppressive therapy used in these three transplant populations over two decades. Furthermore, this is the first study to examine the association between the of type, dose and duration of immunosuppressive therapy and NHL and lip cancer incidence in liver, heart and lung transplant recipients taking competing risk of death into account.

My first set of aims was to quantify the site-specific cancer risk in 4644 liver, heart and lung recipients relative to the general population, to compare the cancer risk profiles by transplant organ type, and to compare the incidence of *de novo* cancer, solid cancer and NHL among recipients of the different transplanted organs after adjustment for age, sex, number of transplants, and calendar year. I identified a total of 499 incident primary cancers during a median of five years follow-up. Over one third of the cohort died, and mortality was particularly high in lung recipients. I observed an excess risk for

16 types of cancer in the entire cohort in comparison to the general population, the majority of which were cancers with a viral aetiology.

I found the overall and site-specific cancer incidence profiles for liver, heart and lung recipients to be generally similar and overall comparable with those of kidney transplant recipients. I did, however, identify some notable and novel differences in cancer risk among transplanted organs. Risk of Merkel cell carcinoma was significantly increased in heart and lung recipients, but not in liver recipients. In addition, lung cancer risk was significantly raised in heart and lung but not liver recipients, risk of melanoma was increased in liver and heart but not lung recipients, and colorectal cancer was elevated in liver and lung but not heart recipients. Furthermore, I showed that the adjusted within-cohort risk of any cancer and of NHL was significantly higher in heart and lung than liver recipients, and the risk of solid cancer was higher in lung than liver recipients. Possible explanations for these differences among organ recipients include variation in the type or intensity (dose) of immunosuppression, variation in predisposing factors such as pre-existing disease or past behaviours, and disparity in statistical power and differences in follow-up time due to variation in mortality rates.

In terms of cancer-related mortality, I observed a significant excess risk of death regardless of the type of transplanted organ, recipient age group or sex. NHL and NMSC were the most common cancer-related causes of death, and the excess risk was attributed predominantly but not exclusively to males and heart and lung recipients. I found that *de novo* cancer was a leading cause of late death, especially in liver and

heart transplantation and in paediatric recipients. These findings were expected given the excess risk of cancer, but they represent the first detailed population-based evidence of the site-specific risk of death from cancer in liver, heart and lung transplant recipients. Unlike some previous SMR data from the United States, the data are robust and particular attention was given to excluding recurrent cancers.

The variation in cancer risk profile by organ type may be driven by the differences in the intensity of immunosuppression, but no prior study has adequately addressed this issue. Importantly, the majority of previous observational studies relied on discharge immunosuppression data, which assumes that recipients remain on the same immunosuppressive regimen throughout follow-up. Furthermore, most studies have known the discharge agents but not their dosages, and very few involved a comprehensive analytic approach that adequately adjusted for the fact that most recipients were on dual therapies.

There is very little published data on the type and dose of immunosuppressive therapy received by recipients of different transplanted organs. To inform and optimise my modelling approach for the association between iatrogenic immunosuppression and cancer risk, I first described and compared the types and doses of immunosuppressive agents received by my cohort. I observed a complex set of relationships, including the expected changes in drug utilisation by calendar year, but also changes by time since transplantation and variation among transplant organ type. I found that the median dose of cyclosporine and mycophenolate reduced with increasing time since transplantation for all transplant recipients; the median dose of azathioprine declined

for heart and lung recipients but not liver, and the median dose of tacrolimus for liver and lung recipients but not heart. I found that liver recipients were more likely to receive CNI monotherapy relative to their cardiothoracic counterparts. I showed antibody induction therapy was more prevalent in heart and lung transplant recipients, whereas liver recipients were more likely to receive antibody rejection therapy.

With respect to maintenance immunosuppressive therapy, I compared the dose of individual immunosuppressive agents by organ type after adjustment for important confounders. Liver recipients consistently received significantly lower doses of azathioprine compared to heart and lung recipients. In contrast, the relative doses of other agents among transplanted organs varied by time since transplantation. For example, the dose of cyclosporine was lower in heart compared to lung recipients at one year after transplantation, while there was no difference in tacrolimus, but a higher dose of azathioprine at one and five years, and no difference in mycophenolate. These findings clearly demonstrate the need to take into account changes in the type and dose of immunosuppressive therapy when examining the relationship between immunosuppressive therapy and associated complications, such as cancer.

I examined the relationship between immunosuppression and the two most commonly occurring cancers in my cohort, NHL and lip cancer. I examined several different longitudinal measures of immunosuppressive therapy, specifically, the current receipt and the current and mean dose of individual immunosuppressive agents. While the current receipt and the current dose represent individual real-time drug exposure, the mean dose approach is believed to be preferable because it captures individuals'

overall dose of immunosuppressive therapy prior to an event (cancer) or the end of follow-up. Using these measures and an analytical approach that took into account all relevant measured confounders, the dose of other agents, and death as a competing risk, I produced novel findings.

I showed that a higher dose of azathioprine conferred a moderate dose-related increased risk of both NHL and lip cancer. This indicated that in addition to immunosuppression *per se,* an individual immunosuppressive agent also contributes to the increased risk of cancer after solid organ transplantation. For the first time I was able to demonstrate that the risk of NHL and lip cancer was no different for liver, heart and lung recipients after complete adjustment for the dose of individual immunosuppressive agents. This result confirms the long-standing hypothesis that differences in the risk of certain cancers among different transplanted organs is driven by organ-specific differences in the extent of iatrogenic immunosuppression. In addition, I confirmed the bimodal pattern of NHL after solid organ transplantation, and the substantial increased risk of early NHL in those exposed to muromonab-CD3 induction antibody therapy. Furthermore, I verified that duration of immunosuppression and increasing age are independent risk factors for late NHL and lip cancer, and I also confirmed that smoking is a strong risk factor for lip cancer. Earlier transplant era significantly increased lip cancer risk, but I observed no association with sex.

Previous observational studies demonstrated that current use of azathioprine is related to an increased risk of lymphoma and cutaneous SCC in transplant recipients

and patients with autoimmune diseases such as IBD. These findings, in conjunction with biological evidence that this agent is carcinogenic for NHL and NMSC, strongly suggest that use of azathioprine should be avoided. On the other hand, cyclosporine has long been suspected to be carcinogenic for NHL and NMSC, but my results do not support this assessment.

In concordance with previous studies, I found no strong evidence that mycophenolate or tacrolimus are associated with risk of NHL and lip cancer; however there were limitations to the extent to which they could be rigorously assessed. These agents, together with mTOR inhibitors, need to be examined in larger cohorts with longitudinal dose data.

Some key limitations of my work must be noted. This was an observational study, I was reliant on the quality, completeness and availability of medical records, and the clinical data were obtained retrospectively. As it was a national cohort and data were collected from 18 transplant units across Australia, I was unable to collect all factors that may influence the choice of, and changes in, the type and dose of immunosuppressive therapy. My study covered the period from 1984 to 2006, the results of which may or may not be generalisable to current clinical practice. I am also unable to exclude residual confounding due to unmeasured confounders and inadequate adjustment for the dose of tacrolimus. In addition, the statistical power of my risk factor models was limited because of the relatively small numbers of incident cancers and thus I may have been unable to detect significant differences among transplanted organs and immunosuppressive agents. My dataset had a substantial

proportion of missing data, but reassuringly the main findings were comparable between imputed and original data. Finally, it is essential that my work be validated in larger population-based cohorts using contemporary clinical data.

In summary, my findings of the cancer incidence profiles in liver, heart and lung transplant recipients provide population-based evidence to aid in the identification of recipient subgroups at high risk of cancer. Close monitoring of recipients for early cancer-related signs and symptoms is recommended, particularly for cancers with a viral aetiology such as NHL in those Epstein-Barr virus seronegative at transplantation. Differences in cancer incidence among different transplanted organs inform targeted cancer screening. For example, liver recipients with primary sclerosing cholangitis or ulcerative colitis should undergo annual colonoscopies. In addition, all transplant recipients should be examined regularly for lip cancer and NMSC, and be encouraged to adopt lifestyle changes to minimise their sun exposure and adopt sun protection practices. High-risk patient subgroups include those older at transplantation, with a history of smoking, and long-term survivors who have been on continuous immunosuppressive therapy for many years.

Indications and future directions

My findings on the carcinogenic role of azathioprine in NHL and lip cancer risk support the current clinical guidelines and practice of replacing this agent with other agents or minimising the dose where possible. For those recipients who do not have an alternative choice of immunosuppression, close cancer surveillance is recommended.

In addition, the association between the dose of immunosuppression and increased risk of NHL and lip cancer reinforces the importance of minimising the overall degree of immunosuppression in the long-term management of recipients. Although cyclosporine is classified as a human carcinogen, my findings of a non-significant effect of cyclosporine on the risk of NHL and lip cancer warrant further investigation as this agent is still widely used in transplantation. Finally, I have developed exposure measurement and risk factor modelling approaches that may be utilised in future observational studies examining the association between cancer risk and immunosuppression in solid organ transplantation. Future population-based longitudinal studies, with contemporary clinical data estimating the role of immunosuppression in risk of cancers that may not be related to an infectious agent as well as in the management of transplant recipients diagnosed with cancer, will further improve patient outcomes.

Appendix I

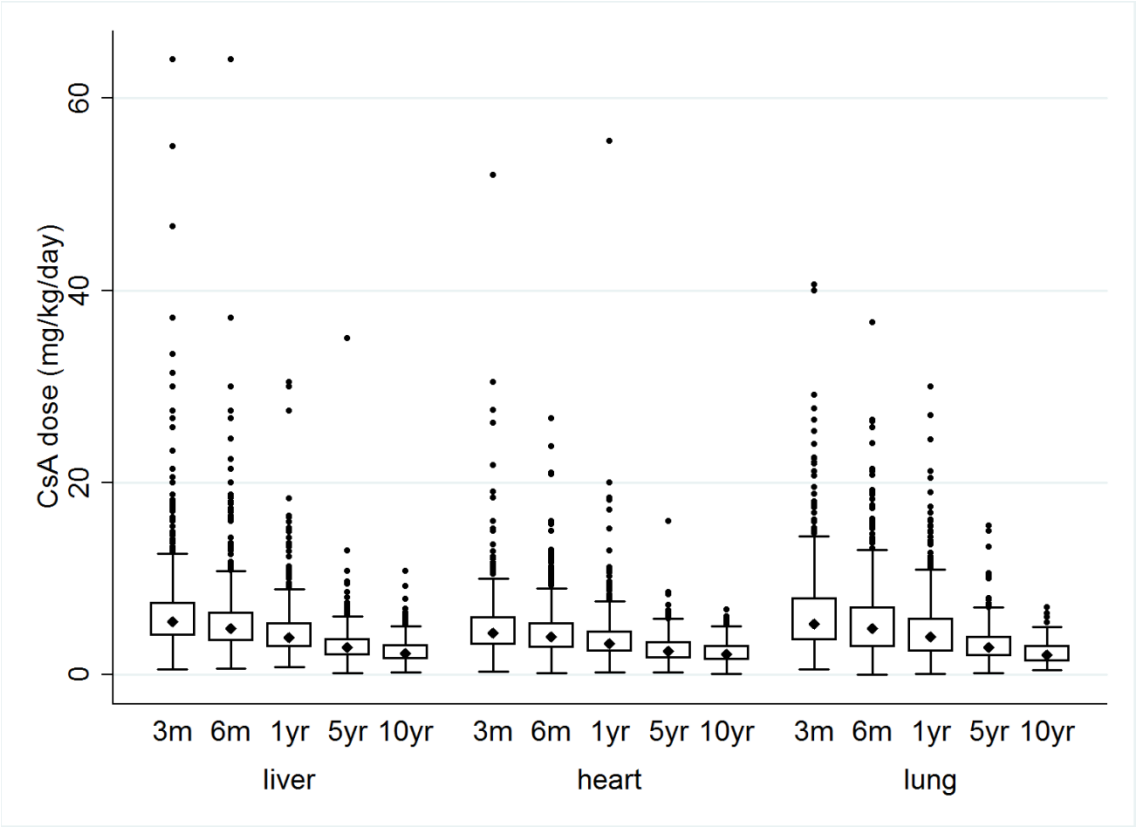


Figure 5 - 12. Change in median (IQR) cyclosporine dose (mg/kg/day) by time since transplantation and organ type, with outliers

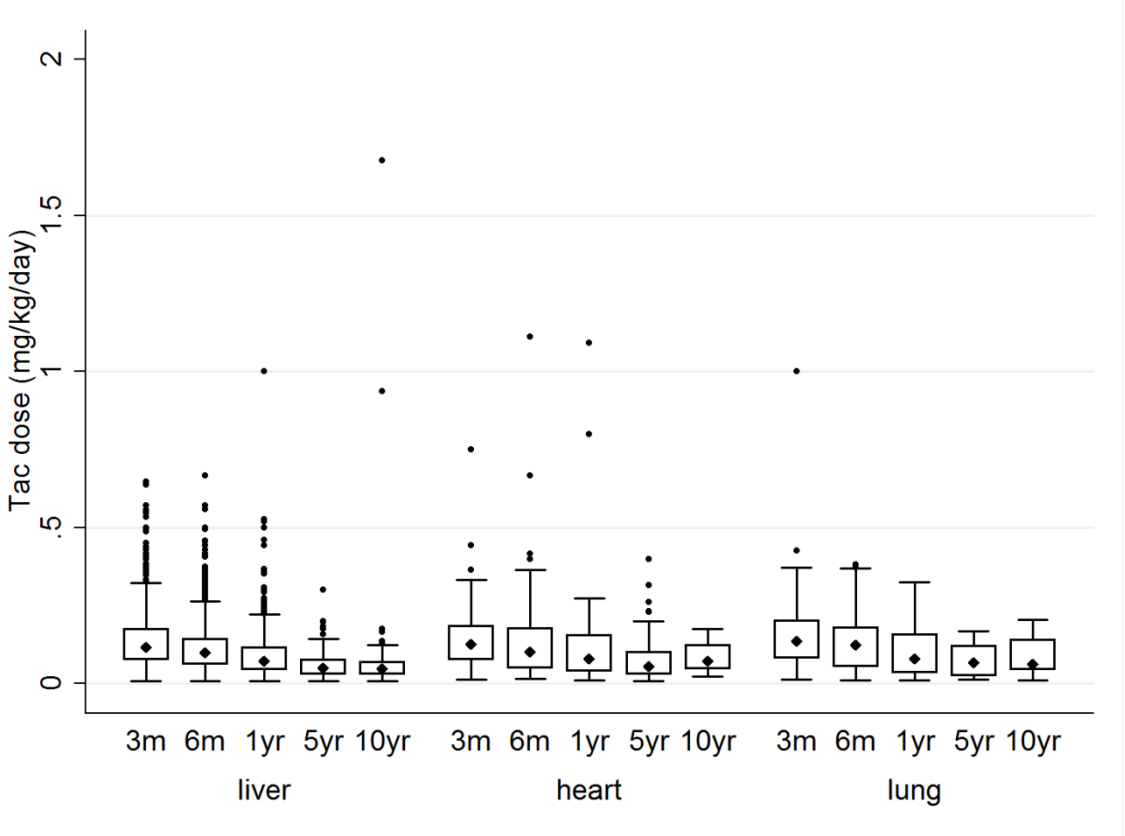


Figure 5 - 13. Change in median (IQR) tacrolimus dose (mg/kg/day) by time since transplantation and organ type, with outliers

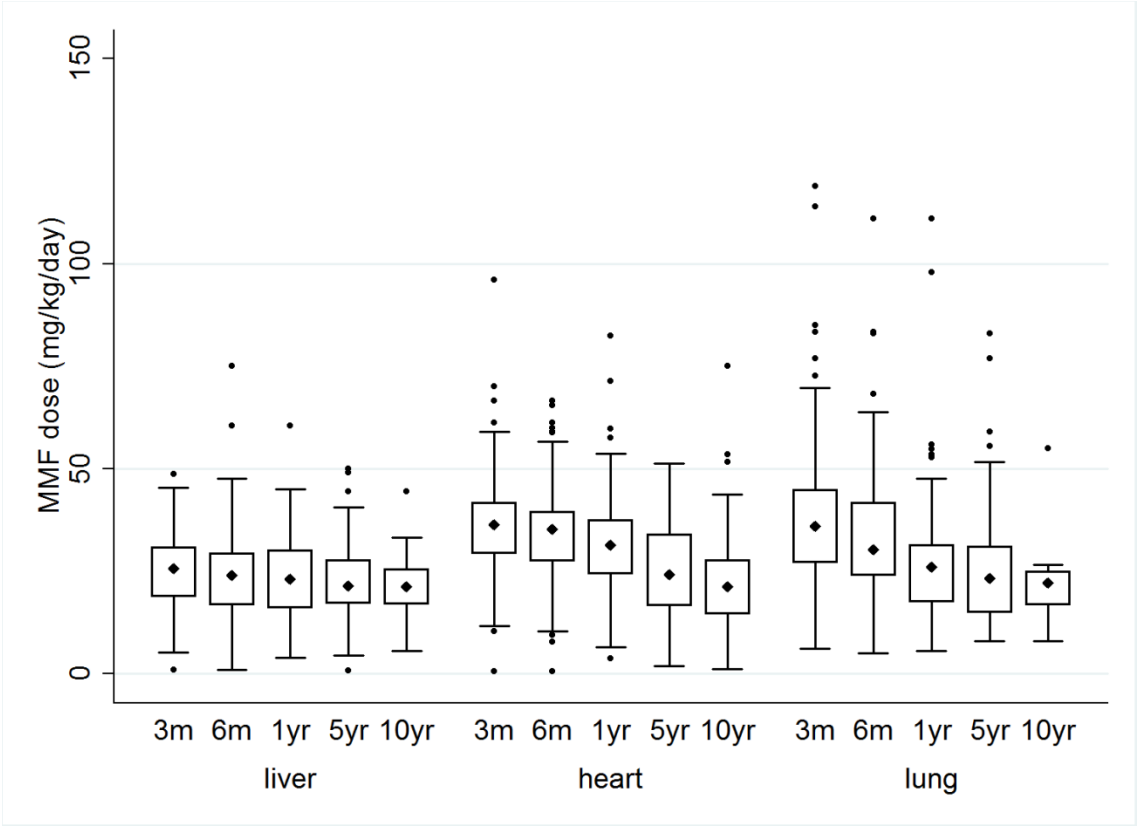


Figure 5 - 14. Change in median (IQR) mycophenolate dose (mg/kg/day) by time since transplantation and organ type, with outliers

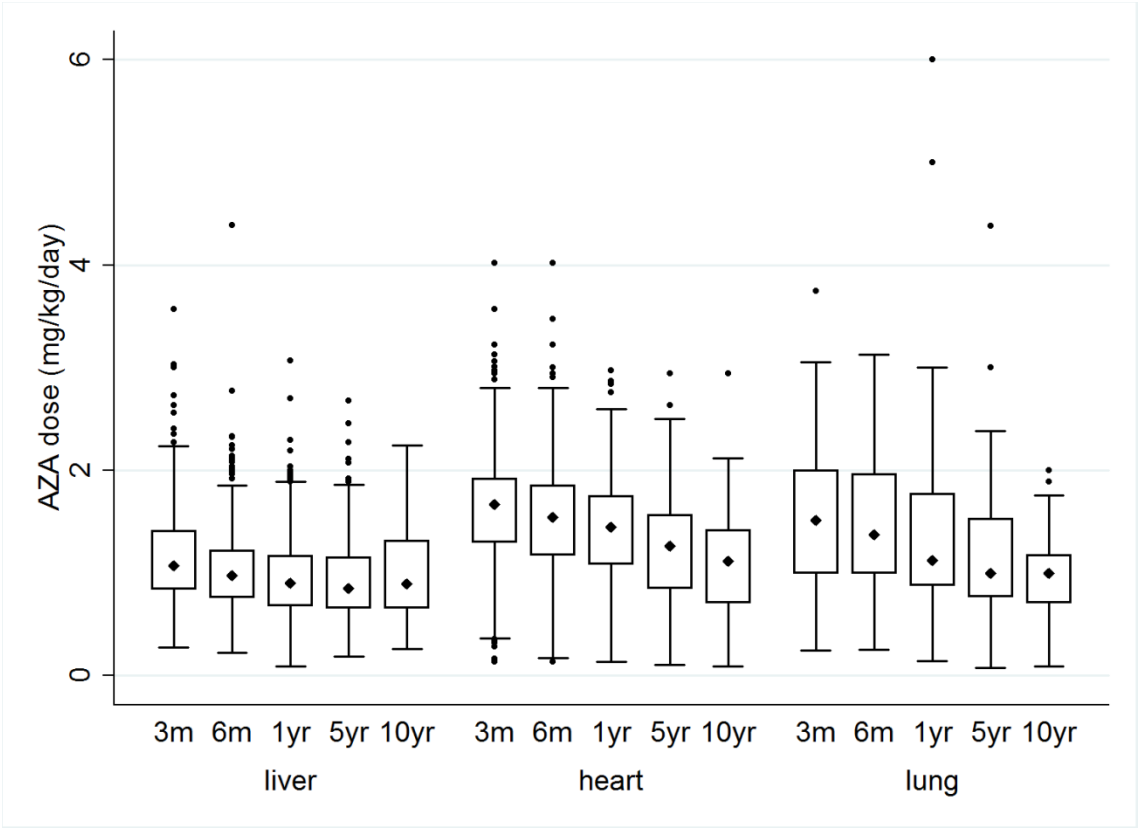


Figure 5 - 15. Change in median (IQR) azathioprine dose (mg/kg/day) by time since transplantation and organ type, with outliers

Appendix II

American Journal of Transplantation 2013; 13: 174–183
Wiley Periodicals Inc.

doi: 10.1111/j.1600-6143.2012.04302.x

Comparison of *De Novo* Cancer Incidence in Australian Liver, Heart and Lung Transplant Recipients

R. Na[a], A. E. Grulich[b], N. S. Meagher[a], G. W. McCaughan[c], A. M. Keogh[d] and C. M. Vajdic[a,*]

[a]*Adult Cancer Program, Lowy Cancer Research Centre, Prince of Wales Clinical School, University of New South Wales, Sydney, Australia*
[b]*Kirby Institute, University of New South Wales, Sydney, Australia*
[c]*The Centenary Research Institute, Australian National Liver Transplant Unit Royal Prince Alfred Hospital and University of Sydney, Sydney, Australia*
[d]*St. Vincent's Hospital, Sydney, Australia*
**Corresponding author: Claire M. Vajdic, claire.vajdic@unsw.edu.au*

Population-based evidence on the relative risk of *de novo* cancer in liver and cardiothoracic transplant recipients is limited. A cohort study was conducted in Australia using population-based liver (n = 1926) and cardiothoracic (n = 2718) registries (1984–2006). Standardized incidence ratios (SIRs) were computed by cancer type, transplanted organ and recipient age. Cox regression models were used to compare cancer incidence by transplanted organ. During a median 5-year follow-up, the risk of any cancer in liver and cardiothoracic recipients was significantly elevated compared to the general population (n = 499; SIR = 2.62, 95%CI 2.40–2.86). An excess risk was observed for 16 cancer types, predominantly cancers with a viral etiology. The pattern of risk by cancer type was broadly similar for heart, lung and liver recipients, except for Merkel cell carcinoma (cardiothoracic only). Seventeen cancers (10 non-Hodgkin lymphomas), were observed in 415 pediatric recipients (SIR = 23.8, 95%CI 13.8–38.0). The adjusted hazard ratio for any cancer in all recipients was higher in heart compared to liver (1.29, 95%CI 1.03–1.63) and lung compared to liver (1.65, 95%CI 1.26–2.16). Understanding the factors responsible for the higher cancer incidence in cardiothoracic compared to liver recipients has the potential to lead to targeted cancer prevention strategies in this high-risk population.

Key words: Cancer, epidemiology, heart transplantation, liver transplantation, lung transplantation, pediatric recipients

Abbreviations: aHR, adjusted hazard ratio; ANZLTR, Australian and New Zealand Liver Transplant Registry; ANZCOTR, Australian and New Zealand Cardiothoracic Organ Transplant Registry; ACD, Australian Cancer Database; BCC, basal cell carcinoma; EBV, Epstein–Barr virus; HPV, human papilloma virus; HR, hazard ratio; IQR, interquartile range; MCC, Merkel cell carcinoma; NDI, National Death Index; NHL, non-Hodgkin lymphoma; NMSC, nonmelanoma skin cancer; PSC, primary sclerosing cholangitis; SCC, squamous cell carcinoma; SIR, standardized incidence ratio; UC, ulcerative colitis.

Received 08 June 2012, revised 10 August 2012 and accepted for publication 29 August 2012

Introduction

Liver and cardiothoracic transplantation are established procedures for patients with end-stage organ failure. In Australia, excellent outcomes are achieved, with 1-year graft survival rates of 80–88% (1,2). Cancer, however, has become the leading cause of death in liver and heart transplant recipients surviving for more than 5 years with a functioning graft (1–4). Immunosuppression is the primary risk factor for cancer in the transplanted population, as reinforced by the remarkably similar cancer profile in those with HIV/AIDS (5).

Population-based cohort studies show a 2.5- to 3-fold excess risk of cancer after solid organ transplantation compared to the general population (5,6). As it is the most common form of transplantation, kidney recipients dominate this estimate, and the spectrum of cancer risk for recipients of other organs is less clear (7–9). Non-population-based estimates suggest that the risk of non-Hodgkin lymphoma (NHL) is highest in lung transplant recipients, followed by heart and liver (10), but the age- and sex-adjusted relative risk of lymphoma and solid cancer between these transplanted organs has not been assessed. Furthermore, few studies have reported cancer incidence by indication for transplantation, and population-level data on cancer in pediatric recipients are scarce (11). A comparison of cancer risk in different subsets of the transplanted population will provide insights into cancer etiology, and thus cancer prevention, in the context of immune suppression. Quantifying and understanding differences in cancer incidence between recipients of different transplanted organs will generate evidence-based clinical strategies for minimizing cancer risk, and for identifying high-risk patient subsets that would benefit most from these interventions.

We estimated the incidence of *de novo* cancer in Australian liver, heart and lung transplant recipients over a 23-year period. We computed cancer risk relative to the general population, and compared cancer risk for recipients of different transplanted organs.

Materials and Methods

Study population

Our study population comprised Australian residents who received a liver or cardiothoracic transplant in Australia between 1984 and 2006. Transplant recipients were registered on the Australian and New Zealand Liver Transplant Registry (ANZLTR) or the Australia and New Zealand Cardiothoracic Organ Transplant Registry (ANZCOTR). These population-based registries recorded all liver and cardiothoracic transplantations since 1985 and 1984, respectively. They systematically recorded the name, date of birth, sex and primary indication for transplantation.

Non-Australian residents were excluded from the study population as they are not eligible for cancer registration in Australia. Patients with a history of cancer prior to transplantation (n = 367), including those whose indication for transplantation was a hepatobiliary tumor (n = 93), were not excluded (6,7,12,13). However, these patients did not contribute person-years at risk for that cancer type. A sensitivity analysis was performed to assess the impact of excluding patients with a history of cancer. In addition, all cancers and person-years follow-up time within 30 days of transplantation (8,14–17) were not included in our analyses. This exclusion was necessary to avoid counting as incident those cancers that were prevalent at transplantation but not registered until they were histopathologically diagnosed in the explanted organ (13). Patients who received a combined liver and kidney transplant (n = 23), and those who received a combined liver, heart and lung transplant (n = 3), were classified as liver transplant recipients; those who received a combined heart and lung transplant (n = 137) were classified as lung transplant recipients.

Data collection

Deaths and incident cancers were ascertained by record linkage with population-based administrative health datasets. Deaths were obtained from the National Death Index (NDI; 1980–2006). Cancers were identified from the Australian Cancer Database (ACD), a register of incident primary invasive neoplasms, other than basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) of the skin which are not ascertained by the Australian population cancer registries. The date of diagnosis, topography and morphology was ascertained for each primary neoplasm diagnosed in the cohort between 1982 and 2006. The Australian cancer registries apply international rules when registering multiple primary cancers. Solid cancers were classified according to the International Classification of Diseases (ICD), 10th revision, while hematopoietic neoplasms and Kaposi sarcomas were classified according to the ICD for Oncology, 3rd edition.

Registrant's name, sex, date of birth, date of death and state of residence were used during record linkage that was performed utilizing an established probabilistic algorithm. A linkage probability or weight was given to each potential matching record pair, and a subset of paired records underwent clerical review.

Cancer incidence rates for the Australian population were obtained from the Australian Cancer Database by 5-year age group, sex, calendar year and state, for 1982–2006.

Ethical approval was obtained and the requirement for informed participant consent was waived because the researchers received only de-identified data.

Data analysis

Cancer incidence: Person-years of follow-up accrued from 30 days post-transplantation until the date of cancer diagnosis, death, age 80, or December 31, 2006, whichever occurred first. Crude and age- and sex-standardized cancer incidence rates (ASR), standardized to the 1996 Australian population, and 95% confidence intervals (CIs; based on the normal approximation to the binomial distribution) were calculated using annual Australian population estimates obtained from the Australian Bureau of Statistics. These rates were computed for any cancer, solid cancers and lymphomas for pediatric recipients (0–15 years) and adult (16+ years) recipients, by transplanted organ.

Risk of cancer relative to the general population: Cancer incidence rates in transplant recipients were compared with the Australian general population using the standardized incidence ratio (SIR), defined as the ratio of the observed and the expected numbers of cancers. The likelihood ratio method was used to calculate 95% CIs for cancers with ≥10 expected cases, while exact CIs were used for cancers with <10 expected cases (18). The expected numbers of incident cancers were calculated by multiplying cohort person-years at risk by the corresponding 5-year age-, sex-, state- and calendar year-specific cancer incidence rates for the Australian population. The exception was Kaposi sarcoma, where 1982 population rates were applied to avoid the impact of AIDS on the incidence of this cancer.

SIRs were computed for the entire cohort and by transplanted organ, recipient age at transplantation (pediatric: ≤15 years), current age (time dependent, 0–39, 40–55, ≥55 years) and primary indication for transplantation. We compared patterns of cancer risk for the different patient subgroups but could not compare SIRs statistically because of the heterogeneity in subgroup age and sex distributions (19).

Comparison of cancer risk by transplanted organ: Within the cohort of all transplant recipients, the risk of any cancer, solid cancers and lymphomas was estimated for pediatric recipients and all recipients. A marginal Cox model, the Wei–Lin–Weissfeld (WLW) model, was applied to enable inclusion of multiple cancers for a recipient (20). Hazard ratios (HR) with 95% confidence intervals (CI) were calculated comparing the incidence of cancer by transplanted organ, adjusted for age at transplant, sex, multiple transplants (one, more than one; time-dependent variable) and calendar period (1984–1989, 1990–1997, 1998–2006). All variables other than the time-dependent covariate satisfied the proportional hazards assumption.

Analyses were performed using SAS® software v9.2 (SAS Institute Inc., Cary, NC, USA). Person-years were calculated using the %*stratify* macro (21) and the WLW marginal model was computed using the PHREG program.

Results

The eligible cohort comprised 4644 transplant recipients; 1926 (41%) liver, 1518 (33%) heart and 1200 (26%) lung (Table 1). Second or higher order transplants were received by 162 (4%) patients. Recipients were followed up for a total of 29 713 person-years. The median duration of follow-up was 5.2 years (interquartile range, IQR 2.0–9.9) and it was highest for heart and lowest for lung recipients. Approximately 9% of patients (n = 415) received a liver or heart transplant before the age of 15 years; no pediatric

Table 1: Characteristics of recipients of liver and cardiothoracic transplants in Australia between 1982 and 2006

	Liver[1]			Heart			Lung[2]		
		Person-years			Person-years			Person-years	
Characteristics	No. (%)	Total	Median[3]	No. (%)	Total	Median[3]	No. (%)	Total	Median[3]
Total	1926 (100)	12703	6.0 (2.2–10)	1518 (100)	11 719	7.1 (2.9–12)	1200 (100)	5292	3.3 (1.5–6.5)
Sex									
Male	1191 (61.8)	7078	4.9 (1.8–8.9)	1218 (80.2)	9 494	7.2 (3.1–12)	641 (53.4)	2827	3.3 (1.5–6.3)
Female	735 (38.2)	5624	6.9 (2.7–12)	300 (19.8)	2 225	6.4 (2.6–11)	559 (46.6)	2464	3.4 (1.4–6.5)
Age at first transplantation (years)									
0–9	248 (13.0)	1906	6.7 (2.7–13)	39 (2.6)	324	8.5 (3.6–13)	1 (0.1)	0.5	–
10–19	104 (5.4)	950	9.5 (3.8–14)	88 (5.8)	639	5.8 (2.0–12)	72 (6.0)	330	3.1 (1.3–6.5)
20–29	93 (4.8)	735	7.1 (3.1–12)	111 (7.3)	793	5.7 (2.8–10)	212 (17.7)	1003	3.6 (1.5–7.5)
30–39	174 (9.0)	1450	8.2 (3.7–13)	168 (11.0)	1 457	8.2 (2.8–14)	189 (15.7)	852	3.1 (1.4–7.1)
40–49	540 (28.0)	3371	5.3 (2.1–9.4)	337 (22.2)	2 920	8.2 (3.9–13)	245 (20.4)	1306	4.4 (1.8–7.7)
50–59	591 (30.7)	3467	4.8 (1.7–9.0)	587 (38.7)	4 517	7.5 (3.1–12)	385 (32.1)	1512	3.1 (1.2–6.0)
≥60	176 (9.1)	824	3.6 (1.5–6.9)	188 (12)	1 067	4.8 (1.7–8.7)	96 (8.0)	288	2.2 (1.2–4.3)
Primary indication for transplantation									
Nonischemic cardiomyopathy	–			692 (45.6)	5 521	7.5 (2.9–12)	–		
Ischemic heart disease	–			582 (38.3)	4 442	6.9 (3.2–12)	–		
COPD[4]	–			–			349 (29.0)	1446	3.5 (1.6–6.0)
Obstructive lung disease	–			–			398 (33.2)	1719	3.2 (1.5–6.4)
Congenital heart disease	–			76 (5)	605	7.9 (2.8–13)	54 (4.5)	359	5.3 (2.1–12)
Viral hepatitis	494 (25.6)	2529	4.1 (1.7–7.9)	–			–		
Hepatobiliary tumor	93 (4.8)	350	2.3 (0.8–5.0)	–			–		
Autoimmune-related liver disease	442 (23)	3374	7.1 (2.6–12)	–			–		
Alcoholic liver disease	223 (11.6)	1406	5.8 (2.6–9.2)	–			–		
Congenital biliary disease	189 (9.8)	1571	7.7 (2.9–13)	–			–		
Miscellaneous	485 (25.5)	3472	6.3 (2.5–11)	168 (11.0)	1 150	5.7 (2.5–11)	399 (33.2)	1767	3.0 (1.2–6.8)

[1]Liver transplant (n = 1900), combined liver and kidney transplant (n = 23), or combined liver, heart and lung transplant (n = 3).
[2]Lung transplant (n = 1063) or combined lung and heart transplant (n = 137).
[3]Median and interquartile range;
[4]Chronic obstructive pulmonary disease.

patients received a lung transplant. The median age at first transplantation was 47 years (IQR 33–55) and it was highest for lung and lowest for liver recipients. Overall, 66% of recipients were male, and the proportion of males by transplanted organ ranged from 53% to 80%. The most common primary indication for transplantation was viral hepatitis (26% of liver), nonischemic cardiomyopathy (46% of heart) and obstructive lung disease (33% of lung).

After transplantation we observed 499 (10.7%) incident primary cancers in 463 patients; 33 patients had multiple cancers. The median age at diagnosis of first cancer was 57 years (IQR 49–63). The most frequently occurring cancers were NHL (n = 100), lip cancer (n = 58) and cutaneous melanoma (n = 50). The crude overall cancer incidence rate was 1679 per 100 000 person-years and the ASR was 1693 per 100 000 (95% CI 832–2553). Rates for all cancers, solid cancers and lymphomas for pediatric and adult recipients by transplanted organ are shown in Table 2.

Cancer risk relative to the general population: all transplant recipients

Risk of any cancer was significantly higher in transplant recipients than the matched Australian population (SIR = 2.62, 95% CI 2.40–2.86). SIRs were greater than unity for 16 different cancers (Figure 1), and relative risk was highest for Kaposi sarcoma, then cancer of the vulva, lip, nonmelanoma skin cancer (NMSC; excluding BCC and SCC) and salivary gland, followed by NHL. Most (n = 6, 75%) salivary gland cancers were squamous cell carcinomas. Risk of some epithelial cancers common in the general population, including prostate, breast and pancreas, was not significantly elevated. Risk of all HPV-related anogenital cancers (cervix, vulva, vagina, anus and penis) was increased (SIR = 6.03, 95% CI 3.12–10.5), as was the risk of all cancers causally related to alcohol consumption (oral cavity, pharynx, esophagus, colorectum, liver, larynx and breast) (22) (SIR = 1.49, 95% CI 1.18–1.86).

Table 2: Cancer incidence in Australian pediatric and adult liver and cardiothoracic transplant recipients

	Pediatric recipients (0–15 years)[1]	Adult recipients (≥16 years)			
		Liver, heart, or lung	Liver	Heart	Lung[2]
Crude incidence rate per 100 000					
Any cancer[3]	516	1825	1404	2195	1873
Solid cancers[3]	152	1431	1140	1749	1332
Lymphoma[4]	364	397	273	446	541
Age- and sex-standardized incidence rate (95% CI)					
Any cancer[3]	468 (233–702)	2060 (944–3176)	1149 (889–1409)	3038 (1534–4542)	2268 (1371–3165)
Solid cancers[3]	156 (14–299)	1701 (588–2814)	931 (696–1166)	2508 (1022–3995)	1714 (860–2569)
Lymphoma[4]	311 (124–498)	361 (272–450)	222 (111–334)	530 (299–761)	553 (271–836)
Standardized incidence ratio (95% CI)					
Any cancer[3]	23.8 (13.8–38.0)	2.54 (2.32–2.78)	2.29 (1.94–2.69)	2.69 (2.36–3.04)	4.28 (3.49–5.19)
Solid cancers[3]	51.3 (16.6–120)	2.26 (2.03–2.49)	2.04 (1.69–2.43)	2.35 (2.04–2.70)	3.36 (2.63–4.22)
Lymphoma[4]	88.5 (45.7–154)	7.82 (6.33–9.53)	5.60 (3.59–8.33)	6.99 (5.02–9.48)	16.8 (11.1–24.4)

[1] Liver and heart transplantations only.
[2] Lung or combined heart and lung transplantation.
[3] Excluding BCC and SCC of the skin.
[4] Includes non-Hodgkin lymphoma, Hodgkin lymphoma and lymphoma not otherwise specified.

Cancer risk relative to the general population: by transplanted organ

Relative to the general population, the risk of any cancer was more than twofold for liver (SIR = 2.20, 95% CI 1.87–2.57) and heart (SIR = 2.64, 95% CI 2.32–2.98) recipients, and more than threefold for lung recipients (SIR = 3.70, 95% CI 3.01–4.48). The risk of lip cancer, NHL and cancer of unknown primary site were significantly elevated for recipients of all three transplanted organs (Supporting Table 1). When recipients with a history of cancer were excluded from the cohort the key findings were unchanged.

The pattern of cancer risk by transplanted organ was similar, with some noteworthy exceptions (Figure 2). Risk of NMSC (other than BCC and SCC) was significantly elevated in both heart (SIR = 32.8, 95% CI 17.9–55.0) and lung (SIR = 61.4, 95% CI 24.7–126) recipients, but there were no incident cases in liver recipients. Seventeen of the 21 skin cancers in cardiothoracic patients were Merkel cell carcinoma (MCC; SIR = 103, 95% CI 60.4–166). In contrast, melanoma risk was significantly increased in liver (SIR = 2.13, 95% CI 1.22–3.46) and heart (SIR = 3.04, 95% CI 2.03–4.36) but not lung (SIR = 1.64, 95% CI 0.53–3.83) recipients. Colorectal cancer risk was significantly raised in liver (SIR = 2.40, 95% CI 1.49–3.68) and lung (SIR = 2.58, 95% CI 1.12–5.09) recipients, but not in heart (SIR = 0.99, 95% CI 0.54–1.63). Only heart and lung transplant recipients exhibited significantly elevated risk of lung cancer (SIR = 2.18, 95% CI 1.39–3.22 and SIR = 3.82, 95% CI 1.65–3.53, respectively).

Cancer risk relative to the general population: by recipient age

Seventeen cases of cancer, including 10 NHL, were observed in 415 pediatric recipients, resulting in SIRs exceeding 20 and 70 for any cancer and lymphoma, respectively (Table 2). Cancer of the vulva, colorectum and breast, as well as NHL and Hodgkin lymphoma, all occurred at significantly increased risk. The median age at diagnosis was 7 years (IQR 6–17) for lymphoma and 30 years (IQR 16–32) for solid cancer.

When considering current or attained age for all recipients combined, a significant excess risk of any cancer, lip cancer, NHL and melanoma was observed for all age groups (Table 3). Colorectal cancer risk was significantly increased in the 0–39 year and 40–55 year age groups and was of borderline significance in the >55 year age group. A significantly elevated risk of skin and lung cancer was observed only for those aged at least 55 years, and risk of cancer of unknown primary site was increased for those more than 40 years of age.

Cancer risk relative to the general population: by indication for transplantation

For liver and cardiothoracic recipients, cancer risk was significantly increased regardless of the indication for transplantation, except for those receiving a liver on account of a hepatobiliary tumor (n = 93; Table 4). After liver transplantation, colorectal cancer risk was increased in those with autoimmune-related liver disease (n = 12; SIR = 4.49, 95% CI 2.32–7.84) but not in those without (SIR = 1.48, 95% CI 0.68–2.82). Similarly, it was increased in those with primary sclerosing cholangitis (PSC, n = 10; SIR = 9.58, 95% CI 4.59–17.6) but not in those without (SIR = 1.43, 95% CI 0.71–2.56), and in those with PSC and ulcerative colitis (UC) (n = 5; SIR = 12.5, 95% CI 4.06–29.1), but not in those without (SIR = 1.32, 95% CI 0.63–2.42).

Comparison of cancer risk by transplanted organ

Pediatric heart recipients were at higher risk of any cancer (aHR 3.10, 95% CI 1.01–9.47) and lymphoma (aHR 6.67, 95% CI 2.37–18.8) compared to liver recipients. For all transplant recipients, the risk of any cancer and of

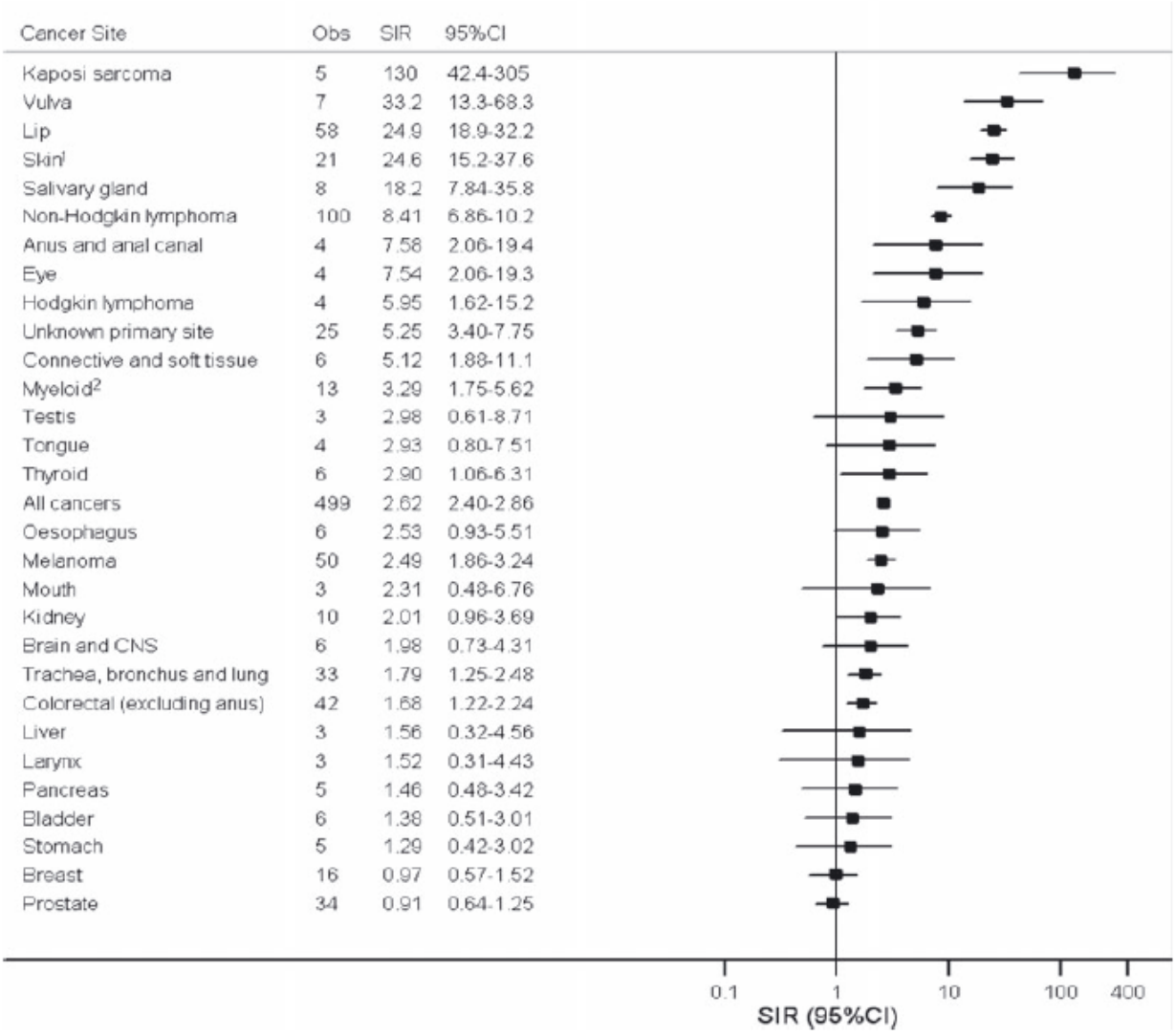


Figure 1: Site-specific cancer risk for Australian liver and cardiothoracic transplant recipients relative to the general population. [1]NMSC, excluding BCC and SCC. [2]Myeloid neoplasms including lymphoid/myeloid not otherwise specified.

lymphoma was significantly greater for heart and lung recipients compared to liver recipients (Table 5), whereas the risk of solid cancer was only increased in lung compared to liver recipients. Solid cancer risk was also increased in lung compared to heart transplant recipients (aHR 1.41, 95% CI 1.03–1.93); there was no significant difference in the risk of any cancer (aHR 1.28, 95% CI 0.98–1.69) or lymphoma (aHR 1.11, 95% CI 0.65–1.89).

Discussion

In this population-based study we found a significantly higher risk of any *de novo* cancer for both heart and lung transplant recipients compared to liver transplant recipients after adjusting for age, sex, multiple transplantations and calendar period of transplantation. An increased risk was also observed for lymphomas and solid cancers, but the excess risk of solid cancers was restricted to lung recipients compared to both liver and heart recipients. Understanding the factors responsible for the greater cancer incidence in heart and lung as compared to liver transplant recipients has the potential to lead to targeted cancer prevention strategies. Compared to the general population matched for age, sex, calendar year and state of residence, the risk of cancer was also increased, from 2.2-fold (liver), to 2.6-fold (heart) and 3.7-fold (lung). The pattern of site-specific risk by transplanted organ was broadly similar, confirming the critical role of immunosuppression in posttransplantation cancer risk. Exceptions to this

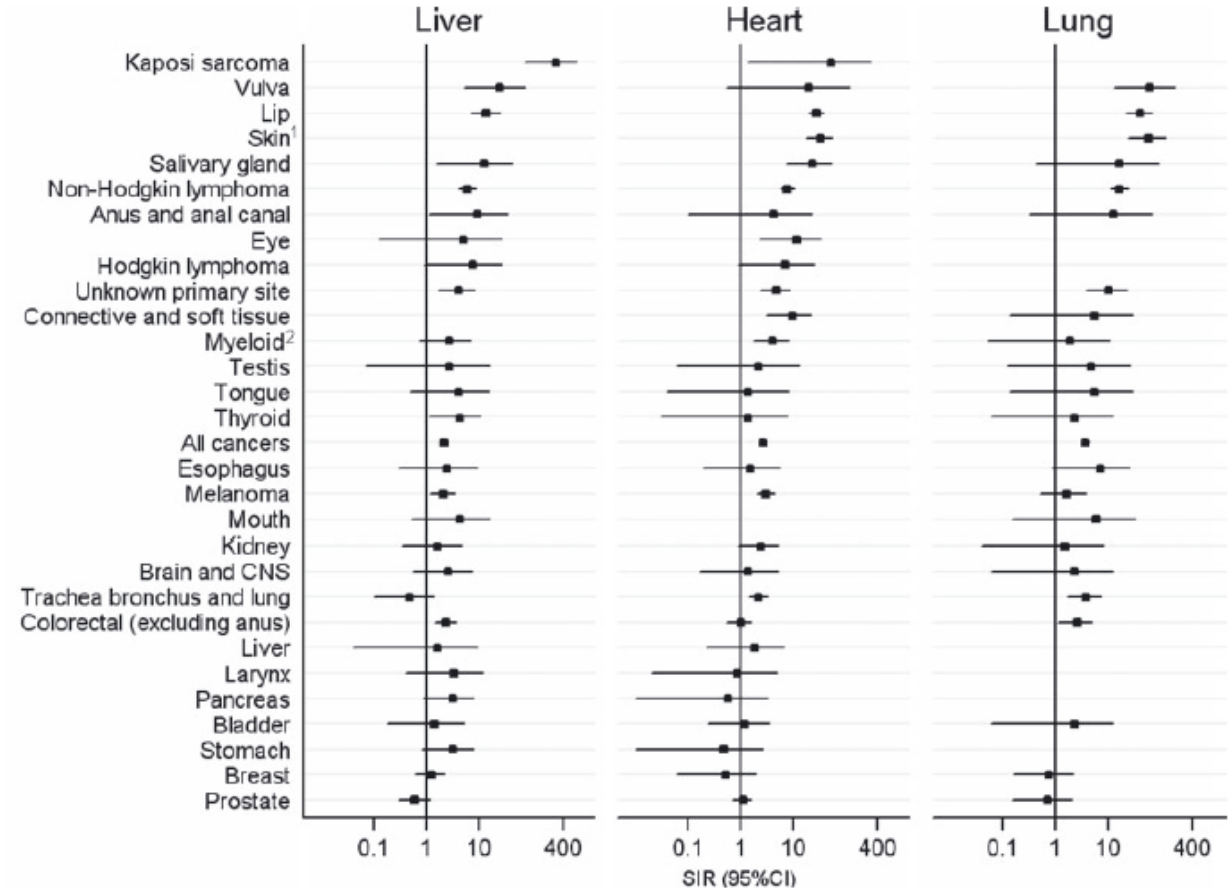


Figure 2: Site-specific cancer risk by transplanted organ relative to the general population. [1]NMSC, excluding BCC and SCC of skin. [2]Myeloid neoplasms including lymphoid/myeloid not otherwise specified.

pattern also suggest patient subgroups at high risk for specific cancer types.

Population-based evidence on the relative risk of cancer in recipients of different types of solid organ is limited. A Swedish study showed an increased risk of NHL for liver, heart and lung transplant recipients relative to kidney transplant recipients, after adjustment for age, sex, year and follow-up time (23). Our twofold higher risk of lymphoma in heart and in lung recipients compared to liver recipients is in line with findings from the Collaborative Transplant Study (10). Our study presents the first population-based evidence of an excess risk of any cancer in heart and lung recipients compared to liver recipients, and a significant increased risk of solid cancer in lung compared to heart and liver transplant recipients.

The key explanation for the difference in cancer risk by transplanted organ is variation in the intensity or type of immunosuppression (15,24,25). Several studies have found an association between posttransplantation cancer risk and receipt of induction therapy with lymphocyte depleting antibodies or maintenance immunosuppression with specific agents (10,26–30). There are no published data directly comparing the dose and type of immunosuppressive agents for liver, heart and lung transplant recipients in Australia. However, Australian clinical transplantation practice has generally followed international trends, with a lower prevalence of induction therapies and lower overall immunosuppressive dose for liver transplant recipients compared to heart and lung recipients (15,31). In addition to differences in the extent and type of immunosuppression by transplanted organ, other factors may also play a role, either independent of or interacting with immunosuppression. These include patient factors, such as prevalent or acquired infection by carcinogenic agents, autoimmune disease, carcinogenic behaviors and genetic predisposition to cancer, as well as inherent biological differences in the transplanted tissue. A greater volume of lymphoid tissue in the lung compared to other organs, and thus greater potential for the transmission of donor lymphocytes infected with Epstein–Barr virus (EBV), has been suggested to explain the higher NHL risk in lung compared to liver transplant recipients (32).

Table 3: Cancer risk by current age for the most frequent incident cancers in Australian recipients of liver and cardiothoracic transplants

	Age 0–39 years				Age 40–55 years				Age >55 years			
Cancer type	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI
All cancers[1]	59	5.14	11.5	8.74–14.8	131	36.7	3.57	2.99–4.22	310	148	2.09	1.87–2.33
Lip	2	0.13	15.9	1.92–57.3	24	0.68	35.4	22.7–52.7	32	1.53	20.9	14.3–29.6
Skin[2]	1	0.04	23.7	0.60–132	1	0.18	5.49	0.14–30.6	19	0.63	30.2	18.2–47.2
Non-Hodgkin lymphoma	24	0.42	56.8	36.4–84.5	38	2.55	14.9	10.5–20.5	38	8.92	4.26	3.01–5.84
Unknown primary site	1	0.05	18.4	0.47–103	5	0.80	6.22	2.02–14.5	19	3.75	5.07	3.05–7.91
Melanoma	7	1.29	5.42	2.18–11.2	14	5.77	2.42	1.33–4.07	29	13.0	2.23	1.51–3.14
Lung	0	-	-	-	5	2.46	2.03	0.66–4.74	28	15.8	1.77	1.19–2.50
Colorectal[3]	3	0.21	14.1	2.91–41.2	9	4.11	2.19	1.00–4.16	30	20.7	1.45	0.99–2.03
Prostate	0	–	–	–	1	2.63	0.38	0.01–2.12	33	34.7	0.95	0.66–1.31

Obs, observed number of cancers; Exp, expected number of cancers; SIR, standardized incidence ratio.
[1] Excluding BCC and SCC of the skin.
[2] NMSC, excluding BCC and SCC of the skin.
[3] Excluding anal cancer.

Table 4: Cancer risk by indication for transplantation in Australian recipients of liver or cardiothoracic transplants

		All cancers[1]			
Transplanted organ(s)	Primary indication	Obs	Exp	SIR	95% CI
Liver	Viral hepatitis	30	16.8	1.79	1.22–2.50
	Hepatobiliary tumor	2	2.13	0.94	0.11–3.39
	Autoimmune-related liver disease	60	21.4	2.80	2.15–3.56
	Alcoholic liver disease	24	12.6	1.91	1.24–2.77
	Congenital biliary disease	4	0.51	7.83	2.13–20.0
	Miscellaneous liver disease	33	16.0	2.06	1.44–2.85
Cardiothoracic	Non-ischemic cardiomyopathy	105	36.4	2.88	2.36–3.47
	Ischemic heart disease	116	50.0	2.32	1.91–2.78
	Congenital heart disease	12	2.11	5.67	3.04–9.51
	Obstructive lung disease	23	3.62	6.35	4.03–9.53
	Chronic obstructive pulmonary disease	35	12.6	2.78	1.96–3.81
	Miscellaneous cardiothoracic disease	55	15.9	3.46	2.63–4.46

Obs, observed number of cancers; Exp, expected number of cancers; SIR, standardized incidence ratio.
[1] Excluding BCC and SCC of the skin.

When the liver and cardiothoracic transplant recipients were considered together, our data agree with prior evidence showing a wide-ranging excess cancer risk relative to the general population (5,6), especially cancers with a viral cause. The pattern of site-specific cancer risk by transplanted organ was broadly similar, and also largely consistent with prior population-based evidence for liver (7,9,12,15,33–36) and heart (8,15,37) transplantation. Our study adds to existing evidence showing an increased risk of NHL, Kaposi sarcoma, colorectal cancer, lip cancer and cancer of unknown primary site after liver transplantation. Novel findings were an excess risk of cutaneous melanoma and cancer of the thyroid, vulva, anus and salivary gland. We did not confirm previously published findings of an excess risk of cancer of the liver (6), lung (12,15,33), oral cavity (15) or kidney (34) after liver transplantation.

Our estimate for colorectal cancer risk after liver transplantation (SIR = 2.40, 95% CI 1.49–3.68) is similar to a meta-estimate for prior population-based studies (SIR = 2.6, 95% CI 1.7–4.1) (38). PSC is an indication for liver transplantation, and 60–80% of individuals with PSC also have inflammatory bowel disease, predominantly UC. It is established that patients with UC are at high risk of colorectal cancer (39,40), and findings from a single retrospective study suggest that transplantation may not alter this inherently high risk (41). In our study, an excess risk of colorectal cancer was confined to liver transplant recipients with a history of PSC and UC. These data support the use of screening in these patients to enable the early diagnosis of UC and colorectal adenomas.

Consistent with prior population-based studies (6,8,15), we identified an excess risk of NHL, lip cancer and lung cancer in heart transplant recipients. However, we did not observe an increased risk of kidney (6,8,15), oral cancer (8,15) or multiple myeloma (8,15). We quantified the excess risk of cutaneous melanoma in Australian heart transplant recipients (42), and we also identified novel associations; an elevated risk of MCC, myeloid neoplasms and cancer of the salivary gland, eye and unknown primary site. The increased risk of MCC, a neuroendocrine skin cancer possibly associated with infection by Merkel cell polyomavirus (MCPyV) (43), is in broad agreement with the only prior

Table 5: Risk factors for cancer in Australian recipients of liver and cardiothoracic transplants

	Adjusted hazard ratio (95% CI)[1]		
	All cancers	All solid cancers	All lymphomas
Age at first transplantation (single years)	1.04 (1.03–1.05)	1.05 (1.04–1.06)	1.00 (0.98–1.01)
Sex			
Male	1.00 (ref)	1.00 (ref)	1.00 (ref)
Female	0.80 (0.63–1.00)	0.74 (0.56–0.97)	0.99 (0.63–1.56)
Number of transplants[2]			
One	1.00 (ref)	1.00 (ref)	1.00 (ref)
More than one	0.78 (0.36–1.69)	0.56 (0.21–1.50)	1.09 (0.34–3.45)
Calendar period of first transplantation			
1984–1989	0.96 (0.72–1.29)	1.15 (0.83–1.59)	0.51 (0.25–1.07)
1990–1997	1.00 (ref)	1.00 (ref)	1.00 (ref)
1998–2006	0.73 (0.57–0.94)	0.73 (0.55–0.97)	0.69 (0.42–1.12)
Transplanted organ			
Liver	1.00 (ref)	1.00 (ref)	1.00 (ref)
Heart	1.29 (1.03–1.63)	1.15 (0.88–1.49)	1.89 (1.14–3.14)
Lung	1.66 (1.27–2.17)	1.61 (1.17–2.21)	2.10 (1.25–3.54)

[1]Adjusted for age at transplant (single years), sex, number of transplants (as a time-dependent covariate) and calendar year at first transplantation.
[2]Time dependent; yes from second transplant.

estimate posttransplantation, an SIR of 66 for Finnish kidney transplant recipients (44). The recent report of an excess risk of Merkel cell carcinoma in US HIV/AIDS patients (SIR = 11, 95% CI 6.3–17) (45) supports an association between risk of this cancer and immunosuppression. However, the absence of MCCs in our cohort of liver transplant recipients suggests that MCC risk may not be related to immunosuppression *per se*, and that the intensity or types of immunosuppression or other factors are likely to be important.

In our study, lung transplantation was associated with an increased risk of NHL, MCC and cancer of the vulva, lip, lung, colorectum and unknown primary site. Our findings thus confirm prior population-based evidence of an excess risk of NHL and lung cancer (6,15), but not anal cancer (15).

There are scarce data on cancer risk in pediatric liver and heart transplant recipients (7,11). We found an increased risk of NHL, skin cancer and vulvar cancer, concurring with a Swedish pediatric cohort consisting mostly of kidney transplant recipients (11). However, the excess risk of colorectal cancer, breast cancer and Hodgkin lymphoma we observed have not previously been reported, and require validation in larger cohorts. Notably, most solid cancers occurred in adulthood, highlighting the need for increased clinical surveillance during this phase of life. The striking increased risk of NHL in childhood has consistently been observed (8,46) and has been associated with EBV seroconversion (47) and intensity of immunosuppression (48). As a result, antiviral prophylaxis and targeted monitoring of EBV viral load in peripheral blood is recommended for high-risk pediatric patients (49).

Several potential limitations must be considered when interpreting our findings. The risk of basal cell and squamous cell skin cancer could not be estimated because these neoplasms are not recorded by all Australian cancer registries; consequently, our estimates for 'any cancer' risk exclude these cancers. In addition, it is possible that a small number of cancers were donor derived. Moreover, this study relied upon record linkage between transplant registries and routinely collected administrative data, and some false positive and negative linkages do occur. Nevertheless, the record linkage is highly sensitive and specific (50), and linkage errors are unlikely to occur differentially by transplanted organ or cancer type. On the other hand, a potential bias would arise if there was differential participation in national cancer screening programs (breast, cervical and colorectal cancer) by transplant recipients compared to the general population, or by transplanted organ. While there is some evidence that Australian kidney transplant recipients undergo more screening (51), with the exception of patients with PSC, there is no data on screening rates for liver, heart and lung transplant recipients. Furthermore, surveillance bias is unlikely to explain differences between transplanted organs in the risk of other solid cancers or of lymphomas. While linkage accuracy is known, there has been no formal validation of linkage completeness, and we were unable to censor upon migration from Australia. Nevertheless, given the high quality of Australian cancer registries, we expect to have identified most incident cancers. Finally, we did not adjust the p-value for statistical significance ($p < 0.05$) to take into account multiple statistical tests, and thus it is possible that some of our findings may be due to chance.

The key strengths of this study are the population basis for inclusion of transplant recipients and for ascertaining deaths and cancers. The use of identical methods for transplant recipients, the general population, and for the different transplanted organs, enabled unbiased comparison of risk and also minimized the influence of selection

bias and loss to follow-up. The study included site-specific cancer risk estimates for the largest population-based series of nonkidney pediatric transplant recipients published to date. The relatively large population size also allowed the risk of some rare cancers, such as MCC, to be estimated. Finally, the systematic recording of indication for transplantation by the transplant registries allowed insight into patient subgroups at high risk.

Conclusions

This study found evidence of a higher risk of cancer in heart and lung compared to liver transplant recipients in Australia. Understanding the factors responsible for these associations is expected to lead to strategies that reduce the cancer burden facing this high-risk patient group. Knowledge of the cancer profile by transplanted organ and patient age will facilitate early detection and improve patient outcomes.

Acknowledgments

This study was funded by the National Health and Medical Research Council (ID510254). A.G. is supported by an NHMRC principal research fellowship (ID568819). C.V. is supported by a National Health and Medical Research Council Career Development Fellowship (ID1023159) and a Cancer Institute New South Wales Career Development Fellowship (ID10/CDF/242). R.N. is supported by a Translational Cancer Research Network PhD scholarship top-up award.

We thank the Australian and New Zealand Liver Transplant Registry, the Australian and New Zealand Cardiothoracic Organ Transplant Registry, and also the Australian Institute of Health and Welfare for conducting the data linkage.

We thank: Phyllis Larkins (Royal Prince Alfred Hospital, Sydney); Geraldine Lipka (Princess Alexandra Hospital, Brisbane); Cassandra Kastaneas (St Vincent's Hospital, Sydney); Vicki Jermyn and Brooke Andersen (The Children's Hospital, Westmead); Jo Maddicks-Law, Nicole Ostenfeld, Sara Gray and Muhtashimuddin Ahmed (Prince Charles Hospital, Brisbane); Kerrie Beale (Royal Childrens Hospital, Brisbane); Libby John, Nicole Williams (Flinders Medical Centre, Adelaide); Kathryn Marshall, Ailsa Cowie, Connie Kambanaros, Jasmin Board and Colleen Farrell (Alfred Hospital, Melbourne); Lyn Crellin, Kathe Beyerle, Kate Schurmann, Anne Shipp, Janette McEwan, Danielle Kamolins, Angie Wood and Hollie Gilmore (Royal Childrens Hospital, Melbourne); Julie Pavlovic and Betheia Lele (Austin Hospital, Melbourne); Sharon Lawrence, Clare Wood and Sharlene Beinke (Royal Perth Hospital); Barb Chester, Judith Bull, Joanne Plummer, Nikki Copland and Megan O-Dea (Sir Charles Gairdner Hospital, Perth).

The following lead investigators participated in this study: George Alex, Glenda Balderson, Peter Bergin, John Chen, Weng Chin, Lawrence Dembo, Pamela Dilworth, Looi Ee, Allan Glanville, Winita Hardikar, Peter Hopkins, George Javorsky, Garry Jeffrey, Robert Jones, Bronwyn Levvey, Steven Lynch, Michael Musk, Ross Pettersson, Greg Snell, Michael Stormon and Robert Weintraub.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

References

1. ANZLTR. ANZLT Registry Report 2010 Australia and New Zealand Liver Transplant Registry. Available at: http://www.anzltr.org/statistics.html. Accessed October 3, 2012.
2. ANZCOTR. The Australia and New Zealand Cardiothoracic Organ Transplant Registry Fifteenth Annual Report 1984–2010. Available at: http://www.anzcotr.org.au/. Accessed October 2, 2012.
3. Stehlik J, Edwards LB, Kucheryavaya AY, et al. The registry of the international society for heart and lung transplantation: Twenty-seventh official adult heart transplant report—2010. J Heart Lung Transplant 2010; 29: 1089–1103.
4. Watt KDS, Pedersen RA, Kremers WK, Heimbach JK, Charlton MR. Evolution of causes and risk factors for mortality post-liver transplant: Results of the NIDDK long-term follow-up study. Am J Transplant 2010; 10: 1420–1427.
5. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: A meta-analysis. Lancet 2007; 370: 59–67.
6. Engels EA, Pfeiffer RM, Fraumeni JF, et al. Spectrum of cancer risk among US solid organ transplant recipients. JAMA 2011; 306: 1891–1901.
7. Aberg F, Pukkala E, Hockerstedt K, Sankila R, Isoniemi H. Risk of malignant neoplasms after liver transplantation: A population-based study. Liver Transplant 2008; 14: 1428–1436.
8. Jiang Y, Villeneuve PJ, Wielgosz A, Schaubel DE, Fenton SS, Mao Y. The incidence of cancer in a population-based cohort of Canadian heart transplant recipients. Am J Transplant 2010; 10: 637–645.
9. Jiang Y, Villeneuve PJ, Fenton SS, Schaubel DE, Lilly L, Mao Y. Liver transplantation and subsequent risk of cancer: Findings from a Canadian cohort study. Liver Transplant 2008; 14: 1588–1597.
10. Opelz G, Dohler B. Lymphomas after solid organ transplantation: A collaborative transplant study report. Am J Transplant 2004; 4: 222–230.
11. Simard JF, Baecklund E, Kinch A, et al. Pediatric organ transplantation and risk of premalignant and malignant tumors in Sweden. Am J Transplant 2011; 11: 146–151.
12. Oo YH, Gunson BK, Lancashire RJ, Cheng KK, Neuberger JM. Incidence of cancers following orthotopic liver transplantation in a single center: Comparison with national cancer incidence rates for England and Wales. Transplantation 2005; 80: 759–764.
13. Vajdic CM, McDonald SP, McCredie MR, et al. Cancer incidence before and after kidney transplantation. JAMA 2006; 296: 2823–2831.
14. Adami J, Gabel H, Lindelof B, et al. Cancer risk following organ transplantation: A nationwide cohort study in Sweden. Br J Cancer 2003; 89: 1221–1227.
15. Collett D, Mumford L, Banner NR, Neuberger J, Watson C. Comparison of the incidence of malignancy in recipients of different types of organ: A UK registry audit. Am J Transplant 2010; 10: 1889–1896.
16. Quinlan SC, Landgren O, Morton LM, Engels EA. Hodgkin lymphoma among US solid organ transplant recipients. Transplantation 2010; 90: 1011–1015

17. Quinlan SC, Pfeiffer RM, Morton LM, Engels EA. Risk factors for early-onset and late-onset post-transplant lymphoproliferative disorder in kidney recipients in the United States. Am J Hematol 2011; 86: 206–209.
18. Swift MB. Comparison of confidence intervals for a poisson mean—Further considerations. Commun Stat Theory Methods 2009; 38: 748–759.
19. Rothman KJ, Greenland S, Lash TL. Modern Epidemiology. 3rd Ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2008; p. 254.
20. Wei LJ, Lin DY, Weissfeld L. Regression analysis of multivariate incomplete failure time data by modeling marginal distributions. J Am Stat Assoc 1989; 84: 1065–1073.
21. Rostgaard K. Methods for stratification of person-time and events—A prerequisite for poisson regression and SIR estimation. Epidemiol Perspect Innov 2008; 5: 7.
22. Secretan B, Straif K, Baan R, et al. A review of human carcinogens—part E: Tobacco, areca nut, alcohol, coal smoke, and salted fish. Lancet Oncol 2009; 10: 1033–1034.
23. Fernberg P, Edgren G, Adami J, et al. Time trends in risk and risk determinants of non-hodgkin lymphoma in solid organ transplant recipients. Am J Transplant 2011; 11: 2472–2482.
24. Euvrard S, Kanitakis J, Pouteil-Noble C, et al. Comparative epidemiologic study of premalignant and malignant epithelial cutaneous lesions developing after kidney and heart transplantation. J Am Acad Dermatol 1995; 33: 222–229.
25. Ong CS, Keogh AM, Kossard S, Macdonald PS, Spratt PM. Skin cancer in Australian heart transplant recipients. J Am Acad Dermatol 1999; 40: 27–34.
26. Caillard S, Lamy FX, Quelen C, et al. Epidemiology of posttransplant lymphoproliferative disorders in adult kidney and kidney pancreas recipients: Report of the french registry and analysis of subgroups of lymphomas. Am J Transplant 2012; 12: 682–693.
27. van Leeuwen MT, Grulich AE, Webster AC, et al. Immunosuppression and other risk factors for early and late non-hodgkin lymphoma after kidney transplantation. Blood 2009; 114: 630–637.
28. Dantal J, Hourmant M, Cantarovich D, et al. Effect of long-term immunosuppression in kidney-graft recipients on cancer incidence: Randomised comparison of two cyclosporin regimens. Lancet 1998; 351: 623–628.
29. Opelz G, Henderson R. Incidence of non-hodgkin lymphoma in kidney and heart transplant recipients. Lancet 1993; 342: 1514–1516.
30. Swinnen LJ, Costanzo-Nordin MR, Fisher SG, et al. Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. N Engl J Med 1990; 323: 1723–1728.
31. Wiesner RH, Fung JJ. Present state of immunosuppressive therapy in liver transplant recipients. Liver Transpl 2011; 17: S1-S9.
32. Cockfield SM. Identifying the patient at risk for post-transplant lymphoproliferative disorder. Transpl Infect Dis 2001; 3: 70–78.
33. Finkenstedt A, Graziadei IW, Oberaigner W, et al. Extensive surveillance promotes early diagnosis and improved survival of de novo malignancies in liver transplant recipients. Am J Transplant 2009; 9: 2355–2361.
34. Haagsma EB, Hagens VE, Schaapveld M, et al. Increased cancer risk after liver transplantation: A population-based study. J Hepatol 2001; 34: 84–91.
35. Jain AB, Yee LD, Nalesnik MA, et al. Comparative incidence of de novo nonlymphoid malignancies after liver transplantation under tacrolimus using surveillance epidemiologic end result data. Transplantation 1998; 66: 1193–1200.
36. Baccarani U, Piselli P, Serraino D, et al. Comparison of de novo tumours after liver transplantation with incidence rates from Italian cancer registries. Dig Liver Dis 2010; 42: 55–60.
37. Kellerman L, Neugut A, Burke B, Mancini D. Comparison of the incidence of de novo solid malignancies after heart transplantation to that in the general population. Am J Cardiol 2009; 103: 562–566.
38. Sint Nicolaas J, Tjon AS, Metselaar HJ, Kuipers EJ, de Man RA, van Leerdam ME. Colorectal cancer in post-liver transplant recipients. Dis Colon Rectum 2010; 53: 817–821.
39. Lieberman DA. Screening for colorectal cancer. N Engl J Med 2009; 361: 1179–1187.
40. Danese S, Fiocchi C. Ulcerative colitis. N Engl J Med 2011; 365: 1713–1725.
41. Hanouneh IA, Macaron C, Lopez R, Zein NN, Lashner BA. Risk of colonic neoplasia after liver transplantation for primary sclerosing cholangitis. Inflamm Bowel Dis 2012; 18: 269–274.
42. Roithmaier S, Haydon AM, Loi S, et al. Incidence of malignancies in heart and/or lung transplant recipients: A single-institution experience. J Heart Lung Transplant 2007; 26: 845–849.
43. Schrama D, Ugurel S, Becker JC. Merkel cell carcinoma: Recent insights and new treatment options. Curr Opin Oncol 2012; 24: 141–149
44. Koljonen V, Kukko H, Tukiainen E, et al. Incidence of merkel cell carcinoma in renal transplant recipients. Nephrol Dial Transplant 2009; 24: 3231–3235.
45. Lanoy E, Dores GM, Madeleine MM, Toro JR, Fraumeni JF, Jr., Engels EA. Epidemiology of nonkeratinocytic skin cancers among persons with AIDS in the United States. AIDS 2009; 23: 385–393.
46. Villeneuve PJ, Schaubel DE, Fenton SS, Shepherd FA, Jiang Y, Mao Y. Cancer incidence among Canadian kidney transplant recipients. Am J Transplant 2007; 7: 941–948.
47. Katz BZ, Pahl E, Crawford SE, et al. Case–control study of risk factors for the development of post-transplant lymphoproliferative disease in a pediatric heart transplant cohort. Pediatr Transplant 2007; 11: 58–65.
48. Schubert S, Renner C, Hammer M, et al. Relationship of immunosuppression to Epstein-Barr viral load and lymphoproliferative disease in pediatric heart transplant patients. J Heart Lung Transplant 2008; 27: 100–105.
49. Lee TC, Savoldo B, Rooney CM, et al. Quantitative EBV viral loads and immunosuppression alterations can decrease PTLD incidence in pediatric liver transplant recipients. Am J Transplant 2005; 5: 2222–2228.
50. Grulich AE, Wan X, Coates M, Day P, JM. Kaldor. Validation of a non-identifying method of linking cancer and AIDS register data. J Epidemiol Biostat 1996; 1: 207–212.
51. Wong G, Webster AC, Chapman JR, Craig JC. Reported cancer screening practices of nephrologists: Results from a national survey. Nephrol Dial Transplant 2009; 24: 2136–2143.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1: Site-specific SIRs for Australian transplant recipients by transplant type (Figure 2)

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

American Journal of Transplantation 2013; 13: 1296–1304
Wiley Periodicals Inc.

doi: 10.1111/ajt.12192

De novo Cancer-Related Death in Australian Liver and Cardiothoracic Transplant Recipients

R. Na[a], A. E. Grulich[b], N. S. Meagher[a], G. W. McCaughan[c], A. M. Keogh[d] and C. M. Vajdic[a,*]

[a]*Adult Cancer Program, Lowy Cancer Research Centre, Prince of Wales Clinical School, University of New South Wales, Sydney, Australia*
[b]*Kirby Institute, University of New South Wales, Sydney, Australia*
[c]*The Centenary Research Institute, Australian National Liver Transplant Unit Royal Prince Alfred Hospital and University of Sydney, Sydney, Australia*
[d]*St Vincent's Hospital, Sydney, Australia*
[*]*Corresponding author: Claire M. Vajdic, claire.vajdic@unsw.edu.au*

Evidence is sparse on the relative mortality risk posed by *de novo* cancers in liver and cardiothoracic transplant recipients. A retrospective cohort study was conducted in Australia using population-based liver (n = 1926) and cardiothoracic (n = 2718) registries (1984–2006). Standardized mortality ratios (SMRs) were computed by cancer type, transplanted organ, recipient age and sex. During a median 5-year follow-up, *de novo* cancer-related mortality risk in liver and cardiothoracic recipients was significantly elevated compared to the matched general population (n = 171; SMR = 2.83; 95% confidence interval [95%CI], 2.43–3.27). Excess risk was observed regardless of transplanted organ, recipient age group or sex. Non-Hodgkin lymphoma was the most common cancer-related death (n = 38; SMR = 16.6; 95%CI, 11.87–22.8). The highest relative risk was for nonmelanocytic skin cancer (n = 23; SMR = 49.6, 95%CI, 31.5–74.5), predominantly in males and in recipients of heart and lung transplants. Risk of death from *de novo* cancer was high in pediatric recipients (n = 5; SMR = 41.3; 95%CI, 13.4–96.5), four of the five deaths were non-Hodgkin lymphoma. *De novo* cancer was a leading cause of late death, particularly in heart and liver transplantation. These findings support tailored cancer prevention strategies, surveillance to promote early detection, and guidelines for managing immunosuppression once cancer occurs.

Key words: Cancer, mortality, heart transplantation, liver transplantation, lung transplantation, pediatric transplantation

Abbreviations: ANZLTR, Australian and New Zealand Liver Transplant Registry; ANZCOTR, Australian and New Zealand Cardiothoracic Organ Transplant Registry; ACD, Australian Cancer Database; BCC, basal cell carcinoma; EBV, Epstein Barr virus; HPV, human papilloma virus; IQR, interquartile range; NDI, National Death Index; NHL, non-Hodgkin lymphoma; SCC, squamous cell carcinoma; SMR, standardized mortality ratio.

Received 16 October 2012, revised 14 December 2012 and accepted for publication 03 January 2013

Introduction

Immunosuppressive therapy after solid organ transplantation is widely reported to be associated with a two- to threefold increased risk of cancer (1–3), but comparatively few studies have quantified the risk of death from cancer in this population (4–8). Furthermore, some of these studies did not exclude deaths from recurrent cancers (6–8), and were limited by a large proportion of deaths of unknown cause (6,7). Documenting the extent and pattern of risk for *de novo* cancer-related death in transplant recipients will guide the identification of high-risk patient subgroups, potentially leading to further improvements in long-term survival.

We aimed to quantify the overall and site-specific risk of death from *de novo* cancer in Australian liver and cardiothoracic transplant recipients over a 23-year period using a population-based record linkage study design.

Materials and Methods

Study population

Our study included all Australian recipients of cardiothoracic and liver transplants between 1984 and 2006. Transplant recipients were registered on the Australian and New Zealand Liver Transplant Registry (ANZLTR) or the Australian and New Zealand Cardiothoracic Organ Transplant Registry (ANZCOTR). These national population-based registries recorded all liver and cardiothoracic transplantations since 1985 and 1984, respectively, and systematically record demographic and clinical data about each recipient.

Recipients with single (n = 4482) and second or and higher-order transplants (n = 162) were included in the analyses. Non-Australian recipients and patients who died within 30 days of transplantation (n = 287) were excluded from the study population. Patients with a history of cancer before transplantation (n = 367), including those whose indication for transplantation was a hepatobiliary tumor (n = 93), were not excluded because these patients were at risk of death from a different *de novo* cancer. However, these patients did not contribute person-years at risk for death from these cancers. A sensitivity analysis was performed to

assess the impact of excluding patients with a history of cancer (before 30-day posttransplantation). Patients who received a combined liver and kidney transplant (n = 23), and those who received a combined liver, heart and lung transplant (n = 3), were classified as liver transplant recipients; those who received a combined heart and lung transplant (n = 137) were classified as lung transplant recipients.

Death and cancer ascertainment

Deaths were identified by record linkage with the National Death Index, a registry of all deaths in Australia since 1980, or from the transplant registry. The underlying cause of death was available for deaths ascertained from the National Death Index, coded to the 9th International Classification of Diseases, 9th revision (ICD-9), between 1984 and 1997, and ICD-10 from 1998 onwards. Deaths identified from the transplant registries alone had an unknown underlying cause and thus could not be included in cause-specific analyses. Cancers were ascertained by record linkage with the Australian Cancer Database (ACD), a register of incident primary invasive neoplasms in Australian residents. The date of diagnosis, topography and morphology was ascertained for each cancer diagnosed between 1984 and 2006. Record linkage was performed by the Australian Institute of Health and Welfare utilizing an established probabilistic record linkage algorithm (9).

Australian population mortality rates for any cancer and site-specific cancers were obtained from the Australian Institute of Health and Welfare by five-year age group, sex, calendar year and State or Territory, for 1984–2006.

Ethical approval was obtained and the requirement for informed participant consent was waived because the researchers received only de-identified data.

Classification of underlying cause of death

The underlying cause of death recorded on Australian death certificates is defined as "the disease or injury, which initiated the train of morbid events leading directly to death, or the circumstances of the accident or violence which produced the fatal injury". For each death the cause was classified according to homogeneous groups defined by the Australian Bureau of Statistics and in accordance with WHO-ICD rules (10). Deaths were further classified as cancer-related, noncancer related, or unknown cause. Cancer-related deaths were considered recurrent if the cancer was diagnosed before transplantation or during the 30-day posttransplantation period. Cancers diagnosed during this period were almost certainly prevalent at transplantation and some were not registered until they were histopathologically diagnosed in the explanted organ. If the cancer attributed to the death was diagnosed more than 30 days after transplantation the cause of death was considered a *de novo* cancer. Some patients (n = 48) died from cancer but did not have a linked registered cancer, or they were registered with a cancer that was not attributed to their death. In these cases, if the cause of death was a liver cancer and there was a history of gallbladder cancer before transplantation or within 30 days of transplantation, the death was classified as a recurrent cancer (n = 2). All other cancer-related deaths, including those due to nonmelanocytic skin cancer (hereafter called skin cancer; C44, n = 14), which is not recorded by the Australian cancer registries, were classified as *de novo* cancer deaths.

Statistical Analysis

Mortality rates

All deaths were included in the calculation of overall mortality rates, whereas deaths due to an unknown cause (n = 69) were excluded from the cause-specific mortality rates. Person-years follow-up accrued from 30 days posttransplantation until the date of death, age 80 years, or December 31, 2006, whichever occurred first. Crude and age- and sex-standardized overall and cause-specific mortality rates (ASMR), standardized to the 1996 Australian population, and 95% confidence intervals (CIs; based on the normal approximation to the binomial distribution) were calculated using annual Australian population estimates from the Australian Bureau of Statistics. Cause-specific mortality rates were computed according to transplanted organ for any cancer, cardiovascular disease, respiratory disease, endocrine disease, nutritional and metabolic diseases, digestive disease and infectious disease.

Survival analyses

Unadjusted overall survival probabilities by transplanted organ were estimated by the Kaplan–Meier method and compared using the log-rank test. Survival probabilities were computed from 30-days after transplantation to the date of death or December 31, 2006. The cumulative incidence of *de novo* cancer deaths was calculated, treating other deaths as a competing risk.

De novo *cancer mortality risk relative to general population*

De novo cancer mortality rates in transplant recipients were compared with rates in the Australian general population using the standardized mortality ratio (SMR), defined as the ratio of the observed and the expected numbers of cancer-related deaths. The likelihood ratio method was used to calculate 95%CIs for deaths with more than 10 expected cases, whereas exact CIs were used for deaths with less than 10 expected cases (11). The expected numbers of cancer deaths were calculated by multiplying cohort person-years at risk by the corresponding five-year age-, sex-, state- and calendar year-specific cancer mortality rates for the Australian population. SMRs were computed for the entire cohort and by transplanted organ, sex and age at transplantation (tertiles). SMRs were not compared statistically because of the heterogeneity in subgroup age and sex distributions (12).

Analyses were performed using SAS® software v9.2 (SAS Institute Inc., Cary, NC, USA). Person-years and SMRs were calculated using the %*stratify* macro written in SAS® (13). The Kaplan–Meier and cumulative incidence curves were generated using STATA, Version 11.2 (StataCorp, College Station, TX, USA).

Results

The eligible cohort comprised 4644 transplant recipients; 1926 (41%) liver, 1518 (33%) heart and 1200 (26%) lung. The median duration of follow-up was 5.2 years (interquartile range, IQR 2.0–9.9), and 1558 deaths were observed over a total of 29 713 person-years. The median age at

death was 53 years (IQR 41–60). The highest number of deaths from any cause was observed in lung transplant recipients (n = 590), followed by heart (n = 564) and liver (n = 404) recipients. There were 77 deaths in 415 pediatric liver and heart transplant recipients; the median age at death was 14 years (IQR 4–18), and most occurred either during the first year posttransplant (n = 28, 36.4%) or more than 5 years posttransplant (n = 28, 36.4%).

Underlying cause of death

There were a total of 1265 noncancer related deaths, led by cardiovascular disease (n = 410, 32.4%), respiratory disease (n = 235, 18.6%), and endocrine, nutritional and metabolic disease (n = 168, 13.3%). The leading causes of death corresponded to the underlying cause of end-stage organ disease, that is, digestive disease for liver transplantation, cardiovascular disease for heart transplantation, and respiratory disease for lung transplantation.

A total of 224 transplant recipients died of cancer; 171 (11%) deaths were *de novo* cancer deaths and 53 (3.4%) were recurrent cancer deaths. The median time between the first registered cancer and *de novo* cancer-related death was 7.1 months (IQR 1.2–24). Non-Hodgkin lymphoma (NHL; n = 38, 22.2%) was the most common cause of *de novo* cancer death, followed by cancer of unknown primary site (n = 25, 14.6%). For those who survived more than 5 years after transplantation (n = 2395, 51.6%), *de novo* cancer was the 2nd leading cause of death in heart (n = 72) transplant recipients, the 3rd leading cause of death in liver (n = 26) and the 4th leading cause of death in lung (n = 16) transplant recipients (Table S2). Five *de novo* cancer deaths occurred in pediatric transplant recipients, and the median time between transplantation and death was 6.6 years (IQR 6.4–7.6). For the 248 pediatric patients who survived more than five years after transplantation, *de novo* cancer was the leading (liver recipients) or the 2nd leading cause of death (heart recipients).

Of the 53 recurrent cancers (23% of all cancer-related deaths), 41 were recurrent liver cancers in liver transplant recipients. The median time between the first liver transplantation and recurrent liver cancer related death was 1.8 years (IQR 0.8–3.7).

Survival analyses

The overall probability of survival for liver, heart and lung transplant recipients is shown in Figure 1(A). Compared to all other causes of death combined, the cumulative incidence of deaths due to *de novo* cancer increased steadily over time (Figures 1B–D). More than 5 years after transplantation, the rate of increase in cancer-related deaths seemed steeper for heart compared to liver and lung transplant recipients.

Crude and age–sex standardized mortality rates

Overall, the crude mortality rate was 5243 per 100 000 and the age–sex standardized mortality rate (ASMR) was 4948 (95%CI, 4052–5843) per 100 000. The crude mortality rate for *de novo* cancer related death was 575 per 100 000 and the ASMR was 359 (95%CI, 283–435) per 100 000. Liver transplant recipients had the lowest overall mortality rate (ASMR 2647; 95%CI, 2291–3002) and lowest rate of *de novo* cancer mortality (ASMR 260; 95%CI, 149–370). The crude mortality rate for recurrent liver cancer in liver transplant recipients was 322 per 100 000 and the ASMR was 268 (95%CI, 162–375) per 100 000.

The ASMR for *de novo* cancer deaths was 121 (95%CI, 38.6–204) per 100 000 up to 2 years after transplantation, 258 (95%CI, 143–373) between 2 and 5 years, and 554 (95%CI, 402–707) more than 5 years after transplantation. The ASMR for NHL was 77.5 (95%CI, 9.05–146) per 100 000 during the first 2 years, 53.3 (95%CI, 2.94–104) between 2 and 5 years, and 172 (95%CI, 70.9–273) beyond 5 years posttransplantation.

Mortality risk compared to the general population: all transplant recipients

Transplant recipients were at 10-fold higher risk of death from any cause compared to the general population (SMR = 10.8; 95%CI, 10.3–11.4). The 171 *de novo* cancer deaths corresponded to a 2.8-fold higher risk compared to the general population. Figure 2 shows the risk of death by cancer site for sites with at least three deaths. The risk of death was significantly elevated for skin cancer, NHL, melanoma, cancer of unknown primary site, liver cancer, connective and soft tissue cancer and lung cancer (Figure 2). There was no excess risk of death due to the most common epithelial cancers in the general population, specifically colon (n = 6; SMR = 1.19; 95%CI, 0.44–2.59), prostate (n = 3; SMR = 1.00; 95%CI, 0.21–2.94), and breast cancer (n = 1; SMR = 0.33; 95%CI, 0.01–1.82). The risk estimates did not change when those with a history of cancer before transplantation were excluded (data not shown).

Mortality risk compared to the general population: by age and sex

Of the 77 deaths in pediatric transplant recipients, five were attributed to *de novo* cancer (SMR = 41.3; 95%CI, 13.4–96.5). The median age at *de novo* cancer death was 17 (IQR 8–20) years. Of the 10 pediatric recipients diagnosed with NHL after transplantation, 7 died and in four cases (3 heart and 1 liver transplant recipient) the deaths were attributed to NHL.

The risk of death from any *de novo* cancer was increased at least twofold regardless of age at transplantation (Table 1). A significant excess risk was also observed for skin cancer, NHL and melanoma for the three age groups examined. The risk of death from cancer of unknown primary site was only increased in those more than 40 years of age at transplantation.

An excess risk of death from any *de novo* cancer was observed for both males and females (Table 2). Both sexes

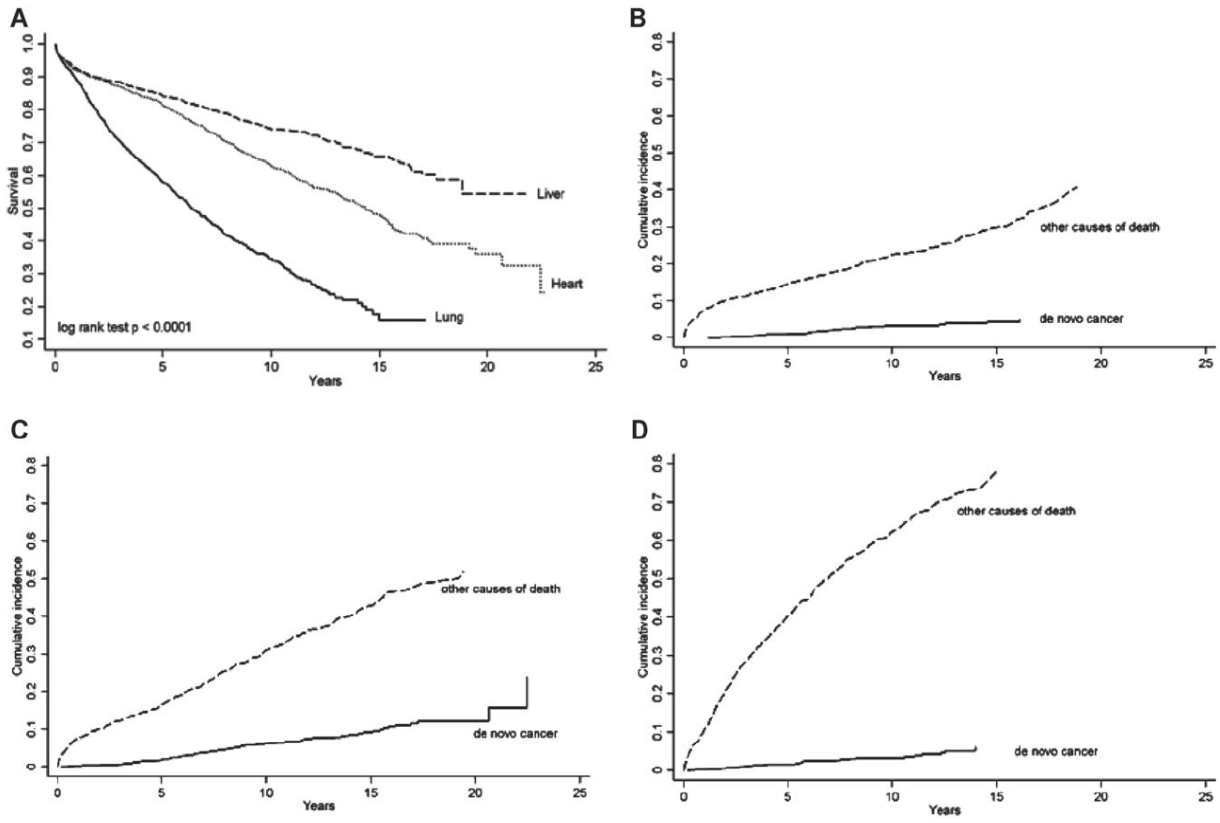


Figure 1: Survival curves by transplanted organ for Australian liver and cardiothoracic transplant recipients. (A) Kaplan–Meier curve by transplanted organ; (B) cumulative incidence by causes of death for liver transplant recipients; (C) cumulative incidence by causes of death for heart transplant recipients; (D) cumulative incidence by causes of death for lung transplant recipients.

also exhibited an increased risk of death due to NHL and cancer of unknown primary site. All skin cancer deaths were observed in males, and a significantly elevated risk of death due to melanoma and lung cancer was also only observed in males.

Mortality risk compared to the general population: by transplanted organ

The risk of death due to any *de novo* cancer was twofold for liver (SMR = 1.96; 95%CI, 1.42–2.61), threefold for heart (SMR = 3.05; 95%CI, 2.49–3.7) and more than fourfold for lung transplant recipients (SMR = 4.41; 95%CI, 3.02–6.23; Figure 3). The risk of death due to NHL and cancer of unknown primary site was significantly increased for all three transplanted organs. An excess risk of mortality due to skin cancer was observed for both heart (SMR = 66.1; 95%CI, 39.2–104) and lung (SMR = 86.4; 95%CI, 23.6–221) but not liver (SMR = 6.91; 95%CI, 0.17–38.5) recipients. Risk of melanoma-related death was significantly increased after liver (SMR = 5.26; 95%CI, 1.43–13.5) and heart (SMR = 8.81; 95%CI, 4.22–16.2) but not lung (SMR = 3.67; 95%CI, 0.09–20.4) transplantation. An increased risk of death due to lung cancer was only observed in lung transplant recipients (SMR = 3.98; 95%CI, 1.46–8.66). There was no excess risk of *de novo* liver cancer death for liver, heart or lung transplant recipients. Lung transplant recipients exhibited a significantly increased risk of death from *de novo* cancer at all time periods posttransplantation (Table 3). A significantly increased risk of death from *de novo* cancer was only observed beyond 2 years after transplantation for liver and heart transplant recipients.

Discussion

In this national population-based cohort study we found a 2.8-fold excess risk of death from *de novo* cancer in liver and cardiothoracic transplant recipients compared to the matched general population. This risk estimate is similar in magnitude to the excess risk of incident cancer in this cohort (SIR 2.62), a finding that is predominantly attributed to immunosuppression (14). The excess risk of death from *de novo* cancer was observed regardless of recipient sex and age at transplantation, and was greatest

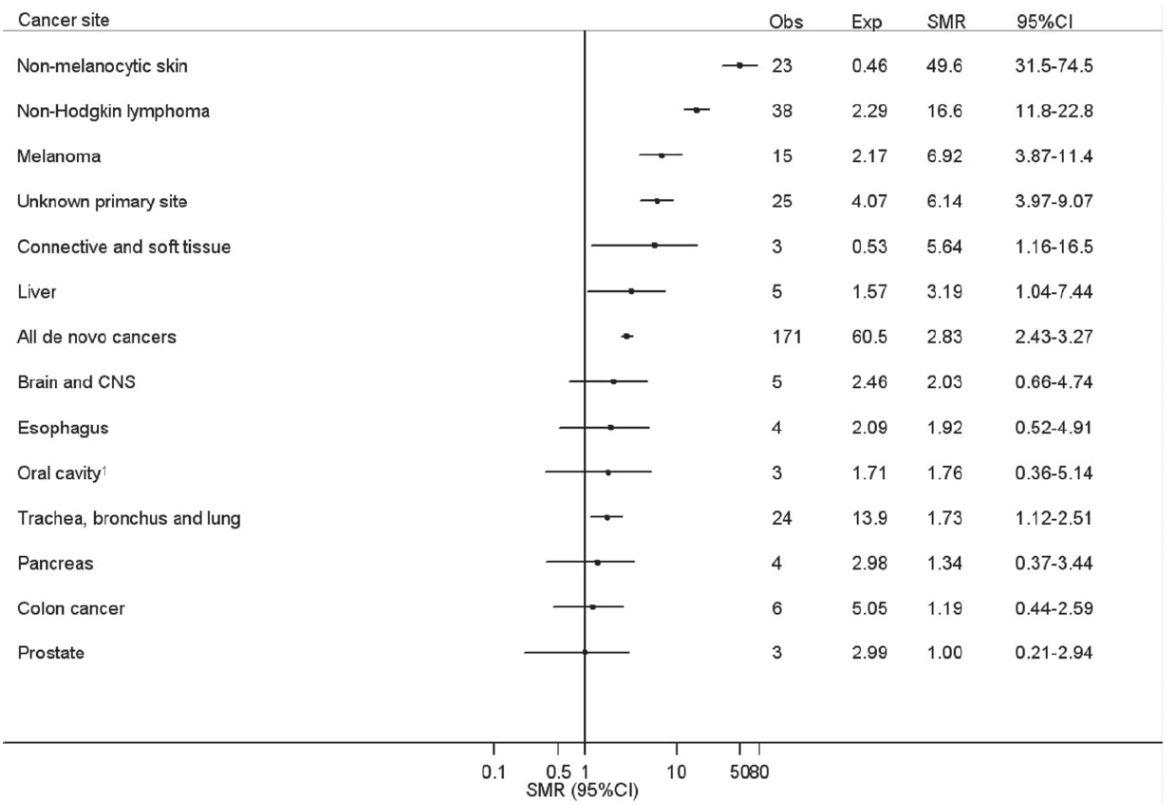


Figure 2: Site-specific cancer mortality risk relative to the general population for Australian liver and cardiothoracic transplant recipients. [1]Oral cavity (C00-C14).

Table 1: Site-specific cancer mortality risk relative to the general population by age at transplantation

	Age at transplantation (years)								
	0–40 years			41–52 years			53–73 years		
Cancer	Obs	SMR	95%CI	Obs	SMR	95%CI	Obs	SMR	95%CI
All *de novo* cancers	27	11.1	7.30–16.1	67	3.74	2.92–4.71	77	1.92	1.52–2.38
Skin cancer[1]	4	237	64.5–606	10	79.4	38.1–146	9	28.1	12.8–53.3
NHL[2]	13	103	54.8–176	12	16.9	8.71–29.5	13	8.96	4.77–15.3
Melanoma	3	16.8	3.46–49.0	7	9.37	3.77–19.3	5	4.02	1.31–9.39
Unknown primary site	0	–		12	10.2	5.29–17.9	13	4.72	2.51–8.07
Trachea, bronchus and lung	0	–		9	2.39	1.09–4.53	15	1.52	0.85–2.51

Obs = observed number of cancer deaths; SMR = standardized mortality ratio.
[1]Nonmelanocytic skin cancer.
[2]Non-Hodgkin lymphoma.

for lung transplant recipients. NHL was the most common cancer-related death. *De novo* cancer was a leading cause of late death, particularly in heart and liver transplantation, and in pediatric recipients. The excess mortality from cancer in this population reinforces the need for tailored cancer prevention strategies, evidence-based surveillance to promote early detection and guidelines for managing immunosuppression and cancer treatment after diagnosis.

There are relatively few prior estimates of cancer-related mortality after solid organ transplantation. Our estimate of a 1.96-fold risk of death from *de novo* cancer after liver transplantation is similar to that observed in a single-center Spanish study (1990–2001) that also excluded recurrent cancers (2.93; 95%CI, 1.56–5.02; Ref. 4). A 2.3-fold risk of death from any cancer (*de novo* and recurrent) was reported in a population-based study of kidney transplant recipients in Hong Kong (1972–2011;

Appendices

Table 2: Site-specific cancer mortality risk relative to the general population by sex

	Male			Female		
Cancer	Obs	SMR	95%CI	Obs	SMR	95%CI
All *de novo* cancers	143	3.07	2.59–3.60	28	2.02	1.36–2.86
Skin cancer[1]	23	54.4	34.5–74.5	0	–	–
NHL[2]	30	16.8	11.3–23.9	8	16.0	6.92–31.6
Melanoma	14	7.72	4.22–13.0	1	2.81	0.07–15.7
Unknown primary site	21	6.65	4.12–10.2	4	4.38	1.19–11.2
Trachea, bronchus and lung	20	1.72	1.07–2.59	4	1.73	0.47–4.44

Obs = observed number of cancer deaths; SMR = standardized mortality ratio.
[1]Nonmelanocytic skin cancer.
[2]Non-Hodgkin lymphoma.

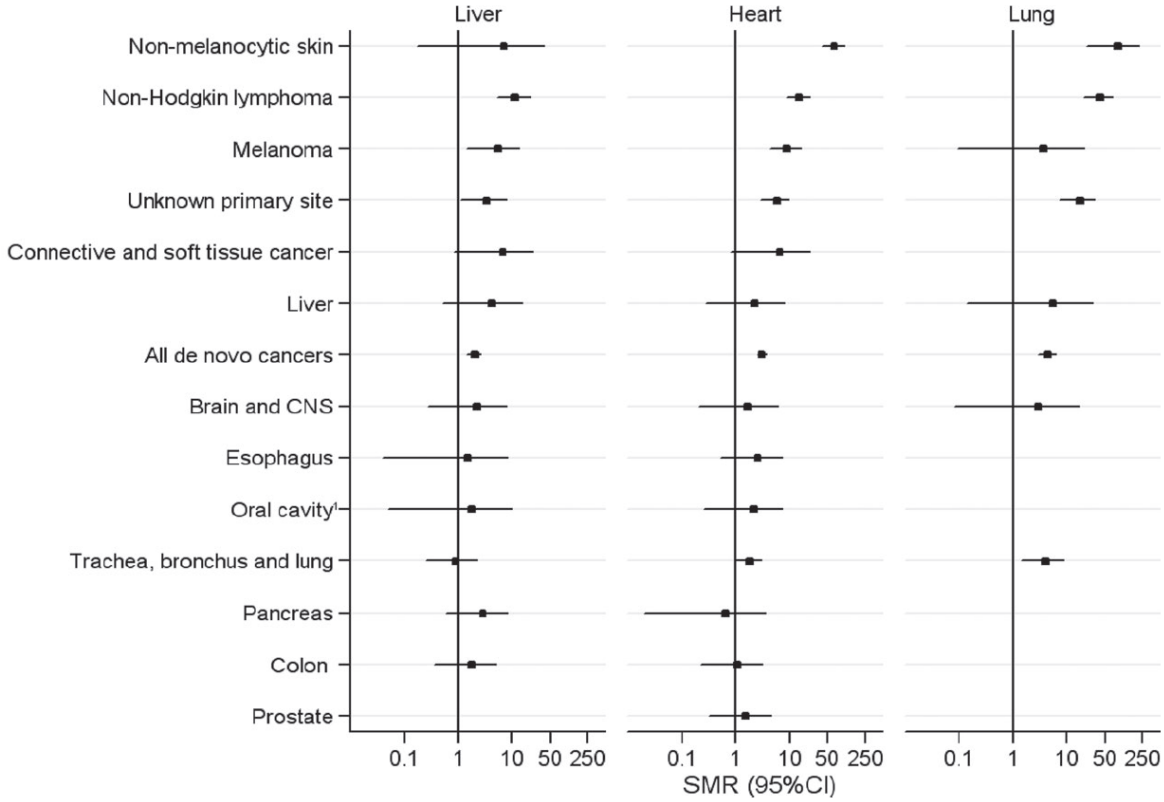


Figure 3: Site-specific *de novo* cancer mortality risk relative to the general population by transplanted organ. [1]Oral cavity (C00-C14).

Table 3: Risk of mortality due to *de novo* cancer and NHL by time since transplant and transplanted organ

		Liver transplant			Heart transplant			Lung transplant		
Cancer	Years since transplant	Obs	SMR	95%CI	Obs	SMR	95%CI	Obs	SMR	95%CI
All *de novo* cancers	<2	3	0.70	0.15–2.06	7	1.46	0.59–3.01	7	2.98	1.20–6.15
	2–5	12	2.08	1.08–3.64	17	2.28	1.33–3.66	9	3.68	1.68–6.98
	≥5	27	2.37	1.58–3.37	73	3.74	2.94–4.66	16	6.50	3.71–10.5
NHL[1]	<2	1	5.99	0.15–33.4	3	15.6	3.22–45.6	5	55.4	18.0–129
	2–5	2	8.95	1.08–32.3	3	10.3	2.13–30.1	3	31.5	6.50–92.2
	≥5	6	14.3	5.23–31.0	12	16.6	8.61–29.1	3	33.6	6.93–98.3

SMR = standardized mortality ratio.
[1]Non-Hodgkin lymphoma.

Ref. 8). On the other hand, no excess risk of cancer death was observed in a population-based study of 164 078 kidney transplant recipients registered on the United States Renal Data System (1990–2004; Ref. 6). However, this finding is likely to have underestimated cancer deaths because cause of death was unknown for 41% of recipients and all of these deaths were classified as noncancer related.

The site-specific pattern of cancer-related death by transplanted organ very closely mirrored that observed for incident cancers in this cohort (14). Specifically, risk of death from NHL and cancer of unknown primary site was elevated for all three transplanted organs; risk of death from skin cancer was increased in heart and lung but not liver recipients, risk of death from melanoma was increased in liver and heart but not in lung transplant recipients, and risk of death from lung cancer was confined to heart and lung transplant recipients. Furthermore, there was no excess risk of death due to prostate or breast cancer in any transplanted group. In contrast to the cancer incidence profile, we observed no excess risk of death attributed to colon cancer (n = 6) or to lip/oral cancer (n = 3).

There are no prior estimates of the risk of death from NHL in transplant recipients relative to the general population. However, in keeping with previous studies (15,16), NHL was the most common cause of cancer-related death in this cohort. This may reflect the high incidence of this neoplasm in this patient group rather than a poor treatment response, as a single study found no difference in NHL survival for transplant recipients and the Surveillance, Epidemiology and End Results (SEER) general population (1964–2007; Ref. 17). NHL in transplant recipients is managed by reducing immunosuppression, chemotherapy with or without rituximab, antiviral therapy for Epstein-Barr viral (EBV)-positive patients, surgery and radiation (18,19).

Skin cancer carried the highest relative risk of cancer-related death in our cohort (SMR = 50). This estimate is very similar to the risk of death from cutaneous squamous cell carcinoma (SCC) in Swedish solid organ transplant recipients (1970–1997; SMR = 52.2; 95%CI, 21.0–107.6), predominantly kidney (5). Although the skin cancer histology was not recorded in our data, a previous Australian study of cardiothoracic transplant recipients found that 95% of nonmelanocytic skin cancers were SCC (20). We did not find an excess risk of mortality from skin cancer in liver transplant recipients, which is consistent with prior evidence that most deaths in this patient group occur from noncutaneous neoplasms (4). This finding, like the different patterns in cancer incidence by transplanted organ, is likely to be largely related to the less intensive immunosuppressive therapy received by liver compared to cardiothoracic transplant recipients (3,21,22).

For pediatric transplant recipients, most deaths of any cause occurred during the first year and more than 5 years after transplantation. *De novo* cancer was the leading cause of late death, the risk relative to the general population was around 40-fold and most cancer-related deaths were attributed to NHL. In the only prior study to assess mortality in pediatric transplant recipients with NHL, heart transplant recipients had significantly lower overall survival compared to kidney or liver transplant recipients (23). Given the relatively high mortality of pediatric recipients with NHL, serial monitoring of EBV load has been recommended as a screening strategy for high-risk patients, and may improve outcomes (23–26).

The increased incidence and mortality from *de novo* cancer in solid organ transplant recipients justifies patient education about the need for sun protection and monthly self skin examinations, and early reporting of new signs and symptoms. Our findings indicate that males are at greatest risk of dying from nonmelanoma skin cancer and melanoma. Clinically, cancer risk may be reduced by minimizing immunosuppression, viral prophylaxis, EBV viral load monitoring, and targeted cancer screening in high-risk patients (18,19,27). Significantly greater non-skin cancer–related survival and overall survival was reported for a period of intensive cancer surveillance (2002–2007) compared to historical surveillance (1982–2001) in Austrian liver transplant recipients (28). However, there was no adjustment for either cancer treatment or improvements in cancer outcomes over time. The intensive surveillance was an annual chest and abdominal CT scan, urological evaluation (including prostate specific antigen [PSA] test), gynaecological evaluation (including Papanicolau smear and mammography), dermatological screening and 3-yearly colonoscopy except in patients with a history of adenoma or inflammatory bowel disease (1-yearly). Nevertheless, as incidence rates of prostate and breast cancer are not increased in organ transplant recipients, there is no evidence to suggest the need for screening practices for these malignancies that would differ from the general population.

The strengths of this study include the population-based design and the relatively large size, allowing stratification by organ and cancer type. A limitation is that some of the cancer-related deaths did not have a matching linked cancer registry record; however, most of these were skin cancers, the majority of which are not notifiable neoplasms in Australia. Also, there is the potential for differential misclassification of the underlying cause of death in individuals with multiple morbidities (such as those with end-stage organ disease and cancer), and this may have led to an underestimation of the true risk of cancer-related death in this patient group (29). Furthermore, 4% of deaths were due to an unknown cause, and these cases were not included in the cause-specific analyses. Despite these limitations, the rank order of cause-specific mortality was in accordance with previous reports (15,30,31).

Conclusions

Our study quantifies the risk of *de novo* cancer-related mortality in liver and cardiothoracic transplant recipients, overall and in relation to organ type, time since transplantation and recipient age and sex. This population-based data provides additional momentum to calls for a review of guidelines for cancer prevention and screening for solid organ transplant recipients, and the generation of robust evidence in these areas (27).

Acknowledgments

This study was funded by the National Health and Medical Research Council (ID510254). Andrew Grulich is supported by an NHMRC principal research fellowship (ID568819). Claire Vajdic is supported by a National Health and Medical Research Council Career Development Fellowship (ID1023159) and a Cancer Institute New South Wales Career Development Fellowship (ID10/CDF/2–42). Renhua Na is supported by a Translational Cancer Research Network (TCRN) PhD Scholarship Top-up Award. The TCRN is a translational cancer research centre program funded by the Cancer Institute NSW.

We thank the Australian and New Zealand Liver Transplant Registry, the Australian and New Zealand Cardiothoracic Organ Transplant Registry, and also the Australian Institute of Health and Welfare for conducting the data linkage.

We thank: Phyllis Larkins (Royal Prince Alfred Hospital, Sydney); Geraldine Lipka (Princess Alexandra Hospital, Brisbane); Cassandra Kastaneas (St Vincent's Hospital, Sydney); Vicki Jermyn and Brooke Andersen (The Children's Hospital, Westmead); Jo Maddicks-Law, Nicole Ostenfeld, Sara Gray and Muhtashimuddin Ahmed (Prince Charles Hospital, Brisbane); Kerrie Beale (Royal Childrens Hospital, Brisbane); Libby John, Nicole Williams (Flinders Medical Centre, Adelaide); Kathryn Marshall, Ailsa Cowie, Connie Kambanaros, Jasmin Board, and Colleen Farrell (Alfred Hospital, Melbourne); Lyn Crellin, Kathe Beyerle, Kate Schurmann, Anne Shipp, Janette McEwan, Danielle Kamolins, Angie Wood and Hollie Gilmore (Royal Childrens Hospital, Melbourne); Julie Pavlovic and Betheia Lele (Austin Hospital, Melbourne); Sharon Lawrence, Clare Wood and Sharlene Beinke (Royal Perth Hospital); Barb Chester, Judith Bull, Joanne Plummer, Nikki Copland and Megan O-Dea (Sir Charles Gairdner Hospital, Perth).

The following lead investigators participated in this study: George Alex, Glenda Balderson, Peter Bergin, John Chen, Weng Chin, Lawrence Dembo, Pamela Dilworth, Looi Ee, Allan Glanville, Winita Hardikar, Peter Hopkins, George Javorsky, Gary P Jeffrey, Robert Jones, Bronwyn Levvey, Steven Lynch, Michael Musk, Ross Pettersson, Greg Snell, Michael Stormon, and Robert Weintraub.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

References

1. Vajdic CM, McDonald SP, McCredie MR, et al. Cancer incidence before and after kidney transplantation. JAMA 2006; 296: 2823–2831.
2. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: A meta-analysis. Lancet 2007; 370: 59–67.
3. Collett D, Mumford L, Banner NR, Neuberger J, Watson C. Comparison of the incidence of malignancy in recipients of different types of organ: A UK registry audit. Am J Transplant 2010; 10: 1889–1896.
4. Herrero JI, Lorenzo M, Quiroga J, et al. De novo neoplasia after liver transplantation: An analysis of risk factors and influence on survival. Liver Transpl 2005; 11: 89–97.
5. Lindelof B, Jarnvik J, Ternesten-Bratel A, Granath F, Hedblad MA. Mortality and clinicopathological features of cutaneous squamous cell carcinoma in organ transplant recipients: A study of the Swedish cohort. Acta Derm Venereol 2006; 86: 219–222.
6. Kiberd BA, Rose C, Gill JS. Cancer mortality in kidney transplantation. Am J Transplant 2009; 9: 1868–1875.
7. Foster BJ, Dahhou M, Zhang X, Platt RW, Hanley JA. Change in mortality risk over time in young kidney transplant recipients. Am J Transplant 2011; 11: 2432–2442.
8. Cheung CY, Lam MF, Chu KH, et al. Malignancies after kidney transplantation: Hong Kong renal registry. Am J Transplant 2012; 12: 3039–3046.
9. Jaro MA. Probabilistic linkage of large public health data files. Stat Med 1995; 14: 491–498.
10. Benden C, Aurora P, Edwards LB, et al. The registry of the international society for heart and lung transplantation: Fourteenth pediatric lung and heart-lung transplantation report 2011. J Heart Lung Transplant 2011; 30: 1123–1132.
11. Swift MB. Comparison of confidence intervals for a Poisson mean—Further considerations. Commun Stat Theory Methods 2009; 38: 748–759.
12. Rothman KJ, Greenland S, Lash TL. Modern epidemiology. 3rd Ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2008; p. 254.
13. Rostgaard K. Methods for stratification of person-time and events—A prerequisite for poisson regression and SIR estimation. Epidemiol Perspect Innov 2008; 5: 1–16.
14. Na R, Grulich AE, Meagher NS, McCaughan G, Keogh AM, Vajdic CM. Comparison of de novo cancer incidence in Australian liver, heart and lung transplant recipients. Am J Transplant 2013; 13: 174–183.
15. Watt KDS, Pedersen RA, Kremers WK, Heimbach JK, Charlton MR. Evolution of causes and risk factors for mortality post-liver transplant: Results of the NIDDK long-term follow-up study. Am J Transplant 2010; 10: 1420–1427.
16. Metcalfe MJ, Kutsogiannis DJ, Jackson K, et al. Risk factors and outcomes for the development of malignancy in lung and heart-lung transplant recipients. Can Respir J 2010; 17: e7–13.
17. Knight JS, Tsodikov A, Cibrik DM, Ross CW, Kaminski MS, Blayney DW. Lymphoma after solid organ transplantation: Risk, response to therapy, and survival at a transplantation center. J Clin Oncol 2009; 27: 3354–3362.
18. Campistol JM, Cuervas-Mons V, Manito N, et al. New concepts and best practices for management of pre- and post-transplantation cancer. Transplant Rev (Orlando) 2012; 26: 261–279.

19. Reshef R, Vardhanabhuti S, Luskin MR, et al. Reduction of immunosuppression as initial therapy for posttransplantation lymphoproliferative disorder. Am J Transplant 2011; 11: 336–347.
20. Veness MJ, Quinn DI, Ong CS, et al. Aggressive cutaneous malignancies following cardiothoracic transplantation: The Australian experience. Cancer 1999; 85: 1758–1764.
21. Euvrard S, Kanitakis J, Pouteil-Noble C, et al. Comparative epidemiologic study of premalignant and malignant epithelial cutaneous lesions developing after kidney and heart transplantation. J Am Acad Dermatol 1995; 33: 222–229.
22. Ong CS, Keogh AM, Kossard S, Macdonald PS, Spratt PM. Skin cancer in Australian heart transplant recipients. J Am Acad Dermatol 1999; 40: 27–34.
23. Taj MM, Hadzic N, Height SE, et al. Long-term outcome for immune suppression and immune related lymphoproliferative disorder: Prospective data from the United Kingdom children's leukaemia and cancer group registry 1994–2004. Leuk Lymphoma 2012; 53: 842–848.
24. Schubert S, Abdul-Khaliq H, Lehmkuhl HB, et al. Diagnosis and treatment of post-transplantation lymphoproliferative disorder in pediatric heart transplant patients. Pediatr Transplant 2009; 13: 54–62.
25. Kerkar N, Morotti RA, Madan RP, et al. The changing face of post-transplant lymphoproliferative disease in the era of molecular EBV monitoring. Pediatr Transplant 2010; 14: 504–511.
26. Tsai DE, Douglas L, Andreadis C, et al. EBV PCR in the diagnosis and monitoring of posttransplant lymphoproliferative disorder: Results of a two-arm prospective trial. Am J Transplant 2008; 8: 1016–1024.
27. Webster AC, Wong G, Craig JC, Chapman JR. Managing cancer risk and decision making after kidney transplantation. Am J Transplant 2008; 8: 2185–2191.
28. Finkenstedt A, Graziadei IW, Oberaigner W, et al. Extensive surveillance promotes early diagnosis and improved survival of de novo malignancies in liver transplant recipients. Am J Transplant 2009; 9: 2355–2361.
29. Sarfati D, Blakely T, Pearce N. Measuring cancer survival in populations: Relative survival vs cancer-specific survival. Int J Epidemiol 2010; 39: 598–610.
30. Stehlik J, Edwards LB, Kucheryavaya AY, et al. The registry of the international society for heart and lung transplantation: Twenty-eighth adult heart transplant report 2011. J Heart Lung Transplant 2011; 30: 1078–1094.
31. Christie JD, Edwards LB, Kucheryavaya AY, et al. The registry of the international society for heart and lung transplantation: Twenty-eighth adult lung and heart-lung transplant report 2011. J Heart Lung Transplant 2011; 30: 1104–1122.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1: Characteristics of total deaths by transplanted organ

Table S2: Causes of death over five years after transplantation for Australian liver and cardiothoracic transplant recipients

References

Aarbakke, J., G. Janka-Schaub and G. B. Elion (1997). "Thiopurine biology and pharmacology." Trends Pharmacol Sci 18(1): 3-7.

Aberg, F., E. Pukkala, K. Hockerstedt, et al. (2008). "Risk of malignant neoplasms after liver transplantation: a population-based study." Liver Transpl 14(10): 1428-1436.

Adami, J., H. Gabel, B. Lindelof, et al. (2003). "Cancer risk following organ transplantation: a nationwide cohort study in Sweden." Br J Cancer 89(7): 1221-1227.

Adcock, I. M., G. Caramori and K. Ito (2006). "New insights into the molecular mechanisms of corticosteroids actions." Curr Drug Targets 7(6): 649-660.

Adu, D., P. Cockwell, N. J. Ives, et al. (2003). "Interleukin-2 receptor monoclonal antibodies in renal transplantation: meta-analysis of randomised trials." BMJ 326(7393): 789.

AIHW and AACR (2012). Cancer in Australia: an overview, 2012. Canberra, AIHW.

Albert, A. and J. A. Anderson (1984). "On the existence of maximum likelihood estimates in logistic regression models." Biometrika 71(1): 1-10.

Allen, U. D., J. K. Preiksaitis and AST Infectious Diseases Community of Practice (2013). "Epstein-barr virus and posttransplant lymphoproliferative disorder in solid organ transplantation." Am J Transplant 13(s4): 107-120.

Allison, A. C. and E. M. Eugui (1996). "Purine metabolism and immunosuppressive effects of mycophenolate mofetil (MMF)." Clin Transplant 10(1 Pt 2): 77-84.

Allison, P. (2000). "Multiple imputation for missing data: a cautionary tale." Sociol Methods Res 28: 301 - 309.

Allison, P. (2001). *Missing data.* Thousand Oaks, CA: Sage, Sage University Papers Series on Quantitative Applications in the Social Sciences.

Alter, M. J. (2006). "Epidemiology of viral hepatitis and HIV co-infection." J Hepatol 44, Supplement 1(0): S6-S9.

Amornphimoltham, P., K. Leelahavanichkul, A. Molinolo, et al. (2008). "Inhibition of mammalian target of rapamycin by rapamycin causes the regression of carcinogen-induced skin tumor lesions." Clin Cancer Res 14(24): 8094-8101.

ANZCOTR (2012). The Australia and New Zealand Cardiothoracic Organ Transplant Registry Seventeenth annual report 1984 - 2012. Available at http://www.anzcotr.org.au/. Accessed Nov 1, 2013.

ANZDATA (2009). ANZDATA Registry Report 2009. Availabel at http://www.anzdata.org.au/v1/annual_reports_download.html. Accessed Nov 1, 2013. S. McDonald, L. Excell and B. livingston.

ANZLTR (2012). ANZLT Registry Report 2012 Australia and New Zealand Liver Transplant Registry. Available at http://www.anzltr.org/statistics.html. Accessed Nov 1, 2013. S. V. Lynch and G. A. Balderson.

Ariyawardana, A. and N. W. Johnson (2013). "Trends of lip, oral cavity and oropharyngeal cancers in Australia 1982-2008: overall good news but with rising rates in the oropharynx." BMC Cancer 13: 333.

Athar, M., R. Agarwal, Z. Y. Wang, et al. (1991). "All-trans retinoic acid protects against conversion of chemically induced and ultraviolet B radiation-induced skin papillomas to carcinomas." Carcinogenesis 12(12): 2325-2329.

References

Baccarani, U., P. Piselli, D. Serraino, et al. (2010). "Comparison of de novo tumours after liver transplantation with incidence rates from Italian cancer registries." Dig Liver Dis 42(1): 55-60.

Baehner, R., G. Magrane, R. Balassanian, et al. (2000). "Donor origin of neuroendocrine carcinoma in 2 transplant patients determined by molecular cytogenetics." Hum Pathol 31(11): 1425-1429.

Barozzi, P., C. Bonini, L. Potenza, et al. (2008). "Changes in the immune responses against human herpesvirus-8 in the disease course of posttransplant Kaposi sarcoma." Transplantation 86(5): 738-744.

Beatty, P. R., S. M. Krams, C. O. Esquivel, et al. (1998). "Effect of cyclosporine and tacrolimus on the growth of Epstein-Barr virus-transformed B-cell lines." Transplantation 65(9): 1248-1255.

Beaugerie, L., N. Brousse, A. M. Bouvier, et al. (2009). "Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study." Lancet 374(9701): 1617-1625.

Benden, C., P. Aurora, L. B. Edwards, et al. (2011). "The registry of the international society for heart and lung transplantation: fourteenth pediatric lung and heart-lung transplantation report 2011." J Heart Lung Transplant 30(10): 1123-1132.

Bernatsky, S., J. L. Lee and E. Rahme (2007). "Non-Hodgkin's lymphoma—meta-analyses of the effects of corticosteroids and non-steroidal anti-inflammatories." Rheumatology 46(4): 690-694.

Berner, A., R. Holm, A. Naess, et al. (1993). "p53 protein expression in squamocellular carcinomas of the lip." Anticancer Res 13(6B): 2421-2424.

Beveridge, T., P. Krupp and C. McKibbin (1984). "Lymphomas and lymphoproliferative lesions developing under cyclosporin therapy." Lancet 1(8380): 788.

Birkeland, S. A. and S. Hamilton-Dutoit (2003). "Is posttransplant lymphoproliferative disorder (PTLD) caused by any specific immunosuppressive drug or by the transplantation per se?" Transplantation 76(6): 984-988.

Birkeland, S. A. and H. H. Storm (2002). "Risk for tumor and other disease transmission by transplantation: a population-based study of unrecognized malignancies and other diseases in organ donors." Transplantation 74(10): 1409-1413.

Boratynska, M. and D. Smolska (2008). "Inhibition of mTOR by sirolimus induces remission of post-transplant lymphoproliferative disorders." Transpl Int 21(6): 605-608.

Borel, J. F., C. Feurer, H. U. Gubler, et al. (1976). "Biological effects of cyclosporin A: a new antilymphocytic agent." Agents Actions 6(4): 468-475.

Botha, P., R. Peaston, K. White, et al. (2008). "Smoking after cardiac transplantation." Am J Transplant 8(4): 866-871.

Boyle, P. and D. M. Parkin (1991). "Cancer registration: principles and methods. statistical methods for registries." IARC Sci Publ(95): 126-158.

Brem, R. and P. Karran (2012). "Multiple forms of DNA damage caused by UVA photoactivation of DNA 6-thioguanine." Photochem Photobiol 88(1): 5-13.

Brennan, D. C., J. A. Daller, K. D. Lake, et al. (2006). "Rabbit antithymocyte globulin versus basiliximab in renal transplantation." N Engl J Med 355(19): 1967-1977.

Brenner, M. and V. J. Hearing (2008). "The protective role of melanin against UV damage in human skin." Photochem Photobiol 84(3): 539-549.

Brewer, J. D., O. R. Colegio, P. K. Phillips, et al. (2009). "Incidence of and risk factors for skin cancer after heart transplant." Arch Dermatol 145(12): 1391-1396.

Briggs, J. D. (2001). "Causes of death after renal transplantation." Nephrol Dial Transplant 16(8): 1545-1549.

Bucks, C. M., J. A. Norton, A. C. Boesteanu, et al. (2009). "Chronic antigen stimulation alone is sufficient to drive CD8+ T cell exhaustion." J Immunol 182(11): 6697-6708.

Buell, J. F., T. G. Gross and E. S. Woodle (2005). "Malignancy after transplantation." Transplantation 80(2 Suppl): S254-264.

Bugelski, P. J., A. Volk, M. R. Walker, et al. (2010). "Critical review of preclinical approaches to evaluate the potential of immunosuppressive drugs to influence human neoplasia." Int J Toxicol 29(5): 435-466.

Bustami, R. T., A. O. Ojo, R. A. Wolfe, et al. (2004). "Immunosuppression and the risk of post-transplant malignancy among cadaveric first kidney transplant recipients." Am J Transplant 4(1): 87-93.

Cadet, J., T. Douki, D. Gasparutto, et al. (2003). "Oxidative damage to DNA: formation, measurement and biochemical features." Mutat Res 531(1-2): 5-23.

Cadet, J., J. L. Ravanat, G. R. Martinez, et al. (2006). "Singlet oxygen oxidation of isolated and cellular DNA: product formation and mechanistic insights." Photochem Photobiol 82(5): 1219-1225.

Caillard, S., V. Dharnidharka, L. Agodoa, et al. (2005). "Posttransplant lymphoproliferative disorders after renal transplantation in the United States in era of modern immunosuppression." Transplantation 80(9): 1233-1243.

Caillard, S., F. X. Lamy, C. Quelen, et al. (2012). "Epidemiology of posttransplant lymphoproliferative disorders in adult kidney and kidney pancreas recipients: report of the French registry and analysis of subgroups of lymphomas." Am J Transplant 12(3): 682-693.

References

Caillard, S., C. Lelong, F. Pessione, et al. (2006). "Post-transplant lymphoproliferative disorders occurring after renal transplantation in adults: report of 230 cases from the French Registry." Am J Transplant 6(11): 2735-2742.

Campistol, J. M., V. Cuervas-Mons, N. Manito, et al. (2012). "New concepts and best practices for management of pre- and post-transplantation cancer." Transplant Rev (Orlando) 26(4): 261-279.

Campistol, J. M., J. Eris, R. Oberbauer, et al. (2006). "Sirolimus therapy after early cyclosporine withdrawal reduces the risk for cancer in adult renal transplantation." J Am Soc Nephrol 17(2): 581-589.

Canning, M. T., S. L. Nay, A. V. Pena, et al. (2006). "Calcineurin inhibitors reduce nuclear localization of transcription factor NFAT in UV-irradiated keratinocytes and reduce DNA repair." J Mol Histol 37(5-7): 285-291.

Capello, D., S. Rasi, P. Oreste, et al. (2009). "Molecular characterization of post-transplant lymphoproliferative disorders of donor origin occurring in liver transplant recipients." J Pathol 218(4): 478-486.

Casabonne, D., A. Lally, L. Mitchell, et al. (2009). "A case-control study of cutaneous squamous cell carcinoma among Caucasian organ transplant recipients: The role of antibodies against human papillomavirus and other risk factors." Int J Cancer 125(8): 1935-1945.

Cazes, B. (1963). "1st world attempt at homotransplantation of the liver in man (Denver, Colorado, USA)." Presse Med 71: 1695-1696.

Chadban, S. J., K. A. Barraclough, S. B. Campbell, et al. (2012). "KHA-CARI guideline: KHA-CARI adaptation of the KDIGO clinical practice guideline for the care of kidney transplant recipients." Nephrology (Carlton) 17(3): 204-214.

References

Chalastanis, A., V. Penard-Lacronique, M. Svrcek, et al. (2010). "Azathioprine-induced carcinogenesis in mice according to msh2 genotype." J Natl Cancer Inst 102(22): 1731-1740.

Chang, S. H., G. R. Russ, S. J. Chadban, et al. (2008). "Trends in adult post-kidney transplant immunosuppressive use in Australia, 1991-2005." Nephrology (Carlton) 13(2): 171-176.

Chapman, J. R., A. C. Webster and G. Wong (2013). "Cancer in the transplant recipient." Cold Spring Harb Perspect Med 3(7).

Cherikh, W. S., H. M. Kauffman, M. A. McBride, et al. (2003). "Association of the type of induction immunosuppression with posttransplant lymphoproliferative disorder, graft survival, and patient survival after primary kidney transplantation." Transplantation 76(9): 1289-1293.

Cheung, C. Y., M. F. Lam, K. H. Chu, et al. (2012). "Malignancies after kidney transplantation: Hong Kong renal registry." Am J Transplant 12(11): 3039-3046.

Christie, J. D., L. B. Edwards, A. Y. Kucheryavaya, et al. (2012). "The registry of the international society for heart and lung transplantation: 29th adult lung and heart-lung transplant report—2012." J Heart Lung Transplant 31(10): 1073-1086.

Christie, J. D., L. B. Edwards, A. Y. Kucheryavaya, et al. (2011). "The registry of the international society for heart and lung transplantation: twenty-eighth adult lung and heart-lung transplant report 2011." J Heart Lung Transplant 30(10): 1104-1122.

Clayton, P. A., S. Campbell, S. J. Chadban, et al. (2013). Transplantation: ANZDATA registry report 2012. S. McDonald, P. A. Clayton and K. Hurst. Adelaide, South Australia, Australia and New Zealand Dialysis and Tranaplant Registry.

References

Cockfield, S. M. (2001). "Identifying the patient at risk for post-transplant lymphoproliferative disorder." Transpl Infect Dis 3(2): 70-78.

Cohen, J. I. (2000). "Epstein-Barr virus infection." N Engl J Med 343(7): 481-492.

Collett, D., L. Mumford, N. R. Banner, et al. (2010). "Comparison of the incidence of malignancy in recipients of different types of organ: a UK registry audit." Am J Transplant 10(8): 1889-1896.

Collins, M. G., E. Teo, S. R. Cole, et al. (2012). "Screening for colorectal cancer and advanced colorectal neoplasia in kidney transplant recipients: cross sectional prevalence and diagnostic accuracy study of faecal immunochemical testing for haemoglobin and colonoscopy." BMJ 345: e4657.

Corbett, C., M. J. Armstrong and J. Neuberger (2012). "Tobacco smoking and solid organ transplantation." Transplantation 94(10): 979-987.

Costanzo, M. R., A. Dipchand, R. Starling, et al. (2010). "The international society of heart and lung transplantation guidelines for the care of heart transplant recipients." J Heart Lung Transplant 29(8): 914-956.

Crespo-Leiro, M. G., L. Alonso-Pulpon, J. A. Vazquez de Prada, et al. (2008). "Malignancy after heart transplantation: incidence, prognosis and risk factors." Am J Transplant 8(5): 1031-1039.

Crosthwaite, N., D. Teale, C. Franklin, et al. (1996). "p53 protein expression in malignant, pre-malignant and non-malignant lesions of the lip." J Clin Pathol 49(8): 648-653.

Czerninski, R., A. Zini and H. D. Sgan-Cohen (2010). "Lip cancer: incidence, trends, histology and survival: 1970–2006." Br J Dermatol 162(5): 1103-1109.

Dalia, S., J. Chavez, J. J. Castillo, et al. (2013). "Hepatitis B infection increases the risk of non-Hodgkin lymphoma: a meta-analysis of observational studies." Leuk Res 37(9): 1107-1115.

Danese, S. and C. Fiocchi (2011). "Ulcerative colitis." N Engl J Med 365(18): 1713-1725.

Danpanich, E. and B. L. Kasiske (1999). "Risk factors for cancer in renal transplant recipients." Transplantation 68(12): 1859-1864.

Dantal, J., M. Hourmant, D. Cantarovich, et al. (1998). "Effect of long-term immunosuppression in kidney-graft recipients on cancer incidence: randomised comparison of two cyclosporin regimens." Lancet 351(9103): 623-628.

de Graaf, Y. G., H. Rebel, A. Elghalbzouri, et al. (2008). "More epidermal p53 patches adjacent to skin carcinomas in renal transplant recipients than in immunocompetent patients: the role of azathioprine." Exp Dermatol 17(4): 349-355.

de la Cruz-Merino, L., F. Henao Carrasco, D. Vicente Baz, et al. (2011). "Immune microenvironment in colorectal cancer: a new hallmark to change old paradigms." Clin Dev Immunol 2011: 174149.

de Visscher, J. G., M. Schaapveld, R. Otter, et al. (1998). "Epidemiology of cancer of the lip in The Netherlands." Oral Oncol 34(5): 421-426.

de Visscher, J. G. and I. van der Waal (1998). "Etiology of cancer of the lip. a review." Int J Oral Maxillofac Surg 27(3): 199-203.

de Visscher, J. G. A. M., J. N. Bouwes Bavinck and I. van der Waal (1997). "Squamous cell carcinoma of the lower lip in renal-transplant recipients: Report of six cases." Int J Oral Maxillofac Surg 26(2): 120-123.

Denton, M. D., C. C. Magee and M. H. Sayegh (1999). "Immunosuppressive strategies in transplantation." The Lancet 353(9158): 1083-1091.

References

Dharnidharka, V. R., P.-L. Ho, D. M. Stablein, et al. (2002). "Mycophenolate, tacrolimus and post-transplant lymphoproliferative disorder: a report of the north American pediatric renal transplant cooperative study." Pediatr Transplant 6(5): 396-399.

Dharnidharka, V. R., K. E. Lamb, J. A. Gregg, et al. (2012). "Associations between EBV serostatus and organ transplant type in PTLD risk: an analysis of the SRTR national registry data in the United States." Am J Transplant 12(4): 976-983.

Dipchand, A. I., R. Kirk, L. B. Edwards, et al. (2013). "The registry of the international society for heart and lung transplantation: Sixteenth official pediatric heart transplantation report—2013; focus theme: Age." J Heart Lung Transplant 32(10): 979-988.

Donders, A. R. T., G. J. M. G. van der Heijden, T. Stijnen, et al. (2006). "Review: A gentle introduction to imputation of missing values." J Clin Epidemiol 59(10): 1087-1091.

Dotti, G., R. Fiocchi, T. Motta, et al. (2000). "Epstein-Barr virus-negative lymphoproliferate disorders in long-term survivors after heart, kidney, and liver transplant." Transplantation 69(5): 827-833.

Dotti, G., R. Fiocchi, T. Motta, et al. (2002). "Lymphomas occurring late after solid-organ transplantation: influence of treatment on the clinical outcome." Transplantation 74(8): 1095-1102.

Ducloux, D., P. L. Carron, J. M. Rebibou, et al. (1998). "CD4 lymphocytopenia as a risk factor for skin cancers in renal transplant recipients." Transplantation 65(9): 1270-1272.

Duncan, F. J., B. C. Wulff, K. L. Tober, et al. (2007). "Clinically relevant immunosuppressants influence UVB-induced tumor size through effects on inflammation and angiogenesis." Am J Transplant 7(12): 2693-2703.

References

Dunn, O. J. (1964). "Multiple comparisons using rank sums." Technometrics 6(3): 241-252.

Duvoux, C., G.-P. Pageaux, C. Vanlemmens, et al. (2002). "Risk factors for lymphoproliferative disorders after liver transplantation in adults: an analysis of 480 patients." Transplantation 74(8): 1103-1109.

Dworkin, A. M., K. L. Tober, F. J. Duncan, et al. (2009). "Chromosomal aberrations in UVB-induced tumors of immunosuppressed mice." Genes Chromosomes Cancer 48(6): 490-501.

Einollahi, B., M. Lessan-Pezeshki, M. H. Nourbala, et al. (2009). "Kaposi's sarcoma following living donor kidney transplantation: review of 7,939 recipients." Int Urol Nephrol 41(3): 679-685.

Elliott, A. C. and L. S. Hynan (2011). "A SAS® macro implementation of a multiple comparison post hoc test for a Kruskal–Wallis analysis." Computer Methods and Programs in Biomedicine 102(1): 75-80.

Engels, E. A., M. Frisch, J. J. Goedert, et al. (2002). "Merkel cell carcinoma and HIV infection." Lancet 359(9305): 497-498.

Engels, E. A., R. M. Pfeiffer, J. F. Fraumeni, et al. (2011). "Spectrum of cancer risk among US solid organ transplant recipients." JAMA 306(17): 1891-1901.

Engels, J. M. and P. Diehr (2003). "Imputation of missing longitudinal data: a comparison of methods." J Clin Epidemiol 56(10): 968-976.

European best practice guidelines for renal transplantation (2002). "Section IV: Long-term management of the transplant recipient. IV.6.2. Cancer risk after renal transplantation. Skin cancers: prevention and treatment." Nephrol Dial Transplant 17 Suppl 4: 31-36.

References

European Medicines Agency (2009). Guideline on clinical investigation of immunosuppressants for solid organ transplantation. E. M. Agency. London, UK: 1-15.

European Medicines Agency (2010). Guideline on missing data in confirmatory clinical trials. EMA/CPMP/EWP/1776/99.

Euvrard, S., J. Kanitakis and A. Claudy (2003). "Skin cancers after organ transplantation." N Engl J Med 348(17): 1681-1691.

Euvrard, S., J. Kanitakis, C. Pouteil-Noble, et al. (1995). "Comparative epidemiologic study of premalignant and malignant epithelial cutaneous lesions developing after kidney and heart transplantation." J Am Acad Dermatol 33(2 Pt 1): 222-229.

Faull, R. J., P. Hollett and S. P. McDonald (2005). "Lymphoproliferative disease after renal transplantation in Australia and New Zealand." Transplantation 80(2): 193-197.

Feng, H., M. Shuda, Y. Chang, et al. (2008). "Clonal integration of a polyomavirus in human Merkel cell carcinoma." Science 319(5866): 1096-1100.

Fernberg, P., G. Edgren, J. Adami, et al. (2011). "Time trends in risk and risk determinants of non-Hodgkin lymphoma in solid organ transplant recipients." Am J Transplant 11(11): 2472-2482.

Fine, J. and R. J. Gray (1999). "A proportional hazards model for the subdistribution of a competing risk." J Am Stat Assoc 94(446): 496-509.

Finkenstedt, A., I. W. Graziadei, W. Oberaigner, et al. (2009). "Extensive surveillance promotes early diagnosis and improved survival of de novo malignancies in liver transplant recipients." Am J Transplant 9(10): 2355-2361.

Fishman, J. A. (2007). "Infection in solid-organ transplant recipients." N Engl J Med 357(25): 2601-2614.

References

Fishman, J. A., V. Emery, R. Freeman, et al. (2007). "Cytomegalovirus in transplantation - challenging the status quo." Clin Transplant 21(2): 149-158.

Fishman, J. A. and P. A. Grossi (2014). "Donor-derived infection-the challenge for transplant safety." Nat Rev Nephrol advance online publication.

Fishman, J. A. and N. C. Issa (2010). "Infection in organ transplantation: risk factors and evolving patterns of infection." Infect Dis Clin North Am 24(2): 273-283.

Fortina, A. B., S. Piaserico, A. L. Caforio, et al. (2004). "Immunosuppressive level and other risk factors for basal cell carcinoma and squamous cell carcinoma in heart transplant recipients." Arch Dermatol 140(9): 1079-1085.

Foster, B. J., M. Dahhou, X. Zhang, et al. (2011). "Change in mortality risk over time in young kidney transplant recipients." Am J Transplant 11(11): 2432-2442.

Friedlaender, M. M., D. Rubinger, E. Rosenbaum, et al. (2002). "Temporary regression of Merkel cell carcinoma metastases after cessation of cyclosporine." Transplantation 73(11): 1849-1850.

Gane, E. J., B. C. Portmann, N. V. Naoumov, et al. (1996). "Long-term outcome of hepatitis C infection after liver transplantation." N Engl J Med 334(13): 815-820.

Gao, S. Z., S. V. Chaparro, M. Perlroth, et al. (2003). "Post-transplantation lymphoproliferative disease in heart and heart-lung transplant recipients: 30-year experience at Stanford University." J Heart Lung Transplant 22(5): 505-514.

Garibyan, L. and D. E. Fisher (2010). "How sunlight causes melanoma." Curr Oncol Rep 12(5): 319-326.

Gensler, H. L. and H. Chen (1991). "Enhanced growth and experimental metastasis of chemically induced tumor in ultraviolet irradiated syngeneic mice." Photochem Photobiol 53(5): 695-698.

Ghobrial, I. M., T. M. Habermann, W. R. Macon, et al. (2005). "Differences between early and late posttransplant lymphoproliferative disorders in solid organ transplant patients: are they two different diseases?" Transplantation 79(2): 244-247.

Glover, M. T., J. J. Deeks, M. J. Raftery, et al. (1997). "Immunosuppression and risk of non-melanoma skin cancer in renal transplant recipients." The Lancet 349(9049): 398.

Goh, A. and A. Vathsala (2011). "Native renal cysts and dialysis duration are risk factors for renal cell carcinoma in renal transplant recipients." Am J Transplant 11(1): 86-92.

Greenberg, J. N. and F. O. Zwald (2011). "Management of skin cancer in solid-organ transplant recipients: A multidisciplinary approach." Dermatol Clin 29(2): 231-241, ix.

Grosse, Y., R. Baan, K. Straif, et al. (2009). "A review of human carcinogens-Part A: pharmaceuticals." Lancet Oncol 10(1): 13-14.

Grulich, A. E., V. Bataille, A. J. Swerdlow, et al. (1996). "Naevi and pigmentary characteristics as risk factors for melanoma in a high-risk population: a case-control study in New South Wales, Australia." Int J Cancer 67(4): 485-491.

Grulich, A. E., M. T. van Leeuwen, M. O. Falster, et al. (2007). "Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis." Lancet 370(9581): 59-67.

Grulich, A. E., X. Wan and M. Coates (1996). "Validation of a non-identifying method of linking cancer and AIDS register data." J Epidemiol Biostat 1: 207-212.

Guiguet, M., F. Boué, J. Cadranel, et al. (2009). "Effect of immunodeficiency, HIV viral load, and antiretroviral therapy on the risk of individual malignancies (FHDH-ANRS CO4): a prospective cohort study." Lancet Oncol 10(12): 1152-1159.

References

Gutierrez-Dalmau, A. and J. M. Campistol (2007). "Immunosuppressive therapy and malignancy in organ transplant recipients: a systematic review." Drugs 67(8): 1167-1198.

Guttmann, R. D. (1979). "Renal transplantation." N Engl J Med 301(18): 975-982.

Haagsma, E. B., V. E. Hagens, M. Schaapveld, et al. (2001). "Increased cancer risk after liver transplantation: a population-based study." J Hepatol 34(1): 84-91.

Hachem, R. R. (2009). "Lung allograft rejection: diagnosis and management." Curr Opin Organ Transplant 14(5): 477-482.

Hakala, T. R., T. E. Starzl, J. T. Rosenthal, et al. (1983). "Cadaveric renal transplantation with cyclosporin-A and steroids." Transplant Proc 15(1): 465-470.

Halloran, P. F. (2004). "Immunosuppressive drugs for kidney transplantation." N Engl J Med 351(26): 2715-2729.

Hamelin, R., C. Borie, M. Raphael, et al. (2004). "An immunogenic process leading to cancer in the context of immunodeficiency." Cell Cycle 3(9): 1130-1132.

Hanouneh, I. A., C. Macaron, R. Lopez, et al. (2012). "Risk of colonic neoplasia after liver transplantation for primary sclerosing cholangitis." Inflamm Bowel Dis 18(2): 269-274.

Hartevelt, M. M., J. N. Bavinck, A. M. Kootte, et al. (1990). "Incidence of skin cancer after renal transplantation in The Netherlands." Transplantation 49(3): 506-509.

Hatton, J. L., A. Parent, K. L. Tober, et al. (2007). "Depletion of CD4+ cells exacerbates the cutaneous response to acute and chronic uvb exposure." J Invest Dermatol 127(6): 1507-1515.

Heidt, S., D. L. Roelen, C. Eijsink, et al. (2008). "Effects of immunosuppressive drugs on purified human B cells: evidence supporting the use of MMF and rapamycin." Transplantation 86(9): 1292-1300.

Heinbokel, T., A. Elkhal, G. Liu, et al. (2013). "Immunosenescence and organ transplantation." Transplant Rev 27(3): 65-75.

Henry, M. L. and A. Rajab (2002). "The use of basiliximab in solid organ transplantation." Expert Opin Pharmacother 3(11): 1657-1663.

Herrero, J. I., A. España, J. Quiroga, et al. (2005). "Nonmelanoma skin cancer after liver transplantation. study of risk factors." Liver Transpl 11(9): 1100-1106.

Herrero, J. I., M. Lorenzo, J. Quiroga, et al. (2005). "De novo neoplasia after liver transplantation: an analysis of risk factors and influence on survival." Liver Transpl 11(1): 89-97.

Hill, R. B., Jr., B. E. Dahrling, 2nd, T. E. Starzl, et al. (1967). "Death after transplantation; an analysis of sixty cases." Am J Med 42(3): 327-334.

Hofbauer, G. F., N. R. Attard, C. A. Harwood, et al. (2012). "Reversal of UVA skin photosensitivity and DNA damage in kidney transplant recipients by replacing azathioprine." Am J Transplant 12(1): 218-225.

Hojo, M., T. Morimoto, M. Maluccio, et al. (1999). "Cyclosporine induces cancer progression by a cell-autonomous mechanism." Nature 397(6719): 530-534.

Humar, A. and D. L. Dunn (2010). Chapter 11. Transplantation. Schwartz's Principles of Surgery, 9e. F. C. Brunicardi, D. K. Andersen, T. R. Billiar et al. New York, NY, The McGraw-Hill Companies.

IARC (1987). "Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1 to 42." IARC Monogr Eval Carcinog Risks Hum Suppl 7: 1-440.

References

IARC (2012). Biological agents. Volume 100 B. A review of human carcinogens. Lyon, France.

IARC (2012). "Personal habits and indoor combustions. Volume 100 E. A review of human carcinogens." IARC Monogr Eval Carcinog Risks Hum 100(Pt E): 1-538.

IARC, Ed. (2012). Pharmaceuticals. Volume 100 A. A review of human carcinogens. IARC Monogr Eval Carcinog Risks Hum.

IARC (2012). "Radiation." IARC Monogr Eval Carcinog Risks Hum 100(Pt D): 7-303.

Imamura, N., M. Nakano, A. Kawase, et al. (1973). "Synergistic action of N-nitrosobutylurea and azathioprine in induction of leukemia in C57BL mice." Gann 64(5): 493-498.

Ingvar, Å., K. E. Smedby, B. Lindelöf, et al. (2010). "Immunosuppressive treatment after solid organ transplantation and risk of post-transplant cutaneous squamous cell carcinoma." Nephrol Dial Transplant 25(8): 2764-2771.

Ito, A., M. Mori and M. Naito (1989). "Induction of uterine hemangioendothelioma and lymphoma in (C57BL/6N x C3H/2N)F1 mice by oral administration of azathioprine." Jpn J Cancer Res 80(5): 419-423.

Jackson, R. C., G. Weber and H. P. Morris (1975). "IMP dehydrogenase, an enzyme linked with proliferation and malignancy." Nature 256(5515): 331-333.

Jain, A. B., L. D. Yee, M. A. Nalesnik, et al. (1998). "Comparative incidence of de novo nonlymphoid malignancies after liver transplantation under tacrolimus using surveillance epidemiologic end result data." Transplantation 66(9): 1193-1200.

Jaro, M. A. (1995). "Probabilistic linkage of large public health data files." Stat Med 14(5-7): 491-498.

Jensen, P., S. Hansen, B. Moller, et al. (1999). "Skin cancer in kidney and heart transplant recipients and different long-term immunosuppressive therapy regimens." J Am Acad Dermatol 40(2 Pt 1): 177-186.

Jiang, Y., P. J. Villeneuve, S. S. Fenton, et al. (2008). "Liver transplantation and subsequent risk of cancer: findings from a Canadian cohort study." Liver Transpl 14(11): 1588-1597.

Jiang, Y., P. J. Villeneuve, A. Wielgosz, et al. (2010). "The incidence of cancer in a population-based cohort of Canadian heart transplant recipients." Am J Transplant 10(3): 637-645.

Kanitakis, J. and S. Euvrard (2013). "Transplantation: donor-derived skin cancer in a kidney transplant recipient." Nat Rev Nephrol 9(12): 702-703.

Kaplan, J. E., C. Benson, K. K. Holmes, et al. (2009). "Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America." MMWR Recomm Rep 58(RR-4): 1-207; quiz CE201-204.

Karran, P. and N. Attard (2008). "Thiopurines in current medical practice: molecular mechanisms and contributions to therapy-related cancer." Nat Rev Cancer 8(1): 24-36.

Kasiske, B. L., J. J. Snyder, D. T. Gilbertson, et al. (2004). "Cancer after kidney transplantation in the United States." Am J Transplant 4(6): 905-913.

Kasiske, B. L., M. A. Vazquez, W. E. Harmon, et al. (2000). "Recommendations for the outpatient surveillance of renal transplant recipients. American Society of Transplantation." J Am Soc Nephrol 11 Suppl 15: S1-86.

References

Katz, B. Z., E. Pahl, S. E. Crawford, et al. (2007). "Case–control study of risk factors for the development of post-transplant lymphoproliferative disease in a pediatric heart transplant cohort*." Pediatr Transplant 11(1): 58-65.

Kawahara, T., S. Asthana and N. M. Kneteman (2011). "m-TOR inhibitors: what role in liver transplantation?" J Hepatol 55(6): 1441-1451.

Kellerman, L., A. Neugut, B. Burke, et al. (2009). "Comparison of the Incidence of de novo solid malignancies after heart transplantation to that in the general population." Am J Cardiol 103(4): 562-566.

Kelly, D. A., J. C. Bucuvalas, E. M. Alonso, et al. (2013). "Long-term medical management of the pediatric patient after liver transplantation: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation." Liver Transpl 19(8): 798-825.

Kelly, G. E., W. Meikle and A. G. Sheil (1987). "Scheduled and unscheduled DNA synthesis in epidermal cells of hairless mice treated with immunosuppressive drugs and UVB-UVA irradiation." Br J Dermatol 117(4): 429-440.

Kerkar, N., R. A. Morotti, R. P. Madan, et al. (2010). "The changing face of post-transplant lymphoproliferative disease in the era of molecular EBV monitoring." Pediatr Transplant 14(4): 504-511.

Kew, C. E., 2nd, R. Lopez-Ben, J. K. Smith, et al. (2000). "Postransplant lymphoproliferative disorder localized near the allograft in renal transplantation." Transplantation 69(5): 809-814.

Kiberd, B. A., C. Rose and J. S. Gill (2009). "Cancer mortality in kidney transplantation." Am J Transplant 9(8): 1868-1875.

Kidney Disease: Improving Global Outcomes Transplant Work, G. (2009). "KDIGO clinical practice guideline for the care of kidney transplant recipients." Am J Transplant 9 Suppl 3: S1-155.

Kinch, A., E. Baecklund, C. Backlin, et al. (2014). "A population-based study of 135 lymphomas after solid organ transplantation: The role of Epstein-Barr virus, hepatitis C and diffuse large B-cell lymphoma subtype in clinical presentation and survival." Acta Oncologica 53(5): 669-679.

King, G. N., C. M. Healy, M. T. Glover, et al. (1995). "Increased prevalence of dysplastic and malignant lip lesions in renal-transplant recipients." N Engl J Med 332(16): 1052-1057.

Kinlen, L. J., A. G. Sheil, J. Peto, et al. (1979). "Collaborative United Kingdom-Australasian study of cancer in patients treated with immunosuppressive drugs." Br Med J 2(6203): 1461-1466.

Kirk, A. D. (2006). "Induction Immunosuppression." Transplantation 82(5): 593-602

Kirk, A. D., W. S. Cherikh, M. Ring, et al. (2007). "Dissociation of depletional induction and posttransplant lymphoproliferative disease in kidney recipients treated with alemtuzumab." Am J Transplant 7(11): 2619-2625.

Klipa, D., N. Mahmud and N. Ahsan (2010). "Antibody immunosuppressive therapy in solid organ transplant: Part II." mAbs 2(6): 607-612.

Knight, J. S., A. Tsodikov, D. M. Cibrik, et al. (2009). "Lymphoma after solid organ transplantation: risk, response to therapy, and survival at a transplantation center." J Clin Oncol 27(20): 3354-3362.

Koljonen, V., H. Kukko, E. Tukiainen, et al. (2009). "Incidence of Merkel cell carcinoma in renal transplant recipients." Nephrol Dial Transplant 24(10): 3231-3235.

References

Koshiol, J., K. Pawlish, M. T. Goodman, et al. (2014). "Risk of hepatobiliary cancer after solid organ transplant in the United States." Clin Gastroenterol Hepatol 12(9): 1541-1549 e1543.

Kotlyar, D. S., J. D. Lewis, L. Beaugerie, et al. (2014). "Risk of lymphoma in patients with inflammatory bowel disease treated with azathioprine and 6-mercaptopurine: a meta-analysis." Clin Gastroenterol Hepatol.

Kotton, C. N. and J. A. Fishman (2005). "Viral infection in the renal transplant recipient." J Am Soc Nephrol 16(6): 1758-1774.

Kotton, C. N., D. Kumar, A. M. Caliendo, et al. (2013). "Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation." Transplantation 96(4): 333-360.

Krynitz, B., G. Edgren, B. Lindelöf, et al. (2013). "Risk of skin cancer and other malignancies in kidney, liver, heart and lung transplant recipients 1970 to 2008—A Swedish population-based study." Int J Cancer 132(6): 1429-1438.

Kuppers, R. (2003). "B cells under influence: transformation of B cells by Epstein-Barr virus." Nat Rev Immunol 3(10): 801-812.

LaCasce, A. S. (2006). "Post-Transplant Lymphoproliferative Disorders." The Oncologist 11(6): 674-680.

Lampros, T. D., A. Cobanoglu, F. Parker, et al. (1998). "Squamous and basal cell carcinoma in heart transplant recipients." J Heart Lung Transplant 17(6): 586-591.

Lanoy, E., G. M. Dores, M. M. Madeleine, et al. (2009). "Epidemiology of nonkeratinocytic skin cancers among persons with AIDS in the United States." AIDS 23(3): 385-393.

References

Lebbe, C., C. Legendre and C. Frances (2008). "Kaposi sarcoma in transplantation." Transplant Rev (Orlando) 22(4): 252-261.

Lee, T. C., B. Savoldo, C. M. Rooney, et al. (2005). "Quantitative EBV Viral Loads and Immunosuppression Alterations can Decrease PTLD Incidence in Pediatric Liver Transplant Recipients." Am J Transplant 5(9): 2222-2228.

Lennard, L., S. Thomas, C. I. Harrington, et al. (1985). "Skin cancer in renal transplant recipients is associated with increased concentrations of 6-thioguanine nucleotide in red blood cells." Br J Dermatol 113(6): 723-729.

Levi, M. E., D. Kumar, M. Green, et al. (2014). "Considerations for screening live kidney donors for endemic infections: a viewpoint on the UNOS policy." Am J Transplant 14(5): 1003-1011.

Libertiny, G., C. J. Watson, D. W. Gray, et al. (2001). "Rising incidence of post-transplant lymphoproliferative disease in kidney transplant recipients." Br J Surg 88(10): 1330-1334.

Lieberman, D. A. (2009). "Screening for colorectal cancer." N Engl J Med 361(12): 1179-1187.

Lim, W. H., J. Eris, J. Kanellis, et al. (2014). "A systematic review of conversion from calcineurin inhibitor to mammalian target of rapamycin inhibitors for maintenance immunosuppression in kidney transplant recipients." Am J Transplant epub ahead of print.

Lim, W. H., R. M. Turner, J. R. Chapman, et al. (2014). "Acute rejection, T-cell–depleting antibodies, and cancer after transplantation." Transplantation 97(8): 817-825.

References

Lindelof, B., J. Jarnvik, A. Ternesten-Bratel, et al. (2006). "Mortality and clinicopathological features of cutaneous squamous cell carcinoma in organ transplant recipients: a study of the Swedish cohort." Acta Derm Venereol 86(3): 219-222.

Lindenfeld, J., G. G. Miller, S. F. Shakar, et al. (2004). "Drug therapy in the heart transplant recipient: Part i: Cardiac rejection and immunosuppressive drugs." Circulation 110(24): 3734-3740.

Liptak, P. and B. Ivanyi (2006). "Primer: histopathology of calcineurin-inhibitor toxicity in renal allografts." Nat Clin Pract Nephrol 2(7): 398-404;.

Long, M. D., H. H. Herfarth, C. A. Pipkin, et al. (2010). "Increased risk for non-melanoma skin cancer in patients with inflammatory bowel disease." Clin Gastroenterol Hepatol 8(3): 268-274.

López-Pintor, R. M., G. Hernández, L. de Arriba, et al. (2011). "Lip cancer in renal transplant patients." Oral Oncology 47(1): 68-71.

Loren, A. W., D. L. Porter, E. A. Stadtmauer, et al. (2003). "Post-transplant lymphoproliferative disorder: a review." Bone Marrow Transplant 31(3): 145-155.

Lucey, M. R. (2014). "Liver transplantation for alcoholic liver disease." Nat Rev Gastroenterol Hepatol 11(5): 300-307.

Lucey, M. R., N. Terrault, L. Ojo, et al. (2013). "Long-term management of the successful adult liver transplant: 2012 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation." Liver Transpl 19(1): 3-26.

Madeleine, M. M., J. L. Finch, C. F. Lynch, et al. (2013). "HPV-related cancers after solid organ transplantation in the United States." Am J Transplant 13(12): 3202-3209.

Mahmud, N., D. Klipa and N. Ahsan (2010). "Antibody immunosuppressive therapy in solid-organ transplant: Part I." mAbs 2(2): 148-156.

Majewski, M., M. Korecka, J. Joergensen, et al. (2003). "Immunosuppressive TOR kinase inhibitor everolimus (RAD) suppresses growth of cells derived from posttransplant lymphoproliferative disorder at allograft-protecting doses." Transplantation 75(10): 1710-1717.

Majewski, M., M. Korecka, P. Kossev, et al. (2000). "The immunosuppressive macrolide RAD inhibits growth of human Epstein-Barr virus-transformed B lymphocytes in vitro and in vivo: A potential approach to prevention and treatment of posttransplant lymphoproliferative disorders." Proc Natl Acad Sci U S A 97(8): 4285-4290.

Maluccio, M., V. Sharma, M. Lagman, et al. (2003). "Tacrolimus enhances transforming growth factor-beta1 expression and promotes tumor progression." Transplantation 76(3): 597-602.

Marcus, J. L., C. R. Chao, W. A. Leyden, et al. (2014). "Prostate cancer incidence and prostate-specific antigen testing among HIV-positive and HIV-negative men." J Acquir Immune Defic Syndr 66(5): 495-502.

Martin, P., A. DiMartini, S. Feng, et al. (2014). "Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation." Hepatology 59(3): 1144-1165.

Martinu, T., E. N. Pavlisko, D. F. Chen, et al. (2011). "Acute allograft rejection: cellular and humoral processes." Clin Chest Med 32(2): 295-310.

Matin, R. N., D. Mesher, C. M. Proby, et al. (2008). "Melanoma in Organ Transplant Recipients: Clinicopathological Features and Outcome in 100 Cases." Am J Transplant 8(9): 1891-1900.

Matsuda, S. and S. Koyasu (2000). "Mechanisms of action of cyclosporine." Immunopharmacology 47(2-3): 119-125.

Mbulaiteye, S. M. and E. A. Engels (2006). "Kaposi's sarcoma risk among transplant recipients in the United States (1993-2003)." Int J Cancer 119(11): 2685-2691.

McGreevy, K. M., S. R. Lipsitz, J. A. Linder, et al. (2009). "Using median regression to obtain adjusted estimates of central tendency for skewed laboratory and epidemiologic data." Clin Chem 55(1): 165-169.

McGregor, J. M., R. J. Berkhout, M. Rozycka, et al. (1997). "p53 mutations implicate sunlight in post-transplant skin cancer irrespective of human papillomavirus status." Oncogene 15(14): 1737-1740.

McGuire, B. M., P. Rosenthal, C. C. Brown, et al. (2009). "Long-term management of the liver transplant patient: recommendations for the primary care doctor." Am J Transplant 9(9): 1988-2003.

McLaughlin, K., S. Wajstaub, P. Marotta, et al. (2000). "Increased risk for posttransplant lymphoproliferative disease in recipients of liver transplants with hepatitis C." Liver Transpl 6(5): 570-574.

Meier-Kriesche, H. U., G. Friedman, M. Jacobs, et al. (1999). "Infectious complications in geriatric renal transplant patients: comparison of two immunosuppressive protocols." Transplantation 68(10): 1496-1502.

Meier-Kriesche, H. U., S. Li, R. W. G. Gruessner, et al. (2006). "Immunosuppression: evolution in practice and trends, 1994–2004." Am J Transplant 6(5p2): 1111-1131.

Melosky, B., M. Karim, A. Chui, et al. (1992). "Lymphoproliferative disorders after renal transplantation in patients receiving triple or quadruple immunosuppression." J Am Soc Nephrol 2(12): S290-294.

Mertens, H. G., G. Hertel, P. Reuther, et al. (1981). "Effect of immunosuppressive drugs (azathioprine)." Ann N Y Acad Sci 377(1): 691-699.

Metcalfe, M. J., D. J. Kutsogiannis, K. Jackson, et al. (2010). "Risk factors and outcomes for the development of malignancy in lung and heart-lung transplant recipients." Can Respir J 17(1): e7-13.

Miller, A. J. and M. C. Mihm, Jr. (2006). "Melanoma." N Engl J Med 355(1): 51-65.

Mistrikova, J., M. Mrmusova, V. Durmanova, et al. (1999). "Increased neoplasm development due to immunosuppressive treatment with FK-506 in BALB/C mice persistently infected with the mouse herpesvirus (MHV-72)." Viral Immunol 12(3): 237-247.

Mita, M. M., A. Mita and E. K. Rowinsky (2003). "The molecular target of rapamycin (mTOR) as a therapeutic target against cancer." Cancer Biol Ther 2(4 Suppl 1): S169-177.

Mohty, M. (2007). "Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond." Leukemia 21(7): 1387-1394.

Moloney, F. J., H. Comber, P. O'Lorcain, et al. (2006). "A population-based study of skin cancer incidence and prevalence in renal transplant recipients." Br J Dermatol 154(3): 498-504.

Molyneux, G., F. M. Gibson, C. M. Chen, et al. (2008). "The haemotoxicity of azathioprine in repeat dose studies in the female CD-1 mouse." Int J Exp Pathol 89(2): 138-158.

Moore, P. S. and Y. Chang (2010). "Why do viruses cause cancer? Highlights of the first century of human tumour virology." Nat Rev Cancer 10(12): 878-889.

Moore, S. R., N. W. Johnson, A. M. Pierce, et al. (1999). "The epidemiology of lip cancer: a review of global incidence and aetiology." Oral Diseases 5(3): 185-195.

Morath, C., J. Beimler, G. Opelz, et al. (2010). "An integrative approach for the transplantation of high-risk sensitized patients." Transplantation 90(6): 645-653.

Morton, L. M., O. Landgren, N. Chatterjee, et al. (2007). "Hepatitis C virus infection and risk of posttransplantation lymphoproliferative disorder among solid organ transplant recipients." Blood 110(13): 4599-4605.

Morton, M., B. Coupes, S. A. Roberts, et al. (2014). "Epstein–barr virus infection in adult renal transplant recipients." Am J Transplant 14(7): 1619-1629.

Mosli, M., K. Croome, K. Qumosani, et al. (2013). "The effect of liver transplantation for primary sclerosing cholangitis on disease activity in patients with inflammatory bowel disease." Gastroenterol Hepatol (N Y) 9(7): 434-441.

Muellenhoff, M. W. and J. Y. Koo (2012). "Cyclosporine and skin cancer: an international dermatologic perspective over 25 years of experience. A comprehensive review and pursuit to define safe use of cyclosporine in dermatology." J Dermatolog Treat 23(4): 290-304.

Muirhead, R. and D. M. Ritchie (2007). "Partial regression of Merkel cell carcinoma in response to withdrawal of azathioprine in an immunosuppression-induced case of metastatic Merkel cell carcinoma." Clin Oncol (R Coll Radiol) 19(1): 96.

Murphy, G. M. (2009). "Ultraviolet radiation and immunosuppression." Br J Dermatol 161 Suppl 3: 90-95.

Muthukkumar, S., T. M. Ramesh and S. Bondada (1995). "Rapamycin, a potent immunosuppressive drug, causes programmed cell death in B lymphoma cells." Transplantation 60(3): 264-270.

References

Myron Kauffman, H., M. A. McBride, W. S. Cherikh, et al. (2002). "Transplant tumor registry: donor related malignancies." Transplantation 74(3): 358-362.

Na, R., A. E. Grulich, N. S. Meagher, et al. (2013). "Comparison of de novo cancer incidence in Australian liver, heart and lung transplant recipients." Am J Transplant 13(1): 174-183.

Na, R., M. A. Laaksonen, A. E. Grulich, et al. (2014). "Immunosuppression therapies for a national cohort of Australian liver, heart, and lung transplant recipients, 1984 to 2006." Am J Transplant Submitted.

Nagai, M., Y. Natsumeda, Y. Konno, et al. (1991). "Selective up-regulation of type II inosine 5'-monophosphate dehydrogenase messenger RNA expression in human leukemias." Cancer Res 51(15): 3886-3890.

Nalesnik, M. A., E. S. Woodle, J. M. Dimaio, et al. (2011). "Donor-transmitted malignancies in organ transplantation: assessment of clinical risk." Am J Transplant 11(6): 1140-1147.

Nepomuceno, R. R., C. E. Balatoni, Y. Natkunam, et al. (2003). "Rapamycin inhibits the interleukin 10 signal transduction pathway and the growth of Epstein Barr virus B-cell lymphomas." Cancer Res 63(15): 4472-4480.

Neto, J. S., R. Pugliese, E. A. Fonseca, et al. (2012). "Four hundred thirty consecutive pediatric living donor liver transplants: Variables associated with posttransplant patient and graft survival." Liver Transpl 18(5): 577-584.

Nguyen, T., P. M. Vacek, P. O'Neill, et al. (2009). "Mutagenicity and potential carcinogenicity of thiopurine treatment in patients with inflammatory bowel disease." Cancer Res 69(17): 7004-7012.

Nordlund, J. J. (2007). "The melanocyte and the epidermal melanin unit: an expanded concept." Dermatol Clin 25(3): 271-281, vii.

Norman, D. J. (1995). "Mechanisms of action and overview of OKT3." Ther Drug Monit 17(6): 615-620.

O'Donovan, P., C. M. Perrett, X. Zhang, et al. (2005). "Azathioprine and UVA light generate mutagenic oxidative DNA damage." Science 309(5742): 1871-1874.

O'Neill, J. O., L. B. Edwards and D. O. Taylor (2006). "Mycophenolate mofetil and risk of developing malignancy after orthotopic heart transplantation: analysis of the transplant registry of the International Society for Heart and Lung Transplantation." J Heart Lung Transplant 25(10): 1186-1191.

O'Reilly Zwald, F. and M. Brown (2011). "Skin cancer in solid organ transplant recipients: Advances in therapy and management: Part I. Epidemiology of skin cancer in solid organ transplant recipients." J Am Acad Dermatol 65(2): 253-261.

Olagne, J., S. Caillard, M. P. Gaub, et al. (2011). "Post-transplant lymphoproliferative disorders: determination of donor/recipient origin in a large cohort of kidney recipients." Am J Transplant 11(6): 1260-1269.

Oliveira, V. D., H. Zankl and T. Rath (2004). "Mutagenic and cytotoxic effects of immunosuppressive drugs on human lymphocyte cultures." Exp Clin Transplant 2(2): 273-279.

Olland, A. B. M., P.-E. Falcoz, N. Santelmo, et al. (2014). "Primary lung cancer in lung transplant recipients." Ann Thorac Surg 98(1): 362-371.

Ong, C. S., A. M. Keogh, S. Kossard, et al. (1999). "Skin cancer in Australian heart transplant recipients." J Am Acad Dermatol 40(1): 27-34.

Oo, Y. H., B. K. Gunson, R. J. Lancashire, et al. (2005). "Incidence of cancers following orthotopic liver transplantation in a single center: comparison with national cancer incidence rates for England and Wales." Transplantation 80(6): 759-764.

Opelz, G., V. Daniel, C. Naujokat, et al. (2009). "Epidemiology of pretransplant EBV and CMV serostatus in relation to posttransplant non-Hodgkin lymphoma." Transplantation 88(8): 962-967.

Opelz, G., V. Daniel, C. Naujokat, et al. (2007). "Effect of cytomegalovirus prophylaxis with immunoglobulin or with antiviral drugs on post-transplant non-Hodgkin lymphoma: a multicentre retrospective analysis." Lancet Oncol 8(3): 212-218.

Opelz, G. and B. Dohler (2004). "Lymphomas after solid organ transplantation: a collaborative transplant study report." Am J Transplant 4(2): 222-230.

Opelz, G. and B. Dohler (2010). "Impact of HLA mismatching on incidence of posttransplant non-hodgkin lymphoma after kidney transplantation." Transplantation 89(5): 567-572.

Opelz, G. and R. Henderson (1993). "Incidence of non-hodgkin lymphoma in kidney and heart transplant recipients." The Lancet 342(8886-8887): 1514-1516.

Opelz, G., C. Naujokat, V. Daniel, et al. (2006). "Disassociation between risk of graft loss and risk of non-hodgkin lymphoma with induction agents in renal transplant recipients." Transplantation 81(9): 1227-1233.

OPTN/SRTR (2012). OPTN/SRTR 2011 annual data report. Rockville, MD, US Department of Health and Human Services.

Ozturk, S., T. K. Ayna, K. Cefle, et al. (2008). "Effect of cyclosporin A and tacrolimus on sister chromatid exchange frequency in renal transplant patients." Genet Test 12(3): 427-430.

Parker, A., K. Bowles, J. A. Bradley, et al. (2010). "Management of post-transplant lymphoproliferative disorder in adult solid organ transplant recipients – BCSH and BTS Guidelines." Br J Haematol 149(5): 693-705.

Parker, A., K. Bowles, J. A. Bradley, et al. (2010). "Diagnosis of post-transplant lymphoproliferative disorder in solid organ transplant recipients – BCSH and BTS Guidelines." Br J Haematol 149(5): 675-692.

Parkin, D. M., S. L. Whelan, J. Ferlay, et al., Eds. (2002). Cancer incidence in five continents. Lyon (Fraqnce), IARC.

Pascual, J. (2007). "Post-transplant lymphoproliferative disorder—the potential of proliferation signal inhibitors." Nephrol Dial Transplant 22(suppl 1): i27-i35.

Penn, I. (1990). "Cancers complicating organ transplantation." N Engl J Med 323(25): 1767-1769.

Penn, I. and M. R. First (1999). "Merkel's cell carcinoma in organ recipients: Report of 41 cases1." Transplantation 68(11): 1717-1721.

Penn, I., W. Hammond, L. Brettschneider, et al. (1969). "Malignant lymphomas in transplantation patients." Transplant Proc 1(1): 106-112.

Perea-Milla Lopez, E., R. M. Minarro-Del Moral, C. Martinez-Garcia, et al. (2003). "Lifestyles, environmental and phenotypic factors associated with lip cancer: a case-control study in southern Spain." Br J Cancer 88(11): 1702-1707.

Perrett, C. M., C. A. Harwood, J. M. McGregor, et al. (2010). "Expression of DNA mismatch repair proteins and MSH2 polymorphisms in nonmelanoma skin cancers of organ transplant recipients." Br J Dermatol 162(4): 732-742.

Pillai, A. A. and J. Levitsky (2009). "Overview of immunosuppression in liver transplantation." World J Gastroenterol 15(34): 4225-4233.

Pintilie, M. (2007). "Analysing and interpreting competing risk data." Stat Med 26(6): 1360-1367.

Piselli, P., G. Busnach, F. Citterio, et al. (2009). "Risk of kaposi sarcoma after solid-organ transplantation: Multicenter study in 4767 recipients in italy, 1970-2006." Transplant Proc 41(4): 1227-1230.

Polesel, J., G. M. Clifford, M. Rickenbach, et al. (2008). "Non-Hodgkin lymphoma incidence in the Swiss HIV Cohort Study before and after highly active antiretroviral therapy." AIDS 22(2): 301-306 310.1097/QAD.1090b1013e3282f2705d.

Ponticelli, C. (2014). "Basiliximab: efficacy and safety evaluation in kidney transplantation." Expert Opin Drug Saf 13(3): 373-381.

Quinlan, S. C., R. M. Pfeiffer, L. M. Morton, et al. (2011). "Risk factors for early-onset and late-onset post-transplant lymphoproliferative disorder in kidney recipients in the United States." Am J Hematol 86(2): 206-209.

Rafferty, P., D. Egenolf, K. Brosnan, et al. (2012). "Immunotoxicologic effects of cyclosporine on tumor progression in models of squamous cell carcinoma and B-cell lymphoma in C3H mice." J Immunotoxicol 9(1): 43-55.

Rama, I. and J. M. Grinyo (2010). "Malignancy after renal transplantation: the role of immunosuppression." Nat Rev Nephrol 6(9): 511-519.

Ramsay, H. M., A. A. Fryer, C. M. Hawley, et al. (2002). "Non-melanoma skin cancer risk in the Queensland renal transplant population." Br J Dermatol 147(5): 950-956.

Ramsay, H. M., A. A. Fryer, C. M. Hawley, et al. (2003). "Factors associated with nonmelanoma skin cancer following renal transplantation in Queensland, Australia." J Am Acad Dermatol 49(3): 397-406.

Reinke, P., E. Fietze, S. Ode-Hakim, et al. (1994). "Late-acute renal allograft rejection and symptomless cytomegalovirus infection." Lancet 344(8939-8940): 1737-1738.

Reitz, B. A. and E. B. Stinson (1982). "Cardiac transplantation--1982." JAMA 248(10): 1225-1227.

Reitz, B. A., J. L. Wallwork, S. A. Hunt, et al. (1982). "Heart-lung transplantation: successful therapy for patients with pulmonary vascular disease." N Engl J Med 306(10): 557-564.

Reshef, R., S. Vardhanabhuti, M. R. Luskin, et al. (2011). "Reduction of immunosuppression as initial therapy for posttransplantation lymphoproliferative disorder." Am J Transplant 11(2): 336-347.

Robson, R., J. M. Cecka, G. Opelz, et al. (2005). "Prospective registry-based observational cohort study of the long-term risk of malignancies in renal transplant patients treated with mycophenolate mofetil." Am J Transplant 5(12): 2954-2960.

Roithmaier, S., A. M. Haydon, S. Loi, et al. (2007). "Incidence of malignancies in heart and/or lung transplant recipients: a single-institution experience." J Heart Lung Transplant 26(8): 845-849.

Rojas, I. G., A. Martinez, U. Brethauer, et al. (2009). "Actinic cheilitis: epithelial expression of COX-2 and its association with mast cell tryptase and PAR-2." Oral Oncol 45(3): 284-290.

Rosen, H. R. (2003). "Hepatitis C virus in the human liver transplantation model." Clin Liver Dis 7(1): 107-125.

Rosenthal, J. T., T. R. Hakala, S. Iwatsuki, et al. (1983). "Cadaveric renal transplantation under cyclosporine-steroid therapy." Surg Gynecol Obstet 157(4): 309-315.

Rothman, K. J., S. Greenland and T. L. Lash (2008; p.254). Modern Epidemiology. Philadelphia, PA, Lippincott Williams & Wilkins.

Rubin, D. (1978). "Multiple imputations in sample surveys - a phenomenological bayesian approach to nonrepsonse." Proceedings of the Survey Research Methods Section of the American Statistical Association: 20 - 34.

Rubin, J., N. Ayoub, F. Kaldas, et al. (2012). "Management of recurrent hepatocellular carcinoma in liver transplant recipients: a systematic review." Exp Clin Transplant 10(6): 531-543.

Ruttens, D., S. E. Verleden, P. C. Goeminne, et al. (2014). "Smoking resumption after lung transplantation: standardised screening and importance for long-term outcome." Eur Respir J 43(1): 300-303.

Sánchez-Fueyo, A. and T. B. Strom (2011). "Immunologic basis of graft rejection and tolerance following transplantation of liver or other solid organs." Gastroenterology 140(1): 51-64.

Sarfati, D., T. Blakely and N. Pearce (2010). "Measuring cancer survival in populations: relative survival vs cancer-specific survival." Int J Epidemiol 39(2): 598-610.

Scherer, M., B. Banas, K. Mantouvalou, et al. (2007). "Current concepts and perspectives of immunosuppression in organ transplantation." Langenbecks Arch Surg 392(5): 511-523.

Schober, T., T. Framke, H. Kreipe, et al. (2013). "Characteristics of early and late PTLD development in pediatric solid organ transplant recipients." Transplantation 95(1): 240-246.

Schrama, D., S. Ugurel and J. C. Becker (2012). "Merkel cell carcinoma: recent insights and new treatment options." Curr Opin Oncol 24(2): 141-149.

Schubert, S., H. Abdul-Khaliq, H. B. Lehmkuhl, et al. (2009). "Diagnosis and treatment of post-transplantation lymphoproliferative disorder in pediatric heart transplant patients." Pediatr Transplant 13(1): 54-62.

Schubert, S., C. Renner, M. Hammer, et al. (2008). "Relationship of immunosuppression to Epstein-Barr viral load and lymphoproliferative disease in pediatric heart transplant patients." J Heart Lung Transplant 27(1): 100-105.

Schulz, T. F. (2009). "Cancer and viral infections in immunocompromised individuals." Int J Cancer 125(8): 1755-1763.

Secretan, B., K. Straif, R. Baan, et al. (2009). "A review of human carcinogens--Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish." Lancet Oncol 10(11): 1033-1034.

Seem, D. L., I. Lee, C. A. Umscheid, et al. (2013). "Excerpt from PHS guideline for reducing HIV, HBV and HCV transmission through organ transplantation." Am J Transplant 13(8): 1953-1962.

Serraino, D., C. Angeletti, M. P. Carrieri, et al. (2005). "Kaposi's Sarcoma in Transplant and HIV-infected Patients: An Epidemiologic Study in Italy and France." Transplantation 80(12): 1699-1704.

Sgro, C. (1995). "Side-effects of a monoclonal antibody, muromonab CD3/orthoclone OKT3: bibliographic review." Toxicology 105(1): 23-29.

Shimizu, M., A. Adachi, S. Zheng, et al. (2004). "Detection of various types of human papillomavirus DNA, mainly belonging to the cutaneous-group, more frequently in normal tissue than in squamous cell carcinomas of the lip." J Dermatol Sci 36(1): 33-39.

Shpilberg, O., J. Wilson, T. L. Whiteside, et al. (1999). "Pre-transplant immunological profile and risk factor analysis of post-transplant lymphoproliferative disease

development: the results of a nested matched case-control study." Leuk Lymphoma 36(1-2): 109-121.

Shuda, M., H. Feng, H. J. Kwun, et al. (2008). "T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus." Proc Natl Acad Sci U S A 105(42): 16272-16277.

Simard, J. F., E. Baecklund, A. Kinch, et al. (2011). "Pediatric Organ Transplantation and Risk of Premalignant and Malignant Tumors in Sweden." Am J Transplant 11(1): 146-151.

Singh, S. and K. D. Watt (2012). "Long-term medical management of the liver transplant recipient: What the primary care physician needs to know." Mayo Clin Proc 87(8): 779-790.

Sint Nicolaas, J., V. De Jonge, E. W. Steyerberg, et al. (2010). "Risk of colorectal carcinoma in post-liver transplant patients: A systematic review and meta-analysis." Am J Transplant 10(4): 868-876.

Sint Nicolaas, J., A. S. Tjon, H. J. Metselaar, et al. (2010). "Colorectal cancer in post-liver transplant recipients." Dis Colon Rectum 53(5): 817-821.

Smith, C. C., G. E. Archer, E. J. Forster, et al. (1999). "Analysis of gene mutations and clastogenicity following short-term treatment with azathioprine in MutaMouse." Environ Mol Mutagen 34(2-3): 131-139.

Staatz, C. and S. Tett (2007). "Clinical pharmacokinetics and pharmacodynamics of mycophenolate in solid organ transplant recipients." Clin Pharmacokinet 46(1): 13-58.

Starzl, T. E., T. L. Marchioro, K. N. Vonkaulla, et al. (1963). "Homotransplantation of the Liver in Humans." Surg Gynecol Obstet 117: 659-676.

Starzl, T. E., T. L. Marchioro and W. R. Waddell (1963). "The reversal of rejection in human renal homografts with subsequent development of homograft tolerance." Surg Gynecol Obstet 117: 385-395.

Stehlik, J., L. B. Edwards, A. Y. Kucheryavaya, et al. (2010). "The Registry of the International Society for Heart and Lung Transplantation: twenty-seventh official adult heart transplant report--2010." J Heart Lung Transplant 29(10): 1089-1103.

Stehlik, J., L. B. Edwards, A. Y. Kucheryavaya, et al. (2012). "The registry of the international society for heart and lung transplantation: 29th official adult heart transplant report—2012." J Heart Lung Transplant 31(10): 1052-1064.

Stehlik, J., L. B. Edwards, A. Y. Kucheryavaya, et al. (2011). "The registry of the international society for heart and lung transplantation: twenty-eighth adult heart transplant report 2011." J Heart Lung Transplant 30(10): 1078-1094.

Suarez, F., O. Lortholary, O. Hermine, et al. (2006). "Infection-associated lymphomas derived from marginal zone B cells: a model of antigen-driven lymphoproliferation." Blood 107(8): 3034-3044.

Susal, C. and G. Opelz (2013). "Current role of human leukocyte antigen matching in kidney transplantation." Curr Opin Organ Transplant 18(4): 438-444.

Swann, P. F., T. R. Waters, D. C. Moulton, et al. (1996). "Role of postreplicative DNA mismatch repair in the cytotoxic action of thioguanine." Science 273(5278): 1109-1111.

Swerdlow, S. H., E. Campo, N. L. Harris, et al. (2008). WHO classification of tumours of haematopoietic and lymphoid tissues, fourth edition. Lyon, IARC.

Swift, M. B. (2009). "Comparison of confidence intervals for a poisson mean - further considerations." Commun Stat Theory Methods 38(5): 748-759.

Swinnen, L. J., M. R. Costanzo-Nordin, S. G. Fisher, et al. (1990). "Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients." N Engl J Med 323(25): 1723-1728.

Swinnen, L. J. and R. I. Fisher (1993). "OKT3 monoclonal antibodies induce interleukin-6 and interleukin-10: a possible cause of lymphoproliferative disorders associated with transplantation." Curr Opin Nephrol Hypertens 2(4): 670-678.

Swinnen, L. J., M. LeBlanc, T. M. Grogan, et al. (2008). "Prospective study of sequential reduction in immunosuppression, interferon alpha-2B, and chemotherapy for posttransplantation lymphoproliferative disorder." Transplantation 86(2): 215-222.

Taj, M. M., N. Hadzic, S. E. Height, et al. (2012). "Long-term outcome for immune suppression and immune related lymphoproliferative disorder: prospective data from the United Kingdom children's leukaemia and cancer group registry 1994-2004." Leuk Lymphoma 53(5): 842-848.

Tanner, J. E. and J. Menezes (1994). "Interleukin-6 and Epstein-Barr virus induction by cyclosporine A: potential role in lymphoproliferative disease." Blood 84(11): 3956-3964.

Taylor, A. L., R. Marcus and J. A. Bradley (2005). "Post-transplant lymphoproliferative disorders (PTLD) after solid organ transplantation." Crit Rev Oncol Hematol 56(1): 155-167.

Taylor, A. L., C. J. E. Watson and J. A. Bradley (2005). "Immunosuppressive agents in solid organ transplantation: mechanisms of action and therapeutic efficacy." Crit Rev Oncol Hematol 56(1): 23-46.

Taylor, D. O., L. B. Edwards, M. M. Boucek, et al. (2006). "Registry of the international society for heart and lung transplantation: twenty-third official adult heart transplantation report--2006." J Heart Lung Transplant 25(8): 869-879.

Tessmer, C. S., L. V. Magalhaes, E. Keitel, et al. (2006). "Conversion to sirolimus in renal transplant recipients with skin cancer." Transplantation 82(12): 1792-1793.

Thibodeau, E. A. and J. A. D'Ambrosio (1997). "Measurement of lip and skin pigmentation using reflectance spectrophotometry." Eur J Oral Sci 105(4): 373-375.

Thorley-Lawson, D. A. and A. Gross (2004). "Persistence of the epstein–barr virus and the origins of associated lymphomas." N Engl J Med 350(13): 1328-1337.

Tripp, C. S., E. A. G. Blomme, K. S. Chinn, et al. (2003). "Epidermal COX-2 induction following ultraviolet irradiation: Suggested mechanism for the role of COX-2 inhibition in photoprotection." J Invest Dermatol 121(4): 853-861.

Tsai, D. E., L. Douglas, C. Andreadis, et al. (2008). "EBV PCR in the diagnosis and monitoring of posttransplant lymphoproliferative disorder: results of a two-arm prospective trial." Am J Transplant 8(5): 1016-1024.

Tsai, D. E., C. L. Hardy, J. E. Tomaszewski, et al. (2001). "Reduction in immunosuppression as initial therapy for posttransplant lymphoproliferative disorder: analysis of prognostic variables and long-term follow-up of 42 adult patients." Transplantation 71(8): 1076-1088.

Turner, J. J., L. M. Morton, M. M. D. Linet, et al. (2010). "InterLymph hierarchical classification of lymphoid neoplasms for epidemiologic research based on the WHO classification (2008): update and future directions." Blood 116(20): e90-e98.

Vajdic, C. M., G. W. McCaughan and A. E. Grulich (2012). "Cancer risk after organ transplantation." JAMA 307(7): 663-664.

Vajdic, C. M., S. P. McDonald, M. R. McCredie, et al. (2006). "Cancer incidence before and after kidney transplantation." JAMA 296(23): 2823-2831.

Vajdic, C. M. and M. T. van Leeuwen (2009). "Cancer incidence and risk factors after solid organ transplantation." Int J Cancer 125(8): 1747-1754.

Vajdic, C. M., M. T. van Leeuwen, A. C. Webster, et al. (2009). "Cutaneous melanoma is related to immune suppression in kidney transplant recipients." Cancer Epidemiol Biomarkers Prev 18(8): 2297-2303.

Vallat, L., Y. Benhamou, M. Gutierrez, et al. (2004). "Clonal B cell populations in the blood and liver of patients with chronic hepatitis C virus infection." Arthritis Rheum 50(11): 3668-3678.

van Leeuwen, M. T., A. E. Grulich, S. P. McDonald, et al. (2009). "Immunosuppression and other risk factors for lip cancer after kidney transplantation." Cancer Epidemiol Biomarkers Prev 18(2): 561-569.

van Leeuwen, M. T., A. E. Grulich, A. C. Webster, et al. (2009). "Immunosuppression and other risk factors for early and late non-Hodgkin lymphoma after kidney transplantation." Blood 114(3): 630-637.

van Leeuwen, M. T., A. C. Webster, M. R. McCredie, et al. (2010). "Effect of reduced immunosuppression after kidney transplant failure on risk of cancer: population based retrospective cohort study." BMJ 340: c570.

Veness, M. J., D. I. Quinn, C. S. Ong, et al. (1999). "Aggressive cutaneous malignancies following cardiothoracic transplantation: the Australian experience." Cancer 85(8): 1758-1764.

Villeneuve, P. J., D. E. Schaubel, S. S. Fenton, et al. (2007). "Cancer incidence among Canadian kidney transplant recipients." Am J Transplant 7(4): 941-948.

Vukadinovic, M., Z. Jezdic, M. Petrovic, et al. (2007). "Surgical management of squamous cell carcinoma of the lip: analysis of a 10-year experience in 223 patients." J Oral Maxillofac Surg 65(4): 675-679.

Walsh, S. B., J. Xu, H. Xu, et al. (2011). "Cyclosporine a mediates pathogenesis of aggressive cutaneous squamous cell carcinoma by augmenting epithelial-mesenchymal transition: Role of TGFβ signaling pathway." Mol Carcinog 50(7): 516-527.

Warnakulasuriya, S. (2009). "Causes of oral cancer--an appraisal of controversies." Br Dent J 207(10): 471-475.

Watt, K., B. Veldt and M. Charlton (2009). "A practical guide to the management of HCV infection following liver transplantation." Am J Transplant 9(8): 1707-1713.

Watt, K. D., R. A. Pedersen, W. K. Kremers, et al. (2009). "Long-term probability of and mortality from de novo malignancy after liver transplantation." Gastroenterology 137(6): 2010-2017.

Watt, K. D. S., R. A. Pedersen, W. K. Kremers, et al. (2010). "Evolution of causes and risk factors for mortality post-liver transplant: results of the NIDDK long-term follow-up study." Am J Transplant 10(6): 1420-1427.

Weber, G., J. C. Hager, M. S. Lui, et al. (1981). "Biochemical programs of slowly and rapidly growing human colon carcinoma xenografts." Cancer Res 41(3): 854-859.

Webster, A. C., E. G. Playford, G. Higgins, et al. (2004). "Interleukin 2 receptor antagonists for renal transplant recipients: a meta-analysis of randomized trials." Transplantation 77(2): 166-176.

Webster, A. C., L. P. Ruster, R. McGee, et al. (2010). "Interleukin 2 receptor antagonists for kidney transplant recipients." Cochrane Database Syst Rev(1): CD003897.

Webster, A. C., G. Wong, J. C. Craig, et al. (2008). "Managing cancer risk and decision making after kidney transplantation." Am J Transplant 8(11): 2185-2191.

Wei, L. J., D. Y. Lin and W. L. (1989). "Regression analysis of multivariate incomplete failure time data by modeling marginal distributions." J Am Stat Assoc(84): 1065-1073.

Wenczl, E., G. P. Van der Schans, L. Roza, et al. (1998). "(Pheo)melanin photosensitizes UVA-induced DNA damage in cultured human melanocytes." J Invest Dermatol 111(4): 678-682.

White, I. R., P. Royston and A. M. Wood (2011). "Multiple imputation using chained equations: Issues and guidance for practice." Stat Med 30(4): 377-399.

Wiesner, R. H. and J. J. Fung (2011). "Present state of immunosuppressive therapy in liver transplant recipients." Liver Transpl 17(S3): S1-S9.

Wisgerhof, H. C., L. Hameetman, C. P. Tensen, et al. (2009). "Azathioprine-induced microsatellite instability is not observed in skin carcinomas of organ transplant recipients." J Invest Dermatol 129(5): 1307-1309.

Wong, G., A. C. Webster, J. R. Chapman, et al. (2009). "Reported cancer screening practices of nephrologists: results from a national survey." Nephrol Dial Transplant 24(7): 2136-2143.

Wu, X., B.-C. Nguyen, P. Dziunycz, et al. (2010). "Opposing roles for calcineurin and ATF3 in squamous skin cancer." Nature 465(7296): 368-372.

Wulff, B. C., D. F. Kusewitt, A. M. VanBuskirk, et al. (2008). "Sirolimus reduces the incidence and progression of UVB-induced skin cancer in SKH mice even with co-administration of cyclosporine A." J Invest Dermatol 128(10): 2467-2473.

Yajima, M., K.-I. Imadome, A. Nakagawa, et al. (2009). "T cell-mediated control of Epstein-Barr virus infection in humanized mice." J Infect Dis 200(10): 1611-1615.

Yajima, Y., H. Sueki, T. Oguro, et al. (2008). "Effects of oral administration of ciclosporin A on skin carcinogenesis: a study using the two-stage carcinogenesis protocol in mice." Clin Exp Dermatol 33(4): 478-483.

Yarosh, D. B., A. V. Pena, S. L. Nay, et al. (2005). "Calcineurin inhibitors decrease DNA repair and apoptosis in human keratinocytes following ultraviolet B irradiation." J Invest Dermatol 125(5): 1020-1025.

Yokota, K., T. J. Gill and H. Shinozuka (1989). "Effects of oral versus topical administration of cyclosporine on phorbol ester promotion of murine epidermal carcinogenesis." Cancer Res 49(16): 4586-4590.

Young, L. S. and A. B. Rickinson (2004). "Epstein-Barr virus: 40 years on." Nat Rev Cancer 4(10): 757-768.

Yuan, B. and Y. Wang (2008). "Mutagenic and Cytotoxic Properties of 6-Thioguanine, S6-Methylthioguanine, and Guanine-S6-sulfonic Acid." J Biol Chem 283(35): 23665-23670.

Yusen, R. D., J. D. Christie, L. B. Edwards, et al. (2013). "The registry of the international society for heart and lung transplantation: Thirtieth adult lung and heart-lung transplant report—2013; focus theme: Age." J Heart Lung Transplant 32(10): 965-978.

Zazgornik, J., P. Schmidt, H. Kopsa, et al. (1979). ""Triple infections" (fungal, bacterial and viral) in immunosuppressed renal transplant recipients." Int Urol Nephrol 11(2): 145-150.