Immunosuppression and post-transplant lifetime cancer risk among solid organ transplant recipients in Quebec

by

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BART: Bayesian Additive Regression Trees

CI: Confidence Interval

CIHI: Canadian Institute for Health Information

CNI: Calcineurin Inhibitors

CORR: Canadian Organ Replacement Register

CsA: Cyclosporine

DAG: Directed Acyclic Graph

FDA: Food and Drug Administration

IA: Immunosuppressive Agents

IARC: International Agency for Research on Cancer

ICD-9: International Classification of Disease 9th revision

ICD-10: International Classification of Disease 10th revision

KTR: Kidney Transplant Recipients

LTR: Liver Transplant Recipients

Med- ECHO: *Maintenance et exploitation des données pour l'étude de la clientèle hospitalière*

NMSC: Non- Melanoma Skin Cancer

PATE: Population Average Treatment Effect

RAMQ: *Régie de l'assurance maladie du Québec*

SOT: Solid Organ Transplantation

SRR: Standardised Risk Ratio

TMLE: Targeted Minimum Loss Estimation

Abstract

Introduction: Solid Organ Transplantation (SOT) is a treatment of choice for individuals who face end-stage organ failure. Annually, over 2800 Canadians and 500 Quebecers undergo solid organ transplantation. This group experiences 2-4 times higher lifetime cancer risk, post-transplantation, compared to the general population. This heightened risk has been attributed to the use of immunosuppressive agents (IA's). With the anticipated increase in cancer incidence in Quebec in the coming decade, there is a significant gap in understanding the impact of IAs on cancer risk among SOT recipients in the province.

This thesis work has the overarching aim of documenting the post-transplantation lifetime cancer burden among SOT recipients in Quebec and investigating the role of immunosuppressive agents in contributing to this burden.

Specifically, the objectives are:

Objective 1: 1. a) To estimate the age and sex standardized post-transplant lifetime cancer incidence rate among SOT recipients in Quebec, from 1997-2016; 1. b) To compare the cancer burden, calculated as the age and sex standardized incidence, between SOT recipients and the general population of Quebec from 1997 to 2016.

Objective 2: To estimate the average treatment effect of modern-era IA, compared to early-era IA, on the post-transplant lifetime risk of developing any cancer among SOT recipients.

Methodology: We constructed a retrospective cohort study by linking two provincial-level administrative healthcare databases from 1997 to 2016: i) the *Régie de l'assurance maladie du Québec* (RAMQ), and ii) *Maintenance et exploitation des données pour l'étude de la clientèle hospitalière* (Med-ECHO). We analyzed the pattern of cancer incidence by organ transplanted and computed the overall incidence rate stratified by sex. Age and sex standardized incidence

per 100,000 for the general population of Quebec during the period of 1997-2016, was obtained from the Quebec Cancer Registry. Subsequently, the direct standardization method was used to estimate the standardized risk ratio between our cohort and Quebec general population, while using the 2011 Quebec population as the standard reference population. To estimate the causal effect of being prescribed one of the modern-era IAs (Mycophenolate mofetil, Sirolimus, and Tacrolimus), compared to one of the early-era drugs (Azathioprine and Cyclosporine), we followed the potential outcomes framework for causal inference. Following this framework, to emulate a target trial from observational data we used Bayesian Additive Regression Trees (BART) models for both treatment assignment mechanism and outcome model. BARTs are flexible non-parametric machine learning models shown to perform superior to traditional parametric models in causal inference tasks. We further corrected the estimate of interest (population average treatment effect) following the recommended Targeted Minimum loss-based estimation method. The average reduction in cancer risk (in risk difference scale) attributable to prescribing one of the modern era IA to all SOT recipients in Quebec who were prescribed one of the early era IA, during 1997-2016, and the corresponding 95% credible intervals were estimated from the model.

Results: We identified 6,783 SOT recipients including 4,284 kidney, 1,142 liver, 612 heart, 443 lung, and 392 multiple or other transplants from the linked administrative database. Based on ICD codes, we identified 1,142 post-transplant cancer cases, with an overall incidence rate of 2,436.1 per 100,000 person-years (95%CI; 2,212 -2,486 per 100,000 person-years). Skin cancer was a common cancer among SOT recipients, followed by cancers of the lymphoid and hematopoietic tissue and digestive organs. The age and sex standardized risk ratio indicated a 2.5-to-4.2-fold increase in cancer risk among SOT recipients compared to the general population of Quebec between 1997-2016. In our analytical cohort, 4,892 individuals were prescribed a baseline maintenance therapy regimen, with a median of 6.93 years of follow-up.

During this period, 909 individuals developed at least one primary malignant neoplasm. The SOT recipients who were originally prescribed one of the early-era IAs would have had 4% points lower post-transplant cancer risk if they had been prescribed one of the modern-era IAs, instead (RD= -0.040; 95%CI = -0.049 to -0.030).

Conclusion: SOT recipients in the province of Quebec were at a significantly higher risk for cancer compared to the general population. Switching to modern-era immunosuppressive agents (e.g., Mycophenolate mofetil, Sirolimus, and Tacrolimus), from early-era IAs, may reduce the lifetime risk of any cancers among SOT recipients in Quebec.

Résumé

Introduction : La transplantation d'organe est un traitement de choix pour les personnes en stade terminal d'une insuffisance organique. Chaque année, plus de 2 800 Canadiens et 500 Québécois reçoivent une transplantation d'organe. Les personnes transplantées ont un risque de cancer à vie de 2 à 4 fois plus élevé à la suite d'une transplantation que la population générale. Ce risque accru s'explique par l'utilisation de médicaments immunosuppresseurs (MI). Avec l'augmentation prévue de l'incidence du cancer au Québec au cours de la prochaine décennie, des données probantes pour mieux comprendre l'impact des MI sur le risque de cancer chez les personnes transplantées de la province seront nécessaires.

L'objectif principal de cette thèse est de documenter le fardeau du cancer à vie à la suite d'une transplantation d'organe chez les personnes transplantées du Québec et d'étudier le rôle des médicaments immunosuppresseurs et leur influence sur ce fardeau.

Plus précisément, les objectifs sont les suivants :

Objectif 1 : 1. a) Estimer le taux d'incidence du cancer post-transplantation normalisé selon l'âge et le sexe chez les receveurs d'une transplantation au Québec de 1997 à 2016; 1. b) Comparer les incidences normalisées selon l'âge et le sexe entre les personnes transplantées et la population générale du Québec au cours de la période de 1997 à 2016.

Objectif 2 : Estimer l'effet moyen du traitement des MI de l'ère moderne, par rapport à ceux de l'ère ancienne, sur le risque à vie de développer un cancer après la transplantation chez les receveurs d'organes.

Méthodologie : Nous avons réalisé une étude de cohorte rétrospective en jumelant deux bases de données administratives de santé provinciales de 1997 à 2016 : i) la Régie de l'assurance maladie du Québec (RAMQ) et ii) la Maintenance et exploitation des données pour l'étude de

la clientèle hospitalière (Med-ECHO). Nous avons estimé le taux d'incidence du cancer global, selon l'organe transplanté et par sexe. Le taux d'incidence standardisée selon l'âge et le sexe pour 100 000 personnes dans la population générale du Québec pour la période 1997-2016 a été obtenu auprès du Registre québécois du cancer. Par la suite, la méthode de standardisation directe a été utilisée pour estimer le rapport de risques standardisés entre la cohorte de personnes transplantées et la population générale du Québec, en utilisant la population du Québec de 2011 comme population de référence. Pour estimer l'effet causal de la prescription de l'un des MI de l'ère moderne (Mycophénolate mofétil, Sirolimus et Tacrolimus) par rapport aux MI de l'ère ancienne (azathioprine et cyclosporine), nous avons suivi le cadre des résultats potentiels pour l'inférence causale. En suivant ce cadre et pour simuler un essai clinique à partir de données d'observation, nous avons utilisé des modèles d'arbres de régression additifs bayésiens (BART) à la fois pour le mécanisme d'affectation du traitement et pour le modèle des résultats. Les BART sont des modèles d'apprentissage automatique non paramétriques et flexibles dont les performances sont supérieures à celles des modèles paramétriques traditionnels dans les tâches d'inférence causale. Nous avons ensuite corrigé l'estimation d'intérêt (effet moyen du traitement sur la population) en suivant la méthode d'estimation basée sur la perte *Targeted Minimum* recommandée. La réduction moyenne du risque de cancer (sur l'échelle de la différence de risque) attribuable à la prescription de l'un de MI de l'ère moderne à toutes les personnes transplantées au Québec à qui l'on a prescrit l'un des MI de l'ère ancienne au cours de la période 1997-2016, ainsi que les intervalles de confiance à 95 % correspondants ont été estimés à partir du modèle.

Résultats : Nous avons identifié 6 783 personnes transplantées, dont 4 284 transplantations de rein, 1 142 de foie, 612 de cœur, 443 de poumon et 392 multiples ou autres, à partir de la base de données administratives jumelée. En utilisant les codes CIM, nous avons identifié 1 142 cas de cancer post-transplantation, avec un taux d'incidence globale de 2 436,1 pour

100 000 personnes-années (IC 95 % ; 2 212 – 2 486 pour 100 000 personnes-années). Le cancer de la peau était le cancer le plus fréquent chez les personnes transplantées, suivi par le cancer des tissus lymphoïdes et hématopoïétiques et des organes digestifs. Le rapport de risques standardisés selon l'âge et le sexe indique une augmentation de 2,5 à 4,2 fois du risque de cancer chez les personnes transplantées par rapport à la population générale du Québec entre 1997 et 2016. Dans notre cohorte, 4 892 personnes se sont vu prescrire un traitement à la suite de la transplantation, avec un suivi médian de 6,93 années. Au cours de cette période, 909 personnes ont développé au moins un néoplasme malin primaire. Les personnes transplantées à qui l'on avait initialement prescrit l'un des MI de l'ère précoce auraient eu un risque de cancer post-transplantation inférieur de 4 % si on leur avait plutôt prescrit l'un des MI de l'ère moderne (RD= -0,040; IC à 95 % = -0,049 à -0,030).

Conclusion : Les personnes transplantées de la province de Québec présentaient un risque significativement plus élevé de cancer par rapport à la population générale. Le passage aux MI de l'ère moderne (c.-i.e., Mycophenolate mofetil, Sirolimus, et Tacrolimus), alternativement à ceux de l'ère ancienne pourrait réduire le risque de cancer au cours de la vie chez les receveurs d'organes du Québec.

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Contribution of Authors

Manuscript I

Risk of cancer among adult solid organ transplant recipients in Quebec, Canada: 1997-2016

Theerthika Dillibabu, MSc candidate: Curated the data, carried out the data analysis, visualization, and interpretation of the findings, and wrote the manuscript.

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Manuscript II

The causal effect of modern-era immunosuppressive agents on lifetime cancer risk among solid organ transplant recipients: A Bayesian machine learning approach

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1 Introduction

Solid organ transplantation is the gold standard procedure for treating individuals with end-stage organ failure, significantly improving their survival and quality of life. Since its experimental beginnings in the mid-20th century, transplantation procedures for organs such as the kidney, liver, heart, lungs, and pancreas have undergone significant advancements [1–3]. Globally, more than 130,000 SOTs are performed annually [4], with over 2800 transplants conducted in Canada in 2022 and these numbers have steadily increased over the years [5,6]. The growing number of SOTs underscores the need for effective post-transplantation care, especially in managing the long-term risks associated with immunosuppression, including cancer.

Despite the life-saving benefits of SOT, these recipients face considerable challenges, particularly an increased risk of developing cancers [3]. The post-transplant lifetime cancer risk among the recipients is reported to be 2-4 times higher than that of the general population [3]. This increased risk is multifactorial, including the type of organ transplanted, patient demographics, prior history of malignancy, infection, and the burden of immunosuppression [3,7–11]. For example, large observational studies have reported that Non-Melanoma skin cancer and Non-Hodgkin lymphoma are the most common malignancies observed among SOT recipients [12,13].

In Canada, data on cancer risk among SOT recipients is limited. The latest nationwide study available is based on data that is more than two decades ago. That study, conducted using the Canadian Organ Replacement Registry (CORR) database and the Canadian Cancer Registry from 1981 to 1998, reported an increased cancer risk among kidney [14], liver [10], and heart transplant [11] recipients. Moreover, since 2011, Canadian national cancer statistics have not

included data from Quebec, leading to a lack of recent data on cancer incidence among SOT recipients in Quebec.

The heightened cancer risk among SOT recipients is hypothesized to be due to the lifelong use of immunosuppressive agents (IA). While the suppression of the immune system is essential to avoid organ rejection [15], it also impedes the system's ability to detect and eliminate emerging cancerous cells leading to heightened susceptibility to cancers [16,17]. IAs can be categorized based on its main intended stage of use as i) induction, ii) maintenance, and ii) treatment of the rejection stage IAs [18]. Out of which, the maintenance IAs is the long-term therapy, and are often used for life long, to preserve the graft [19]. These IAs can further be classified based on the transplantation era when they were introduced as the early- and modern-era IAs [20]. Early-era drugs such as azathioprine and cyclosporine popular in early-era transplantation are associated with higher toxicity and carcinogenicity. In contrast, modern-era drugs including sirolimus, mycophenolate mofetil, and tacrolimus, have been developed with improved actions and reduced side effects [21,22]. Most of this evidence on the carcinogenic potential of these IAs comes from observational studies. Although a randomized controlled trial is the ideal study design to estimate the causal effect of IAs on cancer risk, most prior studies are either mechanistic or observational. In addition to the ethical challenges of such trials, they would suffer from poor patient enrollment, high dropouts due to toxicity, and the need for long follow-up periods. Advancements in modern data analytical approaches, specifically methods to emulate a target trial from observational data, may offer a solution [23–25].

The overarching aim of this thesis work is to elucidate the burden of cancer and the role of IAs on cancer risk among SOT recipients in Quebec. This much-needed evidence will provide more insights into managing and mitigating cancer risk among SOT recipients, ultimately enhancing long-term outcomes and patient care in this population. A retrospective cohort study

constructed from two linked administrative databases is used to achieve this aim. This thesis follows a manuscript-based format where the results chapters are formatted as pre-prints of manuscripts investigating specific objectives. The pattern of cancer incidence and the post-transplant cancer risk are reported in manuscript-I. This is followed by the estimation of the causal effect of prescribing one of the modern-era IAs, compared to one of the early-era IAs on post-transplant lifetime cancer risk among SOT recipients of Quebec, in manuscript-II. Separate chapters for a comprehensive literature review, methods, and discussion are also included.

2 Literature Review

2.1 Solid organ transplantation – a historical perspective

Solid organ transplantation (SOT) is a lifesaving treatment for individuals with terminal diseases and irreversible organ failure [2]. In the 20th century, SOT started as an experimental procedure, where an organ including the kidney, heart, liver, lung, and pancreas from a donor (living or deceased) is placed into another body (recipient) [1,2]. The procedure became mainstream after the first successful kidney transplantation between twins was performed in 1954 [26,27]. Followed by the successful lung, pancreas, heart, and liver transplantation in 1963, 1966, 1967, and 1968, respectively [28–31], organ transplantation became a viable and increasingly common medical procedure.

An end-stage renal disease is the most common indication for kidney transplantation; cardiomyopathy, myocarditis, and heart valve defects for heart transplantation; pulmonary hypertension, emphysema, or cystic fibrosis for lung transplantation; end-stage liver cirrhosis for the liver; diabetes mellitus for pancreatic transplantation [15]. Globally, visceral organs including the kidney, liver, and pancreas are more frequently transplanted followed by thoracic organs such as the heart and lungs [15].

In recent decades SOT has improved the overall survival and quality of life of recipients [3]. A study from the United States (US) reported a survival benefit among transplant recipients with a mean of 4.4 life years [32]. SOT has been a clinically successful and cost-effective treatment procedure compared to non-transplant procedures, especially for kidney transplant recipients, where survival is higher than for patients undergoing dialysis [33]. In the US, post-transplant allograft and recipient survival rates were above 90% among kidney transplant recipients within the first year [33]. These prognostic improvements of SOT over the years were possible due to a better understanding of immunological mechanisms that lead to organ rejection,

invention and use of immunosuppressive agents (IA), and advances in surgical and organ preservation techniques [2,15].

2.1.1 Descriptive epidemiology of solid organ transplantation

Worldwide, 130,000 SOTs were performed in 2022, which was 17% less than the previous year. This decrease, in an otherwise increasing number over the past decade [6], was due to the extended care interruptions following the COVID-19 pandemic [4]. According to the Canadian Organ Replacement Registry 2886 organ transplants were performed in 2022. There was an 11% increase compared to the 2020 volume (N=2594) and a 5% increase compared to 2021 (N=2752) [5]. In Quebec, Transplant Quebec reports 569 transplantations in 2023, with the kidney being the most common organ transplanted (N = 317), followed by the lung (N = 111) [34].

A study conducted in the US reported that approximately 2 million life years have been added during a 25-year study period among SOT recipients, with a mean of 4.3 life years per recipient [32]. While the short-term post-transplantation outcomes have improved, the long-term survival is still lagging. For example, among liver transplant recipients the one-year survival rate was 67% in 1980 and increased to 90% in 2014 and this improvement has been consistent over the years [35,36]. Despite improvements in transplant medicine, recipients suffer from various complications involving the cardiovascular, renal, gastrointestinal, and neurological systems. These complications can cause significant morbidity and mortality among the recipients [37]. Additionally, previous studies showed higher mortality rates among the SOT recipients compared to the age-matched general population [38,39].

2.1.2 Canadian data sources on solid organ transplant recipients

Transplant registries and national health databases gather information on organ transplantation. The strategies for collecting and managing this data vary in different countries depending on the health system. In Canada, the Canadian Organ Replacement Registry (CORR) is a national registry for organ failure. It is a longitudinal database that tracks information on kidney transplantations since 1981 and non-kidney transplantations since 1988, in Canada. CORR collects data from hospital dialysis programs, transplant programs, organ donation organizations, and independent health facilities. It monitors patients from their initial treatment for end-stage organ failure until their death (https://www.cihi.ca/en/canadian-organ-replacement-register-corr). The Canadian Institute for Health Information (CIHI) regulates CORR functions including data collection, management, analysis of the long-term trends on organ donation, transplants and dialysis, and reporting. The regional and provincial organ procurement organizations also submit their data to CIHI [40].

In Quebec, transplantation procedures are covered by RAMQ, the provincial health insurance program. Consequently, information about these procedures is recorded in the RAMQ database and the statistics on organ donation are documented annually in Transplant Quebec. In our project, we used the RAMQ database to obtain information on transplantation performed in Quebec. It includes the medical services file containing information on services rendered under the public health insurance plan and billed by health professionals from the claims submitted to RAMQ. The medical code act corresponds to the services provided by the health care professionals according to the billing manuals.

2.2 Immunosuppression in SOT recipients

2.2.1 Evolution of immunosuppressive agents

The long-term outcomes of the procedures remained poor until the impact of immunosuppression was better understood [2]. The goal of post-transplant immunosuppressive agents (IAs) is to prevent graft rejection and reduce the adverse effects of the drugs including drug toxicity, infection, and malignancy with a balance between under and over-immunosuppression [41].

Early attempts at immunosuppression involved radiation and pharmacological agents such as 6-Mercaptopurine, Azathioprine derivative, and Nitrogen mustard [42]. However, these early agents lead to limited improvement in the short and long-term success of transplantation [2]. For example, between 1960 and 1980 only 40-60% of transplanted organs survived one year mark; depending on the population and the organ [43]. Thus, SOT was still considered an experimental procedure until Cyclosporine was introduced in 1978 increasing the one-year survival rate up to 80% [18,44]. Azathioprine, Cyclosporine, and other corticosteroids were the early-era immunosuppressive agents to prevent organ rejection from the 1960s to 1979 [45]. Within a decade after introducing Cyclosporine, several other immunosuppressants were used to reduce the rejection rate and improve the one-year survival rate by up to 90% or more [45]. Further evolution of these modern-era immunosuppressive agents began in the 1980s [46].

2.2.2 Types of agents and mechanisms of action of maintenance immunosuppressive agents

In transplant medicine, IAs are categorized into those used in the induction, maintenance, and treatment of rejection stages [18]. The induction IAs include high-dose corticosteroids, monoclonal antibodies, and polyclonal antibodies administered at transplantation to prevent

acute rejection [47]. Whereas one or more maintenance IAs are prescribed immediately after transplant and often for rest of the life of the patient or the transplant [19,20]. Major groups of maintenance IAs are: i) Corticosteroids; ii) Calcineurin inhibitors (CNI) line Cyclosporine and Tacrolimus; iii) antimetabolites such as Azathioprine and Mycophenolate mofetil; iv) mammalian target of rapamycin (mTOR) inhibitors like Sirolimus and Everolimus; and v) T cell stimulation blockers. Finally, treatment of rejection IAs is used to manage acute rejection episodes, including high-dose corticosteroids, anti-thymocyte globulin, and rituximab [47]. This multipronged approach is crucial for the long-term success of the transplants and recipients must have a lifetime commitment to prevent graft rejection or failure [15]. The focus of this thesis work is on the maintenance of IAs. Specifically, the first post-transplant IA prescribed to the SOT recipients. The following subsection elaborates on the commonly prescribed maintenance IAs, in Canada. A concise summary is also provided in Table 2.1.

Azathioprine

Azathioprine was developed in the 1950s as a prodrug (an inactive drug that is metabolized in the body to produce an active drug) of 6-Mercaptopurine (a purine antimetabolite) and can inhibit DNA and RNA production [48]. It was an important drug used in the early eras of transplantation and was used until replaced by Mycophenolic acid in the 1990s [49]. In recent years, Azathioprine has been prescribed to a relatively small proportion of recipients. A study from the US reported in 2015, that approximately 9% of kidney transplant recipients were prescribed Azathioprine, while in Australia this proportion was 5-7% between 2006-2010 [48,50,51]. Azathioprine is typically indicated for individual's intolerant to Mycophenolic acid due to gastrointestinal side effects or for pregnant women where the medications are contraindicated. It is commonly used in cardiothoracic transplants with a Spanish study reporting that 69% of heart transplant recipients receiving Azathioprine [52]. However, there are

concerns that Azathioprine may increase the risk of skin cancer, compared to other IAs, through photosensitization [21].

Cyclosporine

Cyclosporine (CsA) was introduced in 1978 and was approved by the Food and Drug Administration in 1983. This approval led to its increased acceptance in SOT procedures with significant improvement in graft survival rates [32]. CsA is a fungal product of Beauveria nivea [53,54] which acts on both cell-mediated T-helper lymphocytes and the synthesis of antibodies by lymphocytes. It was revolutionary in the 1980s when CsA was a part of a multidrug regime improving the one-year survival rate up to 89% in kidney transplant recipients and 70% in heart and liver transplants.[55–58]. CsA was also used as a prophylactic agent against organ rejection in kidney, liver, and heart transplants in addition to corticosteroids [53,54]. Adverse effects of the drug involve nephrotoxicity, neurotoxicity, post-transplant diabetes mellitus, and B-cell lymphoma [59].

Mycophenolate mofetil

Mycophenolate mofetil is also an antimetabolite that acts by inhibiting purine base synthesis essential for T and B cell proliferation [59] It is indicated in renal, cardiac, or hepatic transplant recipients [60]. It is more frequently used than Azathioprine [61]. A randomized controlled trial showed lower incidence and longer time till organ rejection among SOT recipients who received Mycophenolate mofetil compared to Azathioprine [62–65]. Although organ rejection outcomes are improved, Mycophenolate mofetil has been shown to impair the gastric and haematological system among its users [59].

Sirolimus

Sirolimus is an mTOR inhibitor that inhibits the G1- S phase of the cell cycle which inhibits lymphocyte proliferation [66,67]. They are used to reduce the prescription of CNI, by using them in combination with CNI or lower doses. Compared to CNI, mTOR inhibitors have less nephrotoxicity. However, mTOR inhibition causes leukopenia, hypercholesteremia, and anaemia [59]. Sirolimus is also associated with hepatic artery thrombosis, graft loss, and increased mortality in liver transplant recipients. Additionally, among lung transplant recipients Sirolimus can increase bronchial anastomosis dehiscence [68].

Tacrolimus

Tacrolimus is a macrolide antibiotic produced from the *Streptomyces tsukubaensis*, which binds with FK506 binding protein (family of proteins that contain peptidyl-prolyl isomerase) to form a complex inhibiting calcineurin [69]. Tacrolimus has been in use since the 1990s after the FDA approval. It has reduced acute rejection rates and better survival rates than Cyclosporine [70]. It also has a better glomerular filtration rate and reduced biopsy-proven rejection than Cyclosporine. However, it has potential side effects such as developing post-transplant diabetes mellites [18].

Table 2.1 *Indications, mechanisms of action, and common adverse effects of common maintenance immunosuppressive agents*

Immunosuppressive agents (class of drugs)	Year of FDA approval	Era of transplantation	Indications	Mechanism of action	Adverse effects
Azathioprine (Antimetabolite)	1950s	Early	Individual's intolerant to Mycophenolic acid due to gastrointestinal side effects. Contra-indication in pregnant women.	Inhibit DNA and RNA production	Increased risk of skin cancer through photosensitization compared to other IAs
Cyclosporine (Calcineurin Inhibitors)	1983	Early	Prophylactic agent against organ rejection in kidney, liver, and heart transplants in addition to corticosteroids	Acts on both cell-mediated T helper lymphocytes and lymphocyte-derived antibody synthesis. It inhibits cyclophilin.	Nephrotoxicity, neurotoxicity, post-transplant diabetes Mellitus, and B-cell lymphoma
Sirolimus (mTOR inhibitors)	1990	Modern	Reduce the prescription of CNI	Inhibits the G1- S phase of the cell cycle which inhibits lymphocyte proliferation.	Hepatic artery thrombosis, graft loss, and increased mortality in liver transplant recipients. Lung transplant recipients: -bronchial anastomosis dehiscence developing post-transplant diabetes mellites
Tacrolimus (Calcineurin Inhibitors)	1994	Modern	Kidney, Liver, lung, and heart transplant.	Binds with FK506 binding protein to form a complex inhibiting calcineurin	
Mycophenolate mofetil (Antimetabolite)	1995	Modern	Renal, cardiac, or hepatic transplant recipients	Acts by inhibiting purine base synthesis essential for T and B cell proliferation	Impair the gastric and haematological system

2.2.3 Cancer risk in SOT recipients

SOT recipients have a higher incidence of cancer post-transplantation compared to the general population [3]. Despite long-term benefits, post-transplantation cancer is the third leading cause of mortality among them [71]. Recipients are at increased risk for many types of solid organ tumors, with the most common being non-melanoma skin cancer (NMSC) and Non-Hodgkin Lymphoma (NHL) [12,13]. The cancer incidence differs by the type of organ transplanted. Overall excess cancer risk is higher in lung transplant recipients and specifically, NHL is higher in cardiothoracic transplants [8,72].

A meta-analysis of 72 cohort studies worldwide has reported 2-5 times the risk of developing cancer among SOT recipients compared to the general population. Specifically, the risk for kidney transplant recipients (KTR) is estimated to be 2- 6 times higher; for liver transplant recipients 2-3 times higher, and for heart transplant recipients 2-10 times higher in comparison with the general population [3].

In Canada, the kidney is the most common solid organ transplant procedure due to a drastic increase in end-stage renal disease [14,23,24]. A nationwide population study conducted by Villeneuve et al from 1981- 1998 reported a 2.5 times risk of developing cancer among KTR compared to the general population. This study also reported the site-specific Standardized Rate Ratios (SIRs) compared to the general population, with lip cancer at 31.3 [95% CI; 23.5-40.8] followed by non-Hodgkins's lymphoma at 8.8 [95% CI; 7.4-10.5] and kidney cancer at 7.3 [95%CI; 5.7- 9.2] [14]. Additionally, studies that have followed patients over a long period show that the risk of cancer remains significantly higher for up to 10 years of kidney transplantation [14,24].

Another study from Canada by Jiang et al. explored cancer risk among liver transplant recipients (LTR), they reported 2.5 times higher risk compared to the general population with

the highest SIR in non-Hodgkins' lymphoma (NHL) at 20.8 [95%CI; 14.9 – 28.3] and colorectal cancer SIR 2.6 [95% CI; 1.4- 4.4] [10]. Similarly, another study by Jiang et al. on Canadian heart transplant recipients reported 2.7 times the risk with SIR of NHL 22.7 [95%CI; 17.3 – 29.3], oral cancer 4.3 [95% CI; 2.1 – 8.0], and lung cancer 2 [1.2-3.0] [11]. These three studies included data from Quebec and the study period was between 1981 and 1998, after which studies from Canada were provincial. According to Park et al, a recent study conducted in Ontario, Canada from 1991- to 2012 reported melanoma is more frequently diagnosed, often at later stages, and thus leads to higher mortality rates compared to non-transplant recipients [73].

2.2.4 Risk factors for cancer among SOT recipients

The cancer risk among SOT recipients is multifactorial and could vary by type of organ transplantation, population, geography, previous history of malignancy, infections, systemic diseases, immunosuppression, etc. [3,7–11]. The common risk factors among them are elaborated below.

Type of organ transplant

Conditions that lead to end organ failure are also linked to an increased risk of cancer or may indicate exposure to potent carcinogens. For example, cardiothoracic organ transplants tend to have a higher incidence of lung cancer [74]. Post-chronic immunosuppression and a previous history of smoking are the most common risk factors among them. Additionally, this group have been shown to develop end-stage pulmonary diseases including idiopathic pulmonary fibrosis and chronic pulmonary disease [74].

Similarly, the risk of liver cancer is elevated in liver transplant recipients and is a common complication among individuals with end-stage liver disease. Interestingly, liver transplant is

also the treatment of choice for individuals with localized liver cancer and liver cirrhosis [75]. Possible theories associated with an increased risk of liver cancer, among these individuals, include the relapse of infection of the Hepatitis B virus, Hepatitis C virus, and diabetes mellitus [76]. Colorectal cancer is also common among LTR which could be due to relapse of ulcerative colitis and IBD [77,78].

Among KTR, evidence of renal cancer is higher. End-stage renal disease may contribute to higher rates of cancer seen in renal transplant recipients as uremia and dialysis are associated with chronic infection and inflammation, impaired immune function, and the retention of carcinogenic compounds [79].

Geography

Predominately most of the studies are from Western countries including the US, Ireland, Finland, Sweden, and Australia [72,80–83]. There are not many reports from Asia or Middle Eastern countries. Risk factors could vary in the population such as genetic variation of infection-related cancer, the prevalence of infection-related oncogenic viruses, and baseline rates of cancer incidence among these populations [84].

Previous history of malignancy

Acuna et al. conducted a meta-analysis involving 32 cohort studies that examined SOT recipients who had a pre-transplant malignancy in remission. Their findings showed that having a pre-transplant malignancy is linked to a higher risk of overall mortality (pooled hazard ratio of 1.51), cancer-specific mortality (pooled hazard ratio of 3.13), and the development of de novo malignancies (pooled hazard ratio of 1.92) after the transplant, compared to recipients without a pre-transplant malignancy [85].

Infections

Immunosuppression can also lead to cancer by facilitating oncogenesis mediated by viruses and bacteria. IAs disrupt the antiviral response of T-cells, resulting in opportunistic chronic viral infections. These oncogenic viruses are linked to some of the most frequent malignancies observed after transplantation [86]. HPV cancers such as oropharyngeal cancers, neoplasm of the vulva, vagina, and cervix; EBV-related cancers like Hodgkin's lymphoma and Non-Hodgkin's lymphoma, Kaposi sarcoma caused by HPV 8, hepatocellular carcinoma by HBV and HCV, gastric cancer by Helicobacter pylori are some of the cancers associated with infections seen in SOT recipients. These incidences are also common in other immunosuppressant populations such as patients diagnosed with HIV/AIDS [87]. Interestingly, SOT recipients show a higher risk of HPV-related cancer compared to HIV and AIDS patients and a lower risk of EBV-related cancers [87].

Immunosuppression burden

Immunosuppressive medications like CNI and Azathioprine have been found to promote cancer development apart from their immunosuppressive actions. Contrastingly medications like Mycophenolate mofetil (MMF)and mTOR inhibitors produce proteins that fight against cancer [88].The following section provides a detailed explanation of the role of immunosuppression burden in cancer risk among SOTs.

2.3 Role of IA in cancer risk among SOT recipients

2.3.1 Pathophysiology

Long-term use of IA following transplantation is associated with a higher risk of developing cancer [16,17]. Long-term use may cause direct damage to cells and their repair mechanisms. Typically, IAs function by decreasing the amount of circulating T lymphocytes, which reduces

the rate of acute rejection and thus promotes graft survival. However, these drugs also impair immune surveillance, and as a side effect the growth and survival of atypical cells may progress unchecked [89]. IAs reduce the immune system's ability to eliminate and detect abnormal cells to proliferate and develop into cancer. Additionally, it increases the risk of cancer associated with ultraviolet radiation exposure. This occurs due to an impaired immune system being less effective in repairing DNA damage caused by UV radiation, leading to an increased number of mutations and cancer development [41,90]. The carcinogenesis of baseline maintenance immunosuppressive agents is summarized in Table 2.2.

Table 2.2 *Common maintenance immunosuppressive agents and their risk of cancer development*

Immunosuppressive drug	Carcinogenesis	Examples of supporting studies
Azathioprine	Increased risk of squamous cell carcinoma in the skin by inhibiting the DNA repair process necessary to correct the damage induced by ultraviolet radiations.	A meta-analysis conducted by Jiyad et al showed a 56% higher risk of squamous cell carcinoma of the skin among SOT recipients who received Azathioprine, compared to other IAs [21].
Cyclosporine	Increases the risk of skin and lymphoid tissue malignancies post-transplantation	A study conducted by Schmidt et al among renal transplant recipients who received cyclosporine during a four-year follow-up period developed malignancies [22].
Mycophenolate mofetil	Antineoplastic agent inhibiting tumor growth	A systematic review comparing Azathioprine and mycophenolate mofetil did not show a significant difference in the risk of cancer among renal and heart transplant recipients [91,92]
Sirolimus	Sirolimus may reduce the risk of malignancy compared to other IAs.	Knoll et al conducted a meta-analysis including 21 RCTs showed a 40% lower risk of cancer and a 56% lower risk of NMSC with sirolimus use compared to the controls [93].
Tacrolimus	Established risk factors for post-transplant lymphoproliferative disorders.	A multicenter study in Spain reported An increase in dose increases the incidence of cancer among liver transplant recipients [94].

2.4 Causal evidence for the role of IA in post-transplant cancer risk

Almost all the evidence for the cancer risk associated with IAs among SOT recipients is from observational studies [51,81,95–98]. Although there are a few clinical trials that investigated short-term toxicity of early-era IAs compared to modern-era IAs, none have investigated de novo malignancy as an outcome [62,64,65,92]. However, the long latency of cancer development reduces the feasibility of randomized controlled trials along with the ethical concerns of randomization [99].

Observational data or Real-World Data (RWD) from electronic or administrative healthcare databases can address this knowledge gap [23–25] under certain assumptions. RWD has been used in various stages of drug development, as encouraged by the 21st Century Cures Act. This act promotes the use of RWD for validating marketing claims post-approval or for exploring additional uses for already approved drugs [100]. For instance, the FDA has launched the RCT DUPLICATE study (Randomized, Controlled Trials Duplicated Using Prospective Longitudinal Insurance Claims: Applying Techniques of Epidemiology) to compare the outcomes of the RCTs with those from the observational studies designed closely to mimic the structure of RCT [101]. The success of these efforts depends on the ability to infer causality from observational data [100].

2.4.1 Causal inference from observational data

One approach to causal inference from observational studies is by emulating a target trial [99]. The potential outcomes framework proposed by Rubin in 1974 [102] helps in emulating a trial from observational data by estimating the counterfactual outcome and thus the individual treatment effect under counterfactual treatments. This framework requires three essential components including the potential outcomes, treatment assignment mechanism, and a model to explain the relationship between the outcomes and covariates. Moreover, several analytical

models exist to operationalize this approach including the inverse propensity score weighted marginal structural models, marginal nested model [103], g-computation [104], and Bayesian approaches to causal inference [105]. Regardless of the analytical strategy, there are a few assumptions to be satisfied before the estimate can be interpreted as causal in nature. These assumptions are elaborated below in the context of this thesis work on the causal role of IA in post-transplant cancer risk among SOT recipients.

First, the stable unit treatment value assumption states that the potential outcomes of SOT recipient are unrelated to the treatment status of another recipient in the study and that the treatment consistently produces a potential outcome. Second. the positivity assumption [106], states that for every participant there is a non-zero probability of being prescribed both modern-era and early-era IAs. Under a sufficiently large sample size, this assumption translates as there exists both treatment and control groups, participants in the data, for all possible values of covariates(confounders). Second, the exchangeability assumption states that the participants who were prescribed modern-era IAs are exchangeable to those who were prescribed early-era drugs. Randomization guarantees this assumption, however, in observational studies, the treatment assignment to control and the treated groups are not randomized and the treatment and control groups differ in terms of pretreatment characteristics which may affect their outcomes (confounders)[107]. However, with modern causal modelling methods, conditional exchangeability is sufficient. That is the potential outcome is independent of the treatment assignment mechanism conditioned on the set of confounders [108,103]. Additionally, the Bayesian approach to causal inference requires the prior independence assumption: that is the parameters of the treatment assignment mechanisms model are independent of the outcome model and do not influence each other [109].

One of the recent developments in the Bayesian approach to causal inference is the use of non-parametric machine learning models for fitting the treatment assignment model and the outcome model. For example, Bayesian Additive Regression Trees (BART) by Chipman, George, and McCulloch [110] have been shown to outperform frequentist approaches of IPTW methods in causal inference competition [111]. BART achieves this by combining the flexibility of regression trees with the rigor of Bayesian methods. BART is particularly useful for capturing complex, non-linear relationships without requiring a pre-specified model form. BART models the response variable as a sum of many regression trees. Each tree captures a different aspect of the data's structure, and the combined prediction is the sum of the individual trees' predictions. This additive approach allows for high flexibility in modelling complex relationships. Because of this flexibility, BART has been shown to be robust against model miss-specification bias in causal inference.

2.5 Evidence for role of IA in cancer risk among SOT recipients from Canada

Only a few studies have investigated the carcinogenic potential of IAs in the Canadian population and often focused on specific types of organ transplants. A study using data from Ontario reported a 12% cumulative incidence of cancer among KTR with Azathioprine and corticosteroid prescription over a 17-year follow-up period from 1981 to 1998, with a limitation of excluding NMSC incidence [14]. Beyond these studies, evidence on the prescription of IAs on cancer risk among SOT recipients in Canada is limited.

A previous study, using the same cohort as this thesis work, from Quebec, examined the association of immunosuppressive agents and cancer among KTR, reporting a higher risk of primary malignant neoplasm in females compared to males with long-term prescription of Mycophenolate mofetil. Additionally, older KTRs exhibited a higher risk of cancer with long-term exposure to Tacrolimus [95].

The primary shortcomings of these previous studies include the subsection of transplant recipients restricted to specific organs, excluding NMSC incidence, and lack of contemporary data, as most studies were before the 2000s. Furthermore, there is a significant gap in clinical trial evidence on the long-term cancer risk associated with IAs in Canada. This highlights the need for updated and comprehensive studies to improve patient outcomes.

In the context of these limitations of previous studies, the rationale for this thesis work is elaborated in the next chapter.

3 Rationale

In 2022, over 2800 Canadians and 500 Quebecers have undergone SOT, marking an increase from 2021 [5,34]. SOT is the treatment of choice for individuals with end-stage organ failure [2]. However, these patients face a 2-4 times higher cancer risk than that of the general population [3]. This increased risk has been hypothesized to be primarily due to the use of maintenance IAs [36].

Many observational studies have examined the impact of IA on cancer risk among SOT recipients but have not explored their causal relationship. Conducting RCT to study the effect of IA on cancer risk is ideal, but long development time for cancer and the need for extended follow-up are some of the significant challenges. Additionally, clinical trials often face increased patient withdrawal due to toxicity and feasibility issues due to poor enrollment. This makes clinical trials very challenging. An alternative approach is to study the causal relationship using observational or Real-World Data from administrative or electronic healthcare records.

In Canada, especially in Quebec, there is a paucity of data regarding cancer risk among SOT recipients. Studies conducted from 1981 to 1998 using the CORR database and the Canadian Cancer Registry highlighted a higher cancer risk among kidney [14], liver [10], and heart transplant [11] recipients. However, since 2011, Canadian national cancer statistics exclude data from Quebec, creating a paucity of recent information on cancer incidence among SOT recipients in the province. This gap highlights a need for studying cancer risk among SOT recipients in Quebec.

4 Objectives of the thesis

The overall aim of the study is to understand the burden of cancer and the role of IA on post-transplant lifetime cancer risk among SOT recipients in Quebec between 1997 to 2016

1. To describe the pattern of cancer and estimate the post-transplant lifetime cancer risk as the overall incidence rate of cancer, stratified by sex for each organ system, among the SOT recipients in Quebec (Manuscript-I).
2. To calculate the standardized risk ratio to compare the post-transplantation cancer risk with the general population in Quebec annually from 1997 to 2016 (Manuscript-I).
3. To estimate the causal effect of switching to modern-era IAs compared to early-era IAs on the post-transplant lifetime cancer risk among the SOT recipients in Quebec (Manuscript-II).

5 Methods

This section describes the methods of the retrospective cohort: the Quebec Transplant Cohort formed by linking the information from two provincial administrative databases between 1997 to 2016. To avoid repetition, further details on methods specific to each objective are provided in the corresponding manuscripts.

5.1 Study design

We employed a retrospective cohort study design from 1997 to 2016. A longitudinal study was necessary to assess the cancer risk, as it takes years for the outcome to occur. This design allowed us to understand the population changes over the years, including the different transplantation eras, the evolution of IA, and the patterns of cancer incidence.

5.2 Data source and cohort formation

We linked two administrative healthcare databases from Quebec namely *Régie de l'assurance maladie du Québec* (RAMQ) database and *Maintenance et exploitation des données pour l'étude de la clientèle hospitalière* (Med-ECHO). RAMQ is a Quebec provincial health insurance plan created in 1969. It contains information on 7 million Quebec residents including individuals above 65 years of age, private and employee insurance plans. This database includes information on demographics, medical code acts for healthcare procedures and services, details of the drugs prescribed, and healthcare costs for patients covered by the RAMQ insurance plan.

MedECHO is a hospital-based healthcare services database created in 1976 in Quebec. It captures information on patients who have been admitted to the hospital including the diagnosis, services provided, intensive care, and interventions. These two databases were linked to form the Quebec Transplant Cohort (QTC). The linkage was done using the "*Numéro*

d'assurance maladie" (NAM), a unique patient's number identifier attributed at birth or when obtaining resident status in Quebec.

5.3 Study population

We defined our cohort as anyone who received a medical code act to any SOT between 1st January 1997 and 31st December 2016 in Quebec and was above the age of 18 years at transplantation. This medical code acts from RAMQ includes one or multiple transplants of kidney, heart, lung, liver, and pancreas and are according to the billing manual, the "*Manuel des médecins spécialistes' (refer to the supplementary table 2 in the appendix).*

5.4 Exposure

IA prescription information was extracted from the RAMQ prescription claims data. SOT recipients were prescribed five types of maintenance IA most often in the database. These IAs include Azathioprine, Cyclosporine, Mycophenolate mofetil, Sirolimus, and Tacrolimus. We extracted the prescription details along with the prescription date from the database. We classified the drugs into early and modern-era drugs to emulate a two-arm (dichotomous treatment) target trial. The early-era IA group consists of recipients who received Azathioprine or Cyclosporine as their first IA post-transplantation and the modern-era IA group includes recipients who received Mycophenolate mofetil, Sirolimus, or Tacrolimus as their first prescribed IA post-transplantation. We used the intention-to-treat approach for our analysis and assumed the patients did not switch to any other IA after their first post-transplant prescription.

5.5 Outcome definition

We identified the primary cancer incidences using the International Classification of Diseases 9th revision (ICD-9) for outcomes identified 1984 to 2006 and ICD-10 for outcomes identified from 2006 to 2016. These ICD codes are recorded by the health professionals and are captured

in the Med-ECHO database (hospital stay diagnostic files) during the study period. Individuals with a pre-transplant history of cancer were excluded from the study. We also excluded any incidences of secondary neoplasms at any site (See supplementary table for the diagnostic codes used and the classification of organs). A previous study on the same cohort confirmed the validity of the data from Med-ECHO and the Quebec cancer registry, *Fichier des tumeurs du Quebec* (FTQ), with Med-ECHO identifying cancer more cases than FTQ [95].

5.6 Data analysis

Objective 1: To describe the pattern of cancer and estimate the post-transplant lifetime cancer risk as the overall incidence rate of cancer and stratified by sex for each organ system among the SOT recipients in Quebec.

To describe the pattern of cancer among the SOT stratified by transplant organ, we considered the individuals at risk from the date of transplantation till the incidence of any primary cancer, death, or administrative end of the study (31st December 2016) whichever occurs first. We estimated the post-transplant lifetime risk of primary neoplasm as an incidence rate by sex. For site-specific cancer risk, cancer diagnosis of any other primary cancer before the site of interest was ignored.

Objective 2: To calculate the standardized risk ratio to compare the post-transplantation cancer risk with the general population in Quebec annually from 1997 to 2016

The standardized risk ratio is a ratio of standardized risk among the SOT recipients and the standardized risk among the general population in Quebec. We used direct standardization to compute the risk estimates in our cohort annually by using the 2011 Quebec population as the standard reference population. Standardized risk estimates for the general population were retrieved from the Quebec Cancer Registry (QCR) (Available from:

https://www.quebec.ca/sante/systeme-et-services-de-sante/organisation-des-services/donnees-systeme-sante-quebecois-services/donnees-cancer) (Figure 5.1). We excluded the Non-Melanoma Neoplasm of Skin to compute the estimates for our cohort as QCR does not capture NMSC.

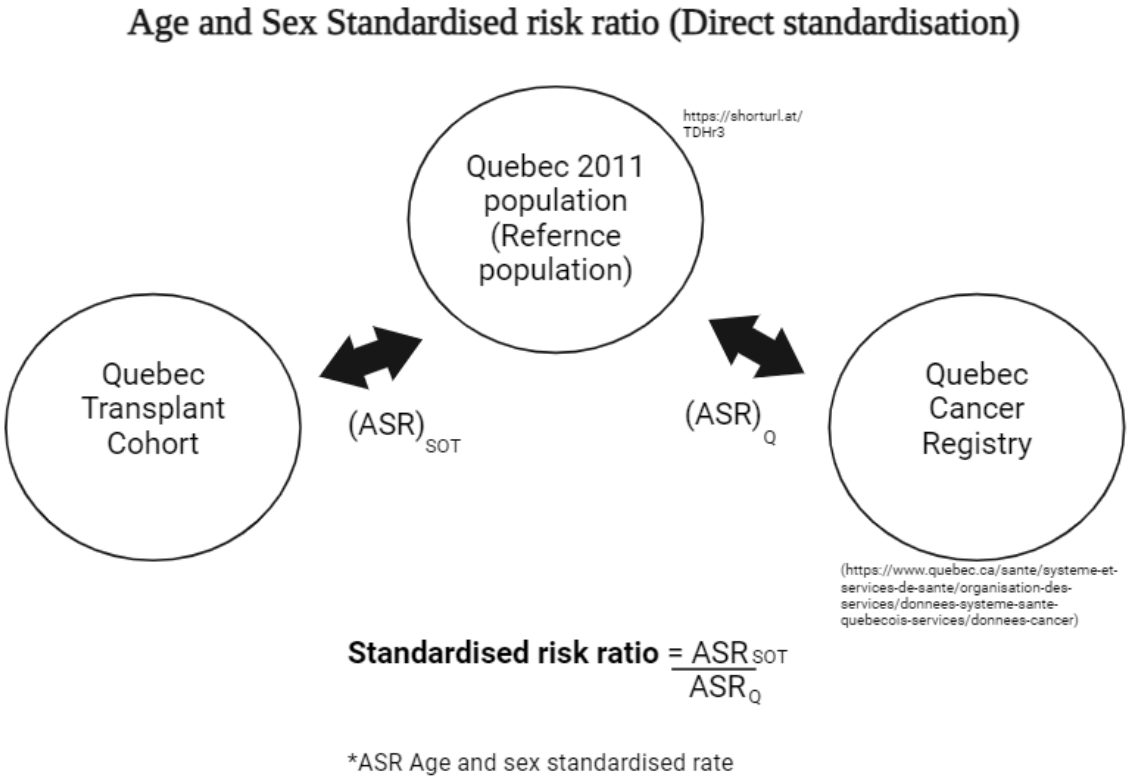
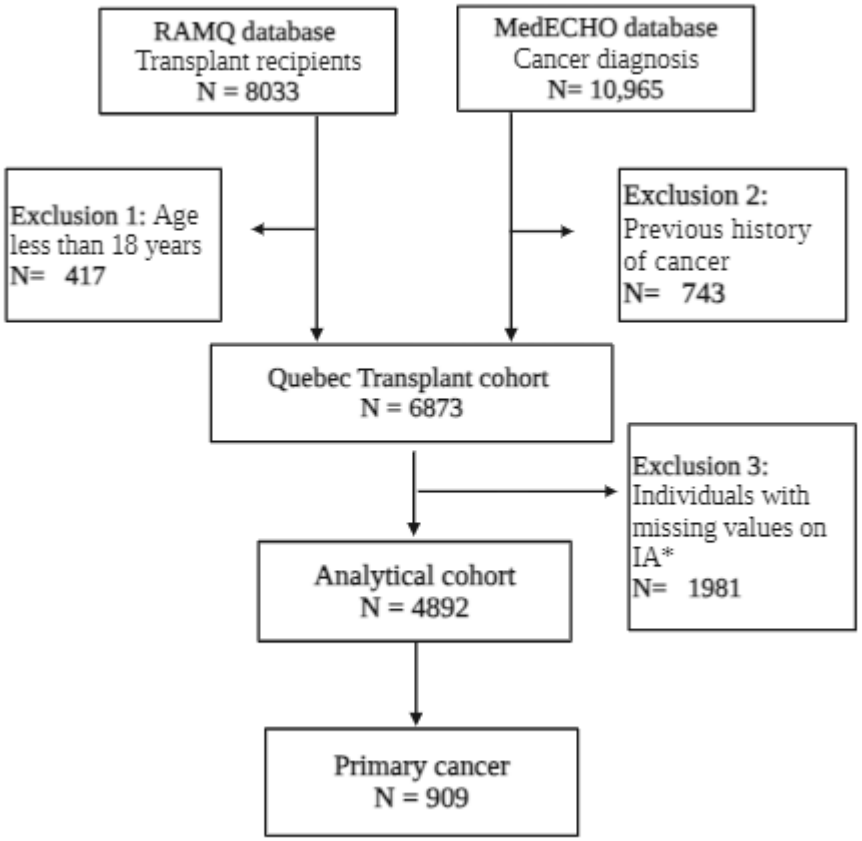


Figure 5.1 *Direct standardization method to estimate the age and sex-standardized risk ratio*

Objective 3: To estimate the causal effect of IA on cancer risk among the SOT recipients in Quebec.

The exclusion criteria entail individuals who have not received IA, whether modern or early-era drugs post-transplantation or are not covered by the RAMQ drug insurance plan. We considered only the first primary cancer diagnosis and excluded the second primary cancer diagnosis (Figure 5.2).

***Figure 5.2**: Study flow diagram*



Using the existing literature, we constructed Directed Acrylic Graphs (DAG) to identify potential confounders, backdoor pathways, and adjustment sets for the analysis that affects the relationship between IA and cancer risk among (Figure 5.3) SOT recipients. DAGs are visual tools that depict the causal and temporal relationships between variables (nodes) along a timeline. Each connection in the graph is directed with a single arrowhead indicating the influence of one variable on another. The acyclic nature of these graphs means they do not contain feedback loops, as a variable cannot influence itself over time. The minimal adjustment set of confounders was identified from the DAG to include in the outcome model. It includes the age at transplantation, sex, type of organ transplanted, year of transplantation, other medications, and other comorbidities taken before transplantation. The information on the

confounders was extracted from the RAMQ database. We utilized the Charlson Comorbidity Index score [112] to measure the burden of the comorbidities among the SOT recipients, based on the diagnoses recorded in the Med-ECHO database.

The potential outcome framework [102] enables to estimate the causal effect of baseline IA prescription on cancer risk among SOT recipients. This framework comprises three key elements: the potential outcomes, the treatment assignment mechanism, and a model that elucidates the relationship between the covariates and the outcomes. We employed Bayesian Additive Regression Trees (BART), a non-parametric machine learning method, to model both the treatment and response variables. BART [113] creates a "sum of trees" model by combining multiple regression trees, allowing it to handle complex non-linearities and multiple interactions. The propensity score, derived from the treatment assignment model, was included as a covariate in the outcome model [108]. In line with recent recommendations for Bayesian causal inference using BART, we estimated individual treatment effects as the difference in posterior predictive distributions of the probability for the outcome under counterfactual treatments for each participant. We then calculated the population average treatment effects (PATE) as a weighted average of these individual treatment effects, using the propensity score as the weight. This process involved adjusting the response surface and propensity scores with a targeted minimum loss-based estimation (TMLE) method as implemented in the bartCause R package [113,114].

Assuming positivity, conditional exchangeability and prior independence, we estimated (a) the PATE, (b) the PATE among the treated (those who in fact were prescribed one of the modern-era IAs), and (c) the PATE among the controls (those who in fact were prescribed one of the early-era IAs). As sensitivity analysis we also calculated these effects without the weighted

averaging approach based on propensity score and without the TMLE correction[105], results of sensitivity analysis are reported in Supplementary Table 4 in the appendix.

For each BART model (propensity score model and outcome model), we fit two hundred regression trees and conducted ten Markov Chain Monte Carlo (MCMC) chains with 5000 burn-in iterations and 1000 samples each. The convergence of the MCMC chains was evaluated using trace plots (Supplementary Figure 3 in the appendix). We report the average treatment effects and their 95% highest posterior density credible interval, following the STROBE guidelines for observational studies[115].

All the data analyses of the thesis were conducted in R studio (Version 4.2.2) using R statistical programming language. The DAG was created using the Dagitty web app.

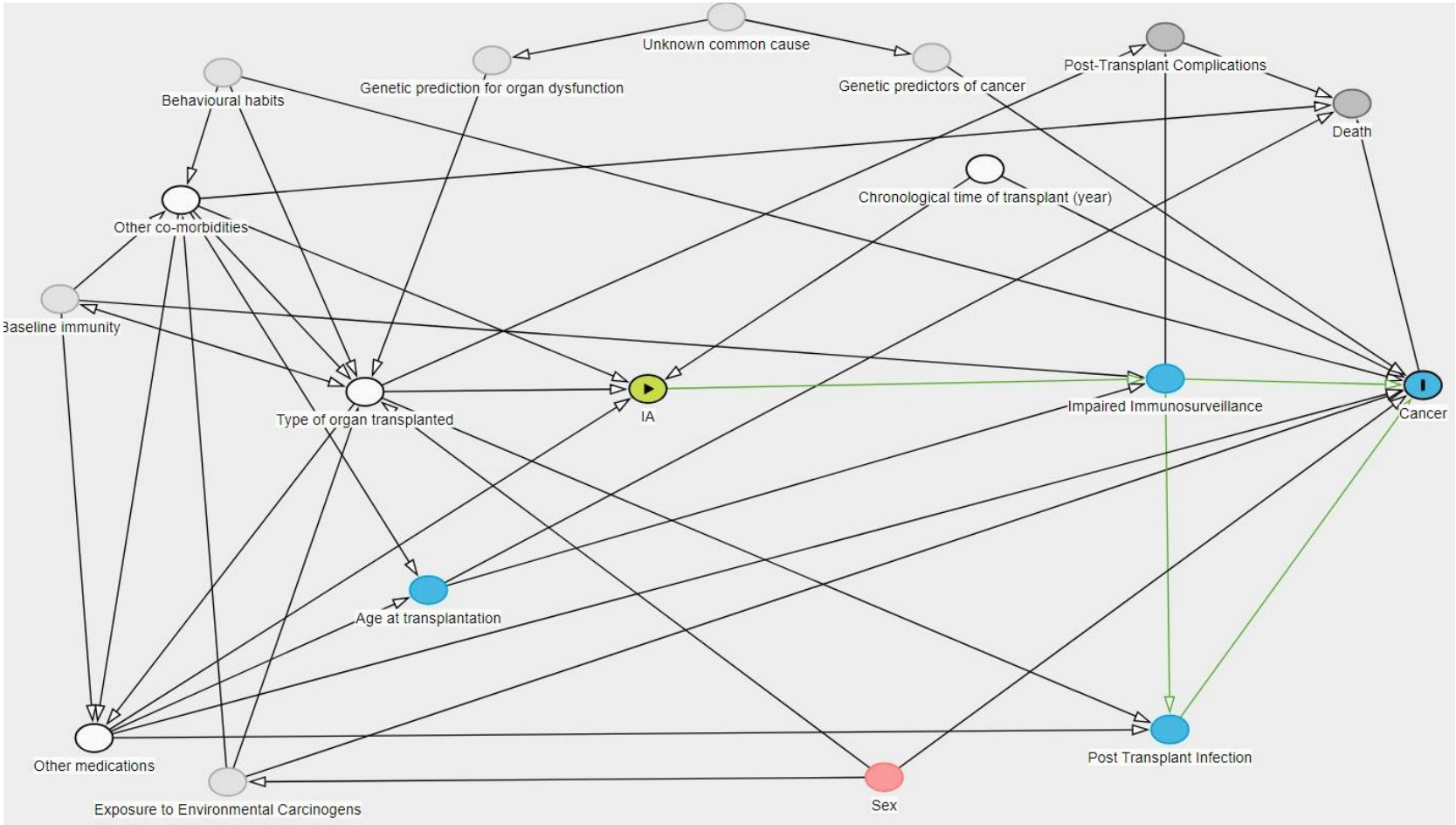


Figure 5.3 *Directed acyclic graph presenting the relationship between IA and cancer risk*

6 Results

The results of this thesis project have been presented through two pre-print manuscripts, each will be submitted to a different peer-reviewed journal as independent research contributions. Consequently, certain concepts and definitions may appear repetitively across various sections of this chapter.

The first manuscript titled *"Risk of cancer among adult solid organ transplant recipients in Quebec, Canada: 1997-2016"* is a short report that focuses on the pattern of cancer incidence and site-specific cancer risk among SOT recipients in Quebec. We have also estimated the age and sex standardized risk ratio to compare the risk with the general population in the province. The findings from the study will provide valuable insights into the specific cancer risks faced by transplant recipients and will inform future healthcare strategies and policies aimed at this population.

6.1 Manuscript 1

Title: Risk of cancer among adult solid organ transplant recipients in Quebec, Canada: 1997-2016

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Short title: Cancer incidence among solid organ transplant recipients in Quebec, Canada

Keywords: Cancer, solid organ transplantation, administrative database, cohort, Canada

Abbreviations:

CORR: Canadian Organ Replacement Register

Med- ECHO- *Maintenance et exploitation des données pour l'étude de la clientèle hospitalière*

RAMQ - *Régie de l'assurance maladie du Québec*

SOT: Solid Organ Transplantation

SRR: Standardised Risk Ratio

Novelty and Impact statements: Findings for the first study analysing 20 years of cancer risk in solid organ transplant (SOT) recipients in Quebec Province, Canada indicate that SOT recipients have a much higher risk of cancer, especially melanoma and other malignant

neoplasm of the skin, compared to the general population. This highlights the need for better cancer screening and prevention in SOT patients.

Abstract

We described the overall cancer risk, among SOT recipients in Quebec, Canada. A 20-year provincial-wide retrospective cohort was created by linking two administrative databases from 1997 to 2016. We identified 6783 SOT recipients, including 4,284 kidney, 1,142 liver, 612 heart, 443 lung, and 392 multiple or other transplants. We described the cancer incidence rate stratified by sex and age and sex standardized risk ratio (SRR) and 95% confidence intervals (CI) by comparing the observed cancer cases in our study population to the number of expected cases in the general population using the Quebec cancer registry. We observed 1,142 cancer cases, resulting in an overall incidence rate of 2436.1 per 100,000 person-years (95% CI; 2212.0 - 2486.2 per 100,000 person-years). Among SOT recipients skin cancer was the most common followed by cancers of lymphoid and hematopoietic tissue and digestive organs. The age and sex SRR showed a 2.5-to-4.2-fold cancer risk between 1997-2016. Our findings showed that SOT recipients in Quebec province were at high risk for cancer compared to the general population. To better understand the variability in cancer risk among SOT recipients in Canada, a nationwide representative study of cancer incidence risk is needed.

Introduction

Solid organ transplantation (SOT), the use of an organ from a deceased or living donor by those experiencing end-stage organ failure due to illness or injury is a cost-effective lifesaving procedure that offers improved quality of life [1]. According to the Canadian Organ Replacement Register (CORR) Annual Report, more than 40,000 Canadians were living with a SOT, and 2,886 individuals received transplants in 2022 [2]. In Quebec, 569 patients benefited from a SOT in 2023 [3].

The improved overall survival in SOT recipients is largely due to immunosuppressive therapy preventing organ rejection [4], its prolonged use has deleterious side effects including the development of de novo post-transplantation malignancies, a major adverse outcome of SOT [1]. According to a recent meta-analysis including 72 cohort studies, the cancer risk in SOT recipients is 2-4-fold compared to the general population[4]. This risk varies according to the type of organ transplanted, predisposed systemic infections and disorders, and history of cancer [5]. Interestingly, some studies have investigated cancers primarily driven by infections, such as oropharyngeal cancers (human papillomavirus (HPV)), and lymphoproliferative tumors (Epstein-Barr virus (EBV)), showing that these cancers are common among SOT recipients. These infection-related cancers are also more elevated among SOT recipients [6,7].

Evidence suggests an increase in cancer incidence among SOT recipients in various countries including Ireland, the United States, Australia, Korea, and Sweden [5,6,8–10]. There is a paucity of data on cancer risk among SOT recipients in Canada. The few nationwide Canadian studies conducted using the CORR database and the Canadian Cancer Registry using data from two decades ago have shown an increased risk of cancers among kidney[11], liver [12], and heart transplant [13] recipients. Similarly, a recent study in Ontario reported a higher incidence of keratinocyte carcinoma (KC) among SOT recipients between 1994 and 2012 linking CORR and the Ontario cancer registry compared to the general population [14]. Importantly, since 2011,

Canadian national cancer statistics have not included data from Quebec, creating a lack of up-to-date information on cancer incidence among SOT recipients in the province. Here we report the overall incidence rate of cancer among SOT recipients from the Quebec province between 1997 and 2016 by sex. Furthermore, we calculated the standardized risk ratio (SRR) to compare cancer risk following transplantation with the general population in the province of Quebec. Providing a better understanding of cancer risk among SOT recipients in Quebec will help inform healthcare strategies and prevention efforts for the high-risk population.

Methods

Study Design, Data Source, and Population

We constructed a 20-year retrospective cohort including all the individuals in Quebec, Canada, who underwent solid organ transplantation using the *Régie de l'assurance maladie du Québec* (RAMQ) database. This database captures information on the healthcare medical service and drug claims. We identified individuals who have undergone kidney, liver, lung, heart, and pancreas transplantation from 1st January 1997 to 31st December 2016 using the medical act codes corresponding to the services provided by the healthcare professionals, according to the billing manual: the "*Manuel des médecins spécialistes".* After excluding individuals less than 18 years of age, we prospectively linked the recipients to the hospital services database (*Maintenance et exploitation des données pour l'étude de la clientèle hospitalière* (Med-ECHO)) to identify the incidence of cancer among the SOT recipients. This database captures all interventions, hospitalizations, and diagnoses received. Further, we excluded SOT recipients who had a pre-transplant diagnosis of any malignancy from the Med-ECHO database, creating the Quebec Transplant Cohort (QTC). These two administrative databases were linked using the "*Numéro d'assurance maladie"* (NAM), a unique patient's number identifier for residents of Quebec.

Outcome of interest

The primary outcome of interest was the post-transplant incidence of any cancer among the SOT recipients during the study period. Using *the International Classification of Diseases 9th revision* (ICD-9) for cases identified between 1984 to 2006 and *10th revision* (ICD-10) coding systems for the period from 2006 to 2016, we identified cancer cases in hospital stay diagnostics files from the Med-ECHO database.

Data analysis

Individuals were considered at risk from the day of transplantation until the incidence of cancer, death, or administrative end of the study (December 31st, 2016) whichever occurred first. We estimated the post-transplant incidence rate of all types of primary neoplasms overall and stratified by organ system and sex. For site-specific cancer risk, diagnosis of any other primary cancer before the site of interest was ignored. To compare the cancer risk of SOT recipients to the general population of Quebec, age and sex SRR were calculated for every follow-up year. For this purpose, first, we obtained the age and sex standardized risk (number of cases per 100,000) in the Quebec population for every year of follow-up from the Quebec Cancer Registry (QCR) (https://www.quebec.ca/sante/systeme-et-services-de-sante/organisation-des-services/donnees-systeme-sante-quebecois-services/donnees-cancer) [15]. Next age and sex standardized risk in the study population was estimated using the 2011 Quebec population as the standard reference population. We used direct standardization as the age and sex-specific rates were available in QCR. Non-melanoma neoplasms of skin were excluded while computing the SRR to avoid overestimation, as QCR does not capture non-melanoma neoplasms of skin. We plotted the ratios to observe the trends in cancer risk over the study period.

Results

Study cohort

After excluding a total of 1,160 individuals with a pre-transplant diagnosis of any malignancy (n=734) and those under 18 years of age (n=417), A total of 6873 individuals received SOT during the study period. The most common organ transplanted was the kidney (62.3%) followed by the liver (16.6%), heart (8.9%), lung (6.5%), and other or multiple transplantations (5.7%). The majority of the SOT recipients were males (63%) with a median age of 51.9 years [IQR; 41.1-60.1] at the time of transplant (Table 6.1)

Table 6.1: *Characteristics of participants at transplantation and post-transplant primary cancer counts in the Quebec Transplant Cohort from 1997- 2016*

Variables	Categories	Total of recipients N= 6873 n (%)	Transplanted organ				
			Kidney N= 4284 n (%)	Liver N= 1142 n (%)	Heart N= 612 n (%)	Lung N= 443 n (%)	Other/Multiple N=392 n (%)
Age at transplant (years)	18 - 34	1052 (15.3)	688 (16.1)	98 (8.6)	85 (13.9)	110 (24.8)	71 (18.1)
	35 - 49	2027 (29.5)	1308 (30.5)	293 (25.7)	152 (24.8)	99 (22.3)	175 (44.6)
	50 - 64	2968 (43.2)	1655 (38.6)	636 (55.7)	333 (54.4)	216 (48.8)	128 (32.7)
	65+	826 (12.0)	633 (14.8)	115 (10.1)	42 (6.9)	18 (4.1)	18 (4.6)
Sex	Male	4329 (63.0)	2663 (62.2)	716 (62.7)	463 (75.7)	233 (52.6)	254 (64.8)
	Female	2544 (37.0)	1621 (37.8)	426 (37.3)	149 (24.3)	210 (64.8)	138 (35.2)
Calendar year of transplantation	1997-2001	1546 (22.5)	1004 (23.4)	290 (25.4)	116 (19.0)	74 (16.7)	62 (15.85)
	2002-2006	1742 (25.3)	1076 (25.1)	300 (26.3)	165 (27.0)	111 (25.1)	90 (23.0)
	2007-2011	1782 (25.9)	1099 (25.7)	283 (24.8)	165 (27.0)	137 (30.9)	98 (25.0)
	2012-2016	1803 (26.2)	1105 (25.8)	269 (23.6)	116 (27.1)	121 (27.3)	142 (36.2)
Follow-up (Years)	Mean ± SD	7.1 ± 5.3	7.7 ± 5.3	6.2 ± 5.60	6.3 ± 5.2	5.3 ± 4.3	6.1 ± 5.4
	Median [IQR]	6.1 [2.5-11.0]	7.0 [3.2-11.8]	5.0 [1.0-10.3]	5.4 [1.3-10.3]	4.5 [2.0-7.7]	4.8 [1.1-9.6]
	0 - 5	3347 (48.7)	1893 (44.2)	645 (56.5)	314 (51.3)	279 (63.0)	216 (55.1)
	6 - 10	1805 (26.3)	1184 (27.6)	244 (21.4)	160 (26.1)	116 (26.2)	101 (25.8)
	11 - 15	1214 (17.7)	837 (19.5)	175 (15.3)	115 (18.8)	36 (8.1)	51 (13.0)
	16 - 20	507 (7.4)	370 (8.6)	78 (6.8)	23 (3.8)	12 (2.7)	24 (6.1)
Cancer Diagnosis	No	5731 (83.3)	3545 (82.7)	926 (81.1)	518 (84.6)	386 (87.2)	354 (90.8)
	Yes	1142 (16.6)	739 (17.2)	216 (18.9)	94 (15.3)	57 (12.8)	36 (9.1)
Cancer site	Melanoma/other malignant neoplasm of skin	335 (29.3)	239 (32.3)	41 (19.0)	25 (26.6)	24 (42.1)	6 (16.7)
	Digestive organs	170 (14.9)	63 (8.5)	91 (42.1)	10 (10.6)	**	**
	Lymphoid, hematopoietic, and related tissue	165 (14.4)	90 (12.2)	34 (15.7)	16 (17.0)	13 (22.8)	12 (33.3)
	Urinary tract	160 (14.0)	136 (18.4)	7 (3.2)	7 (7.4)	**	**
	Respiratory and intrathoracic organs	122 (10.7)	81 (11.0)	14 (6.5)	16 (17.0)	**	**

Male genital organs	55 (4.8)	39 (5.3)	6 (2.8)	6 (6.4)	**	**
Breast	36 (3.2)	24 (3.2)	7 (3.2)	**	**	**
Malignant neoplasm of other and ill-defined sites	20 (1.8)	10 (1.4)	**	**	**	**
Lip, oral cavity, and pharynx	20 (1.8)	11 (1.5)	6 (2.8)	**	**	**
Female genital organs	19 (1.7)	16 (2.2)	**	**	**	**
Malignant neoplasm of thyroid and endocrine gland	17 (1.5)	14 (1.9)	**	**	**	**
Mesothelial and soft tissues	14 (1.2)	8 (1.1)	**	**	**	**
Brain, spinal cord, and CNS	9 (0.8)	8 (1.1)	**	**	**	**

*Abbreviations: SD, Standard deviation; IQR, Interquartile Range.

** To respect confidentiality standards, number of cancers < or equal to 5 were not reported.

[a] Other/multiple category includes recipients with pancreatic transplants or one or more organs transplanted within 30 days of first transplant.

Overall cancer incidence

The median follow-up period was 6.17 years [IQR; 2.5-11.0], during which 1142 individuals (16.6%) developed at least one primary de novo malignancy. Melanoma and other malignant neoplasms of the skin (27.9%) were the most common cancers among kidney, heart, and lung transplant recipients. In contrast, neoplasm of digestive organs was common among liver transplant recipients (Table 6.1). Overall, 14.2% and 18% of the females and males developed post-transplant malignancies, respectively.

Cancer incidence rate stratified by sex

Table 6.2 summarizes cancer incidence rate analysis for each system stratified by sex. While the overall cancer incidence rate was 2346.1 (95% CI; 2212.0 -2486.2) per 100,000 person-years, the rates among males and females were 2561.2 (95% CI; 2384.5 - 2747.6) and 1987.8 (95% CI; 1788.6-2203.2) incidences per 100,000 person-years, respectively. Moreover, compared to females, male SOT recipients had a higher risk for almost all cancer sites examined including neoplasms of the skin, digestive organs, urinary tract, head and neck, mesothelial, and soft tissues. Interestingly, female SOT recipients showed a higher incidence of breast, thyroid, brain, and spinal cord neoplasm than male SOT recipients.

***Table 6.2**: Post-transplant cancer incidence rate among SOT recipients in Quebec stratified by sex, Canada from 1997- 2016*

Cancer site	n (%)	Incidence per 100,000 person-years (95% CI)		
		All transplant recipients	**Female**	**Male**
Melanoma and other malignant neoplasm of skin	335 (29.3)	731.1 (659.0-808.8)	505.2 (409.6-616.2)	738.4 (647.6-838.4)
Digestive Organs	170 (14.9)	368.3 (318.2-424.1)	183.5 (128.5-254)	408.8 (342.5- 484.1)
Lymphoid, hematopoietic, and related tissue	165 (14.4)	368.7 (318.6-424.6)	318.8 (244.4-408.7)	313.15 (255.6-379.8)
Urinary tract	160 (14.0)	331.6 (284-384.8)	184.5 (128.9-254.9)	380.1 (316.1-453.1)
Respiratory and intrathoracic organs	122 (10.7)	299.5 (254.6-350)	229.1 (167.1-306.6)	232.5 (183.5-290.6)
Male genital organs	55 (4.8)	-	-	128.9 (100.1-163.4)[a]
Breast	36 (3.2)	75.7 (54.1-103.1)	179.1(124.7-249)	3.0 (0.1-16.8)
Other and ill-defined sites	20 (1.8)	68 (47.6-94.1)	40.6 (17.5-80)	36.1 (18.7-63.1)
Lip, oral cavity, and pharynx	20 (1.8)	58.57 (39.8-83.1)	30.5 (11.2-66.3)	42.5 (23-70.7)
Female genital organs	19 (1.7)	-	41.6 (26-62.9)[a]	-
Thyroid and endocrine gland	17 (1.5)	45.4 (29.1-67.5)	55.97 (27.94-100.14)	18 (6.7-39.3)
Mesothelial and soft tissues	14 (1.2)	39.6 (24.5-60.6)	10.2 (1.2-36.7)	30.1 (18.6 - 63)
Brain, spinal cord, and CNS	9 (0.8)	20.8 (10.4-37.2)	30.4 (11.2-66.2)	9 (1.9-26.4)
Total	1142	2346.1 (2212-2486.2)	1987.8 (1788.6-2203.2)	2561.2 (2384.5-2747.6)

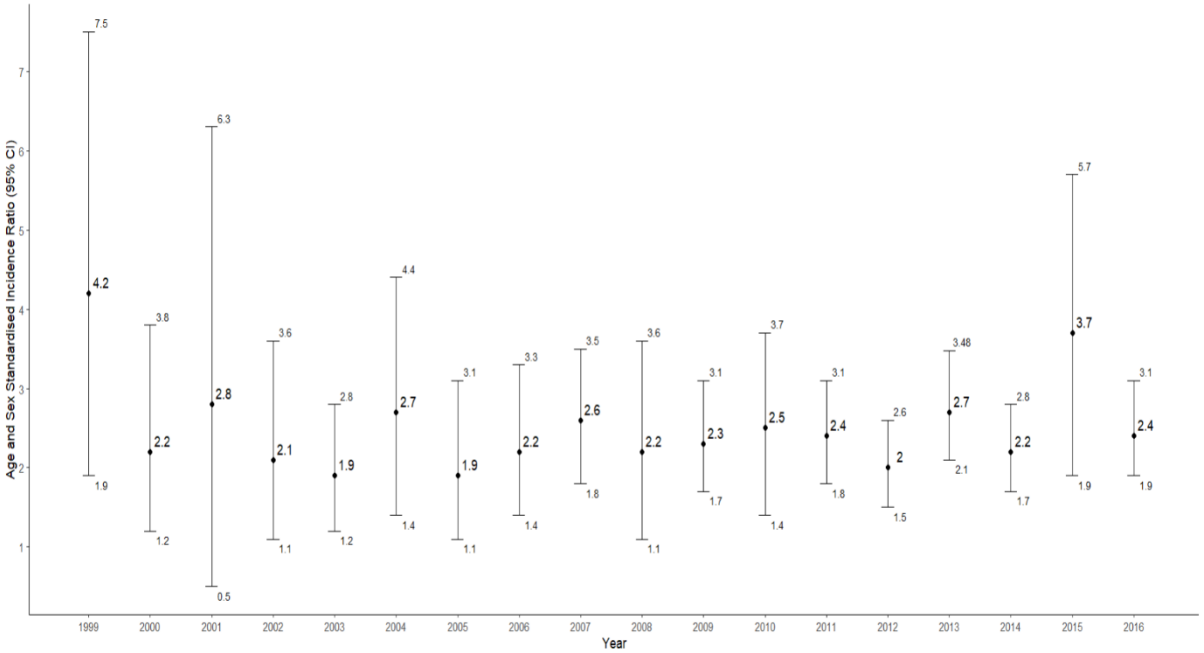
*Abbreviations: CI, confidence interval; CNS, central nervous system

[a] Incidence is presented for the entire cohort but was calculated separately for males and females for sex-specific malignancies.

Standardized risk ratios

Figure 6.1 represents SRR computed using the QCR estimates from 1999 to 2016. In 1997 and 1998, the incident cases in the study population were too low (<10) to provide a reliable estimate of the risk ratio. The SRR ranged from 4.2 (95% CI; 1.9 -7.5) in 1999 to 2.4 (95% CI; 1.9 - 3.1) in 2016. The SRR estimates fluctuated annually, showing both increasing and decreasing trends, with the highest SRR observed in 1999, followed by 2015 at 3.7 (95% CI; 1.9 - 5.7). Overall, the Standardized risk ratio remained approximately above 2.0 for the entire study period, indicating a consistently higher incidence of cancer among SOT recipients compared to the general population.

***Figure 6.1**: Annual standardized risk ratio and 95% confidence intervals of cancer among SOT recipients in the Quebec Transplant cohort comparing to Quebec* cancer registry estimates from 1997-2016.*



* Malignant skin neoplasm was excluded to estimate the risk ratio, as the QCR does not capture malignant skin neoplasm.

Discussion

Our study bridges an important gap in the information on the cancer burden among SOT recipients in Quebec, Canada, over 20 years from 1997 to 2016. Although our results broadly concur with previous Canadian studies [11,13], direct comparisons were limited because most prior research focused on a specific organ transplant. Nevertheless, our findings align with studies from other countries, including Ireland and the United States, which reported increased risk among SOT recipients compared to the general population as reported here [5,6].

Overall Cancer Incidence

Skin cancer was the most common cancer among kidney, lung, and heart transplant recipients followed by cancers of digestive organs and lymphoid and hematopoietic tissue. SOT recipients are also at higher risk of infection-associated cancers, including those related to viral or non-viral infections such as HPV, EBV, and hepatitis B and C viruses [4,16]. Several studies support the risk of skin cancer among SOT recipients including nonmelanoma skin cancer [17]. A systematic review conducted on a randomized control trial on behavioral and pharmaceutical interventions for the prevention of skin cancers in SOT recipients reported that these individuals might have 65-250 times the risk of squamous and basal cell carcinoma compared to the general population [17]. HPV-related infection may also increase the risk of SCC whereas immunosuppressive agents are potentially modifiable risk factors for SCC [4,5].

We categorized all types of lymphoma, leukemia, and other immunoproliferative malignancies under the lymphoid and hematopoietic malignancies category. EBV infection, an oncogenic virus could be associated with non-Hodgkin's Lymphoma (NHL) [4]. A previous Canadian study reported a 20-fold increased risk of NHL among liver transplant recipients [12]. Although viral exposure information was not available in the administrative databases we did observe a higher incidence of digestive organ neoplasm, including those of the esophagus, colon, stomach, liver,

pancreas, anal canal, and other ill-defined digestive organs, among liver transplant recipients. Relapses of the Hepatitis B and C infections and diabetes mellitus are considered to elevate the risk of liver cancer [4]. Increased incidence of ulcerative colitis and Inflammatory bowel disease post-liver transplantation might elevate the risk of colon and colorectal cancer [4,12]. This pattern of infection-related malignancies resembles cancer incidences among AIDS/HIV-infected individuals [6], possibly due to poor immunosurveillance towards oncogenic viruses.

Cancer incidence rate stratified by sex.

Our study identified a male predominance in cancer sites including the skin, digestive organs, urinary, mesothelial, soft tissue, head, and neck. Conversely, females showed high breast, thyroid, brain, and spinal cord incidence rates. Sarah et all [18] reported that the male predominance observed in the general population was less pronounced in SOT recipients. They argued that females have better innate and adaptive immune systems, enhancing their immune surveillance than males. However, the interplay between sex, immune function, age, and cancer risk is complex. Males and females, as well as older and younger individuals, may respond differently to immunosuppressive agents, which can mitigate the sex and age differences in cancer incidence observed in the general population. For example, in kidney transplant recipients, higher cumulative doses of mycophenolate mofetil were associated with greater cancer risk in females than in males. At the same time, tacrolimus posed a greater risk in younger recipients [18,19].

Standardized risk ratio

We observed a 4.2 to 2.5-fold risk of cancer among SOT recipients in Quebec compared to the general population during the period from 1999 to 2016. The cancer incidence has decreased over the years among SOT recipients which could be due to the evolution of IAs. In contrast, new cancer cases have been on the rise among the general population in Canada [20]. This could

be due to the update in diagnostic codes for cancer in 2006, which could capture more cancer cases, resulting in fluctuating risk ratios during the study period.

The strength of our study lies in exploring the cancer risk among SOT recipients in Quebec using high-quality provincial data. We were able to link the two longitudinal administrative databases from Quebec. This is the first study from Quebec to describe the pattern of cancer among SOT recipients, not limited to specific organ transplantation. However, our study is limited to using the RAMQ database, which captures only individuals covered by the insurance, and we did not account for competing outcomes such as non-cancer related death (e.g., infections) or cases lost-to-follow-up. A previous study on the same cohort confirmed the validity of the data from Med-ECHO and the Quebec cancer registry, *Fichier des tumeurs du Quebec* (FTQ), with Med-ECHO identifying cancer more cases than FTQ [19]. Even though this approach is validated, there may not be complete case ascertainment leading to underestimation of incidence in this population. Future studies should incorporate competing risk analysis to more accurately assess cancer risks. Additionally, further research should explore donor characteristics, patients' behavioral history of these patients, genetic predictors of cancer, comprehensive pre-transplantation data, and exposure to environmental carcinogens. Our analysis was unable to stratify the site-specific cancer risk by organ due to low incidence numbers, which limited our ability to examine the effects of varying levels of immunosuppression across different organ

In conclusion, our findings confirm a higher cancer risk among SOT recipients compared to the general population in Quebec highlighting the need for improved surveillance among this group.

Authors' contribution: All authors have made substantial contributions, and edited and approved the final version of the text. Conceptualization and study design: CL & BN. Data curation: TD, CL, SM. Statistical analysis and result interpretation: TD, CL, SM. Supervision: CL, SM. Writing—original draft: TD. Writing—review & editing: TD, CL, BN, SM.

Conflict of Interest: The authors declare that they have no known competing interests that could have appeared to influence the work reported in this manuscript.

Ethics statement: The *Commission d'accès à l'Information,* the *Régie de l'assurance maladie du Québec* (RAMQ), as well as the *Institut de la statistique du Québec* approved the study and gave access to administrative data (reference number: 1015461-S). All the data used for this work were anonymous, the researchers had no information on the population under study. The institutional ethics review board from McGill University and Université de Montréal have approved the study.

Data availability statement: The data that support our findings are not publicly available. Requests should be directed to the corresponding author who will contact the relevant authorities.

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Preface of Manuscript II

In the first manuscript, we demonstrated a heightened cancer risk among SOT recipients in Quebec compared to the general population. In this second manuscript, we aim to investigate the extent to which this increased cancer risk can be attributed to the prescription of immunosuppressive agents (IAs) type. Specifically, we will examine the early and modern-era maintenance IAs and their causal effect on cancer risk among SOT recipients. The findings from this study may provide insights to optimize post-transplant care and potentially mitigate cancer risk in this vulnerable population.

6.2 Manuscript 2

Title: The causal effect of modern-era immunosuppressive agents on lifetime cancer risk among solid organ transplant recipients: A Bayesian machine learning approach

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Short title: The Effect of immunosuppressive agents on cancer risk among solid organ transplant recipients

Keywords: Cancer, solid organ transplantation, causal effect, Bayesian additive regression trees

Abbreviations:

Med- ECHO- *Maintenance et exploitation des données pour l'étude de la clientèle hospitalière*

RAMQ - *Régie de l'assurance maladie du Québec*

SOT: Solid Organ Transplantation

BART: Bayesian additive regression tree

TMLE: Targeted minimum loss estimation

Novelty and Impact statements: This study used a robust causal inference framework to estimate the impact of modern and early-era IAs on cancer risk among solid organ transplant recipients in Quebec. Using comprehensive provincial healthcare databases and advanced statistical methods, our findings show a decrease in cancer risk with modern-era drugs. These findings could inform better post-transplant care and treatment decisions.

Abstract

Background: Over 2,500 Canadians and 500 Quebecers undergo solid organ transplantation (SOT) annually. It is well known that cancer is the leading cause of death in solid organ transplantation recipients (SOTR), attributed to the use of Immunosuppressive agents (IA's). Cancer Incidence is expected to rise in Quebec in the coming years and there is a notable gap in understanding the effect of IAs on cancer risk among SOT in Quebec.

Objective: To estimate the average treatment effect of modern-era IA, compared to early-era IA, on the post-transplant lifetime risk of developing at least one primary cancer among SOT recipients.

Methodology: A retrospective cohort study was created linking two provincial-level administrative healthcare databases from 1997 to 2016: the *Régie de l'assurance maladie du Québec* (RAMQ) and *Maintenance et exploitation des données pour l'étude de la clientèle hospitalière* (Med-ECHO). Individuals who have been prescribed one of the modern-era IAs (Mycophenolate mofetil, Sirolimus, and Tacrolimus) were our treatment group of interest, and individuals who have been prescribed one of the early-era IAs (Azathioprine and Cyclosporine) were our comparator group of interest. We used the potential outcomes framework for causal inference and the Bayesian Additive Regression Trees (BART) models to emulate the target trial. Following an intention-to-treat approach, we estimated the average reduction in cancer risk (in risk difference scale) attributable to prescribing one of the modern era IA to all SOT recipients in Quebec who were prescribed one of the early era IA, during 1997-2016.

Results: The analytic cohort included 4,892 individuals prescribed these IAs as a baseline maintenance therapy regimen. With a median of 6.93 years of follow-up, 909 individuals had developed primary malignant neoplasm. The SOT recipients who were originally prescribed one of the early-era IAs would have had 4% points lower post-transplant cancer risk if they

had been prescribed one of the modern-era IAs, instead (RD= -0.040; 95%CI = -0.049 to -0.030).

Conclusion: Switching to modern-era immunosuppressive agents (e.g., Mycophenolate mofetil, Sirolimus, and Tacrolimus), from early-era IAs, may reduce the post-transplant lifetime risk of cancers among SOT recipients in Quebec.

Background:

Solid organ transplantation (SOT) is a life-saving treatment for individuals facing end-stage organ failure, offering improved long-term survival and enhanced quality of life [1] while being more cost-effective than alternative treatments [2] that prolong the patient's survival [3,4]. More than 2,500 Canadians and 500 Quebecers undergo Solid Organ transplantation annually [5,6]. Almost all of these SOT recipients are prescribed immunosuppressive Agents (IAs) lifelong to prevent organ rejection. However, long-term immunosuppression may lead to an array of associated complications including toxicity, infections, cardiovascular disease, and de novo malignancy[7–10]. For example, a meta-analysis conducted with 72 cohort studies worldwide concluded that the lifetime risk of cancers among SOT recipients is 2 to 4-fold higher than in the general population [3].

Since the invention of immunosuppression therapy, several types of IAs have been developed to reduce such side effects while maintaining the balance between under and over-immunosuppression [11]. For example, modern-era IAs such as Sirolimus, Mycophenolate mofetil, and Tacrolimus provide targeted immunosuppression and reduce the incidence of adverse effects, including cancers [12]. However, certain early-era IAs including Azathioprine and Cyclosporine, are considered carcinogenic by the International Agency for Research on Cancer (IARC) [13] but are still prescribed to SOT recipients across the world including in Canada [10,14,15].

In light of recent reports projecting a substantial increase in the incidence of cancer in North America [16], there is a need to re-evaluate the treatment strategies for this high-risk group. To this end, estimating the causal effect of switching to modern-era IAs, from early-era IAs, for SOT recipients has high utility for informed decision-making. Although several studies have investigated the relationship between immunosuppression and cancer risk among SOT

recipients [10,17], almost all of this evidence is associational in nature. Additionally, there is a notable gap in data concerning the incidence of cancer among SOT recipients in Quebec. The fact that recent Canadian National Cancer statistics do not include data from Quebec further increases the knowledge gap and highlights the need to investigate the question with provincial-level data.

In this manuscript, following the potential outcomes framework, we estimate the average treatment effect of modern-era drugs compared to early-era drugs on post-transplant lifetime cancer risk among SOTR in Quebec from 1997 to 2016 using the Quebec Transplant Cohort (QTC), an administratively linked database.

Methods

Data source, study design, and population.

We constructed the Quebec Transplant Cohort, a provincewide retrospective cohort spanning 20 years by linking two administrative healthcare databases from Quebec, Canada: i) *Régie de l'assurance maladie du Québec* (RAMQ), and ii) *Maintenance et exploitation des données pour l'étude de la clientèle hospitalière* (Med-ECHO).

RAMQ is the Quebec provincial health insurance plan created in 1969, covering approximately 7 million Quebec residents including temporary residents, individuals above 65 years of age, and those covered by private and/or employer-paid insurance plans. RAMQ's insurance claims database includes information on demographics, healthcare procedures and services, details of drugs prescribed, and the healthcare costs, for patients covered by the plan. All healthcare procedures and prescriptions covered by the RAMQ, including SOT, are recorded as medical act codes as described in the billing manual: *Manuel des médecins spécialistes* [18]. We defined our cohort as anyone who received a medical act code related to any SOT between January 1st, 1997, to December 31st, 2016, and was above 18 years of age at transplant. These codes

included transplantation of one or multiple kidneys, liver, lung, heart, or multiple types of organs (See supplementary table 1 for a full list of codes used).

Med-ECHO is a hospital-based healthcare services database of Quebec, created in 1976. This database captures the information on patients admitted to the hospital including diagnosis, services provided, intensive care admissions and services, and any procedural interventions. To identify the cancer incidence among our participants, we prospectively linked the cohort to their information in Med-ECHO using the *Numéro d'assurance maladie* (NAM), a unique healthcare identifier assigned to all individuals settled permanently or temporarily in Quebec (See supplementary figure 1 in the appendix for the study flow diagram).

Exposure and Outcome Definitions

RAMQ database only captures the prescription of a drug. Hence, we define the treatment of interest as the first post-transplant prescription of an IA. Thus, our treatment group is defined as SOT recipients who were prescribed one of the modern-era IAs (Mycophenolate mofetil, Sirolimus, and Tacrolimus) and the control group as those who were prescribed one of the early-era IAs (Azathioprine and Cyclosporine), as the first post-transplant immunosuppression regime. We used the intention-to-treat analysis approach and assumed that the patients did not switch to any other IA after their first prescription. We excluded a proportion (28.8%) of participants for whom no post-transplant IA prescription information was available in the RAMQ database.

The outcome of interest was defined as the earliest post-transplant diagnosis for any primary cancer type. The case ascertainment was done using the International Classification of Diseases (ICD) codes, specifically ICD-9 for the period from 1984 to 2006 and ICD-10 for the period from 2006 to 2016 (See supplementary table 2 in the appendix for the ICD-9 and 10 codes used for cancer incidence identification). Data were extracted from the Med-ECHO repository

excluding those individuals with a pre-transplant history of malignancy. A previous study conducted on the same cohort validated the data from Med-ECHO and the Quebec cancer registry, *Fichier des tumeurs du Quebec (FTQ)*. Med-ECHO captured more cancer incidents than FTQ [19]. Even though this approach is validated, there may not be complete case ascertainment leading to underestimation of incidence in this population. SOT recipients were considered at risk from the date of first transplantation till the earliest of the following events, death, date of diagnosis of primary cancer, or administrative end of the study (December 31, 2016).

Data analysis

Using Directed Acrylic Graphs (DAG) (See supplementary figure 2 in the appendix) we identified the following minimum adjustment set for an unconfounded effect estimation: age of the recipients at transplantation, sex, type of organ transplanted, year of transplantation, comorbidities, and other medications taken before transplantation.

We followed the potential outcome framework [20] to estimate the causal effect of the first post-transplant maintenance IA prescription on cancer risk among SOT recipients. This framework requires three essential components including the potential outcomes, treatment assignment mechanism, and a model that characterizes the relationship between the outcomes and covariates. We employed Bayesian Additive Regression Trees (BART), a non-parametric machine learning approach to model the treatment and the response model. BART fits an additive combination of several regression trees creating a "sum-of-trees" model and can accommodate complex non-linearity and multiple interactions [21]. The propensity score estimated from the treatment assignment model was used as a covariate in the outcome model [21,22]. Following the latest recommendations in the Bayesian approach to causal inference using BART, we estimate the individual treatment effects as the difference between posterior

predictive distributions of probability of the outcome under factual and counterfactual treatments. Followed by the estimation of population average treatment effects as a weighted average of these individual effects, weighted by the propensity score. In this process, the response surface and propensity scores are corrected using a targeted minimum loss-based estimation method (TMLE). [23] This approach is implemented in the bartCause R package [21,24]. With the positivity, conditional exchangeability, and prior independence assumptions, we estimate a) the population average treatment effect (PATE), b) the PATE among the treated (those who were factually prescribed one of the modern-era IAs), and c) the PATE among the controls (those who were factually prescribed one of the early-era IAs). As a sensitivity analysis, we also estimated these effects based on a non-weighted average (propensity score is a covariate in the outcome model, but not used for the weighted average of individual treatment effects) and in addition without the TMLE correction [25] (supplementary table 4 in the appendix).

Two hundred regression trees were fit for each BART model with ten Markov Chain Monte Carlo (MCMC) chains run with 5000 iterations for burn-in and 1000 samples each. The convergence of the MCMC chains was assessed using trace plots (Supplementary Figure 3 in the appendix). The average treatment effects and the corresponding 95% highest posterior density credible intervals are reported. We followed the STROBE reporting guidelines for improved reporting on observational studies [26].

Results:

Cohort characteristics

After excluding children less than the age of 18 (417 individuals), individuals with a previous history of cancer (743 individuals), and individuals with missing values on IA from the RAMQ database (1,981 individuals) (supplementary figure 1 in the appendix) we had 4892 patients

who received SOT from 1st January 1997 to 31st December 2016 available for the analysis. This included 3,159 kidney, 756 liver, 406 heart, and 295 lung transplant recipients. Due to relatively small numbers, individuals who received a pancreatic transplant (2.8%) were categorized along with those who received more than one organ transplant (276). Among the SOT recipients, 63.7% recipients were male, the majority were in the age group of 50-60 years (Table 6.3) and the median follow-up time was 69.3 (IQR 3.14 – 11.62)

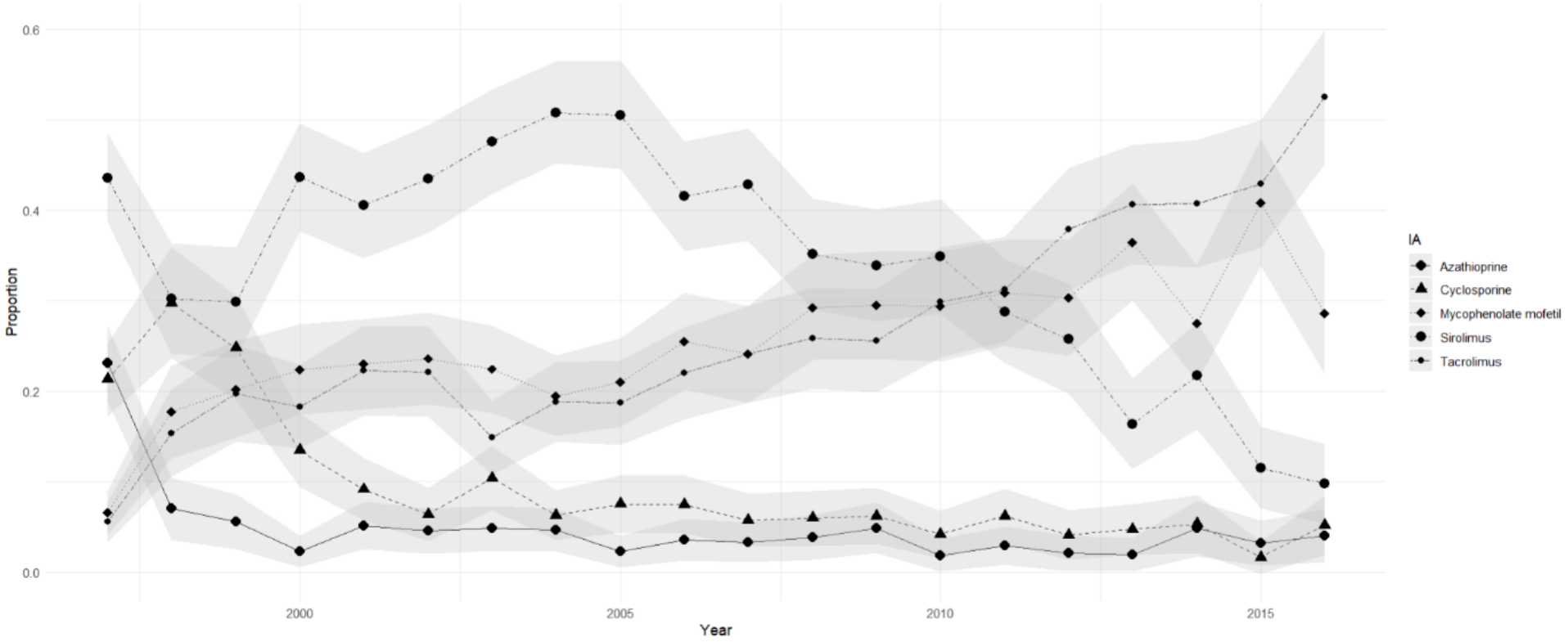
Immunosuppression and cancer incidence

The baseline characteristics of the cohort, stratified by the type of drug prescribed at baseline, are presented in Table 6.3. There were 738 and 4154 individuals who were prescribed early and modern-era IAs, respectively. Temporal variations in the prescription patterns of IAs were observed during the study period (Figures 6.2-a and 6.2-b). For example, Sirolimus was prescribed to 50% of the SOT recipients in 2005 compared to 10% in 2016. Reflecting the shift in prescription patterns of IAs over the years, where there was a downward trend in the proportion of individuals who were prescribed one of the early-era IAs (Azathioprine and Cyclosporine), compared to modern-era IAs (Sirolimus, Tacrolimus, and Mycophenolate mofetil), as the first post-transplant IA from 1997 to 2016.

***Table 6.3**: Characteristics of solid organ transplant recipients by the type of immunosuppressive agents prescribed as the first post-transplant IA, in the province of Quebec from 1997–2016*

	Early-era IA prescribed at baseline			**Modern-era IA prescribed at baseline**				**Overall N= 4892 n (%)**
	Azathioprine N=263 n (%)	**Cyclosporine N=475 n (%)**	**Total N=738 n (%)**	**Mycophenolate mofetil N = 1191 n (%)**	**Sirolimus N= 1758 n (%)**	**Tacrolimus N= 1205 n (%)**	**Total N= 4154 n (%)**	
Age at transplantation, years								
18 – 34	50 (19.0)	82 (17.3)	132 (17.9)	264 (22.2)	122 (6.9)	235 (19.5)	621 (14.9)	753 (15.4)
35 – 49	86 (32.7)	133 (28.0)	219 (29.7)	342 (28.7)	394 (22.4)	318 (26.4)	1054 (25.4)	1273 (26.0)
50 – 64	105 (39.9)	214 (45.1)	319 (43.2)	467 (39.2)	809 (46.0)	534 (44.3)	1810 (43.6)	2129 (43.5)
65+	22 (8.4)	46 (9.7)	68 (9.2)	118 (9.9)	433 (24.6)	118 (9.8)	669 (16.1)	737 (15.1)
Sex								
Male	154 (58.6)	348 (73.3)	502 (68.0)	719 (60.4)	1184 (67.3)	713 (59.2)	2616 (63.0)	3118 (63.7)
Female	109 (41.4)	127 (26.7)	236 (32.0)	472 (39.6)	574 (32.7)	492 (40.8)	1538 (37.0)	1774 (36.3)
Transplanted organ								
Kidney	118 (44.9)	241 (50.7)	359 (38.6)	828 (69.5)	1229 (69.9)	743 (61.7)	2800 (67.4)	3159 (64.6)
Liver	113 (43.0)	127 (26.7)	240 (32.5)	109 (9.2)	124 (7.1)	283 (23.5)	516 (12.4)	756 (15.5)
Heart	7 (2.7)	53 (11.2)	60 (8.1)	84 (7.1)	214 (12.2)	48 (4.0)	346 (8.3)	406 (8.3)
Lung	17 (6.5)	40 (8.4)	57 (7.7)	110 (9.2)	54 (3.1)	74 (6.1)	238 (5.7)	295 (6.0)
Other or multiple	8 (3.0)	14 (2.9)	22 (3.0)	60 (5.0)	137 (7.8)	57 (4.7)	254 (6.1)	276 (5.6)
Follow up, years Median [IQR]	7.47 [2.87 –13.23]	9.79 [4.45 –14.71]	8.80 [3.81 – 14.03]	8.18 [4.02 –12.83]	5.15 [2.13 – 9.23]	7.67 [3.83 –12.40]	6.69 [3.05 – 11.12]	6.93 [3.14 –11.62]
Calendar year of transplantation								
1997-2001	89 (33.8%)	259 (54.5%)	348 (47.2)	329 (27.6%)	220 (12.5%)	289 (24.0%)	838 (20.2%)	1186 (24.2%)
2002-2006	68 (25.9%)	119 (25.1%)	187 (25.3%)	327 (27.5%)	425 (24.2%)	325 (27.0%)	1077 (25.9%)	1264 (25.8%)
2007-2011	51 (19.4%)	63 (13.3%)	114 (15.4%)	297 (24.9%)	551 (31.3%)	311 (25.8%)	1159 (27.9%)	1273 (26.0%)
2012-2016	55 (20.9%)	34 (7.2%)	89 (12.1%)	238 (20.0%)	562 (32.0%)	280 (23.2%)	1080 (26.0%)	1169 (23.9%)
Comorbidity Index (Charlson)								
0-2 (mild)	106 (40.3%)	210 (44.2%)	316 (42.8%)	629 (52.8%)	535 (30.4%)	609 (50.5%)	1773 (42.7%)	2089 (42.7%)
3-4 (moderate)	109 (41.4%)	154 (32.4%)	263 (35.6%)	369 (31.0%)	618 (35.2%)	382 (31.7%)	1369 (33.0%)	1632 (33.4%)
>5 (severe)	48 (18.3%)	111 (23.4%)	159 (21.5%)	193 (16.2%)	605 (34.4%)	214 (17.8%)	1012 (24.4%)	1171 (23.9%)
Other medications								
Yes	197 (74.9%)	276 (58.1%)	473 (64.1%)	688 (57.1%)	1603 (91.2%)	688 (57.1%)	2979 (71.7%)	3452 (70.6%)
No	66 (25.1%)	199 (41.9%)	265 (35.9%)	503 (42.2%)	155 (8.8%)	517 (42.9%)	1175 (28.3%)	1440 (29.4%)

***Figure 6.2**: a) Proportion of individuals prescribed different immunosuppressive agents as the first post-transplant IA by year of transplantation*



b) Proportion of individuals prescribed one of the early- and modern-era IAs as the first post-transplant IA by year of transplantation

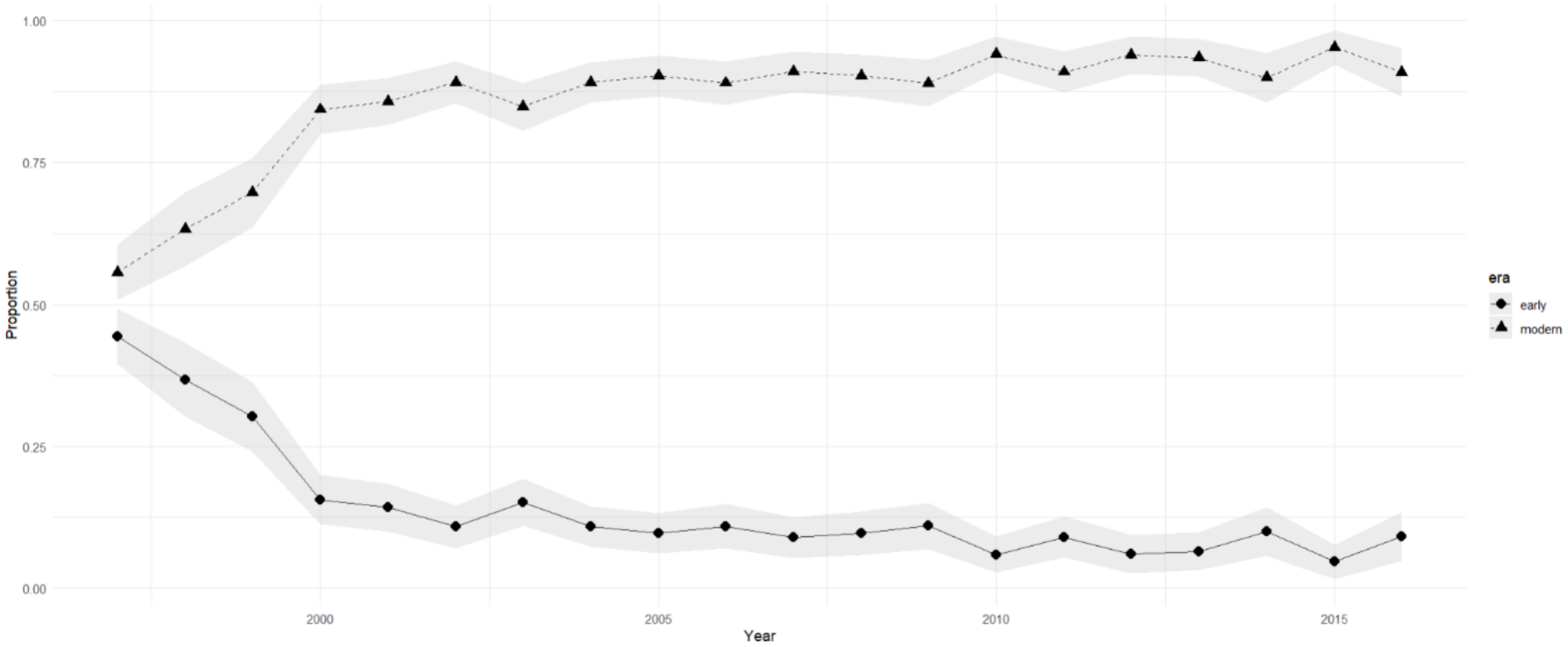
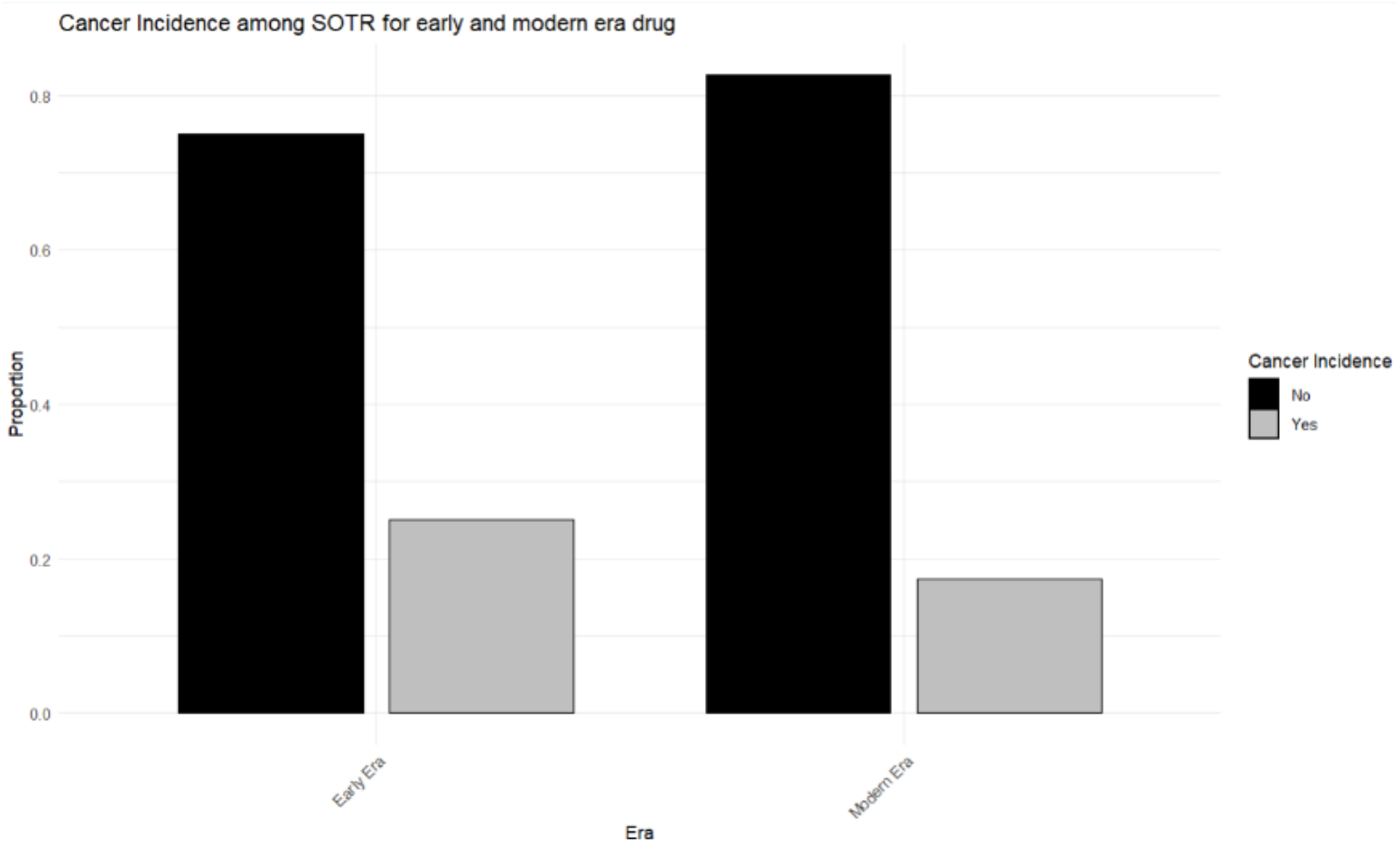
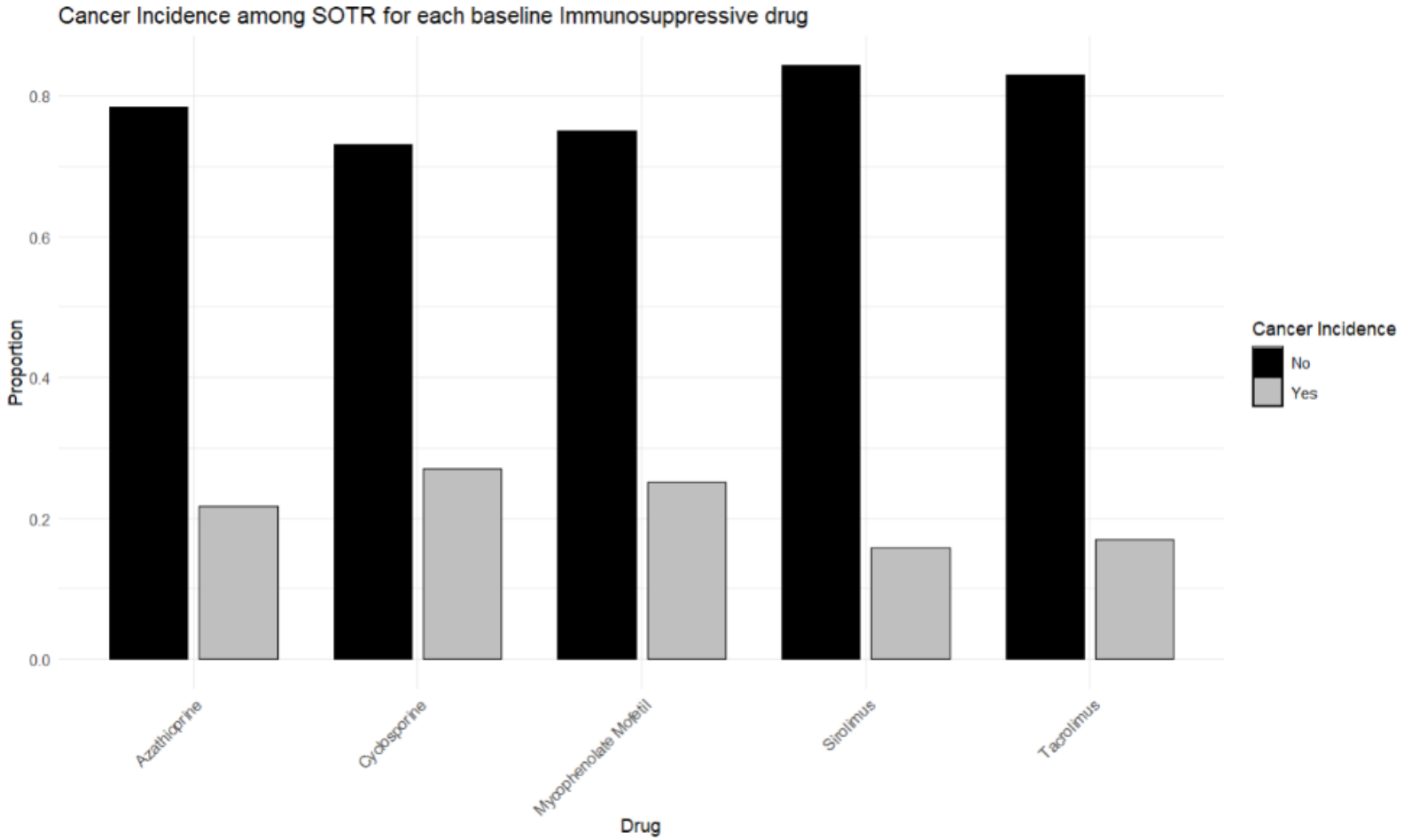


***Figure 6.3**: a) Distribution of cancer cases by the type of first post-transplant IA prescription*



b) Proportion of cancer cases among SOT recipients by prescription of specific IA type



A total of 909 (18.6%) SOT recipients were identified with primary malignant neoplasm. Cancer incidence varied significantly between the two groups (Figure 6.3-a). Among the early-era IA group, 25.1% developed cancer. Whereas the modern era had a lower incidence at 17.4%. The highest cancer incidence was observed among the participants who were prescribed Cyclosporine (26.9%) and the lowest among those who were prescribed Sirolimus (15.8%) (Figure 6.3-b). Melanoma and other malignant neoplasm of the skin (30.6%) were the most common among the SOT recipients, followed by digestive organs (14.9%). Cancer incidence stratified by IA type is summarized in supplementary table 1. The distribution of cancer sites was comparable among the two groups of IAs.

Other medications and comorbidities

A higher percentage of patients in the modern-era IA group (71.7%) were on additional medications compared to the early-era group (64.1%). Mild comorbidities were common among both groups.

Causal effects

The BART model was adjusted for the confounders identified from the DAG: age at transplantation, sex, organ of transplantation, year of transplantation, other comorbidities, and other medications taken before transplantation (Supplementary Figure 2). The average treatment effects estimated are presented in Table 6.4. The average risk of developing at least one primary cancer post-transplantation, if all the recipients were treated with modern-era IAs, would have been 2.4 % points lower compared to if they had been with early-era IAs (ATE= -0.024; CI = - 0.034 to -0.013). The average treatment effect among the treated was -0.020 (ATT = -0.020; CI= -0.035 to -0.007) implying the 2% of the risk reduction among SOT recipients who were prescribed one of the modern-era IAs can be attributed to their treatment. On the other hand, the average risk of developing at least one primary post-transplant cancer, among

SOT recipients who were prescribed one of the early-era IAs would have been 4% points lower if instead they had been prescribed one of the modern-era IAs (ATC= -0.040; CI = -0.049 to -0.030).

***Table 6.4**: Population average treatment effects*

	RD	95% Credible Interval (CI)	Rhat[a]	n.eff[b]
Average treatment effect (ATE)	-0.024	- 0.034 to -0.013	1.003	870.70
Average treatment effect among treated (ATT)	-0.020	-0.035 to -0.007	1.019	700.63
Average treatment effect among controls (ATC)	-0.040	-0.049 to -0.030	1.014	783.97

[a] Rhat nearing 1 suggests a convergence of MCMC chains (Brooks and Gelman 1998)
[b] Number of effective samples. The closer the number of post-warmup samples (1000) the better the posterior distribution approximation.

Discussion:

We estimated the causal effect of IAs on lifetime post-transplant cancer risk among SOT recipients in Quebec using a provincial-wide retrospective cohort study from 1997 to 2016. A total of 909 (18.6%) SOT recipients developed post-transplant malignancies. The IAs have evolved over the years, with the secular trends indicating a decline in the use of early-era drugs. We observed an average of 4% reduction in cancer risk among the early-era group if they had been prescribed modern-era IAs. The incidence of cancer was highest among SOT recipients who had received Cyclosporine and lowest among those who received Sirolimus, as the first post-transplant IA prescription.

Immunosuppression is a key factor for the success of organ transplantation by preventing organ rejection among SOT recipients [27] however, the secondary effect of long-term immunosuppression is the increased risk of malignancy [28]. Important factors that determine the prescription of IAs apart from the patient factors are the transplant era (early or modern era), newer studies on novel IAs, and the scope for clinical trial enrolment [10]. IAs have different

impacts on the risk of cancer post-transplantation [29] and drugs like Azathioprine and Cyclosporine are being identified as human carcinogenic agents by IARC [13]. An association study conducted by Shaw et al in the US between 2000 and 2021 reported Azathioprine, Cyclosporine, and unexpectedly Sirolimus to be associated with an increased risk of malignancy [17]. Similarly, our study also showed an increased incidence of skin cancer among individuals who were prescribed Azathioprine and Mycophenolate mofetil. Azathioprine, a purine antimetabolite IA was used widely until Mycophenolic acid was introduced in the late 1990's [15]. The former has been associated with increased cancer risk, especially skin cancer due to its interference in DNA synthesis and repair [17]. On the other hand, in our study, recipients who were prescribed Sirolimus had a lower incidence of cancer which aligns with the meta-analysis published by Knoll et al [30]. Sirolimus is an mTOR inhibitor that was approved by the FDA in 1999 and for over 15 years it has been effectively IA-associated with low nephrotoxicity. Interestingly Sirolimus has also been demonstrated to possess low anti-tumor properties [29]. Most of the previous studies investigating the relationship between specific types of IAs and cancer risk have been associational studies stemming from observational data.

The increased cancer risk among SOT recipients has been attributed to several factors including long-term immunosuppression, pre-existing comorbidities, and end-organ failure (2,9). Importantly, the effect of IAs on the type and the site of cancer appears to vary significantly depending on the specific organ transplanted (9). However, due to the small sample size for site-specific cancer incidences in our study, we were not able to pursue a stratified analysis by the type of organ transplanted.

IAs are carefully prescribed by balancing graft function and the side effects due to immunosuppression (8). IA prescription may also vary with patient characteristics including predisposed systemic diseases including the history of malignancy, time on dialysis, infections, and harmful behavioral patterns (e.g., tobacco smoking) (10). Furthermore, secular trends may

appear in the prescription patterns of IAs over the years as new drugs are developed. We observed that the proportion of prescriptions of modern-era IAs increased among SOT recipients in the later years of the cohort. Although we have included the year of transplant as a potential confounder in our outcome and propensity score model, this approach may not capture fully the underlying secular trends sufficiently, leading to residual confounding in the results [23]. Furthermore, clinicians may switch the type of IA after an initial phase of testing the acceptance. Although emulating a sequentially randomized trial is an analytical option, the administrative nature of our cohort reduced the potential to capture nuanced information on potential mediators and confounders of these mediators and the outcome. Hence, we used an intention-to-treat analysis approach.

An additional limitation of our study is the missing IA prescription information for 1,981 participants in the RAMQ database, this could be individuals covered by private insurance or those who migrated to a different province for treatment post-transplant. A pan-Canadian study could mitigate this limitation to a certain extent. We also miss information on guidelines and the factors that predict the prescription of IAs, which may have led to confounding by indication bias. However, we believe our propensity score adjusting and weighting approach in the approach using all available confounders in this administrative database, may mitigate this issue to an extent.

Several analytical approaches exist for causal inference from observational studies [31]. We used BART, a flexible nonparametric machine learning model for both propensity score estimation and outcome models. BART has been shown to be superior performing compared to many standard methods in causal inference data science competition [32] and can accommodate complex interactions between the pre-transplant factors and post-transplant immunosuppressive therapy without explicit parametrization from the user [21]. BART requires a few assumptions to be satisfied to ensure its validity. Of which except for the positivity

assumption [33], the rest are unverifiable from the observed data. These assumptions include the prior independence assumption that the parameters of the propensity score model and the outcome model are independent [34]. However, any causal inference from observational data requires these assumptions and is only valid to the extent to which those assumptions are met. Under the feasibility barriers of RCT to investigate this question we believe our approach bridges an important knowledge gap with high-quality administrative data. Lastly, we did not account for competing risk issues such as death [35] which is a common complication among SOT recipients. Nonetheless, our study contributes to the evidence supporting the evolution and optimization of IAs in SOT recipients by using advanced statistical methods to derive meaningful causal inferences from observational data.

Conclusion:

Our results suggest that switching to modern-era drugs can reduce the lifetime risk of cancers among SOT recipients in Quebec. Despite the risk reduction, it may not be feasible to switch recipients to modern-era drugs solely to reduce cancer risk, as these drugs might induce other side effects including acute organ rejection and eventually death. Therefore, a balance between cancer risk and acute organ rejection must be weighed alongside the comorbidities of the recipients. Further studies should expand to a nationwide dataset to conduct sub-group analysis by individual drug. Additionally, administrative databases capturing information on laboratory data, environmental carcinogen exposure, and vaccination history will further inform decision-making.

Authors' contribution: All authors have made substantial contributions including editing and approving the final text. Conceptualization and study design: CL & BN. Data curation: TD, CL, SM. Statistical analysis and result interpretation: TD, CL, TS, SM. Supervision: CL, SM. Writing—original draft: TD. Writing—review & editing: TD, CL, BN, TS, SM.

Conflict of Interest: The authors declare that they have no known competing interests that could have appeared to influence the work reported in this manuscript.

Ethics statement: The *Commission d'accès à l'Information,* the *Régie de l'assurance maladie du Québec* (RAMQ), as well as the *Institut de la statistique du Québec* approved the study and gave access to administrative data (reference number: 1015461-S). All the data used for this work was anonymous, the researchers had no information on the population under study. The institutional ethics review board from McGill University and Université de Montréal have approved the study.

Data availability statement: The data that support our findings are not publicly available. Requests should be directed to the corresponding author who will contact the relevant authorities.

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7 Discussion

The results and findings for each objective are discussed in detail at the end of each manuscript, which may lead to some repetition in this section. This section provides a comprehensive summary of the results, discusses their alignment with the existing literature, and acknowledges the strengths and limitations of the study.

7.1 Summary of our findings

7.1.1 Risk of cancer among adult solid organ transplant recipients in Quebec, Canada: 1997-2016

SOT recipients in Quebec exhibit a higher cancer risk compared to the general population. Previous studies from Canada, utilizing the CORR database and CCR from 1981 to 1988, and recent studies from Ontario highlighted this increased risk [10,11,14,51,73]. Notably, Quebec has been excluded from the National Cancer Statistical Registry since 2011, creating a gap in the data regarding the cancer risk among SOT recipients in Quebec. Our retrospective cohort study from 1997 to 2016 addresses this gap by describing the pattern of cancer incidence, estimating the overall incidence rate of cancer as age and sex-standardized risk ratio to compare the burden with the general population in Quebec.

Our study indicates a pattern of cancer incidence consistent with the existing literature[116], with Melanoma and other skin malignant neoplasms being common. A systematic review conducted on a randomized control trial on behavioral and pharmaceutical interventions for the prevention of skin cancers in SOT recipients noted that these recipients might have a significantly higher risk of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) compared to the general population [116]. Our data also shows a male predominance in several cancer sites including the skin, digestive organs, urinary, mesothelial, soft tissue, head, and neck, whereas females showed higher incidences in the breast, thyroid, brain, and spinal cord cancers. This

aligns with the study from the US, [117] that explored the sex differences in cancer incidence among SOT and suggested that the male predominance in the general population is less pronounced among SOT recipients due to differences in immune surveillance between sexes.

In our cohort, SOT recipients experienced a cancer risk of 4.2 to 2.5 times higher than that of the general population in Quebec. However, over time, the incidence of cancer among SOT recipients has marginally declined, probably due to improvement in IAs. Concurrently, the general population in Canada has seen an increase in new cancer cases [81]. The fluctuations in the risk ratios during the study period may also be partially attributed to the 2006 changes in cancer diagnostic codes, which allowed for the identification of more cancer cases.

7.1.2 The causal effect of immunosuppressive agents on cancer risk among solid organ transplant recipients

Cancer risk among SOT recipients is hypothesized to be linked to immunosuppressive agents. A previous study in Canada examined the prescription of Azathioprine and corticosteroids on cancer risk among KTR in Ontario province [118]. Another study from Quebec also explored IA prescription among KTR [95]. These two studies examined only a subset of transplant recipients. We have included all types of organ transplants including heart, lung, liver, and pancreas. An ideal setting to study the impact of IA on cancer will be to conduct RCT. However, due to the various challenges involved in conducting an RCT, we emulated a target trial with observational data from the Quebec Transplant cohort.

Our findings suggest that the average cancer risk would be 4% points lower if all recipients were treated with modern-era drugs compared to the early-era drugs. We observed that individuals prescribed Cyclosporine showed higher cancer incidence, while those prescribed Sirolimus had lower incidences. Although, over the years, the use of early-era drugs has declined, they are still prescribed in the Quebec system. Additionally, there was a notable increase in skin cancer among individuals taking Azathioprine and Mycophenolate mofetil.

Azathioprine has been linked to a higher skin cancer risk, due to its crucial role in DNA synthesis and repair [98]. Conversely, Sirolimus is associated with low nephrotoxicity and anti-tumor properties [119].

7.2 Methodological considerations

7.2.1 Strengths

The strength of our study includes using high-quality provincial data to investigate the cancer risk among SOT recipients, linking two longitudinal administrative databases from Quebec. It is the first comprehensive study from Quebec covering all organ transplants. To understand the protective effect of modern-era drugs compared to the early-era drugs, we employed BART [110], a flexible nonparametric approach to account for the complexities of the covariates and the outcome. We used propensity score as a covariate to account for the issue of randomization for assigning the treatment among the different groups and un-confoundedness. We further corrected the propensity score and response model using the targeted minimum loss estimation method approach.

7.2.2 Limitations

The study has various limitations. The RAMQ database only includes individuals covered by the insurance, and we did not account for competing outcomes such as death or lost-to-follow-up. Future studies should consider donor characteristics, patient behavioral history, genetic cancer predictors, comprehensive pre-transplantation data, and environmental carcinogen exposure.

As with any other causal inference modeling approach, BART requires assumptions for valid interpretation of estimates as causal in nature. For instance, the positivity assumption [106] verified in our data, shows a non-zero probability of early-era drug prescriptions. However, due to secular trends, early-era drug prescriptions were declining in our cohort. This bias may

have been mitigated to an extent by including the propensity score, which was estimated using the year of transplant, as a covariate in the outcome model [108]. Lack of fine-grained information on confounders may have led to residual confounding and the conditional exchangeability assumption may have been violated [102]. However, this would be a limitation in any causal inference approaches that use administrative-linked datasets. Additional information collection or linking to behavioural surveys may allow us to reduce the impact of this violation.

Additionally, there were missing values for IAs in the RAMQ database, potentially due to death post-transplantation due to complications, private insurance coverage, migration to another province, or rare non-prescription of IAs. We have not accounted for competing risk issues such as death [120]. We also assumed that the individual continued the same drug post-first prescription, despite the possibility of switching to different drugs due to adverse effects of the drugs. Lastly, drugs were categorized by era rather than individual drugs due to the small sample size. However, our results were in consensus with the overall evidence of increased risk associated with early-era drugs reported from observational studies from other countries.

8 Conclusion

Our findings that SOT recipients in Quebec face a higher risk of cancer compared to the general population underscore the necessity for enhanced surveillance. Switching from early-era IAs (Azathioprine and Cyclosporine) to modern-era immunosuppressive agents (e.g., Mycophenolate mofetil, Sirolimus, and Tacrolimus), can reduce the lifetime cancer risk among SOT recipients in Quebec. However, it may not be practical to switch solely to reduce cancer risk, due to potential side effects including acute organ rejection and mortality. A careful balance between cancer risk and rejection risk must be considered along with the recipient's comorbidities. Further research should expand to a larger cohort with the nationwide dataset to analyze individual drug effects and databases capturing laboratory results, environmental carcinogen exposure, and vaccination history to better understand the relationship between IA and cancer risk.

9 References

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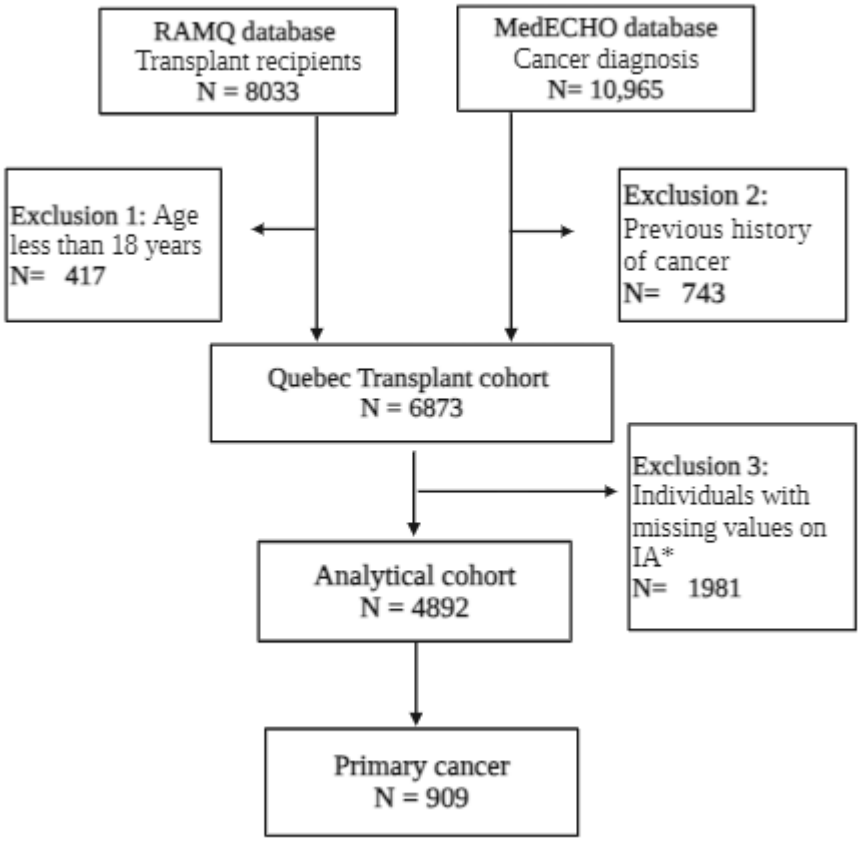
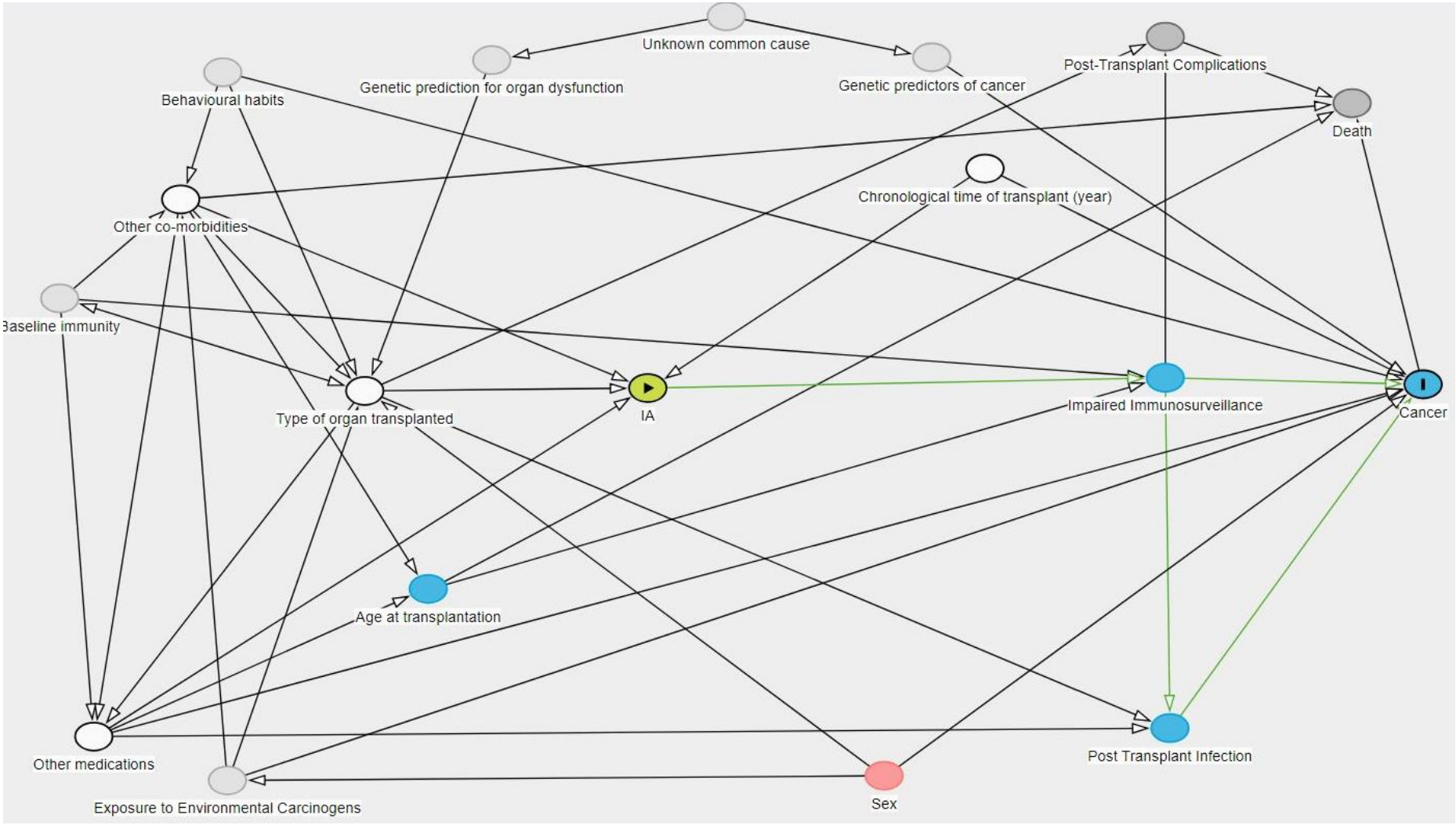
10 Appendix

Supplementary Figure 1: Study flow diagram



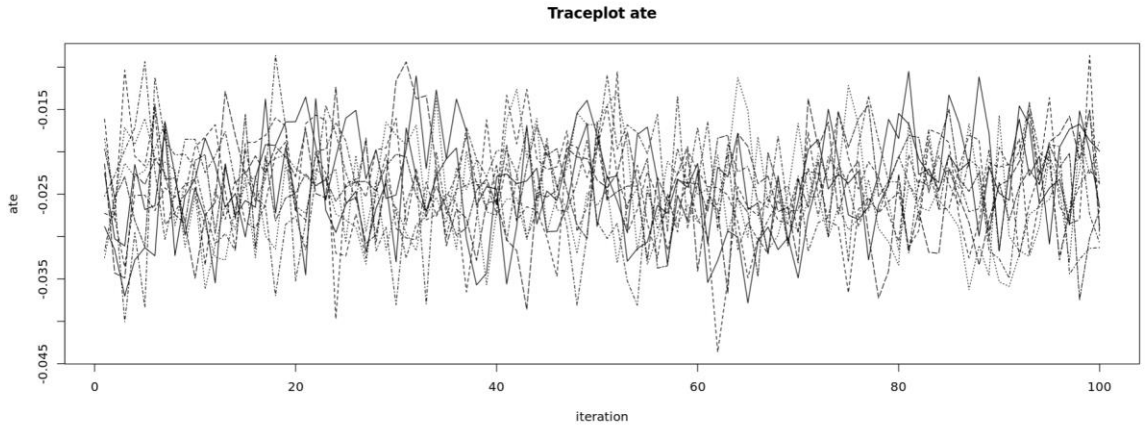
*IAs including azathioprine, cyclosporine, mycophenolate mofetil, sirolimus and tacrolimus.

Supplementary Figure 2: Directed acyclic graph illustrating the causal structural assumptions pertinent to immunosuppression and cancer risk

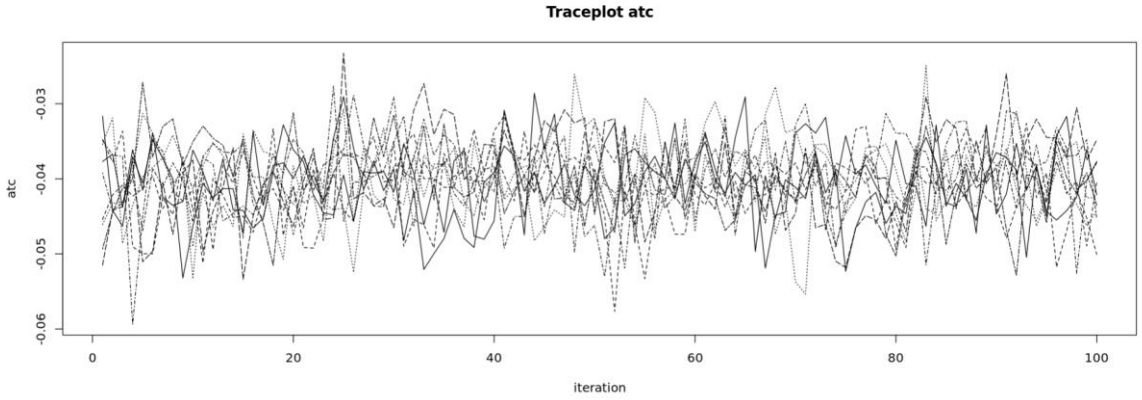


Supplementary Figure 3: MCMC diagnostics plots to assess the efficiency of the model to approximate the posterior distribution of the parameters of interest.

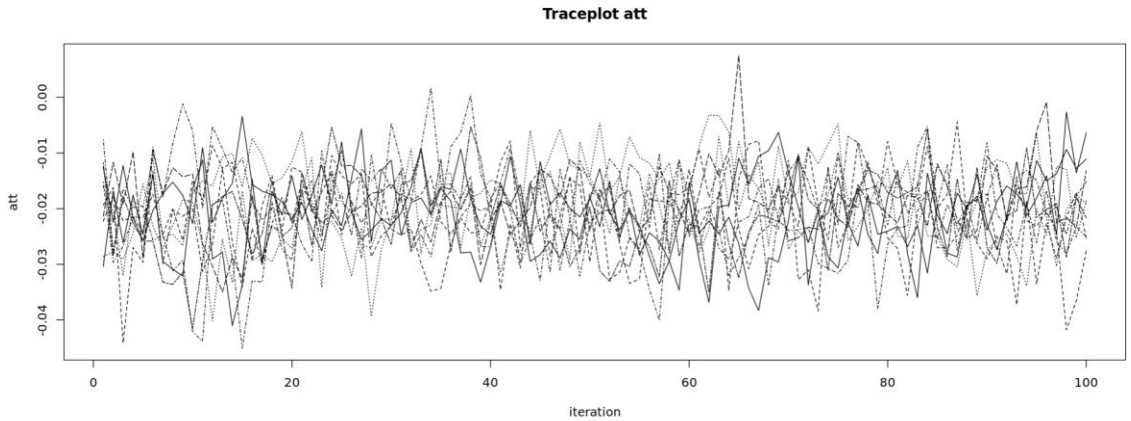
a) Trace plot: It is used to assess the mixing of MCMC chains. There should not be any secular trends where a particular chain is stuck in a certain area of the posterior.
   1. Average treatment effect



   2. Average treatment effect among controls

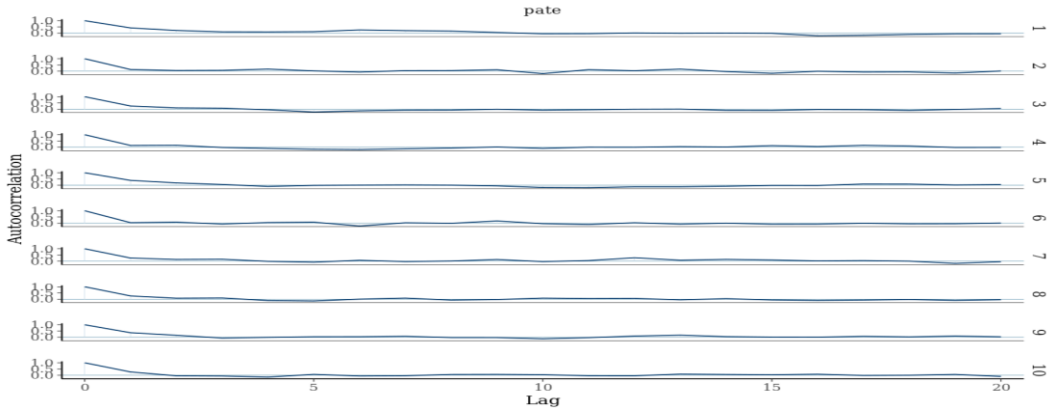


   3. Average treatment effect among the treated

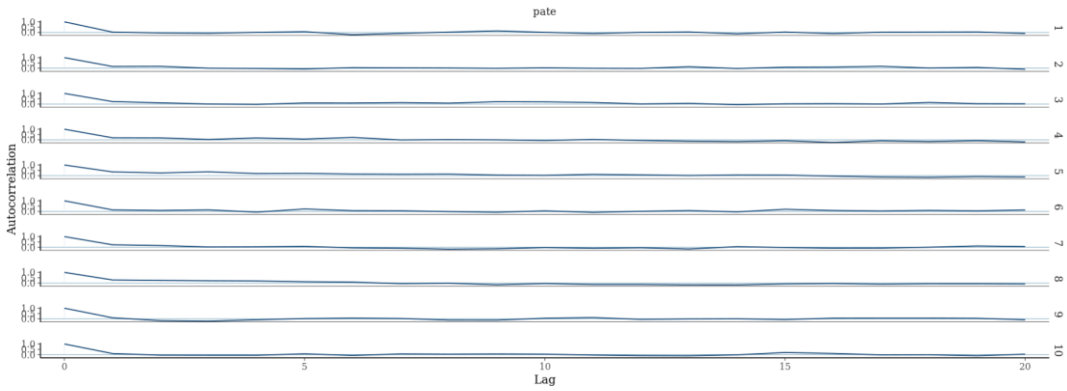


b) Autocorrelation plot: It shows the correlation of the parameter samples with their lagged values. Descending trend that is reduction in autocorrelation with increase in lag is better.

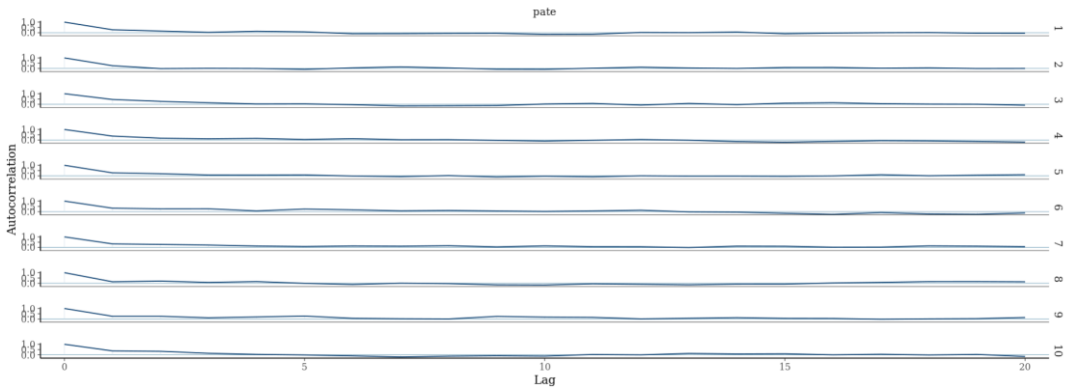
1. Average treatment effect



2. Average treatment effect among controls

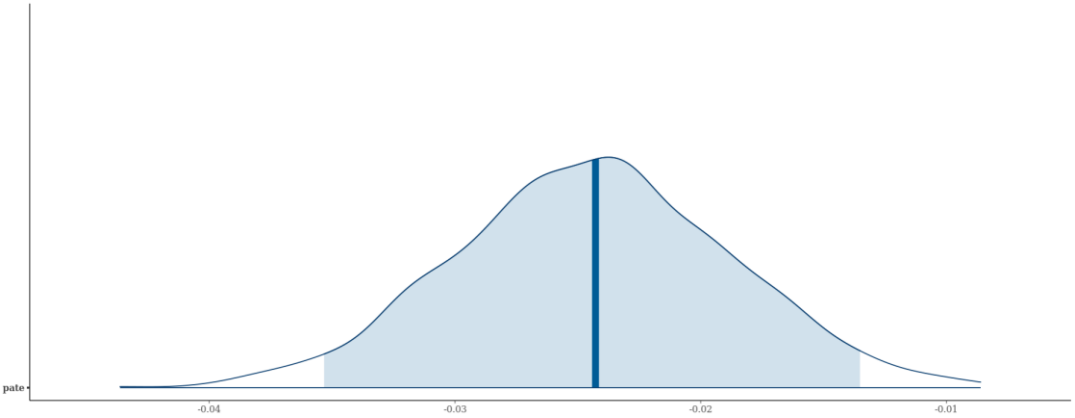


3. Average treatment effect among the treated

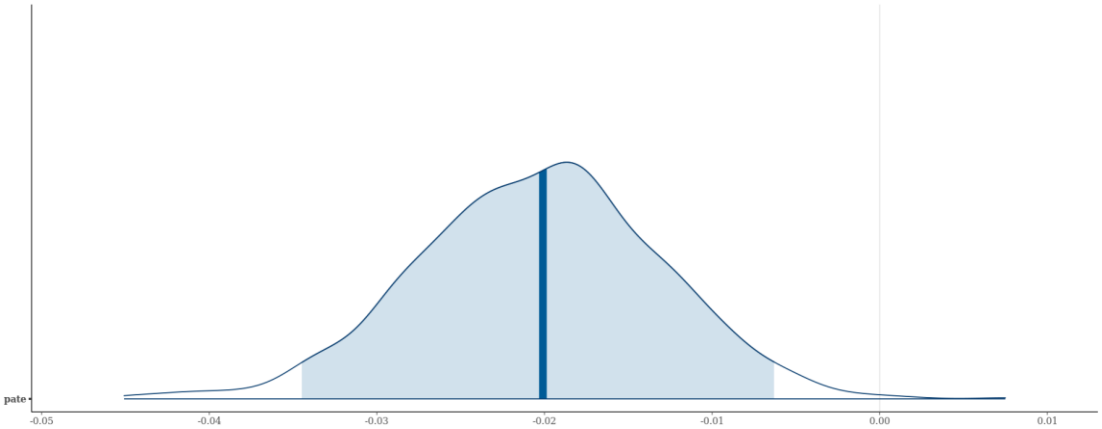


c) Posterior distributions: Density plots with all the chains merged, with 95% Credible intervals shown in shaded areas under the curves.

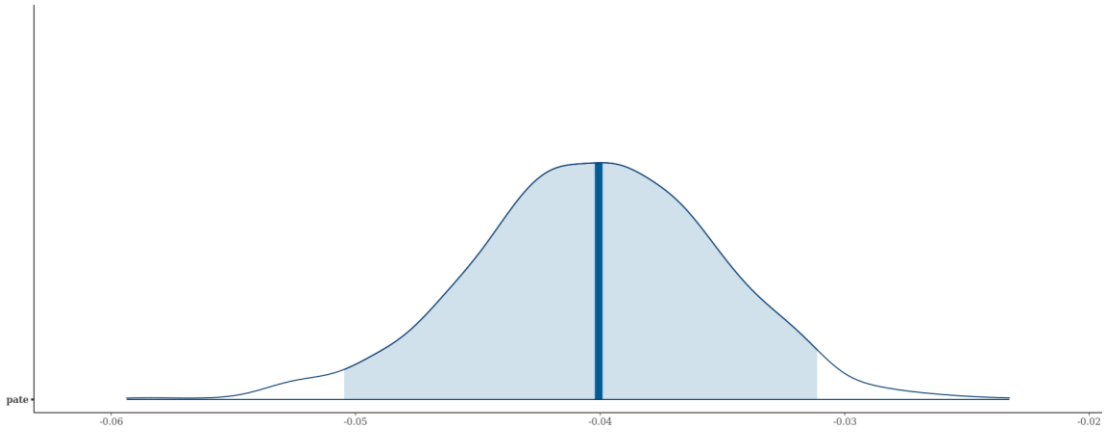
1. Average treatment effect



2. Average treatment effect among treated



3. Average treatment effect among controls



Supplementary table 1: Medical act codes for solid organ transplantation procedures

Organ Transplant	Medical act codes
Lung	4530, 4042, 4043, 4044
Heart	4528
Liver	5450, 5297, 5453, 5506, 5553
Kidney	6221, 6222, 6223, 6092
Pancreas or multiple organs	5416, 5299, 4529

Supplementary Table 2: ICD 9 and ICD 10 codes for each malignant neoplasm

Type of organ	Specific sites	ICD 9 codes	ICD 10 codes
Melanoma and other malignant neoplasm of skin	-	1720 – 1739	C430 – C449
Malignant neoplasm of lymphoid, hematopoietic, and related tissue	Lymphoma, immunoproliferative diseases, and leukemia	2000 – 2898	C810 – C97
Malignant neoplasm of Digestive Organs	Oesophagus, stomach, small intestine, colon, anus, anal canal, liver, intrahepatic bile conduct, gallbladder, pancreas and other ill-defined digestive organs	1500 – 1599	C150 – C269
Malignant neoplasm of Urinary tract	Kidney, renal pelvis, bladder, other and unspecified urinary organ	1880 – 1899	C64 – C689
Malignant neoplasm of Respiratory and intrathoracic organs	Nasal cavity, middle ear, accessory sinuses, larynx, trachea, larynx, bronchus, lung, thymus, heart, mediastinum and pleura	1600 – 1659	C3000 – C399
Malignant neoplasm of male genital organs	Penis, testis, prostate, other and unspecific genital organs	1859 – 1879	C600 – C639
Malignant neoplasm of Breast	-	1740 – 1749	C5000 – C5099
Malignant neoplasm of other and ill-defined sites	-	1950 – 1958	C760 – C809
Head and Neck Cancer including lip, oral cavity, and pharynx	Lip, tongue, floor of mouth, gum, palate, parotid gland, tonsil, minor salivary glands, nasopharynx, hypopharynx, and piriform sinus	1400 – 1498	C000 – C148
Malignant neoplasm of Thyroid and endocrine gland	Thyroid, adrenal, and related glands	1930 – 1949	C73 – C759
Malignant neoplasm of female genital organs	Vulva, uterus, ovary, and other genital organs	1820 – 1849	C510 – C58
Tumors of Mesothelial and soft tissues	Mesothelioma, Kaposi sarcoma, peripheral nerves, and autonomic nervous system, peritoneum, other connective and soft tissues	1991	C450 – C499
Malignant neoplasm of the Brain, spinal cord, and CNS	Eye, adnexa, meninges, brain, spinal cord, cranial nerves, and other central nervous system	1903 – 1929	C690 – C729

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Supplementary Table 3: Cancer Incidence stratified by baseline Immunosuppressive agent prescribed from 1997 to 2016 by SOT recipients

Malignant neoplasm by organ	Early era drugs N = 185 n (%)			Modern era drugs N = 724 n(%)				
	Azathioprine N = 57	**Cyclosporine N = 128**	**Total**	**Mycophenolate mofetil N = 242**	**Sirolimus N= 277**	**Tacrolimus N= 205**	**Total**	**Overall N= 909**
Melanoma and other malignant neoplasm of skin	18 (31.6)	34 (26.6)	52 (28.1)	87 (36.0)	75 (27.1)	64 (31.2)	226 (31.2)	278 (30.6)
Lymphoid, hematopoietic, and related tissue	11 (19.3)	17 (13.3)	28 (15.1)	29 (12.0)	44 (15.9)	29 (14.1)	102 (14.1)	130 (14.3)
Digestive Organs	13 (22.8)	25 (19.5)	38 (20.5)	21 (8.7)	41 (14.8)	35 (17.1)	97 (13.4)	135 (14.9)
Urinary tract	5 (8.8)	18 (14.1)	23 (12.4)	50 (20.7)	36 (13.0)	23 (11.2)	109 (15.1)	132 (14.5)
Respiratory and intrathoracic organs	5 (8.8)	11 (8.6)	16 (8.6)	18 (7.4)	42 (15.2)	12 (5.9)	72 (9.9)	88 (9.7)
Male genital organs	<5	6 (4.7)	8 (4.3)	13 (5.4)	17 (6.1)	10 (4.9)	40 (5.5)	48 (5.3)
Breast	<5	5 (3.9)	7 (3.8)	<5	6 (2.2)	9 (4.4)	19 (2.6)	26 (2.9)
Other and ill-defined sites	<5	<5	<5	<5	<5	8 (3.9)	13 (1.8)	16 (1.8)
Head and Neck Cancer including lip, oral cavity, and pharynx	<5	<5	<5	5 (2.1)	<5	5 (2.4)	13 (1.8)	16 (1.8)
Thyroid and endocrine gland	<5	<5	<5	<5	<5	<5	8 (1.1)	11 (1.2)
Female genital organs	<5	<5	<5	<5	<5	<5	9 (1.2)	11 (1.2)
Mesothelial and soft tissues	<5	<5	<5	<5	5 (1.8)	<5	8 (1.1)	10 (1.1)
Brain, spinal cord, and CNS	<5	<5	<5	<5	<5	<5	8 (1.1)	8 (0.9)

Supplementary Table 4: a) Population average treatment effect estimated by considering propensity score as a covariate in the outcome model without weights averaging or TMLE correction

Population average treatment effect	RD	95% Credible intervals	Rhat	n.eff
Average treatment effect	-0.031	- 0.064 – 0.004	1.01	908
Average treatment effect among treated	-0.030	-0.066 – 0.006	1.01	850
Average treatment effect among controls	-0.035	-0.082 – 0.013	1	1000

b) Population average treatment effect estimated by considering propensity score as a covariate in the outcome model and average effects as weighted average by propensity score but no TMLE correction

Population average treatment effect	RD	95% Credible intervals	Rhat	n.eff
Average treatment effect	-0.030	- 0.062 – 0.001	1.007	848.13
Average treatment effect among treated	-0.030	-0.065 – 9e-04	1.009	794.16
Average treatment effect among controls	-0.035	-0.065 – -0.002	1.002	1087.08