Steroid-free immunosuppression in kidney transplantation

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To courageous kidney transplant recipients

without whom this research would not be possible.

ABSTRACT

Background: Steroids have been successfully used in kidney transplantation for six decades as part of different immunosuppressive protocols. To date, the most frequently used regimen is a combination of tacrolimus, mycophenolate mofetil and prednisolone (a steroid drug), preceded by antibody induction. Chronic use of steroids, however, has several serious side effects, such as diabetes mellitus, weight gain, hypertension, hyperlipidemia, associated with increased cardiovascular risk, and negative impact on patient and graft survival.

This thesis evaluates whether a steroid-free immunosuppressive regimen reduces the early onset of posttransplantation diabetes mellitus (PTDM) and assesses the long-term efficacy, safety, and sustainability of this approach.

Methods: An open-label multicenter randomized controlled trial, the SAILOR trial, compared two immunosuppressive strategies in kidney transplant recipients with low immunologic risk: 1. **Steroid avoidance** arm, i.e., without prednisolone, based on low-dose tacrolimus and mycophenolate mofetil together with antithymocyte globulin induction; 2. **Steroid maintenance** arm with prednisolone, low-dose tacrolimus and mycophenolate mofetil together with basiliximab induction.

Paper I detailed the design of the randomized controlled trial, clinical protocol, and provided the theoretical foundation and hypothesis that steroid avoidance could decrease the incidence of PTDM within the first year post-transplantation. The trial was designed to validate a novel immunosuppressive regimen centered on steroid avoidance and minimization of calcineurin inhibitor.

Paper II presented results from the SAILOR trial, primarily the impact of steroid avoidance on incidence of PTDM, as well as short-term (2 years) efficacy and safety outcomes.

Paper III analyzed retrospectively the impact of steroids on mycophenolic acid exposure during co-administration with tacrolimus in participants of the SAILOR trial.

Paper IV, the SAILOR follow-up observational study, described long-term outcomes (>7 years post-transplantation) in the SAILOR trial participants, and assessed the feasibility and safety of steroid avoidance strategy.

Results: The SAILOR trial demonstrated a comparably low incidence of PTDM, similar incidence of biopsy-proven rejection in both treatment arms, without the steroid-free regimen compromising efficacy and safety at two years. Mycophenolic acid exposure was lower in patients without steroids early post-transplantation, and negatively correlated with body weight. The long-term follow-up showed similar patient survival, death-censored graft survival, kidney function and safety parameters after 7.3 years. Two thirds of participants originally randomized to the steroid avoidance arm remained on steroid-free immunosuppression.

Conclusions: Steroid avoidance combined with low-dose tacrolimus and mycophenolate mofetil is feasible, effective, and safe for immunologically low-risk kidney transplant recipients up to 7 years. A broader application of steroid-free immunosuppression might be beneficial in a carefully selected group of kidney transplant recipients to mitigate the long-term adverse effects of steroid use.

Keywords: kidney transplantation, steroid avoidance, steroid-free protocol, immunosuppression, posttransplantation diabetes mellitus, biopsy-proven rejection, mycophenolic acid exposure

SAMMANFATTNING PÅ SVENSKA

Njurtransplantation är det bästa behandlingsalternativet vid kronisk njursvikt, men kräver livslång behandling med immunhämmande läkemedel för att förebygga avstötning av den transplanterade njuren. Idag används oftast en standardkombination av tacrolimus, mykofenolatmofetil och prednisolon, tillsammans med intravenös antikroppsbehandling vid operation. Steroider (prednisolon) har använts framgångsrikt vid njurtransplantation i decennier, men ett långvarigt bruk har ett flertal allvarliga biverkningar, t.ex. diabetes, viktökning, högt blodtryck och förhöjda blodfetter. Dessa är förknippade med en ökad risk för hjärt-kärlsjukdomar samt en negativ påverkan på både patientens och transplantatets överlevnad.

Syftet med denna avhandling är att undersöka om ett steroidfritt immunhämmande behandlingsregim kan minska den tidiga förekomsten av posttransplantationsdiabetes (PTDM) och om denna regim är effektiv, säker och hållbar både på kort och lång sikt.

Metoder: Vi initierade och genomförde en öppen multicentrisk randomiserad kontrollerad studie, "SAILOR-studien", där vi jämförde två immunhämmande behandlingar för njurtransplanterade med låg immunologisk risk: 1. ***Steroidfri behandling***: utan prednisolon, baserad på lågdos tacrolimus och mykofenolatmofetil, tillsammans med antithymocytglobulin för induktion. 2. ***Standardbehandling***: med prednisolon, lågdos tacrolimus och mykofenolatmofetil tillsammans med basiliximab för induktion.

I **delarbete I** beskrevs den randomiserade studiens utformning, inklusive studieprotokollet, teoretisk bakgrund, samt hypotesen att steroidfri behandling kan minska förekomsten av PTDM under det första året efter njurtransplantation. Tre skandinaviska transplantationscentra deltog: Göteborg, Århus och Malmö.

Delarbete II rapporterade resultaten från SAILOR-studien. Totalt deltog 222 njurtransplanterade, hälften fick behandling utan steroider och hälften med steroider. Förekomsten av PTDM var jämförbart låg i båda behandlingsgrupperna, med bibehållen effektivitet och säkerhet efter 2 år. Biopsiverifierad avstötning förekom i liknande utsträckning, liksom infektioner, kardiovaskulära händelser och maligniteter.

I **delarbete III** undersökte vi retrospektivt effekten av steroider på mykofenolsyrehalter under samtidig behandling med tacrolimus hos deltagare i SAILOR-studien. Lägre halter av mykofenolsyra noterades hos deltagare utan steroider tidigt efter transplantation. Mykofenolsyrehalterna var negativt korrelerade med kroppsvikt.

Delarbete IV, SAILOR-långtidsuppföljningsstudien, utvärderade retrospektivt de långsiktiga resultaten. Deltagarna följdes i 7,3 år efter transplantation. Immunhämmande behandling med lågdos tacrolimus och mykofenolatmofetil utan kortison var effektiv och säker för njurtransplanterade med låg immunologisk risk upp till 7 år. Två tredjedelar av deltagarna som inte fick prednisolon vid transplantation var fortfarande steroidfria efter 7 år.

Slutsats: Våra resultat, i enighet med fynd i andra studier, talar för att betydligt fler utvalda njurtransplanterade med låg immunologisk risk skulle ha nytta av ett steroidfritt protokoll för att undvika de negativa biverkningarna av steroider.

LIST OF PAPERS

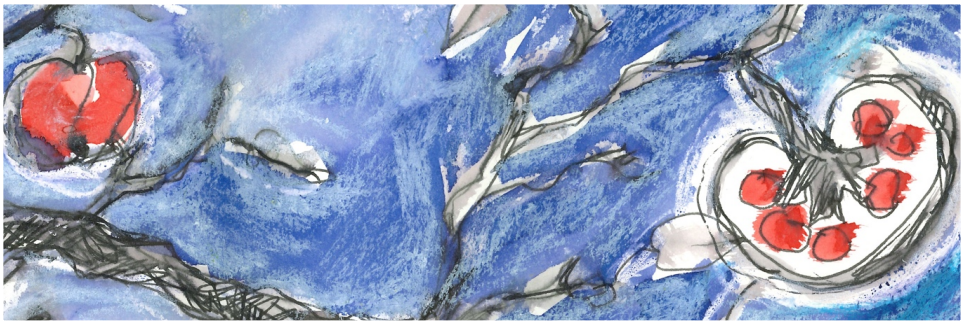
This thesis is based on the following studies, referred to in the text by their Roman numerals.

I Ekberg J., Ekberg H., Jespersen B., Källen R., Skov K., Olausson M., Mjörnstedt L., Lindnér P. **An in-progress, open-label, multi-center study (SAILOR) evaluating whether a steroid-free immunosuppressive protocol, based on ATG induction and a low tacrolimus dose, reduces the incidence of new onset diabetes after transplantation.** Transplantation Research 2014, 3:12

II Ekberg J. and Baid-Agrawal S., Jespersen B., Källén R., Rafael E., Skov K., Lindnér P. **A Randomized Controlled Trial on Safety of Steroid Avoidance in Immunologically Low-Risk Kidney Transplant Recipients.** Kidney Int Rep 2022, 7, 259-269.

III Nourbakhsh N., Ekberg J., Skov K., Daugaard Peters C., Øzbay A., Lindner P., Henrik Buus NH. **Effects of Corticosteroid Treatment on Mycophenolic Acid Exposure in Renal Transplant Patients—Results From the SAILOR Study.** Front. Pharmacol. 2021, September 12, 742444

IV Ekberg J., Aagaard Enevoldsen A-E. Wallquist C., Skov K., Jespersen B., Lindnér P., Baid-Agrawal S. **Steroid avoidance with low-dose tacrolimus is safe and effective at seven years for kidney transplant recipients with low immunologic risk: The SAILOR follow-up study.** Submitted



I

II

III

IV

TABLE OF CONTENTS

ABBREVIATIONS

ADA	American Diabetes Assocoation
ATG	antithymocyte globulin
AUC	area under the curve
AZA	azathioprine
BPAR	biopsy-proven acute rejection
BPR	biopsy-proven rejection
BKV	BK virus
CD	cluster of diferenciation
CNI	calcineurin inhibitor
CS	corticosteroids
CyA	cyclosporine A
CYP3A4	cytochrom P450
dd-cfDNA	donor-derived cell-fee DNA
DGF	delayed graft function
DSA	donor specific antibody
ESWD	early steroid withdrawal
FPG	fasting plasma glucose
FU	follow-up
GFR	glomerular filtration rate
HLA	human leukocyte antigen
iv	intravenous
IL-2R	Interleukin-2 receptor
KTR	kidney transplant recipient
MACE	major adverse cardiovascular event
MMF	mycophenolate mofetil
MPA	mycophenolic acid
mTOR	mammalian target of rapamycin
NODAT	new onset of diabetes after transplantation
OGTT	oral glucose tolerance test
PRA	panel reactive antibody
PTDM	posttransplantation diabetes mellitus
RCT	randomized controlled trial
SA	steroid avoidance
SAILOR	TRIAL OF **S**TEROID **A**VO**I**DANCE AND **LO**W-DOSE CNI BY ATG-INDUCTION IN **R**ENAL TRANSPLANTATION
SM	steroid maintenance
Tac	tacrolimus

1 INTRODUCTION

1.1 Kidney transplantation

Kidney transplantation is the most effective treatment for individuals with end-stage kidney disease, providing higher survival rates and improved quality of life compared to dialysis. Advances in immunosuppressive therapy, diagnostic techniques, and patient management have significantly improved short-term outcomes. For instance, one-year graft survival rates for the first kidney transplant from the deceased donor have progressed from 52% in the 1970s, to 72% after introduction of cyclosporine A[1], and currently stand at 94%, with modern immunosuppression.[2]

Despite these advancements, there has not been a corresponding improvement in long-term graft survival, as annual attrition rates have remained constant at 4%.[3] Approximately 40% of kidney transplants are lost within ten years, contributing to increased morbidity and mortality rates. The etiology of this graft loss is multifactorial, and includes both immunological causes, predominantly chronic rejection, and non-immunological factors such as calcineurin inhibitor (CNI)-induced nephrotoxicity, BK virus (BKV) nephropathy, and donor-related variables.[3]

Premature death with a functioning graft remains a significant cause of graft loss. Although mortality has trended downward over the past two decades, a substantial proportion of kidney transplant recipients (KTR) still die within 10 years with a functioning graft. Among individuals who received their first kidney transplant after 1996, 3.2% died with a functioning graft within the first year, and 22% died over a 10-year period.[4] The leading causes of death were cardiovascular events, followed by infections and malignancies (Figure 1). Improving long-term outcomes continues to be a major challenge.

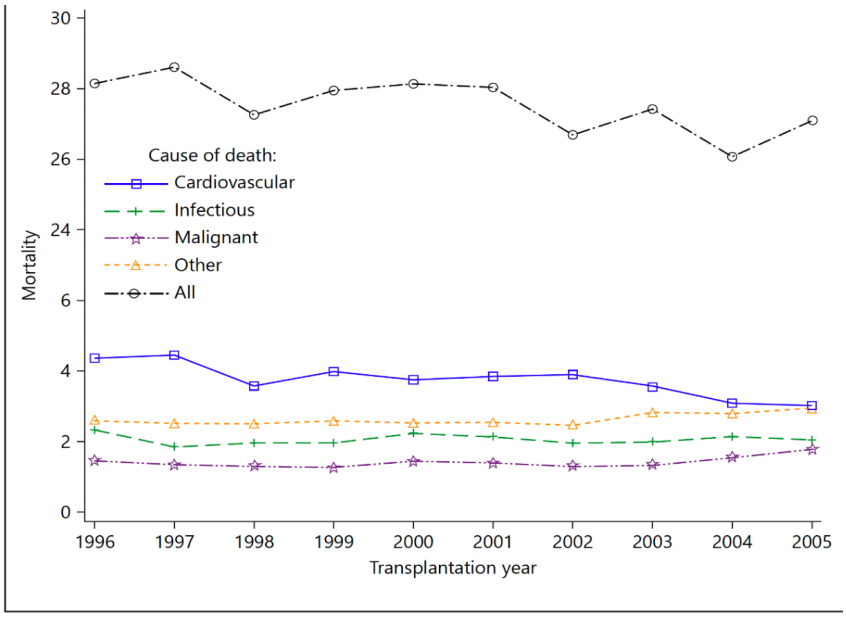


Figure 1. Trends in 10-years all-cause and cause-specific mortality in KTRs in the US. From Awan et al., AJT, 2018.[4] KTR, kidney transplant recipient

1.2 Immunosuppression

The immune system serves as a highly sophisticated and robust defense mechanism, preserving the body's somatic integrity by identifying and neutralizing external threats. It comprises two primary arms: innate immunity provides immediate, non-specific defense against pathogens and injuries, and adaptive immunity offers a more specialized, targeted response against specific antigens or foreign tissue.

After any organ transplantation, immunosuppression is essential to ensure the survival of the transplanted organ by preventing its rejection by the recipient's immune system. Immunosuppressive therapies inhibit various components of the immune response that would otherwise recognize and attack the transplanted tissue as "non-self."

However, the administration of immunosuppressive drugs after transplantation presents a significant clinical dilemma. While necessary to prevent graft rejection, these drugs also impose a burden by increasing the recipient's vulnerability to infections, malignancies, and other complications that contribute to increased morbidity and mortality. The challenge is to balance the intensity of immunosuppression to prevent rejection while minimizing adverse effects.

1.2.1 Induction therapy

Induction therapy is administered just before and shortly after grafting, with the aim to preemptively suppress the natural immune response against an allogenic organ, preventing acute rejection of the graft during the early post-transplant period.

The global standard for induction therapy involves an intravenous (iv) bolus dose of methylprednisolone, and concomitant iv administration of mono- or polyclonal antibodies.[5] Currently, most used antibodies are:[6]

Basiliximab, a humanized monoclonal antibody targeting a group of molecules (CD25) as part of the interleukin-2 receptor (IL-2R) on the surface of T lymphocytes. By inhibition of this receptor, basiliximab blocks the expansion of the clonal T lymphocytes and disrupts the activation and differentiation of T lymphocytes, significantly suppressing their immune response against the transplanted organ.

Antithymocyte globulin (ATG), a polyclonal antibody, designed to target multiple surface antigens expressed on T lymphocytes (e.g., CD2, CD3, CD7, and CD45). By binding to these antigens, ATG induces T-cell apoptosis and complements-mediated lysis, leading to rapid and profound depletion of T lymphocytes. This depletion significantly diminishes the activation and proliferation of T-cells, which are the primary effectors of cell-mediated rejection.

Rituximab, a monoclonal antibody targeting the CD20 receptor on B lymphocytes, key immune cells responsible for antibody production. By binding to CD20, rituximab depletes B lymphocytes, leading to reduction of donor-specific antibodies (DSA), and mitigating antibody-mediated rejection (AMR).

Alemtuzumab, a potent monoclonal antibody, directed against the pan-lymphocyte antigen (CD52) depleting all lymphocytes, incl. T- and B cells. However, the use of this agent is limited due to its off-label status.

Induction with cell-depleting agents like ATG, rituximab and alemtuzumab is often preferred for immunized patients with high levels of antibodies against human leukocyte antigen (HLA), where the immunologic risk of active and chronic active antibody-mediated rejection is elevated.

The site of action is depicted in Figure 2.

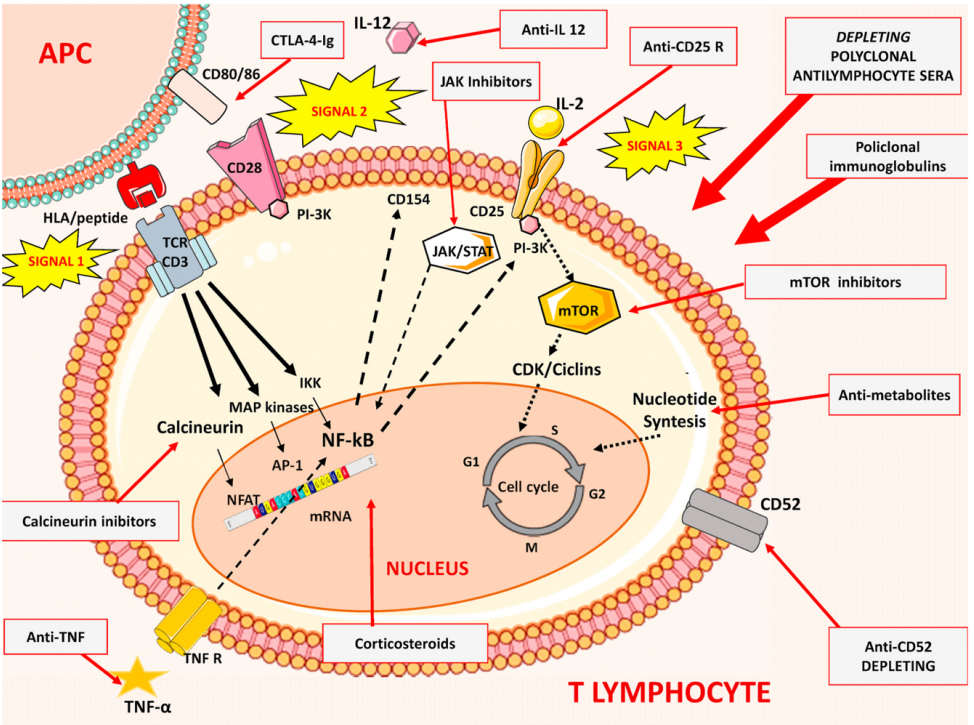


Figure 2. Cell targets on T cells and antigen presenting cells, and their interactions and immunosuppressive agents. Anti-CD25: basiliximab; Anti-CD52: alemtuzumab; CTLA-4-Ig: belatacept. APC, antigen presenting cell; CD, cluster of differentiation;; mTOR, mammalian target of rapamycin; TCR, T-cell receptor; From Meneghini et al., 2021.[6]

1.2.2 Maintenance immunosuppression and its adverse effects

Maintenance immunosuppression is a lifelong therapy typically administered orally to prevent organ rejection.[7,8] The cornerstones of immunosuppression are the following:[6]

Steroids (prednisolone, cortisone) have been used in rheumatology since 1948 and have revolutionized the treatment of inflammatory and autoimmune diseases. Introduced in the early 1960s for organ transplantation, they were used to prevent or treat acute rejection. Steroids interact with transcription factors that play an important role in the synthesis of adhesion molecules and pro-inflammatory cytokines, resulting in both immunosuppressive and anti-inflammatory effects. In high doses, they can mediate regulated apoptosis

However, despite their advantages, long-term use of steroids can lead to significant adverse effects:

- Metabolic disorders, such as hyperglycemia and diabetes, and obesity
- Cardiovascular effects including hypertension and hyperlipidemia

- Effects on bone metabolism like osteoporosis
- Musculoskeletal issues, muscle weakness
- Gastrointestinal complications
- Dermatological disorders
- Ocular effects such as cataracts and glaucoma
- Psychiatric disorders

Calcineurin inhibitors: Cyclosporine A, introduced in the early 1980s, and tacrolimus, introduced in the 1990s, represented revolutionary advancements in transplant medicine due to their potent and selective immunosuppressive properties. While cyclosporine A initially reduced acute rejection rates, tacrolimus, as a second-generation CNI, has largely replaced cyclosporine A (Figure 3) due to its superior efficacy in further reducing the acute rejection rate to 10-20%.[8] Both drugs bind to cytoplasmic proteins called immunophilins, blocking calcineurin, an enzyme crucial for activating nuclear factors that drive T-cell activation and proliferation. CNIs also reduce cytokine production, suppressing the alloimmune response.

CNIs, however, are associated with several serious side effects, including:

- Nephrotoxicity, potentially leading to reduced renal blood flow or damage to kidney transplants with progressive loss of kidney function over time
- Metabolic effects such as hyperglycemia and diabetes mellitus
- Cardiovascular problems, including hypertension and hyperlipidemia
- Neurotoxic effects (particularly with tacrolimus)
- Dermatological issues such as hirsutism and gingival hyperplasia with cyclosporine A

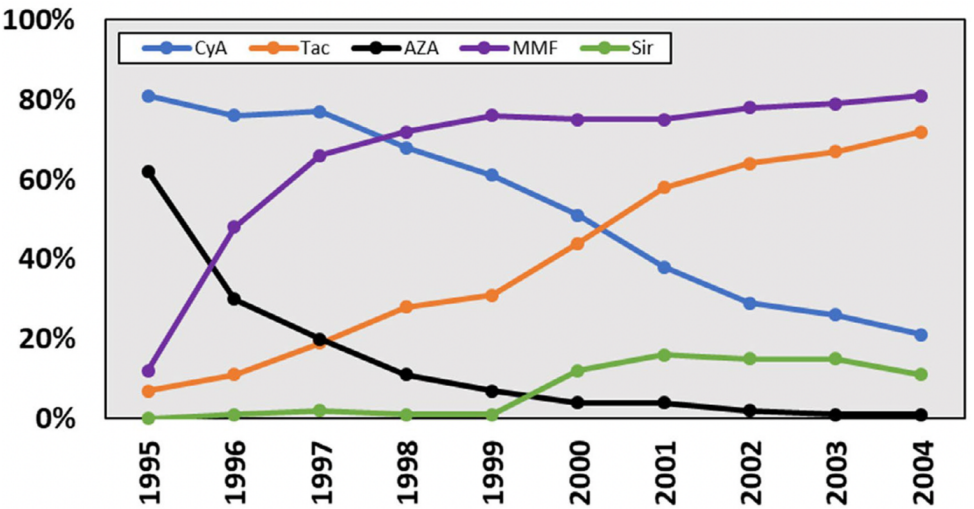


Figure 3. Maintenance immunosuppression utilization in KTR in US 1995 to 2004. AZA, azathioprine; CyA, cyclosporine A, MMF, mycophenolate mofetil; Sir, sirolimus; Tac, tacrolimus. From Pilch et al., 2021[8]

Antimetabolites: Azathioprine was used starting in the early 1960s, and later replaced by mycophenolic acid (MPA) in the 1990s (Figure 3). Antimetabolites inhibit DNA synthesis and cell division of both T and B lymphocytes by targeting purine synthesis, crucial for lymphocyte proliferation. Azathioprine, a purin analog, is metabolized into 6-mercapto-purine, exerting a cytotoxic effect on leukocytes. MPA selectively inhibits the enzyme essential for *de novo* purine synthesis and DNA replication.

Antimetabolites can cause the following adverse effects:

- Gastrointestinal effects, as diarrhea, nausea, abdominal pain, gastrointestinal bleeding
- Bone marrow suppression
- Hepatotoxicity

Inhibitors of the mammalian target of rapamycin (mTOR-i), such as sirolimus and everolimus, introduced in the late 1990s, have expanded the scope of oral immunosuppressive therapy.[9] These agents inhibit the mTOR signaling pathway, which is essential for cell-cycle progression and proliferation, and thus suppress T-cell activation and proliferation. Due to antiproliferative properties, mTOR inhibitors are also utilized in oncology.

Common side effects include:

- Metabolic effects such as hyperlipidemia and hyperglycemia
- Delayed wound healing
- Proteinuria
- Bone marrow suppression
- Skin reactions
- Mouth ulcers

Belatacept: It is the only iv monoclonal antibody approved for chronic maintenance treatment, implemented in the late 2000s. It works by binding to the CD28 ligands on activated antigen-presenting cells, thereby blocking their interaction with the CD28 receptor on T lymphocytes. The process known as co-stimulation plays a crucial role in the activation of T lymphocytes. Although it does not affect kidney function[10], its limited use is attributed to intravenous administration, high costs, and production shortages.

An inherent characteristic shared by all immunosuppressive drugs is their association with an increased risk of infections and, over the long term, a heightened risk of malignancies. The site of action for described agents is illustrated in Figure 2.

1.2.3 Immunosuppressive protocols

The development of immunosuppressive protocols has consistently aimed to balance the efficacy, preventing rejection and extension of graft survival, with mitigation of the risk of overimmunosuppression and the related adverse effects. Maintenance therapy typically involves a combination of two or three immunosuppressive agents to enhance the immunosuppressive effect, while distributing the side effects more evenly.[11]

At present, based on findings from the landmark ELITE-Symphony study, the most widely adopted standard immunosuppressive regimen globally after kidney transplantation involves induction with a monoclonal antibody targeting the IL-2R, followed by a maintenance protocol of low-dose tacrolimus, mycophenolate mofetil, and prednisolone.[12] This regimen has demonstrated superior outcomes in terms of renal function, the lowest rates of biopsy-proven acute rejection (12.3%), and the highest graft survival at one year (94.2%), compared with other 3 regiments, one everolimus-based, and two based on cyclosporine A with or without antibody induction. However, it was also associated with the highest incidence of posttransplantation diabetes mellitus (PTDM).

Over the past two decades, randomized controlled trials (RCTs) have extensively investigated steroid-sparing protocols, including strategies of both steroid avoidance and withdrawal. A systematic Cochrane meta-analysis found that these steroid-sparing regimens increased the rate of acute rejection, but did not significantly impact graft loss or mortality up to five years following transplantation.[13] Drawing definitive conclusions from these studies has been problematic due to the low incidence of events within relatively small cohorts, variations in immunosuppressive protocols used, and the limited duration of follow-ups. Of 45 RTCs, only 17 studies employed the induction with either monoclonal or polyclonal antibody, and only 10 trials used the maintenance immunosuppression based on tacrolimus. Furthermore, long-term data regarding chronic rejection rates with steroid-free regimens, particularly in conjunction with low-dose tacrolimus, are scarce. While the adoption of steroid-sparing approaches remains a desirable goal post-transplant, concerns over increased rates of both acute and chronic rejection impede their widespread implementation. The most significant trials involving steroid-weaning protocols, based on antibody induction and tacrolimus (both standard-dose and low-dose) maintenance are outlined in Table 1, inclusive the current standard of care, ELITE-Symphony study.

Name of study	n FU time	Primary endpoint	Immunosupression	Main study findings	Secondary findings
Current standard of care trial					
ELITE-Symphony Ekberg H, 2007[12]	n=1645 12 months	GFR	1. Standard-CyA, MMF, CS 2. Daclizumab + low-dose CyA, MMF, CS 3. Daclizumab + low-dose tac, MMF, CS 4. Daclizumab + low-dose everolimus, CS	The highest eGFR in arm 3	Arm 3: - lowest BPAR rate - highest GS - highest rate of PTDM
Steroid-weaning trials					
ATLAS Vitko, 2005[14]	n=451 6 months	BPAR	1. IL-2Ri + standard-tac 2. Standard-tac, MMF 3. Standard-tac, MMF, CS	Lowest BPAR in arm 3	Similar patient and graft survival
ATLAS FU Krämer, 2012[15]	n=421 3 years			Similar BPAR at 6m-3y	Similar patient and graft survival
CARMEN Rostaing, 2005[16]	n=538 6 months	BPAR	1. Daclizumab + standard-tac, MMF 2. Daclizumab + standard-tac, MMF, CS	Similar BPAR	Reduced incidence of insulin-dep. PTDM Similar kidney function
Laftavi, 2005[17]	n=60 1 year	BPAR	1. rALG + standard-tac, MMF, CS 2. rALG + standard-tac, MMF, ESWD	Similar BPAR	Similar kidney function Fibrosis in biopsy higher in 2
ADVANCE Mourad, 2017[18]	n=1138 24 weeks	PTDM	1. IL-2Ri + standard-tac, MMF, ESWD 2. IL-2Ri + standard-tac, MMF	Similar PTDM	Higher BPAR in arm 2
ADVANCE FU Pernin, 2023[19]	n=814 5 years			Similar graft and patient survival	Similar rejection-free survival
Harmony Thomusch, 2016[20]	n=615 1 year	BPAR	A. IL-2Ri + low-tac, MMF, CS B. IL-2Ri + low-tac, MMF, ESWD	Similar, low BPAR;	Lower PTDM in arm B and C vs. arm A

Harmony FU Stumpf, 2024[21]	359 5 years		C. ATG + low-tac, MMF, ESWD.	Low BPAR and dc-graft loss independent of ESWD	ESWD had positive impact on patient survival
CS Cessation Woodle, 2008[22]	n= 385 5 years	Death/graft loss/BPAR	1. ATG / IL-2Ri + standard-tac, MMF, ESWD 2. ATG / IL-2Ri + standard-tac, MMF, CS	Composite outcome similar in 2 arms	Higher BPAR in arm 1
CS Cessation FU Woodle, 2021[23]	15.8 years	Graft failure		Similar graft failure from any cause	
3C Haynes, 2014[24]	n=852 12 months	BPAR at 6 months	1. Alemtuzumab + low-tac, MMF 2. IL-2Ri + standard-tac, MMF, CS	Reduced risk of BPAR in arm 1	Similar graft loss and severe infections
INTAC Hannaway, 2011[25]	n=501 36 months	BPAR at 6,12 months	1. Alemtuzumab + stand-tac, MMF (high-risk rec.) 2. ATG + stand-tac, MMF (high-risk rec.) 3. Alemtuzumab + stand-tac, MMF (low-risk rec.) 4. Basiliximab + stand-tac, MMF (low-risk rec.)	Reduced BPAR in arms 1 vs.2 and 3 vs. 4	Lower BPAR at 3 years in arm 3 vs. 4 Similar adverse event rate in all arms
BEST Kaufman, 2021[26]	n=316 24 months	Death/graft loss/eGFR< 45 ml/min	1. Alemtuzumab + belatacept, MMF 2. rATG + belatacept, MMF 3. rATG + tac, MMF	Composit outcome similar in 3 arms	Fewer eGFR<45ml/min and higher BPAR rate in arm 1 and 2 Similar DSA

Table 1. Overview of relevant RCTs applying steroid-weaning strategies. rALG, rabbit-antilymphocyte globulin; ATG, antithymocyte globulin; BPAR, biopsy-proven acute rejection; CyA, cyclosporine A; CS, corticosteroids; DSA, donor specific antibody; ESWD, early steroid withdrawal; GFR, glomerular filtration rate; MMF, mycophenolate mofetil; IL-2Ri, interleukin-2 receptor inhibitor; PTDM, posttransplantation diabetes mellitus; RCT, randomized controlled trial; tac, tacrolimus

1.3 Long-term side-effects of steroids in kidney transplant recipients

Long-term administration of steroid therapy in KTRs has well-recognized adverse effects, with PTDM being the most significant, followed by hypertension, dyslipidemia, and weight gain, that contribute to cardiovascular disease[27], osteoporosis[28] and risk for infections. Steroid-weaning strategies have demonstrated significant improvement in these cardiovascular risk factors[29,30,31] after kidney transplantation.

Steroid dose at year 1 correlates significantly with overall death with functioning graft (Figure 4), death due to cardiovascular disease or infections, and *de novo* osteoporosis.[28,32] Moreover, maintenance steroid dose has a significant direct relationship with death with functioning graft due to cardiovascular disease or infections, even in patients with favorable graft outcomes where steroid treatment might be unnecessary.

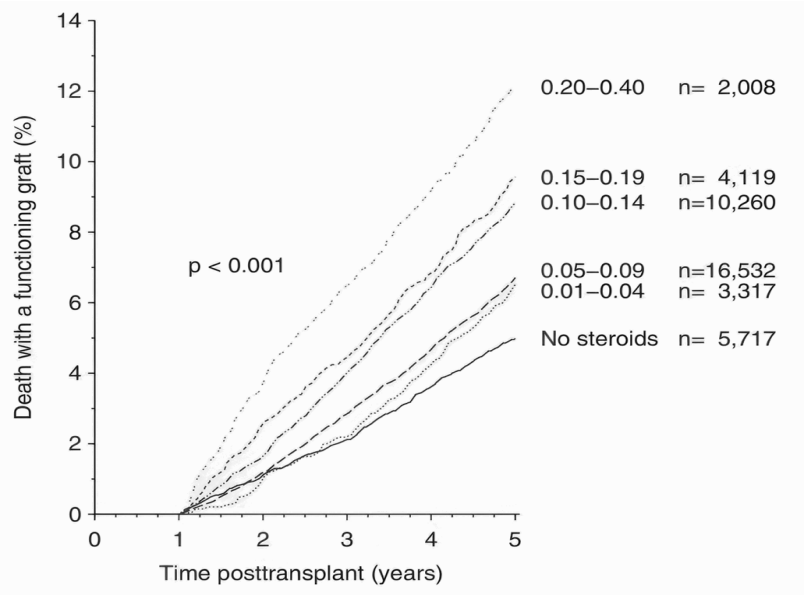


Figure 4. Cumulative incidence of death with a functioning graft during years 2-5 in kidney transplant recipients according to dose (mg/kg/day) of maintenance steroids administered at year 1. From Opelz et al., AJT, 2013[32]

1.4 Posttransplantation diabetes mellitus

The diabetogenic effect of steroids is exacerbated by tacrolimus, which can induce hyperglycemia and PTDM. Steroids are implicated in enhancing insulin resistance, while tacrolimus detrimentally affects insulin secretion in a dose-dependent manner.[33] PTDM

is an independent risk factor for cardiovascular disease (Figure 5)[34-36] and serves as an independent predictor of both graft failure[37] and increased mortality in KTR.[38,39]

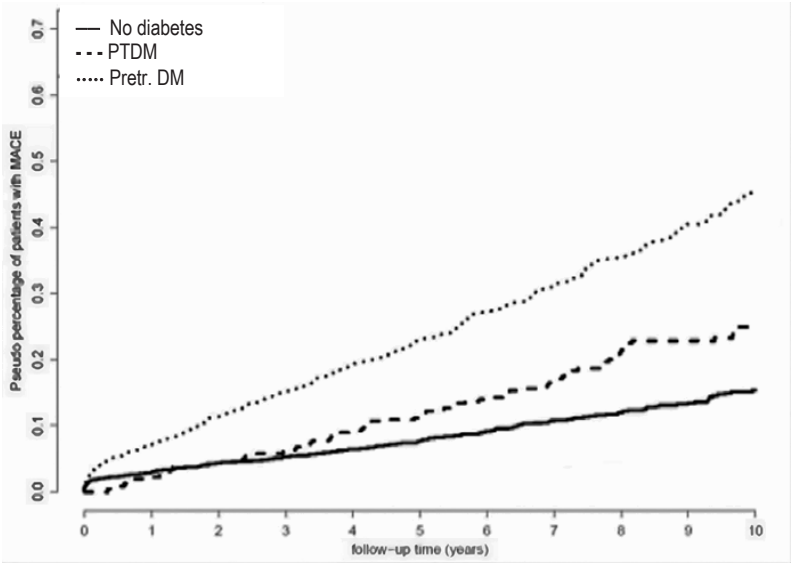


Figure 5. Extended Kaplan-Meier rates for MACE according to diabetes status post-transplant. MACE, major adverse cardiovascular events; PTDM, posttransplantation diabetes mellitus; Pretr. DM, pretransplantation diabetes mellitus. From Lim et al., Transplantation, 2021[36].

The large variability in the reported incidence of PTDM across studies is primarily due to differences in immunosuppressive regimens and the lack of uniform diagnostic criteria. In 2003, a global consensus was reached to adopt the American Diabetes Association (ADA) criteria[40] for diagnosis of PTDM, previously referred to as new-onset diabetes after transplantation (NODAT).[41] By 2013, a broader composite definition of PTDM for clinical trials had been proposed.[42] (Table 2)

ADA criteria of PTDM	**Composite criteria of PTDM**
Symptoms of diabetes or random P-glucose ≥11.1 mmol/L	ADA 2 FPG ≥ 7.0 mmol/L ≥ 30 days apart
Fasting P-glucose ≥7.0 mmol/L	HbA1c ≥ 6.5%
2 hours OGTT P-glucose ≥11.1	Oral hypoglycemic agents ≥ 30 consecutive days
	Insulin ≥ 30 consecutive days

Table 2. PTDM diagnostic criteria.

A comprehensive retrospective analysis has indicated that the incidence of PTDM can range between 30%-37% in patients undergoing standard-dose tacrolimus, MMF, and steroid-based maintenance therapy.[42]
Furthermore, there are other widely recognized risk factors that can contribute to the development of PTDM, such as obesity, age, ethnicity, family history of diabetes, donor source, and hepatitis.[38]

1.5 Effect of steroids on other immunosuppressants

1.5.1 Steroids and MMF

MMF is an inactive pro-drug hydrolyzed to its active form, mycophenolic acid (MPA), and it is metabolized in liver, intestine and kidney to the inactive metabolite MPA glucuronid. The most accurate assessment of MPA exposure is the area under the plasma concentration curve (AUC). A peak is seen after approximately 1-2 hours after oral administration, and the second peak can be measured due to enterohepatic recirculation. There is huge interindividual variability in MPA AUC with the standard MMF dose described. MMF was marketed as a one-dose-suits-all despite evidence supporting concentration-controlled dosing.[43] The pharmacokinetic interaction between steroids and MMF has been less extensively explored, and only in small studies with conflicting results. [44,45] A proposed interference in enhanced MPA metabolism by steroids has not been confirmed in a large prospective study.

1.5.2 Steroids and tacrolimus

Tacrolimus is metabolized by cytochrome P450 (CYP3A4) mainly in the liver. The most likely mechanism of interaction involves the specific enzymatic induction of CYP3A4 and/or P-glycoprotein by steroids. The higher the steroid dosage, the higher the dosage of tacrolimus needed to achieve target trough levels in patients. Interaction is present even when the steroid dosage is low.[46]

2 AIMS

We conducted a RCT OF **S**TEROID **A**VO**I**DANCE AND **LO**W-DOSE CNI BY ATG-INDUCTION IN **R**ENAL TRANSPLANTATION, the SAILOR trial, with an overall aim to validate the concept of steroid avoidance in conjunction with CNI minimization in KTRs, and to assess the short- and long-term outcomes.

Specific aims:

- To establish an open-label multicenter RCT focused on steroid avoidance and concurrent CNI minimization in KTR, aiming to reduce adverse effects while ensuring patient safety.
- To test the primary hypothesis, if steroid avoidance incorporating ATG induction, low-dose tacrolimus, mycophenolate mofetil reduces the incidence of PTDM within the first year following kidney transplantation.
- To evaluate the influence of steroid-free immunosuppressive protocol on the frequency of biopsy-proven rejections.
- To examine the impact of steroids on MPA exposure during concomitant therapy with tacrolimus.
- To assess if steroid avoidance protocol is efficient and safe even on the long-term, specifically 7 years post-transplantation.

Through these specific aims, the project seeks to provide new insights into the benefits and potential risks associated with steroid avoidance, in conjunction with CNI minimization strategies in kidney transplantation.

3 PARTICIPANTS AND METHODS

3.1 Design of the studies

Papers I and II: Investigator-initiated open-label multicenter RCT, conducted in three Scandinavian transplant centers, Gothenburg, Aarhus, and Malmö.

Papers III and IV: Retrospective observational studies of the original cohort included in the SAILOR RCT.

3.2 Study population

Paper I-III: Adult recipients of the first or second kidney transplant from a living or deceased donor with low immunological risk (panel reactive antibodies, PRA <25%) who provided informed written consent were eligible for the study. The main exclusion criteria were the history or diagnosis of diabetes mellitus, ABO-incompatible living donor kidney transplant, and HLA-identical sibling transplant. Recipients were randomized before kidney transplantation to one of the two treatment arms in a 1:1 ratio, stratified by study site and donor status (Figure 6). Participants were included in the study between February 2012 and March 2017. The studies were approved by the Regional Ethical Board of Gothenburg, Sweden (Dnr. 357-12) and Ethical Board for Region Midtjylland, Denmark (Dnr. 1-10-72-211-13). The RCT EudraCT number: 2012-000451-13.

Paper IV: Participants of the original SAILOR RCT were included, except those who withdrew the written consent (Figure 6). Participants who died or experienced graft loss between the original trial and the follow-up study were included in the analyses. Data were collected retrospectively in all three centers between December 2022 and November 2023. The study was approved by the Swedish National Ethical Board (amendment Dnr. 2019-01763; 2022-02860-02) and by the Ethical Board for Region Midtjylland, Denmark (amendment to Dnr. 1-10-72-211-13, from 29-Aug. 2022).

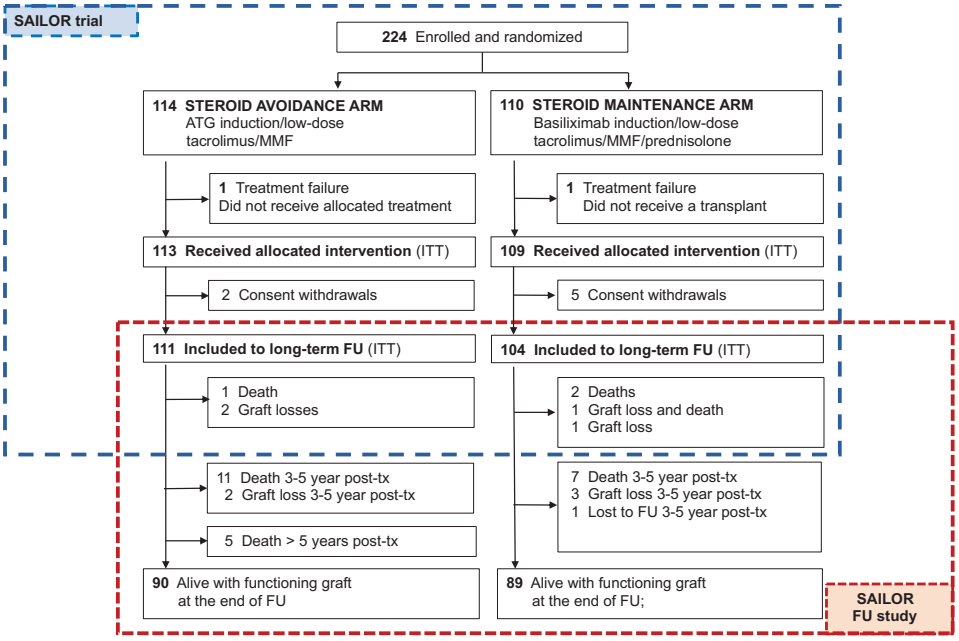


Figure 6. SAILOR trial and SAILOR follow-up study populations. ATG, antithymocyte globulin; FU, follow-up; MMF, mycophenolate mofetil; ITT, intention-to-treat; tx-transplantation. Adapted from Ekberg et al.; submitted 2024

3.3 Immunosuppression

- **Steroid avoidance arm (SA-arm):**
  Induction with antithymocyte globulin (ATG) and methylprednisolone bolus
  Maintenance treatment based on low-dose tacrolimus, and MMF controlled by a single mycophenolic acid area under the curve, without prednisolone

- **Steroid maintenance arm (SM-arm):**
  Induction with basiliximab and methylprednisolone bolus
  Maintenance treatment based on low-dose tacrolimus, MMF controlled by a single MPA AUC, and prednisolone

  Detailed information regarding immunosuppression and the schedule of study visits are shown in Figure 7.

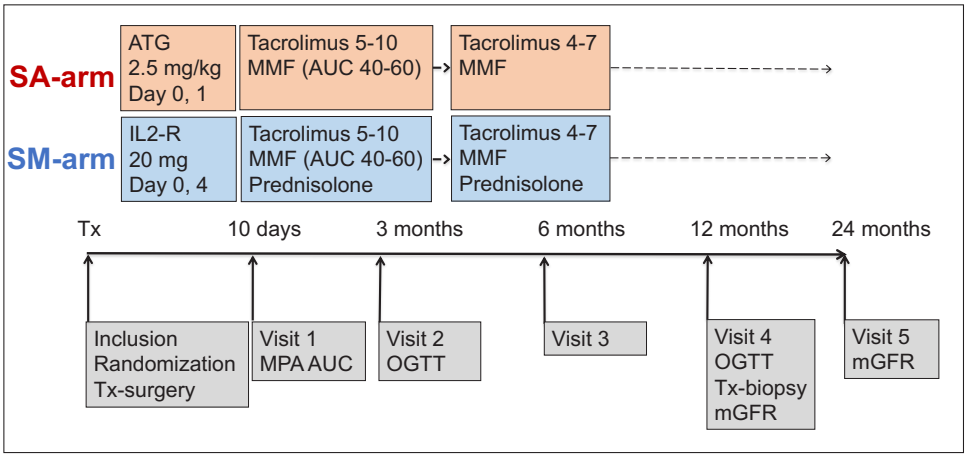


Figure 7. SAILOR trial: immunosuppression and study flow chart. ATG, antithymocyt globulin; AUC, area under the curve; IL-2Ri, interleukin-2 receptor inhibitor; mGFR, measured glomerular filtration rate: MMF, mycophenolate mofetil; OGTT, oral glucose tolerance test; SA-arm, steroid avoidance arm; SM-arm, steroid maintenance arm; Tx, transplantation

3.4 Follow-up

Paper I-III: The duration of the follow-up period was 2 years. Participants were followed at the following 6 time points: at transplantation, day 10, months 3, 6, 12 and 24 (Figure 7). Adverse events were evaluated, monitored and registered at the time of appearance.

Paper IV: The duration of the follow-up period was 10±5 years. Following the initial 2 years in the SAILOR trial, recipients were subsequently monitored in accordance with the practices of each center and data were collected prospectively in transplant registries. Clinical and laboratory data were retrieved at 1, 2, and 5 years post-transplant, and at the most recent clinical visit. Clinical events of intrerest were derived from in-patients clinical records and the National Patient Register (Sweden).

3.5 Endpoints

The study endpoints are listed in Table 3.

	Study	Follow-up	Primary endpoint	Main secondary endpoints
Papper I,II	SAILOR RTC	2 years	Incidence of PTDM[a)] at 1 year	Incidence of BPR[b)] at 1 year Composite measure[c)] at 1,2 years Kidney function Incidence of: - PTDM at 2 years - severe infections - MACE[d)] - malignancies
Papper III	SAILOR RTC	2 years	Effect of CS on MPA AUC	Correlation between MPA AUC and: - MMF dose - body weight
Papper IV	SAILOR FU study	10±5 years	Patient survival Death censored graft survival[e)]	Cummulaive incidence of: - rejection - PTDM - severe infections - MACE - malignancies Kidney function DSA

Table 3. BPR, biopsy-proven rejection; CS, corticosteroids; DSA, donor specific antibody; FU, follow-up; PTDM, posttransplantation diabetes mellitus; MACE, major adverse cardiovascular events; MPA AUC, mycophenolic acid, area under the curve; RTC, randomized controlled trial

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Definition of endpoints:

[a)] **PTDM:** the composite diagnosis was determined, if one of the following was present
- fasting plasma glucose (FPG) ≥ 7.0 mmol/L ≥ 30 days apart;
- 2-h plasma glucose ≥ 11.1 mmol/L in the oral glucose tolerance test (OGTT), scheduled at month 3 and 12
- oral hypoglycemic agent or insulin given ≥ 30 consecutive days

[b)] **Biopsy-proven rejection:** diagnosis of rejection was determined by for cause kidney transplant biopsy at the time of appearance or by protocol biopsy at month 12. The biopsies were assessed according to the Banff 2017 classification[47,48]

[c)] **Kidney function** was assessed by measured glomerular filtration (GFR) rate (paper I) or by estimated GFR (paper IV).

[d)] **MACE** was defined as acute coronary syndrome or myocardial infarction or stroke.

[e)] **Death-censored graft survival** describes the graft survival censored for death with a functioning graft.

3.6 Statistical analysis

Paper I: The sample size calculation was based on the assumption that the incidence of PTDM, defined according to the ADA criteria, will be reduced from an estimated 36% with steroid maintenance to 18% with steroid avoidance. The sample size of 222 subjects was calculated using Fisher´s exact test to achieve 80% power for superiority of the steroid avoidance-arm over the steroid maintenance-arm, with a two-sided type 1 error of 5% and allowing 5% dropout.

Paper II, IV: All analyses were performed using the intention-to-treat (ITT) population, which consisted of all randomized participants who received a kidney transplant, had at least one study treatment and one recorded follow-up.

Incidence of PTDM was analyzed even on the per-protocol (PP) population, i.e. participants who received treatment according to the protocol without major protocol deviations, and completed the study for two years / seven years.

Comparisons were made between the two treatment arms, SA-arm and SM-arm. Continuous variables were reported as means ± standard deviation (SD). Frequencies were reported in absolute numbers or relative %. For comparison between groups, the following tests were used: Fisher´s exact test for binary variables, Mantel-Haenszel chi-squared test for categorical variables, Fisher´s nonparametric permutation test for continuous variables, and t-test for continuous variables. Two-sided p-values < 0.05 were considered statistically significant.

The time-to-event endpoints were analyzed with the Kaplan-Meier method, including the log-rank test.

The calculation of confidence intervals (CI) for continuous variables is based on the assumption of normality. Cox regression analysis was used to identify risk factors and calculate adjusted hazard ratios (aHR) for events. Adjusted models were made by the stepwise selection of independent covariates; the level of significance was 0.05.

Differences between mGFR and the four eGFR equations were tested with the Wilcoxon signed-rank test.

The statistical software SAS 9.4 was used for statistical analyses or GraphPad Prism (©GraphPad Software).

Paper III: Statistical software Stata was used for analyses, multilevel mixed-effects linear regression was used to examine the effect of steroids on MPA AUC.

4 RESULTS

Paper I and Paper II

In total, 222 participants were included, 113 in SA-arm and 109 in SM-arm (Figure 6). The 2-year follow-up completed 108 and 100 participants in SA- and SM- arms, respectively.

The incidence of PTDM at 1 year was similarly low in both treatment arms, 12.4% in SA-arm vs. 18.3% in SM-arm (Figure 10). PTDM occurred early, within the first six months. The survival freedom from PTDM (Figure 8) and the composite endpoint (Figure 9) were similar. The incidence of overall biopsy-proven rejection at 1 year was not statisticaly different, 15% in SA-arm vs. 13.8% in SM-arm (Figure 11).

The other main secondary endpoints - kidney transplant function, incidences of infection, MACE and malignancy - were similar in both treatment arms. At the end of the trial, 64% of participants in the SA-arm remained on steroid-free treatment.

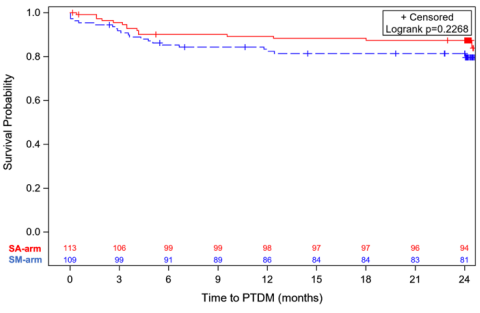

Figure 8. Freedom from PTDM according to study arms. PTDM, posttransplantation diabetes mellitus; SA-arm, steroid avoidance arm; SM-arm, steroid avoidance arm. Adapted from Ekberg et al., 2022[49]

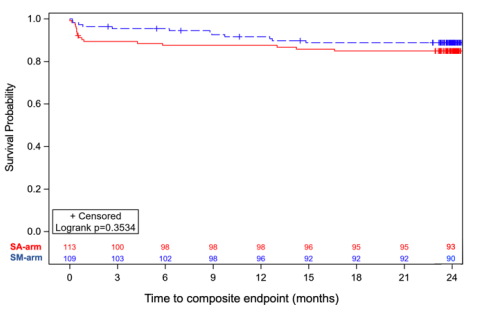

Figure 9. Kaplan- Meier of composite endpoint (BPAR, graft loss, death) according to study arms. SA-arm, steroid avoidance arm; SM-arm, steroid avoidance arm. Adapted from Ekberg et al., 2022[49]

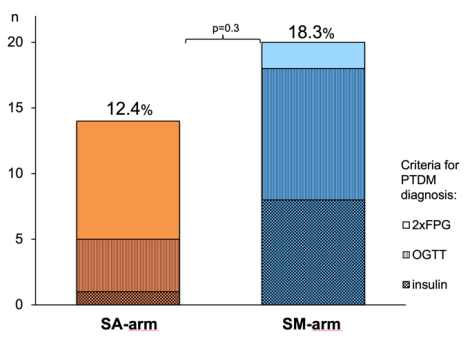

Figure 10. Incidence of PTDM at 1 year, according to study arms and criteria for PTDM diagnosis. FPG, fasting plasma glucose; OGTT, oral glucose tolerance test. SA-arm, steroid avoidance arm, SM-arm, steroid maintenance arm. Adapted from Ekberg et al., 2022[49]

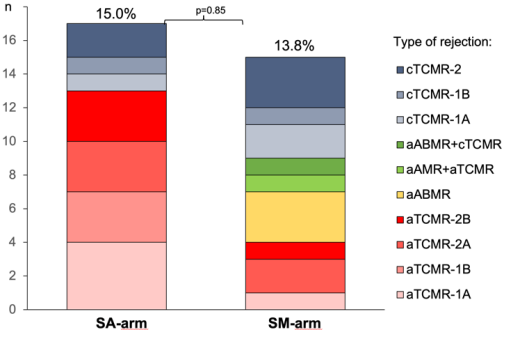

Figure 11. Incidence of biopsy-proven rejection at 1 year, according to study arms, and type of rejection (Banff 2017). a, active; ABMR, antibody-mediated rejection; c, chronic; SA-arm, steroid avoidance; SM-arm, steroid maintenance arm; TCMR, T-cells mediated rejection. Adapted from Ekberg et al., 2022[49]

Paper III

The median MPA AUC at baseline was 53 mg*h/L, and interindividual variation was up to 5-fold on standard MMF dose. Median MPA AUC was 52 mg*h/L in SA-arm and 57 mg*h/L in SM-arm, which was significantly lower at baseline by 12.5% (95% CI; 3.2-20.9%, p=0.01) in participants in SA-arm compared to those in SM-arm. (Figure 12).

The MMF dose was positively corelated with MPA AUC (p=0.001) The body weight was negatively corelated with MPA AUC (linear regression coefficient-0,32, p=0.001) (Figure 13). Tacrolimus trough levels did not affect significantly MPA AUC.

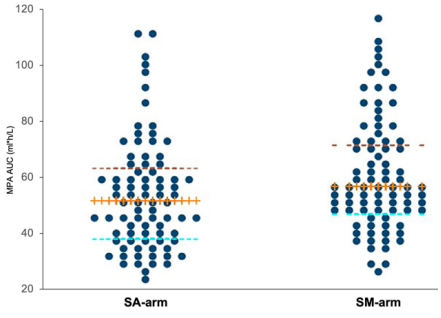

Figure 12. MPA AUC at baseline according to study arms. MPA, mycophenolic acid; AUC, area under the curve. Horizontal line depict the median and interquartile ranges. Adapted from Nourbakhsh, et al., 2021[50]

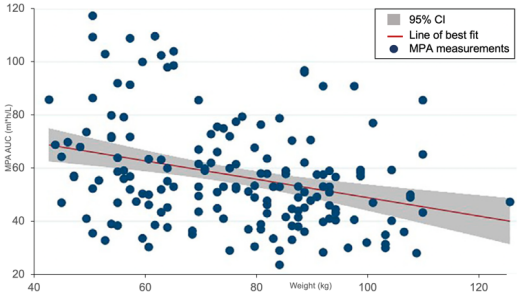

Figure 13. Relationship between MPA AUC and body weight, linear regression. MPA, mycophenolic acid; AUC, area under the curve. Adapted from Nourbakhsh et al., 2021[50]

Paper IV

The follow-up study included a total of 215 participants, with 111 in the SA-arm and 104 in the SM-arm. The mean follow-up time was 7.3 years in both groups.

The efficacy measures, including patient survival (Figure 14), death-censored graft survival (Figure 15), incidence of biopsy-proven rejection, and kidney function were similar in the two treatment arms (Table 4).

The safety parameters, such as the incidence of *de novo* DSA, PTDM, serious infections requiring hospitalization, MACE, and malignancy, did not differ between the two treatment arms.

Two thirds of participants remained on steroid-free maintenance immunosuppression at the end of the follow.up.

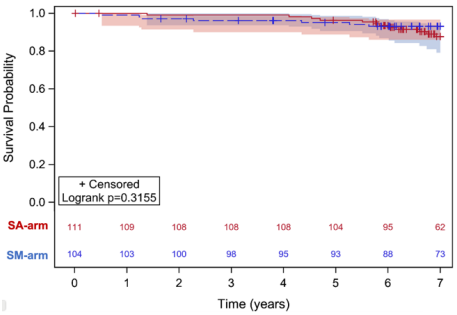

Figure 14. Kaplan-Meier of patient survival according to study arms. SA-arm, steroid avoidance arm; SM, steroid maintenance arm.

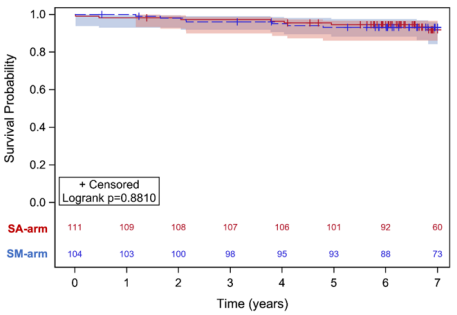

Figure 15. Kaplan-Meier of death-censored graft survival according to study arms. SA-arm, steroid avoidance arm; SM-arm, steroid maintenance arm

	SA-arm n=111	**SM-arm** n=104	**p-value**
FU time (years)	7.3 ± 1.76	7.3 ± 2.03	0.99
Patient survival	0.88 (0.79-0.93)	0.93 (0.86-0.97)	0.32
Death censored-graft survival	0.92 (0.84-0.96)	0.93 (0.86-0.97)	0.88
Any BPR	22 (19.8)	17 (16.3)	0.60
BPR categories	30 (27.0)	25 (24.0)	0.64
Active / acute BPR	16 (13.5)	13 (12.5)	0.70
Chronic BPR	14 (12.6)	12 (11.5)	0.84

Table 4. Main efficacy endpoint. ATG, antithymocyte globulin; BPR, biopsy-proven rejection; FU, follow-up; SA-arm, steroid avoidance arm; SM-arm, steroid maintenance arm

5 DISCUSSION

The studies related to this thesis address a need for establishing the long-term safety and efficacy of a steroid-free, low-tacrolimus based immunosuppressive protocol as an alternative to the current steroid-containing standard of care regimen used in routine clinical practice. Our primary hypothesis was that avoiding steroids could reduce the incidence of PTDM, a recognized risk factor for cardiovascular morbidity and mortality that negatively impacts long-term outcomes. PTDM typically develops within the first six months post-transplant. Therefore, we selected PTDM as a primary outcome of interest, as it can be diagnosed and effectively monitored within a defined timeframe, without compromising the study's rigor as an RCT.

Our concern of implementing two immunosuppressive reduction strategies—steroid avoidance and tacrolimus minimization—was to ensure the long-term efficacy and safety of this regimen.

5.1 Steroid-free immunosuppressive protocol in kidney transplantation – are we there now?

The introduction of modern, effective immunosuppressive drugs for maintenance treatment in combination with induction agents has led to a paradigm shift in transplantation. The focus has shifted from early graft loss caused by acute rejection to concerns about late graft loss due to chronic antibody-mediated rejection, as well as the risk of morbidity and mortality from cardiovascular disease, infections, and malignant diseases. Several earlier RCTs demonstrated the beneficial impact of steroid-weaning strategies on cardiovascular risk factors without compromising short- and mid-term efficacy and safety particularly in KTR at low immunologic risk, despite increased risk of acute rejection. However, despite this evidence, steroid-weaning protocols have not yet been widely adopted, and the majority of recipients receive steroids. In 2022, 68% of KTR in the US remained on steroids[51] and recent European data from the Collaborative

Transplant Study showed that steroids were a part of immunosuppressive regimen for 77% of KTR three years post-transplant (Figure 16).[28] This continued use of steroids may have stemmed from a lack of long-term safety data.

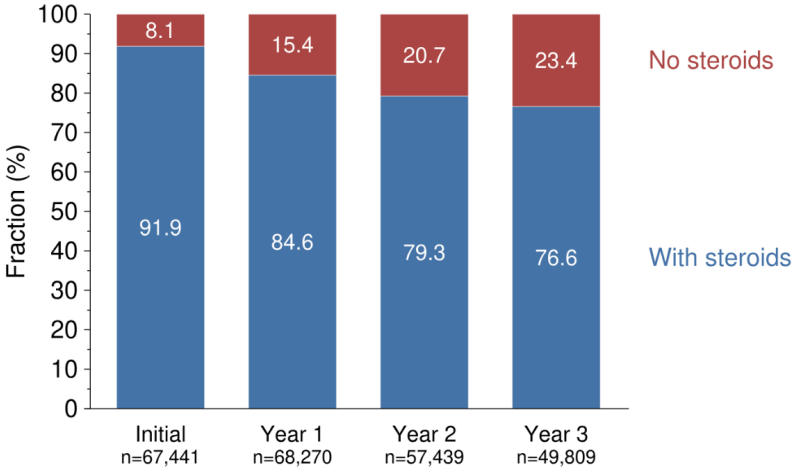

Figure 16. Percentage of adult kidney transplant patients from the transplant years 2000–2022 receiving steroid medication, depending on the time after transplantation. From Collaborative Transplant Study Newsletter 4:2024[28]

The 2009 KDIGO clinical practice guidelines for KTR[5] do not incorporate the recent evidence supporting steroid-minimization strategies. These guidelines, at a moderate evidence level (2B), suggest that "in patients at low immunological risk and who receive induction therapy, corticosteroids could be discontinued during the first week after transplantation".

Our study, along with several other robust RCTs[19,21,23], has now demonstrated significant benefits of steroid-weaning strategies in improving cardiovascular risk factors in KTR at low immunologic risk while maintaining efficacy and safety over extended follow-up periods beyond the typical short- to medium-term focus of earlier studies. Based on these findings, we propose that steroid-weaning approach, even with current regimens using low-dose tacrolimus and MMF following antibody induction, could be advantageous for a broader patient population than currently recognized.

5.2 Who can benefit from steroid-free protocol?

In general, it may be recommended that all non-immunized patients receiving a well-matched kidney transplant should be considered for a steroid-free immunosuppressive

protocol with antibody induction. These patients typically have a low risk of rejection and may gain substantial benefits by avoiding steroid-related adverse effects.[32]

Patients with pre-existing cardiovascular risk factors, such as hypertension, hyperlipidemia, diabetes, metabolic syndrome, or established coronary, cerebrovascular, or peripheral arterial disease, may particularly benefit from a steroid-free approach to reduce further exacerbation of cardiovascular risk.

Patients with pre-diabetes or an elevated risk of developing PTDM could also significantly benefit from steroid avoidance, as PTDM is known to negatively impact both graft and patient survival.[36,37] Additionally, a steroid-free regimen could help patients with established osteoporosis or severe psychiatric disorders by preventing further deterioration of these conditions.

Given the evolving demographics of kidney transplant candidates, which increasingly include older, more complex, and frail patients, steroid-free immunosuppression may also be considered appropriate for these groups. Their generally weaker immune response and lower risk of rejection make them potential candidates for the benefits of steroid avoidance.[52]

5.3 When should the steroid-free protocol be used with caution?

Delayed graft function (DGF)

DGF, a manifestation of acute kidney injury, is a well-recognized risk factor for graft loss.[53] In a large registry study, early steroid withdrawal (ESWD) in recipients with DGF was associated with an increase in rejection, graft failure and mortality, leading to the conclusion that ESWD may lead to worse kidney transplant outcomes in recipients with DGF.[54] One retrospective UK study demonstrated inferior allograft and patient outcomes in patients with DGF who received monoclonal antibody induction and a steroid sparing protocol. Notably, patients with DGF receiving an IL2R antibody had a higher risk of early rejection in compared to those receiving alemtuzumab induction.[55] Until convincing data from RCTs are available, caution should be used when implementing steroid-free protocols in recipients with DGF.

Recurrent disease

IgA nephropathy, the most common form of glomerulonephritis leading to end-stage kidney disease, can recur in the kidney transplant and is associated with reduced graft survival rates. Although steroids are commonly used to treat IgA nephropathy in native kidneys, their protective role in preventing recurrence in transplanted kidneys remains inconclusive. A large US registry study reported a cumulative graft loss incidence of 15.4% over five years due to recurrence of IgA nephropathy, and ESWD was associated with an increased risk of graft loss due to recurrence.[56] Similarly, an Australian registry

study observed a cumulative graft loss incidence 4.3% over 10 years from recurrent IgA, and found that steroid use was associated with a significantly reduced risk of recurrence.[57] In contrast, a multicenter retrospective study reported a 23% cumulative incidence of IgA recurrence over 15 years and found no association between recurrence and type of immunosuppression, including steroids.[58] Further research is essential to elucidate the role of steroids in preventing IgA nephropathy recurrence in kidney transplants. Until more definitive evidence emerges, careful consideration is warranted when using steroid-free protocols in KTR at risk of recurrent disease.

5.4 When should the steroid-free protocol be avoided?

HLA sensitization

Patients who are HLA-sensitized—due to prior organ transplantation, blood transfusion, or pregnancy—often experience longer waiting time for a kidney transplant and are at an increased risk of rejection and graft failure. Their immune system is primed to respond rapidly and aggressively against a new allograft. To alleviate this risk, sensitized patients typically require potent induction and maintenance immunosuppression.[59] Panel-reactive antibody (PRA) levels are a key indicator of immune sensitivity. Immunologic risk is generally categorized as low (PRA <25%), moderate (PRA 25-80%), or high (PRA >80%). The data on steroid-free attempts in sensitized patients are limited, as the major RCTs applying steroid-weaning approach have restricted their study population to recipients with low immunologic risk.[14,16,17,22,31] A large registry study indicated less favorable outcomes for recipients with higher PRA, especially for those with PRA levels exceeding 60%.[60] One small prospective study with 21 sensitized patients showed similar 1-year graft survival when alemtuzumab + tacrolimus mono-therapy was compared with ATG + triple treatment.[61] Another retrospective study from the UK examined a steroid-free protocol in highly sensitized recipients, defined by a calculated reaction frequency >85% and reported favorable one-year outcomes using alemtuzumab as an induction agent, along with low-dose tacrolimus and MMF for maintenance.[62] However, long-term outcome data are not available from any study. Recipients with pre-formed DSA are at a significantly higher risk for active antibody-mediated rejection.[63] To reduce this risk, immunosuppression is generally intensified, making ESWD inadvisable in this group of patients.

ABO-incompatibility

In the setting of living donor ABO-incompatible kidney transplantation, immunosuppressive protocols are typically intensified to overcome the ABO incompatibility barrier. To date, only anecdotal reports have documented the short-term success of ESWD in these patients,[64] underscoring the lack of robust evidence supporting its use in such high-risk transplants.

5.5 What can be applied in our own clinical practice?

Our results from the SAILOR trial, especially with the extended seven years follow-up results, and the findings from other recent RCTs[19,21,23] indicate that the steroid-free immunosuppressive protocol using low-tacrolimus and MMF with antibody induction could benefit a broader range of patients than is currently utilized. These protocols should be routinely considered for most KTR at low immunologic risk, as they offer significant benefits by minimizing the long-term side effects associated with steroid use and enhancing the quality of life.

MMF is typically initiated using a fix dose regimen, with early target concentration intervention currently performed to reach adequate exposure. To further opitimize outcomes, initial MMF dosing could be adjusted based on invidividual charactersitiscs, such as very low or very high body weight, to reduce a huge inter-individual variability observed in drug response.

5.6 Ethical reflections

The SAILOR trial and SAILOR FU study adhered to the ethical principles of the Declaration of Helsinki[65], Good Clinical Practice guidelines, and the International Council for Harmonization guidelines.[66] Signed and dated informed consent has been obtained from each study participant before randomization to the SAILOR trial. The consent could be withdrawn at any time. The information about the observational follow-up after 5 years was provided in the original SAILOR trial information. The later amendment for extension to 10±5 years was approved by the Swedish and Danish authorities. Those individuals who have undergone transplantation at the Transplant Centre in Gothenburg and have provided a new written consent for the intervention will be included in the upcoming follow-up interventional sub-study.

When the SAILOR trial was designed, the primary safety concern was the potential increased risk for rejection associated with reduction of maintenance immunosuppression regimen from three drugs to two. To address this concern, and to minimize risk to study participants, we chose to use a potentially more potent T-cell depleting agent, ATG, as induction therapy, instead of basiliximab.

The protocol biopsy carries a potential risk of bleeding. Standardized safety precautions minimize this risk through pre-biopsy control of haemostasis, blood pressure management, ultrasound guidance during the procedure, and close clinical monitoring afterwards. We have carefully considered that the benefits of protocol biopsy far outweigh the potential risks for participants. The protocol biopsies are promptly evaluated and assist clinicians in their decision-making for potential therapeutic intervention for each individual patient.

6 CONCLUSIONS

The main conclusions of the presented studies in this thesis are following:

- The incidence of PTDM after kidney transplantation was low across the entire cohort from the three Scandinavian transplant centers.
- The rates of PTDM and biopsy-proven rejection were similar between the SA- and SM- arms, both in the short- and long-term.
- The SAILOR trial and SAILOR FU study did not demonstrate either superiority of the steroid avoidance regimen in PTDM incidence or inferiority in rejection rates up to seven years post-transplant.
- Early monitoring of MPA exposure offers valuable information for personalized dosing of MMF.
- A steroid-free immunosuppressive protocol, combined with low-dose tacrolimus, MMF and antibody induction, is effective and safe for KTR with low immunologic risk, both in the short term and over an extended period of up to seven years.

7 FUTURE PERSPECTIVES

7.1 SAILOR FU interventional sub-study

A sub-study of SAILOR FU has been initiated. The aim is to investigate the impact of steroid avoidance on incidence of chronic rejection and incidence of *de novo* DSA. The SAILOR FU study has not provided any indication for that, although the long-term protocol biopsy and consequent DSA controls were not included in the observational study. Therefore, in addition to already retrieved clinical data reported in the SAILOR FU study, a second protocol biopsy, mGFR, and DSA will also be obtained. The study population is limited only to the Gothenburg cohort, and only to participants who have signed a new written consent. The ethical approval has been received, and the study is anticipated be completed in 2025.

7.2 SAILOR iBOX sub-study

iBOX is an integrative risk prediction score for kidney allograft failure, and it has been recognized as a tool for monitoring patients, tailoring the clinical follow-up, and optimization of immunosuppression.[67] The European Medicines Agency has proved the iBox scoring system as a novel secondary efficacy endpoint to compare novel immunosuppressive treatments to the standard of care in kidney transplant clinical trials.[68] The iBOX score is obtained through a computed algorithm, based on eight independent risk parameters (clinical, laboratory, immunological, and histological). The aim of this sub-study is to compute iBOX scores on 1-year SAILOR trial data and compare with the real-life outcomes. Based on the data from SAILOR FU interventional study, we will also apply the iBOX score prospectively on 10-years data for prediction of risk for long-term graft loss.

7.3 Final remarks

The increasing utilization of organs from extended criteria donors or donors after cardiac death has led to an elevated risk for DGF. The choice of induction and maintenance immunosuppression, along with machine perfusion, are modifiable factors that can influence this risk. Alemtuzumab induction may be beneficial in preventing early rejection episodes associated with DGF,[55] but it still remains an off-label drug. Further evidence from RCTs is necessary to identify the most beneficial immunosuppressive regimen for patients with DGF or those at a risk for it.

Specific patient groups (e.g. highly sensitized individuals, recipients of ABO-incompatible kidneys from living donors, and patients with inflammatory kidney diseases at risk of recurrence) require thorough investigation, ideally through prospective trials, before steroid-free strategies can be safely implemented in these populations.

Improved and more precise detection of rejection and personalized immunosuppression are essential to mitigate the risks of both under- and over-immunosuppression. Early and accurate detection of active rejection followed by effective treatment is crucial for the long-term survival of kidney transplants. Traditionally, kidney transplant biopsy has been the gold standard for diagnosing rejection. Recently, a novel non-invasive biomarker, donor derived cell-free DNA (dd-cfDNA), has been developed and proposed for serial monitoring after transplantation for early detection of active rejection.[69] Dd-cfDNA are fragments of DNA released early from the donor graft into recipient circulation in case of cell injury due to rejection. Incorporating dd-cfDNA in routine clinical practice and further studies of steroid avoidance protocols could detect rejection before clinical or laboratory sign of graft dysfunction appear, enabling early, more effective intervention and personalized adjustments in maintenance immunosuppression while reducing unnecessary biopsies.

Finally, to optimize the selection of KTRs who would benefit most from a steroid-free protocol, pre-transplant stratification based on immunological, metabolic, cardiovascular, and infection risks is essential for personalizing treatment both at the time of and after transplantation.

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