Thesis

Comparison of Tacrolimus Formulations in Kidney Transplant Recipients

submitted by

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Declaration of Academic Integrity

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

Graz, 13.02.2024 *Afërdita Gashi, m.p.*

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Zusammenfassung

Einleitung: Der Calcineurininhibitor Tacrolimus gehört zur immunsuppressiven Standardtherapie bei nierentransplantierten PatientInnen. Tacrolimus ist in verschiedenen Formulierungen verfügbar, die unterschiedliche pharmakokinetische Eigenschaften haben. Das Ziel dieser Studie war es die verschiedenen Formulierungen miteinander zu vergleichen.

Methoden: In diese Studie wurden insgesamt achtundachtzig PatientInnen nach Nierentransplantation eingeschlossen. Sechsundfünfzig PatientInnen erhielten Prograf® und zweiunddreißig PatientInnen erhielten Envarsus®. Studienvisiten wurden laut Studienprotokoll eine Woche, zwei Monate und zwölf Monate nach der Nierentransplantation durchgeführt. Weiters wurden Messungen aus den ambulanten Besuchen über die ersten drei Monate verwendet. Akute Abstoßungsreaktionen wurden mittels Nierenbiopsie gesichert. Infektionen mit Zytomegalievirus oder BK-Polyomavirus wurden mittels PCR-Test im Blut nachgewiesen.

Die Variabilität der Tacrolimusspiegel und die Zeit im Zielbereich wurden in vierwöchigen Intervallen für die ersten drei Monate nach der Nierentransplantation bestimmt. Ein schneller Tacrolimus-Metabolismus wurde durch ein Konzentrations-Dosis-Verhältnis von < 1.05 definiert.

Ergebnisse: Der mediane Kreatininwert war signifikant niedriger in der Envarsus® Gruppe sowohl zwei (1.35 ± 0.51; p = 0.02) als auch zwölf (1.36 ± 0.55, p = 0.046) Monate nach Nierentransplantation.

Es kam insgesamt zu 12 akuten Abstoßungen, 3 davon waren „borderline rejections“. Weniger akute Abstoßungen und mehr BK-Polyomavirus Infektionen wurden in der Envarsus® Gruppe beobachtet. Dieses Ergebnis war statistisch nicht signifikant.

Patientinnen mit schneller Metabolisierungsrate zeigten signifikant mehr akute Abstoßungen unabhängig von Behandlungsgruppe (HR 4.5; CI 95% 1.4 – 14.4; p = 0.01). Patientinnen mit schneller Metabolisierungsrate in der Prograf® Gruppe hatten eine höhere Inzidenz von akuten Abstoßungen (HR 5.0; 1.3 – 20.3; p = 0.02). Dieser Effekt war in der Envarsus® Gruppe abgeschwächt (HR 2.72; CI 95% 0.1 – 28.5; p = 0.45).

Eine signifikant höhere Zeit im Zielbereich zeigte sich in der Envarsus® Gruppe zwei Monate nach Nierentransplantation.

Conclusio: Bei Nierentransplantierten mit schnellem Tacrolimus Metabolismus, die *de novo* Envarsus® erhielten, wurden weniger akute Abstoßungsreaktionen und eine höhere Inzidenz von BK-Polyomavirus Infektionen beobachtet. Dieses Ergebnis deutet auf eine potenziell stärkere Immunsuppression unter *de n*ovo Envarsus®. Um einer individualisierten Immunsuppression näher zu kommen, sind weitere Forschung des biologischen Effektes dieser Formulierung sowie die Entwicklung von neuen relevanten Biomarkern notwendig.

Abstract

Introduction: The calcineurin-inhibitor tacrolimus is a standard immunosuppressive agent in kidney transplantation. It is currently available in different formulations, each with a distinct pharmacokinetic profile. Our aim was to evaluate any differences in outcome depending on the tacrolimus formulation, which was prescribed de novo after kidney transplantation.

Methods: For this study fifty-six kidney transplant recipients who received Prograf® and thirty-two kidney transplant recipients who received Envarsus® for induction were included. Study-visits were predefined to take place at one week, two months and twelve months post-transplantation. Routine follow-up checks over the first three months post-transplantation provided additional data. Graft rejections were diagnosed following indication biopsy. Cytomegalovirus and BK polyomavirus infections were identified using blood PCR tests. Tacrolimus intra-patient variability and time in target range were calculated in four-week intervals for the first three months post-transplantation. Furthermore, tacrolimus fast metabolizers were identified using concentration-to-dose ratio.

Results: Two and twelve months post-transplantation median creatinine was lower (1.35 ± 0.51; p = 0.02 and 1.36 ± 0.55, p = 0.046, respectively) and mean eGFR was higher (54.93 ± 17.05; p = 0.07 and 55.53 ± 16.38; p = 0.06) in the Envarsus® treatment group.

A total of 18 acute graft rejections occurred during follow-up, 3 of them were borderline rejections. A trend wise lower rate of graft rejection and higher rate of BK polyomavirus viremia was noticed in the Envarsus® group. This difference was not statistically significant.

Fast metabolizers showed a significantly higher risk of acute graft rejection regardless of treatment group (HR 4.5; CI 95% 1.4 – 14.4; p = 0.01).

Fast metabolizers in the Prograf® group showed a significantly higher risk of acute graft rejection (HR 5.0; 1.3 – 20.3; p = 0.02), this effect was blunted in the Envarsus® treatment group (HR 2.72; CI 95% 0.1 – 28.5; p = 0.45).

Treatment groups did not differ regarding intra-patient variability. Patients in the Envarsus® group demonstrated a significantly higher time in target range at month two post-transplantation.

Conclusion: Induction with *de novo* Envarsus® decreased rejection risk in fast-metabolizer phenotypes potentially due to improved pharmacokinetic profile. The observation of trend wise lower rejection rate and higher BK polyomavirus viremia incidence may reflect a stronger immunosuppressive effect despite targeting similar trough levels with *de novo* Envarsus®. Further biological effect analysis is needed to identify markers potentially guiding immunosuppression.

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Glossary and abbreviations

%	percent
®	registered trademark
ABCB1	ATP-binding cassette subfamily B member 1
ABMR	antibody-mediated rejection
ATG	antithymocyte globulin
ATI	acute tubular injury
ATP	adenosine triphosphate
AUC	area under the curve
AZA	azathioprine
BKV	BK polyomavirus
BKVAN	BK polyomavirus-associated nephropathy
BMI	body mass index
BPAR	biopsy-proven acute rejection
C/D ratio or CDR	concentration-to-dose ratio
C0	trough concentration
CD	cluster of differentiation
CGA	creatinine, glomerular filtration rate and albuminuria
CI	confidence interval
CKD	chronic kidney disease
CMV	cytomegalovirus
CNI	calcineurin-inhibitor
COVID-19	coronavirus disease 2019
CsA	cyclosporine A
CTLA-4	cytotoxic T-lymphocyte associated protein 4
CYP3A	cytochrome P450, family 3, subfamily A
DALY	disability-adjusted life years
DBD	donor after brain death
DC	dendritic cell
DCD	donor after cardiac death
de novo	from the beginning
DGF	delayed graft function

DNA	deoxyribonucleic acid
DSA	donor-specific antibody
eATG	equine antithymocyte globulin
EBV	Epstein-Barr virus
ECD	expanded-criteria donor
eGFR	estimated glomerular filtration rate
ER-Tac	extended-release tacrolimus
ESKD	end-stage kidney disease
et al.	latin: et alii - and others
FKBP	FK506 binding protein
FM	fast metabolizer
GBM	glomerular basement membrane
GBS	Guillan-Barré syndrome
GC	germinal center
GFR	glomerular filtration rate
H&E stain	hematoxylin-eosin stain
HCV	hepatitis C virus
HHV	human herpesvirus
HIS	hospital information system
HLA	human leukocyte antigen
HR	hazard ratio
HSV	herpes simplex virus
IFN-γ	interferon gamma
i-IFTA	inflammation in areas of interstitial fibrosis and tubular atrophy
IL	interleukin
IL2-RA	interleukin-2 receptor antagonist
IM	intermediate metabolizer
IMPDH	inosine monophosphate dehydrogenase
IPV	intrapatient variability
IQR	interquartile range
IR-Tac	immediate-release tacrolimus
KDIGO	Kidney Disease: Improving Global Outcomes

KDPI	kidney donor profile index
KDRI	kidney donor risk index
KRT	kidney replacement therapy
KTR	kidney transplant recipient
KTX	kidney transplantation
LCP-Tac or LCPT	novel once-daily extended-release tacrolimus
M	mean
MDN	median
MDR	multidrug resistant mutation
MHC	major histocompatibility complex
MMF	mycophenolate mofetil
MPA	mycophenolic acid
MR	myogenic response
NaCl	sodium chloride
NAT	nucleic acid testing
NFATC	nuclear factor of activated T cells, cytoplasmic
NF-κB	nuclear factor kappa of activated B cells
NJ	New Jersey
NODM	new-onset diabetes mellitus
PAS stain	periodic acid-Schiff stain
PCR	polymerase chain reaction
Pgp	P glycoprotein
PRR	pattern recognition receptor
PTDM	post-transplant diabetes mellitus
rATG	rabbit antithymocyte globulin
RBF	renal blood flow
RNA	ribonucleic acid
RR	blood pressure
SCD	standard-criteria donor
SD	standard deviation
SM	slow metabolizer
SNGFR	single-nephron glomerular filtration rate
SPSS	statistical package for the social sciences

Tac	tacrolimus
TCMR	T cell-mediated rejection
TDM	therapeutic drug monitoring
TGF	tubuloglomerular feedback
T_H1	T helper 1 cell
TNFα	tumor necrosis factor alfa
TTR	time in target range
US or USA	United States of America
vs.	versus
VZV	varicella zoster virus
YLD	years lived with disability
YLL	years of life lost

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1 Introduction

1.1 Chronic Kidney Disease

1.1.1 Definition and aetiology

Chronic kidney disease (CKD) is described as an abnormal kidney structure or function of the kidney, which persists for over three months, with consequences on patient health. (1) While imaging can capture changes in structure, kidney function impairment can present itself clinically as oedema, hypertension, and changes in urine output. (2) Staging of CKD, also known as CGA staging, is based on causality, glomerular filtration rate (GFR) category and albuminuria category. A decreased GFR starts at < 60 ml/min/1.73 m^2, while a GFR of 15 ml/min/1.73 m^2 is viewed as kidney failure or end-stage kidney disease (ESKD). Albuminuria, which describes the presence of albumin in urine, is an important prognostic factor and therefore indispensable for CKD staging. Albuminuria can be quantified using the albumin excretion rate for 24 hours or the ratio of albumin to creatinine and is classified in categories from A1 to A3. (1)

The risk of developing CKD depends on genetic, demographic, socioeconomic and environmental factors. Important and well-known risk factors for developing CKD are hypertension, diabetes, and obesity. In high-income and middle-income countries diabetes and hypertension are main causes of CKD. In low-income countries CKD occurs mainly because of infections, glomerulonephritis, and inappropriate medication use. (2)

Apart from progression to kidney failure, chronic kidney disease leads to metabolic, endocrine, and cardiovascular complications through various mechanisms. Cardiovascular complications of CKD are the main cause of mortality and include mainly non-atherosclerotic pathologies such as heart failure, atrial or ventricular dysrhythmia and sudden death. (3) It is therefore important to stop or slow down disease progression, diagnose cases of kidney failure early and refer them to specialist kidney services to prevent poorer outcomes. (1)

Chronic kidney disease has a large economic and environmental impact and is the most expensive of chronic diseases because of the need for dialysis and kidney transplantation. (1)
Compared to the majority of other medical therapies, haemodialysis has an evidently higher waste generation profile and a large carbon footprint, especially when considering an expected increase to 5 million people on dialysis treatment by the year 2025. (4)

1.1.2 Epidemiology

The number of patients with CKD worldwide has been estimated to be around 843.6 million, while it is more prevalent in female than male patients. According to 33 population-based representative studies, 10.4% of men and 11.8% of women over 20 years of age are affected by CKD globally. (5)
From 2007 to 2017 the prevalence of CKD increased by 28.8% in females and 25.4% in males. (6) The prevalence of chronic kidney disease differs depending on the race and country. However, it is difficult to evaluate these differences because they result from a combination of several risk factors. (5) In 2017 CKD was ranked in top 30 of all diseases regarding prevalence, mortality, years of life lost (YLL), years lived with disability (YLD) and disability-adjusted life years (DALY, a composite of YLL and YLD). (6)

1.1.3 Pathophysiology

The filtering function of the kidney is located in the glomerular barrier of a nephron. The glomerular filtration barrier is composed of the endothelial cell layer, glomerular basement membrane (GBM), and the filtration slit in the podocyte foot process layer. (7)
Enhanced renal blood flow (RBF) causes a rise in glomerular filtration rate (GFR) as well as metabolic demand by increasing tubular reabsorption. The kidney relies on two mechanisms to match blood flow to the metabolic and functional demand of its tissue. These mechanisms include the myogenic response (MR) and tubuloglomerular feedback (TGF) response. TGF regulates the preglomerular

vascular resistance according to luminal sodium-chloride (NaCl) concentration, which is sensed from the cells of macula densa. (8) High luminal sodium-chloride activates TGF and reduces GFR. (9)

In the case of kidney disease with nephron loss the single nephron glomerular filtration rate (SNGFR) increases as a compensatory mechanism. This is often accompanied by an increase of the intracapillary hydraulic pressure.

Over time, the single-nephron glomerular hyperfiltration leads to permeability changes in the capillary membrane and podocyte injury. The resulting damage and scarring in the kidney filtration units presents as progressive decrease of the overall GFR and proteinuria in an individual. (7)

Proteinuria is not just a consequence of a damaged glomerular barrier and a marker for the level of the existing kidney injury. Data from experimental studies suggest that proteinuria itself leads to the inflammation of glomeruli by upregulation of growth factors, cytokines, vasoactive substances and even activation of complement system. This results in interstitial fibrosis and therefore damage of the kidney filtration function. (10)

1.2 Kidney replacement therapy (KRT)

Progression of CKD to kidney failure can present as progressive uraemia, anaemia, mineral and bone disorder, volume overload with electrolyte imbalances and acidaemia and lead to death without proper treatment. Treatment options include supportive care or kidney replacement therapy either in form of dialysis or kidney transplantation. (11) Initiation of KRT is recommended when one or more of the following are present: (1)

1. Symptoms or signs of kidney failure: serositis, pruritus, acid-base, or electrolyte imbalances
2. Incontrollable blood pressure or volume status
3. Cognitive impairment
4. Unmanageable deterioration of nutritional status

Haemodialysis is the predominant dialysis form and at the same time the most common form of KRT worldwide irrespective of the economic condition in a country. On the other hand, peritoneal dialysis is less available and was estimated to be the

chosen dialysis form in only 11% of patients receiving long-term dialysis worldwide in 2018. The quality of life of patients on long-term dialysis is significantly lower than that of the general population. Despite a declining trend over the past years, the mortality rate of dialysis continues to be very high. Main causes of death in dialysis patients are cardiovascular events and infection. (12)

1.2.1 Kidney transplantation

Kidney transplantation provides a longer and improved quality of life than other forms of kidney replacement therapy. This remains true regardless of the chosen immunosuppressive regimen post-transplantation. (13)
Therefore, all patients who are expected to reach end-stage kidney disease should be informed and considered for kidney transplantation. (14)
Adult patients with a GFR of <20 ml/min/1.73 m^2 and irreversible CKD, expected to progress over the preceding 6-12 months, should be considered for living donor preemptive kidney transplantation. (1)
The Global Observatory on Organ Donation and Transplantation estimated 92 532 kidney transplantations in 2021, making it the most frequent transplanted organ of all 144 302 solid organ transplantations in the same year. These data represented 76% of the global population. In comparison to 2020 the number of kidney transplantations increased by 14.3%. (15)
The Eurotransplant organization, consisting of eight European Union countries, reported 2819 kidney transplantations in 2022. (16) Only in Austria 337 kidney transplantations took place in 2022, while 587 patients were actively waiting. (17)
Generally, the number of patients waiting for a kidney transplantation has always been higher than the number of kidneys available. To overcome the organ shortage, lower quality donor kidneys started to gain attention. Hence, donor kidney quality classified either as standard-criteria donor (SCD) or expanded-criteria donor (ECD). (18)
Later in 2014 the kidney donor profile index (KDPI) system as a quality indicator for deceased donor kidneys was introduced. Following 10 donor characteristics (age, height, weight, ethnicity, history of hypertension or diabetes, cause of death, serum creatinine, hepatitis C virus (HCV), and donation after cardiac death (DCD)), a risk

score of 1 to 100 gets assigned to the donor. This score, known as kidney donor risk index (KDRI), is translated to a percentage which indicates KDPI. Donor characteristics of ECD kidneys can be viewed equivalent to a KDPI of ≥ 85. (18)
It has been shown that donor after cardiac death (DCD) kidneys had a higher rate of delayed graft function (DGF) but do not have increased rate of graft failure or patient mortality when compared to donor after brain death (DBD) kidneys. (18)
Nevertheless, graft and patient survival rates have improved over time independent of recipient dialysis duration, body mass index (BMI), incidence of post-transplant diabetes, recipient and donor age, deceased kidney donations, and degree of HLA presensitization. This has come due to lower acute rejection rates, consequent monitoring for infections and antiviral prophylaxis, more precise cross-matching, and paired-organ transplantation. (19)
Living donor transplantation has been associated with better allograft survival compared to deceased donor kidney allografts. (20)
A big advantage of living donor kidney transplantation is the possibility of pre-emptive implementation without undergoing dialysis. This has made it the preferred form of transplantation in infants and children. (21)

1.3 Immune system – overview

The immune system includes mechanisms to defend the host against foreign organisms which could cause disease. We differentiate between the innate and adaptive immune response based on their specificity and speed of reaction. The innate immunity reacts quickly after pathogen exposure while the adaptive immunity needs several days or weeks to develop. (22)

1.3.1 Innate immunity

The innate response takes place immediately after pathogen exposure and consists of cells (monocytes, granulocytes, and macrophages), complement system, cytokines, and acute phase proteins. Vertebrates have pattern recognition receptors (PRRs) on the cell surface or in the cytoplasm, which serve to distinguish pathogens. They recognize a broad array of molecular patterns such as lipopolysaccharides and lipotechoic acid on bacteria and mannans on the surface

of yeast cells. However, PRRs induce an innate response which lacks precision and attacks also healthy tissues. (22,23)

Macrophages get activated in the very early stages of infection or tissue damage and release cytokines. This stimulates bone marrow and creates neutrophil leucocytosis. Circulating neutrophiles localize at the site of infection with the help of proinflammatory mediators, chemokines, chemoattractants and adhesion molecules. They kill pathogens after they have phagocytosed them.

The complement system is made of at least 20 factors and once activated it kills pathogens by osmotic lysis. Additionally, it aids the inflammatory response by increasing vascular permeability and enabling tissue infiltration with other immune cells and molecules. Other immune cells like eosinophils, basophils, mast cells and natural killer cells do not perform phagocytosis. After recognizing pathogens in distinct ways, they either kill pathogens directly by releasing cytotoxic granules or aid the inflammatory response through the release of mediators. (22)

Cytokines on the other hand, are small molecules and have the function of messengers. Chemokines, interleukins, and colony-stimulating factors are all cytokines with distinct functions. Chemokines are involved in the chemoattraction of cells, interleukins are messengers between leukocytes and colony-stimulating factors induce differentiation and proliferation of stem cells. (23)

1.3.2 Adaptive immunity

The specific immune response is carried out by T and B lymphocytes, which have antigen-specific receptors on their cell surface and thus can react precisely to pathogens and memorize them. Both cell lines are produced in the bone marrow. Afterwards, T cells migrate to the thymus, where their early development takes place. Rearrangement and splicing of multiple DNA segments during this phase, leads to the production of over 10^8 T cell receptors and 10^{10} antibody-specificities. After their early development, T and B cells are still naïve because they haven´t been exposed to their antigens yet. They locate in secondary lymphoid tissues, which are continuously in contact with other tissues via the lymphatic and vascular system. Antigen-presenting cells present antigens to T cells in lymphoid tissues after they have bound them to a self MHC molecule.

Aside from this, stimulation of T cell coreceptors like CD28, CTLA-4 and CD40, is indispensable for T cell activation and proliferation. Without coreceptor stimulation, activated T or B cells would undergo programmed cell death. (22)

T cells differentiate in two types of effector cells: $CD4^+$ T helper cells and $CD8^+$ cytotoxic T cells. The complex of antigen with MHC class I is recognized by $CD8^+$ T lymphocytes, while $CD4^+$ T lymphocytes recognize antigen presented with MHC class II. $CD4^+$ T helper cells secrete cytokines which induce the proliferation of cytotoxic T cells and activate other cells. $CD8^+$ cytotoxic T cells kill the recognized cells directly.

The early development for B cells takes place in the bone marrow. Their activation is similar to that of T cells. The maturation of B cells to antibody-producing cells takes place mostly with the help of T cells, although a few antigens can induce this process directly. Antibodies of B cells neutralize toxins, activate complement system, prevent adherence of microorganisms to mucosal surfaces, mark bacteria for phagocytosis and infected or tumour cells for cytotoxic attack by other immune cells. (22)

1.3.2.1 Alloimmune response

Three signal types are essential to the alloimmune response after organ transplantation:

- Signal 1 describes the interaction of T-cell receptor with major histocompatibility complex (MHC) molecules needed for antigen recognition
- Signal 2 consists of costimulation through binding of CD28 on T cells with CD80 or CD86 on antigen presenting cells
- Signal 3 consists of cytokine mediated signals

Antigen-recognition through T cells can follow a direct, semi-direct or indirect pathway. In direct allorecognition, dendritic cells of the donor travel from the graft to recipient lymph nodes to present peptide-MHC complexes to recipient T-cells. However, donor-derived dendritic cells do not survive for a long time after transplantation. Indirectly, the recipient dendritic cells invade the graft to capture donor antigens and present them later to T cells in secondary lymphoid organs. The semi-direct way describes antigen presentation through recipient dendritic cells to T cells, after they have acquired donor peptide-MHC complexes from donor

extracellular vesicles. (24) Signal 2 presents the simultaneous engagement of CD28 receptors on T cells by dendritic cells. Signal 1 and signal 2 activate three other cascades, including the calcium-calcineurin pathway. This triggers production of molecules, such as interleukin-2, CD154 and CD25. IL-2 and other cytokines further activate the “target of rapamycin” pathway, which stimulates lymphocyte proliferation. (25)

After being exposed to antigens, $CD4^+$ T cells differentiate and take over different functions including support for cytotoxic $CD8^+$ T cells and B cells in antibody production. $CD8^+$ T cells attack foreign cells directly via release of certain molecules or induce apoptosis through interactions on the cell surface.

Cellular allograft rejection following transplantation is mediated by T lymphocytes, while B cells mediate humoral rejection by producing alloantibodies against donor HLA antigens. (24)

Aided by $CD4^+$ T cells activated B cells can develop into short-lived plasma cells, memory B cells, or germinal center (GC) B cells. Short-lived plasma cells are quick in producing antigen-specific antibodies. Memory B-cells can develop independently of T cells and get activated only by polysaccharide antigens.

GC-B cells form germinal centers (GC) together with $CD4^+$ T cells. Subsequently they can develop into long-lived plasma cells, GC-dependent memory B cells or recirculate back into the germinal center.

Memory B cells and long-lived plasma cells are long-term sources of donor-specific antibodies (DSA), which mediate graft rejection. (26)

1.4 Immunosuppressive therapy after kidney transplantation

Immunosuppression in the recipient is essential for allograft tolerance after transplantation and graft function in long-term. The immunosuppressive regimen after kidney transplantation includes the induction and maintenance therapy. The immunosuppressive drug class includes a variety of substances such as glucocorticoids, small molecules, lymphocyte depleting and non-depleting proteins and immunoglobulins. (25)

In this work, we will provide a short description of the most commonly used immunosuppressive drugs in kidney transplant recipients in Europe and USA.

1.4.1 Induction therapy

The induction immunosuppressive therapy takes place before, during or right after transplantation surgery and aims to prevent acute allograft rejection. In this phase, patients receive biological agents in addition to their standard immunosuppressive regimen. (14)

Interleukin-2 receptor antagonists are recommended as first-line induction therapy. The choice between interleukin-2 receptor antagonists (IL2-RA) and T-cell (lymphocyte) depleting antibodies as biological agents, is based on the immunological risk of the kidney transplant recipient. For patients under high immunological risk T-lymphocyte depletion is recommended. (13,14)

1.4.1.1 Interleukin-2 receptor antagonists

The humanized monoclonal antibody Basiliximab targets CD25 or the α-subunit of the IL-2 receptor on the surface of T-lymphocytes. Antagonization of this receptor disrupts the proliferation of activated T-cells. (27)

Basiliximab serves a biological agent in induction therapy and its use in case of an acute cellular rejection is not warranted. (28)

1.4.1.2 Anti-lymphocyte thymoglobulin

Thymoglobulin is used in induction and acute rejection therapy. Through immunization of horses and rabbits with human thymocytes, polyclonal antibodies

against the latter are produced and then extracted as equine (eATG) or rabbit (rATG) antithymocyte globulines. (29)
The polyclonal antibodies predominantly deplete T-cells through complement-dependent lysis and T cell activation-induced apoptosis. To some extent they also target B-cells, neutrophils, and monocytes. (27)
Aside from immunodeficiency, it can cause cytokine-release syndrome thrombocytopenia, serum sickness and allergic reactions. (25)

1.4.2 Maintenance therapy

Maintenance immunosuppression describes the long-term immunosuppressive treatment and includes an early and late phase. The early maintenance period is characterised by tapered immunosuppression usually at three to six months after transplantation. The late maintenance period represents mostly the period beyond three to six months post-transplantation, when immunosuppressive therapy has been tapered to long-term levels. (13)
The KDIGO recommended maintenance immunosuppressive regimen after kidney transplantation includes calcineurin-inhibitors, antiproliferative agents and corticosteroids. (14)

1.4.2.1 Calcineurin-inhibitors (CNI)

Cyclosporine A (CsA) and tacrolimus (Tac) form a complex with cytosolic proteins and inactivate this way the serine-threonine phosphatase calcineurin. The inhibition of calcineurin prevents the dephosphorylation of nuclear factors which induce the transcription of various cytokine genes including interleukin-2. (29)
Adverse effects such as neurotoxicity, infections, hypertension, hyperlipidemia, posttransplant diabetes mellitus and hypertension are common under CNI treatment. (30) While hyperlipidemia, hypertension, gingival hyperplasia, skin changes and hirsutism are more likely to occur under cyclosporine treatment, tremor and post-transplant diabetes are more common with tacrolimus. (25)
Both tacrolimus and cyclosporine are well known for their nephrotoxic effects. Histologic morphology in acute CNI nephrotoxicity include vacuolization, acute arteriolopathy, and thrombotic microangiopathy. In kidney-biopsies with chronic CNI

nephrotoxicity interstitial fibrosis, tubular atrophy, arteriolar hyalinosis, tubular microcalcifications and glomerulosclerosis were found. (31)

1.4.2.2 Glucocorticoids

Receptor-bound effect of glucocorticoids is that they block transcription factors like NF- κB and activated protein-1. This inhibits the production of various cytokines, such as IL-2, IL-5, IL-1 and TNFα. This prevents the differentiation of T-helper cells and leads to their apoptosis, induces the apoptosis of eosinophiles and disrupts the function of macrophages.

Glucocorticoids hinder neutrophile migration, increase their secretion in bone marrow and decrease their apoptosis. These mechanisms induce a leucocytosis. However, the overall impact on neutrophiles is far less pronounced than their effects on other cell lines. (27)

They cause many side effects, which can be dermatological, ophthalmological, endocrinological, metabolic, gastrointestinal, musculoskeletal, and neuropsychiatric. Therefore, in kidney transplantation corticosteroids are either tapered or completely withdrawn after the initial perioperative administration.(28)

In patients with low immunological risk, the rapid withdrawal of steroids did not result in a higher rate of biopsy-proven acute rejection (BPAR) or decreased graft function. (32)

1.4.2.3 Antiproliferative immunosuppressant drugs

Antiproliferative agents inhibit purine base synthesis, which is essential for T- and B-cell proliferation. (28)

1.4.2.3.1 Azathioprine (AZA)

Azathioprine is a prodrug of 6-mercaptopurine, which acts as a purine analog. It inhibits DNA and RNA synthesis and affects immunomodulation by interfering with the de novo purine synthesis. Azathioprine is the treatment of choice in pregnancy setting because it hasn´t been linked with teratogenicity. It´s side effects are bone marrow suppression and gastrointestinal intolerance. (25)

Patients with a reduced activity of the metabolizing enzyme thiopurine-S-methyltransferase due to genetic polymorphism are at risk for bone marrow suppression. (33)

1.4.2.3.2 Mycophenolic acid (MPA)

Mycophenolate mofetil and mycophenolate sodium are prodrugs of mycophenolic acid. They are potent but reversible and noncompetitive inhibitors of the enzyme inosine monophosphate dehydrogenase (IMPDH). These drugs act as cytostatic on T and B cells by limiting the production rate of guanosine nucleotides, supress the formation of antibodies and limit the production of glycoproteins by lymphocytes and monocytes. (28)

Mycophenolate has gastrointestinal and hematologic side effects. The enteric-coated formulation aimed to reduce the gastrointestinal consequences. It has been shown that mycophenolate is better than azathioprine in preventing graft rejection, making it the recommended first-line antiproliferative agent in kidney transplantation. (14,25)

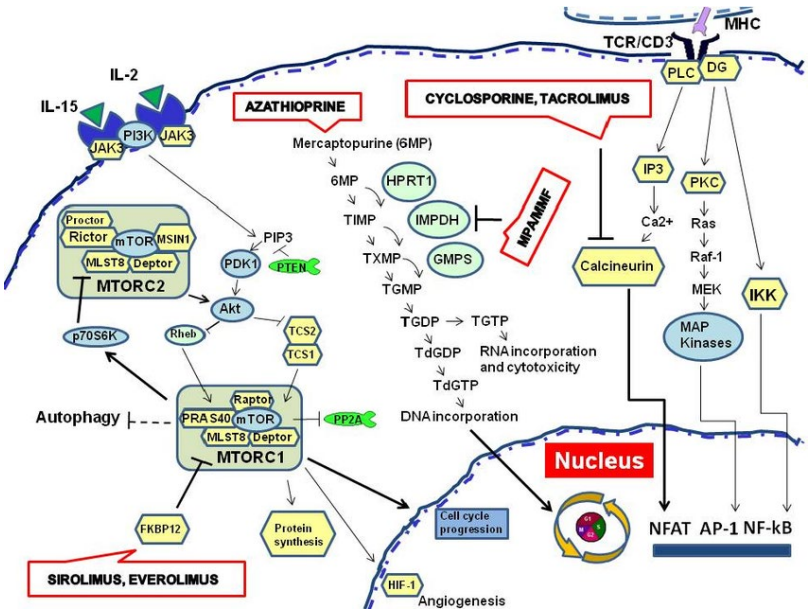


Figure 1: Immunosuppressive agents in kidney transplantation

Source: Zaza G, Granata S, Tomei P, Dalla Gassa A, Lupo A. Personalization of the immunosuppressive treatment in renal transplant recipients: the great challenge in “omics” medicine. International journal of molecular sciences. 2015 Feb 17;16(2):4281-305. (34)

1.5 Tacrolimus

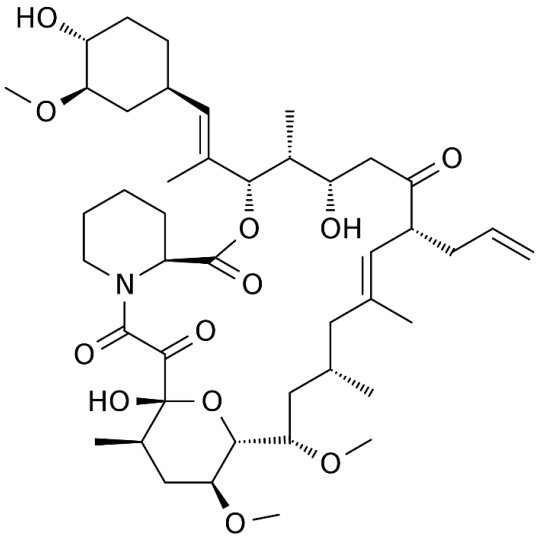


Figure 2: Structure of tacrolimus
Source: www.wikipedia.com/tacrolimus

1.5.1 Pharmacodynamics

The calcineurin-inhibitor tacrolimus (also Tac or FK-506) is a macrolide antibiotic and a product of *Streptomyces tsukubaensis*. (35) This fungus strain was isolated for the first time in 1984 from a soil sample in Tsukuba, Japan. (36) Inside the cell tacrolimus binds to a cytosolic immunophilin, the FK-binding protein (FKBP), inhibiting this way the phosphatase calcineurin. An increase of intracellular Ca^{2+} levels activate calcineurin. An active calcineurin dephosphorylates the nuclear factors of activated T cell (NFAT)c family, NFATC1 and NFATC2. These transcription factors are active when dephosphorylated and move to the cell nucleus. In the nucleus they initiate the transcription of interleukin-2 (IL-2), interferon gamma (IFN-γ) and other cytokine-like genes.
Inhibition of calcineurin prevents the activation of T-helper cells and production of IL-2. Inhibition of IL-2 synthesis leads to decreased clonal proliferation of T cells. (37,38)
In the ELITE-SYMPHONY study the immunosuppressive regimen with steroids, mycophenolic acid (MPA) and low-dose tacrolimus showed better kidney function and the lowest acute rejection rates in comparison to regimens with cyclosporine or

sirolimus. A regimen with tacrolimus also resulted in better allograft survival than with cyclosporine or sirolimus. (30)
Another multicentred study compared two sirolimus based regimens to a regimen with tacrolimus and mycophenolate mofetil (MMF). Despite nephrotoxicity of CNIs, a better graft survival could not be achieved with sirolimus based regimens. (39)
These and other studies which proved the advantage of tacrolimus have led to the replacement of cyclosporine and have made tacrolimus a first-line calcineurin-inhibitor in immunosuppressive regimens after kidney transplantation. (14)

1.5.2 Pharmacokinetics

The first-pass metabolism of calcineurin-inhibitors is regulated mainly by cytochrome P450 enzymes and P-glycoprotein (Pgp).
The intracellular monooxygenases CYP3A5 and CYP3A4 metabolise tacrolimus by various catalysing processes. P-glycoprotein is found in the cell membrane and is ATP-dependent. It works as an efflux pump for tacrolimus by carrying it back to the intestinal lumen.
The second-pass metabolism takes place in the liver and involves demethylation, glucuronidation, sulfation, acetylation, and conjugation processes.
Some among the various clinical aspects which affect tacrolimus metabolism include age, gender, ethnicity, intestinal pathology, food, concomitant use of other drugs, galenic of tacrolimus formulation and time from transplantation. (40–42)
Tacrolimus is known for its high pharmacokinetic variability and narrow therapeutic window. Not only different individuals show different tacrolimus pharmacokinetics, but this variability manifests also within the same patient. While overexposure to tacrolimus poses the risk of drug toxicity, underexposure means increased risk for allograft rejection. This makes its therapeutic drug monitoring (TDM) essential in clinical practice, while taking into consideration that under- and overexposure are possible also within the therapeutic range. (42)
Tacrolimus trough concentration levels (C0) have shown an adequate correlation with the area under the concentration-time curve (AUC) and are clinically used to assess drug exposure. (43)

The current KDIGO clinical practice guideline recommends measurement of 12-hour trough concentrations (C0) for therapeutic drug monitoring. (1) However, this concerns only twice-daily tacrolimus formulations. During treatment with once-daily formulations tacrolimus trough concentrations are measured every 24 hours. (44,45)

However, therapeutic drug monitoring has only limited say since it cannot predict individual dose requirement to reach certain target trough concentrations. It is also dependent on the method used and cannot mirror unbound blood concentrations of tacrolimus. (40,42)

In attempt to simplify the clinical handling of tacrolimus, Thölking et al used the ratio between trough concentration and corresponding daily dose to define three metabolizer groups for tacrolimus. This presented an approach to tailored immunosuppression since fast metabolizers required higher tacrolimus doses to reach target trough concentrations. (46)

Yet, the Tac daily dose is prescribed to the kidney recipient by solely adjusting it to body weight as recommended by the manufacturer. This is accompanied by under- and overexposure especially in the first days after transplantation.

Only recently, a dosing algorithm for tacrolimus (which integrates pharmacokinetics, pharmacogenetics, age, and hematocrit) has been evaluated. Following dose administration according to this algorithm, target Tac trough levels were achieved in 55% of patients on day 5 post-transplantation. Meanwhile, only 20% of patients receiving body weight adjusted Tac doses had reached target Tac levels at the same timepoint. (47)

1.5.3 Pharmacogenomics

While CYP3A4 is a determinant in ciclosporin metabolization, the enzyme CYP3A5 plays a main role for tacrolimus.

Therefore, genetic polymorphisms of these enzymes also influence the pharmacokinetic profile of this drug class. Patients who are CYP3A enzyme expressors (carriers of at least one CYP3A5*1 allele) require higher tacrolimus doses, implying a faster metabolism rate for tacrolimus, than non-expressors (homozygous CYP3A5*3 patients). (41)

The ABCB1 or MDR gene encodes for the efflux pump Pgp and also undergoes many polymorphisms but their association to the pharmacokinetics of tacrolimus could not be proved in most studies and the findings have been inconsistent across different studies. (48,49)
Research outcomes regarding the influence of genetic polymorphisms of CYP3A5 and Pgp on pharmacodynamic aspects including acute rejection, nephrotoxicity, neurotoxicity, new-onset diabetes mellitus and hypertension have not been conclusive. (41)

1.5.4 Tacrolimus formulations

In this work we will further describe three different formulations of tacrolimus: Prograf® (immediate-release tacrolimus, IR-Tac), Advagraf® (extended-release tacrolimus, ER-Tac) and Envarsus® (novel once-daily tacrolimus, LCP-Tac). Immediate-release tacrolimus (IR-Tac) requires a twice-daily administration, while extended-release (ER-Tac) and novel once-daily extended-release (LCP-Tac) tacrolimus formulas require a once-daily administration. (50)

1.5.4.1 IR-Tac, Prograf®

Immediate-release tacrolimus with the market name Prograf® (Astellas Pharma US, Inc., Northbrook IL, Tac-IR), was the initially launched tacrolimus formulation and gained approval for use in kidney transplantation in 1997.
In capsule form, Prograf® is available in 0.5mg, 1mg and 5mg dose strengths.
Its complex pharmacokinetic profile leading to high variability of drug exposure between and within patients has been known for very long. Additionally, side effect profile and patient adherence have been components with room for improvement. For this reason alternative formulations with the aim to ameliorate the aforementioned aspects were developed. (36)

1.5.4.2 ER-Tac, Advagraf®

The very first extended-release (or prolonged-release) formulation of tacrolimus with a once-daily administration was Advagraf® (Astellas Pharma, US, Inc., Northbrook, IL, Tac-ER)). It received approvement for kidney transplantation in 2013. (36) It is also known under other market names depending on the country, due to requirement for a national authorization. (44,51)

The formulation contains, in addition to tacrolimus, specific concentrations of the excipients ethylcellulose, hypromellose and lactose monohydrate. This leads to a controlled water penetration and thus slower drug release. These hard gelatine capsules are available in 0.5mg, 1mg and 5mg of tacrolimus. (51)

An early review compared the pharmacokinetic profile, efficacy, adverse events, and utility of Advagraf® and Prograf®. Even though a conversion ratio of 1:1 is recommended when switching between formulations, studies showed that Prograf® and Advagraf® are not completely equivalent. Lower trough concentrations and overall drug exposure had been achieved with Advagraf® in multiple studies on stable and de novo kidney transplant recipients. This held especially true in the early post-transplantation period. (51)

Contrary to the twice-daily formulation, Advagraf® reaches only one peak blood concentration which still corresponds to the first peak of Prograf®. (52)

The long suggested improved patient compliance with a once-daily tacrolimus formulation was finally confirmed in a belgian trial. Medication intake in patients, who were switched from twice-daily tacrolimus formulation Prograf® to Advagraf®, was monitored using an electronic device. The results showed an improved therapy adherence with Advagraf®.

However, when patients missed a dose after being switched to Advagraf®, the total number of a full day without treatment was higher than under twice-daily Prograf® treatment. (53)

1.5.4.3 LCP-Tac, Envarsus®

Envarsus® (LCP-Tac or LCPT; Envarsus XR [Envarsus in Europe]; Veloxis Pharmaceuticals, Inc., Edison, NJ) is available in three tablet strengths, each of them containing 0.75mg, 1mg and 4mg of the active substance. This product was developed using MeltDose® technology and contains tacrolimus powder dispersed in a polymer matrix of polyglycols. This has promoted a controlled drug release and an improved solubility and bioavailability. (36,45)

The first phase III clinical trial comparing outcomes in KTRs receiving Prograf® to KTRs converted from Prograf® to Envarsus®, showed no differences regarding biopsy-proven acute rejection (BPAR), graft failure, patient death, or loss to follow-up.

Safety profile of Envarsus® was similar to that of Prograf®. Adverse events such as diarrhea, urinary tract infection, increased blood creatinine, nasopharyngitis, headache, upper respiratory tract infection, peripheral edema and hypertension were not more common with Envarsus®. Furthermore, no differences in laboratory parameters (glucose level, leukocyte and platelet count, cholesterol, and triglyceride level, eGFR), opportunistic infections, malignancy, and incidence of new-onset diabetes mellitus (NODM) between treatment groups were noticed. (54) Similar findings were observed for the first year post-transplantation in de novo KTRs receiving Envarsus® when compared to KTRs receiving treatment with Prograf®. (55)

Phase III trials confirmed lower dose requirements and improved bioavailability of Envarsus® compared to Prograf®. Conversion from Prograf® to Advagraf® or Envarsus® did not show any improvement on the incidence rate of diabetes. Kidney function was achieved after converting from Prograf® to Advagraf®. However, adverse events of tacrolimus are related to the level of drug exposure. Since conversion to Envarsus® led to an overall reduction of drug exposure, the reason of better kidney function is not clear. (36)

Comparison of Advagraf® to Envarsus® showed similar safety profiles. Again, lower daily doses were required with Envarsus® than with Advagraf®. Peak concentrations and peak-to trough fluctuations were lower with Envarsus®. (56)

After evaluation of the pharmacokinetic profile of all three formulations, a dose reduction of 30% for Prograf® and 36% for Advagraf® to reach comparable drug exposure after switching to Envarsus® is recommended.

The peak blood concentration of Envarsus® is reached 4-6 hours after administration and is lower than the peak concentrations of the former two formulations. (50)

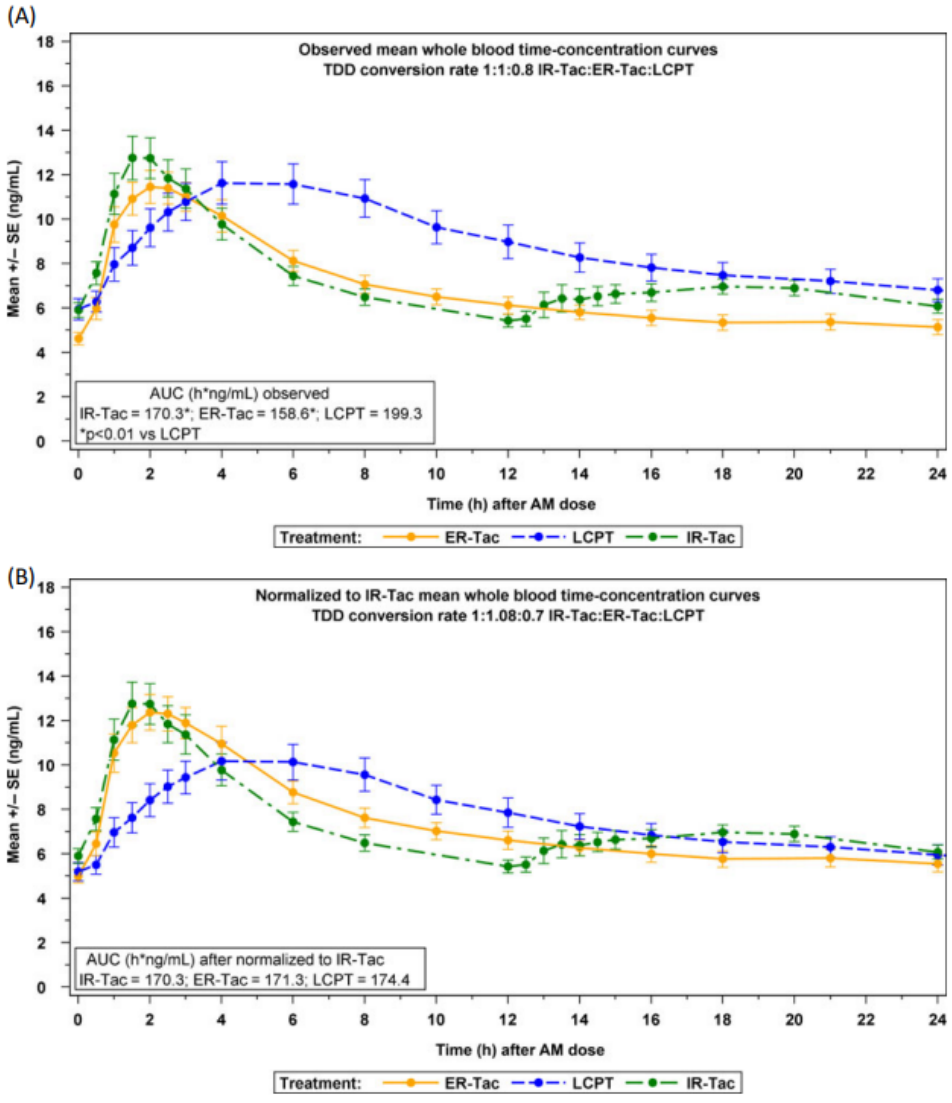


Figure 3: Pharmacokinetic profile of Prograf®, Advagraf® and Envarsus®

Conversion rate between formulations was 1:1:0.8 respectively.

A) Observed mean Tac concentration curves B) Mean Tac concentration curves normalized to area under the curve (AUC). (50)

1.6 Complications in the first year post-transplantation

Primary causes for delayed allograft function vary depending on the time frame after transplantation.

Acute tubular injury (ATI), hyperacute rejection or problems associated with the surgical procedure such as acute vascular thrombosis, dehiscence or leaks of anastomosis, perinephric hematoma or fluid collection characterize the immediate post-transplantation period (≤3 days since implantation).

The early post-transplantation period, which includes the first 3-30 days after transplantation, is shaped by acute cell-mediated rejection, acute antibody-mediated rejection, and calcineurin-inhibitor toxicity.

Apart from acute rejection and calcineurin inhibitor toxicity, allograft dysfunction during the first year after transplantation might also be caused by recurrence of preexisting diseases in the host and by viral infections. (57)

1.6.1 Graft rejection

Biopsy is indispensable for diagnosing the cause of graft dysfunction. The Banff Classification System of Allograft Pathology is internationally the most frequently used classification system to report histopathological changes of kidney grafts. It classifies two main forms of rejection: T cell mediated rejection (TCMR) and antibody-mediated rejection (ABMR). (58,59)

1.6.1.1 Acute graft rejection

In TCMR activation and expansion of T lymphocytes is induced by HLA epitope mismatches and costimulatory molecules and cytokines. Kidney graft interstitium, tubules, arteries of the graft and in severe cases also glomeruli get infiltrated by mononuclear cells, leading to tubulitis, interstitial edema and endarteritis. This from of allograft rejection has been reduced to <10% in the first post-transplantation year owing to improved immunosuppression regimes. (57)

Antibody-mediated rejection (ABMR) is characterized by the presence of donor-specific anti-HLA antibodies (DSA), which are either preformed or develop later after transplantation. Another acknowledged marker for antibody-mediated rejection is positive C4d staining, although it carries a low sensitivity. Non-HLA antibodies stand

among other methods with a diagnostic potential and could be used when anti-HLA antibodies cannot be detected. (60)

Acute ABMR gets induced by high levels of circulating DSA, whereas in chronic ABMR endothelial cells are exposed to gradually increasing levels of DSA. The latter leads to a more moderate cell injury and inflammatory response, enabling onset of graft accommodation and repair mechanisms. (61)

The histological features of acute antibody-mediated rejection involve dilated capillaries, vacuolization of endothelial cells and presence of various activated cells such as monocytes, T cells, macrophages, natural killer cells, neutrophils and eosinophiles. Available treatments for this form of graft rejection are currently plasma exchange, intravenous immunoglobulins, and glucocorticoids. As the long-term outcomes are yet unfavourable, attempts for new treatment options are being made. (60)

Younger recipient, older donor, African-American ethnicity, blood-group incompatibility, number of HLA mismatches, panel reactive antibodies and donor-specific antibodies, cold ischemia time > 24 hours and delayed graft function are all risk factors associated with acute graft rejection. (14)

1.6.1.2 Chronic graft rejection

Chronic graft rejection expresses as a gradual decline in kidney graft function and typically starts at one-year after transplantation.

Inflammation in areas with interstitial fibrosis and tubular atrophy (i-IFTA) are a feature of chronic TCMR. (62)

In TCMR the most observed phenotype was T_H1 cells, supposedly influenced by recipient DCs. Dendritic cells in rejection seem to have a different monocyte precursor than those involved in infection. (24)

Chronic antibody-mediated rejection is recognized mainly as vascular lesions, which in kidney allografts are represented by glomerulopathy and arteriopathy. (60) Chronic ABMR is mediated by DSA which already exist in the KTR or by newly developed de novo DSA. Insufficient immunosuppression or early TCMR enable the development of de novo DSAs. (61)

1.6.2 Virus infections

KTRs are more susceptible to infections than the general population due to the continuous immunosuppressive treatment. Common viral infections in KTRs are infections with cytomegalovirus, BK polyoma virus, Epstein-Barr virus (EBV), Varicella-Zoster virus (VZV) and herpes simplex virus (HSV). (13)

1.6.2.1 Cytomegalovirus infection

Cytomegalovirus (CMV) infection is diagnosed when the virus is detected in peripheral blood. We speak of cytomegalovirus disease when clinical signs and symptoms are also present. General symptoms of CMV disease are fever, malaise, leukopenia, and thrombocytopenia. In tissues it can manifest itself as pneumonia, gastrointestinal disease, chorioretinitis, hepatitis, pancreatitis, encephalitis, myocarditis, nephritis, cystitis, or mucocutaneous disease. (63)

KTRs at higher risk for experiencing a CMV infection or disease are seronegative patients with a CMV-positive transplant, higher donor age, cyclosporine or antilymphocyte antibody treatment and compromised transplant function.(63)

The IgG serostatus is very essential in determining risk of CMV infection or disease. At highest risk are previously unexposed patients (R-) receiving an organ from a donor with a positive CMV serostatus (D+). (64)

For KTRs at high-risk, chemoprophylaxis with oral ganciclovir or valganciclovir is recommended for three months after transplantation or six weeks after treatment with T-cell depleting antibodies. (14)

Treatment in more severe cases consists of intravenous ganciclovir. In persistent and severe cases, a decrease of immunosuppression should be undertaken. Nucleic acid testing (NAT) in plasma and pp65 antigenemia should be utilized to monitor disease and define treatment duration. (14,63)

Apart from lower transplant and recipient survival, cytomegalovirus in KTRs is associated with other pathologies such as activation of other viruses, EBV-related posttransplant lymphoproliferative disease, Human Herpesvirus-8 (HHV 8) -related Kaposi sarcoma, kidney artery disease, posttransplant diabetes mellitus (PTDM), thrombotic microangiopathy and Guillain-Barré syndrome (GBS). (63)

1.6.2.2 BK-polyomavirus infection

BK polyomavirus (BKV) is widespread in the general population and has a prevalence of over 90% by the age of 4 years. In kidney transplant recipients a BK virus infection is either a new infection acquired from the donor kidney or a reactivation of a latent infection. In most cases it occurs in the first year post-transplantation, when immunosuppression is strongest. (65)

The common clinical presentation is viruria, which can progress to viremia. In the first 2 -6 weeks post-transplantation up to 50% of KTRs with viremia can develop BK virus-associated nephropathy (BKVAN). In just a few cases it manifests itself clinically as ureteral stenosis or hemorrhagic cystitis in KTRs. (65)

There is no specific treatment for BK viremia or BKVAN except for lowering of immunosuppression. Antimetabolite agents are the first component which should be reduced or completely withdrawn. In high viral load further persists, target trough concentration of CNIs should be reduced. This way the high rate of graft loss due to BKVAN has been reduced to 15% in the past two decades. Nonetheless, the need for more specific treatment is still present and expected to come in the future. (65)

a) **b)** **c)**

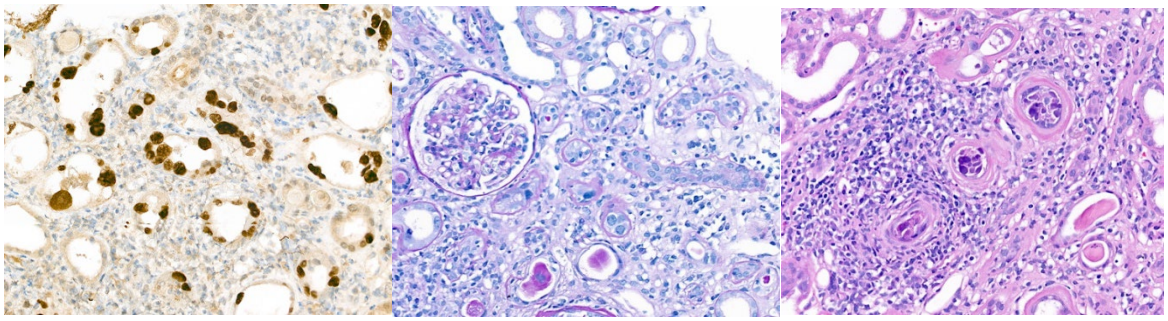


Figure 4: Histologic findings in patients with BKVAN

University clinic of Graz, department of nephrology. a) Decoy cells in H&E; b) Interstitial nephritis in PAS stain; c) Immunohistochemistry of BKVAN

2 Materials and methods

2.1. Study design

We analysed data of 88 patients, who had undergone kidney transplantation between September 2016 and August 2020 at the Transplant Centre Graz of Graz University Hospital as part of a prospective mono-centric observational trial. The aim was to analyse differences between patients treated exclusively with novel one-daily extended-release tacrolimus (LCP-Tac or LCPT, Envarsus®) and patients treated with immediate-release (IR-Tac, Prograf®) or extended-release formulation of tacrolimus (ER-Tac, Advagraf®) among other immunosuppressive medication after kidney transplantation.
This trial was approved by the Ethics Committee of the Medical University of Graz in Austria (vote number: 28-514 ex 15/16).

2.2. Study procedures

Study visits took place 1 week, 2 months and 12 months post-transplantation. Besides predefined study-visits, data from routine follow-ups during the first three months after kidney transplantation were included. Patients were grouped considering the tacrolimus formulation they received starting at one week post-transplantation. Dose-adjustments were permitted to reach pre-defined tacrolimus target trough concentrations throughout the entire follow-up period.

2.3. Study population

Patients recruited for this study were between 18 – 80 years of age, received kidney transplantation for the first time and provided a written informed consent. Pregnancy, immunosuppression up to three months prior to transplantation, kidney transplantation in the past and AB0-incompatible kidney transplant recipients were excluded from this study.

2.4 Data collection

Data for this thesis were collected retrospectively by using the hospital information system (HIS) OpenMEDOCS at LKH University Clinic of Graz. All patient data have been made anonymous for the purposes of this study.

2.5 Statistical analysis

Statistical analysis was performed using *IBM SPSS Statistics Version 28* and *GraphPad Prism Version 10.0.2.*

The Kolmogorov-Smirnov test was used to check for normal distribution of data. The mean and standard deviation were used to present parametric data, whereas non-parametric data were described using the median and interquartile range. Categorical data were shown as absolute and relative frequencies. Differences between groups were analysed by applying student´s t-test for parametric data and Mann-Whitney U test for non-parametric data. Chi-square test was used to analyse categorical data. Time to biopsy-proven acute rejection, cytomegalovirus and BK polyomavirus viremia were analysed using the Kaplan-Meier method and a log-rank analysis was used to assess differences between treatment groups.

The significance level was set at values of probability (p) less than 0.05. All P-values were two-sided.

2.6 Pathological analysis

All kidney allograft rejections were confirmed with kidney biopsies and were classified using the latest BANFF classification system.

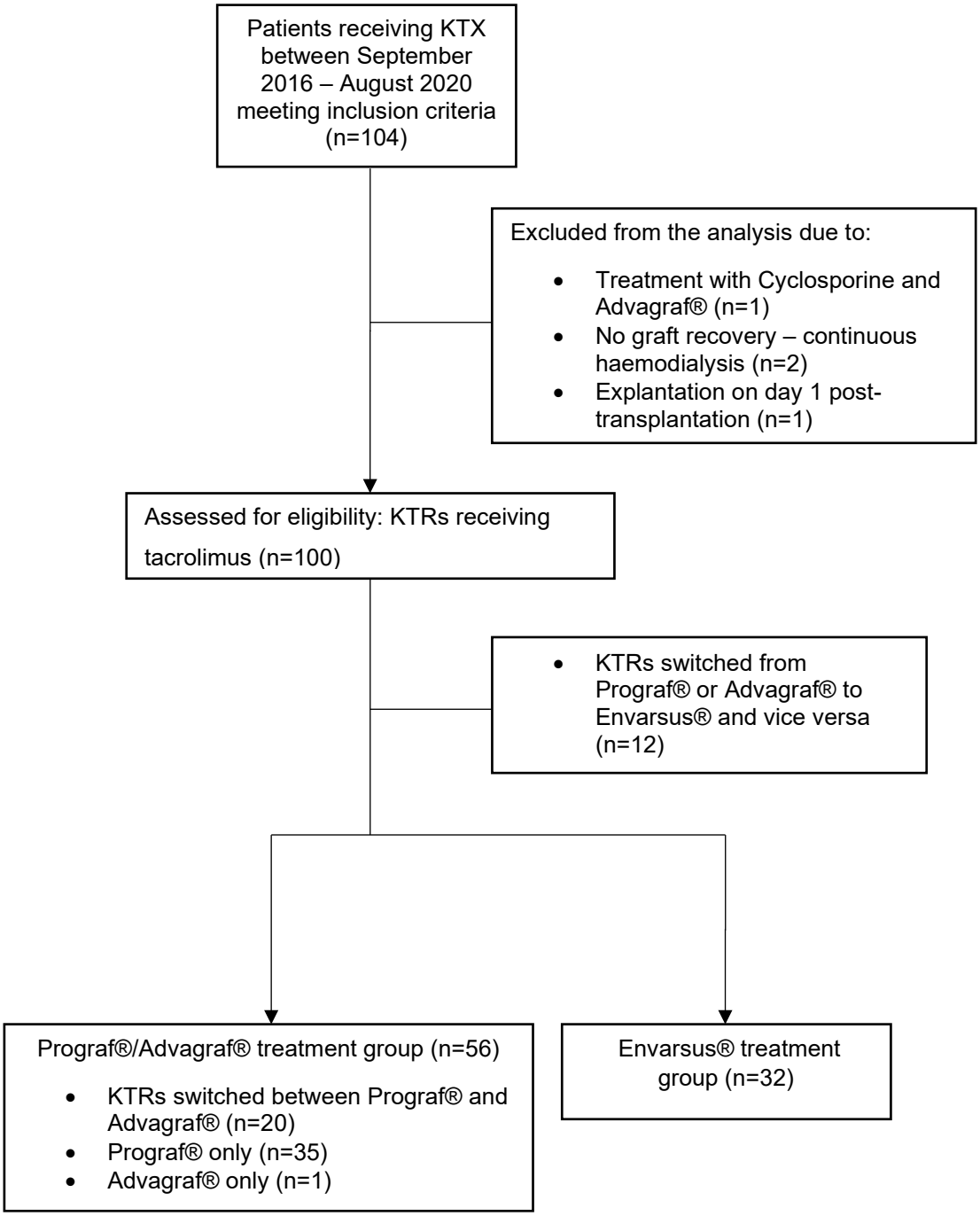


Figure 5: Flow chart of study participant recruitment

Division in two treatment groups was done considering the received tacrolimus formulation from one week post-transplantation. KTX: kidney transplantation; KTRs: kidney transplant recipients.

3 Results

3.1. Baseline characteristics

3.1.1 Prograf®/Advagraf® vs. Envarsus®

A total of 56 patients received treatment with Prograf® or Advagraf®. Nineteen patients were female and 37 male. Out of 32 patients, who received a treatment with Envarsus®, 13 were female and 19 were male. The mean age in the Prograf®/Advagraf® group was 55.4 ± 12.8 years and 59.3 ± 7.8 years in the Envarsus® group. The treatment groups did not differ regarding age, gender, height (170 ± 8.5 cm vs. 171.8 ± 8 cm respectively, p = 0.34) and BMI (27.9 ± 7.2 kg/m^2 vs. 27.8 ± 7.4 kg/m^2 respectively, p = 0.62).

There was a trend wise higher weight in KTRs receiving Envarsus® than Prograf®/Advagraf® (85 ± 25.5 kg vs. 79.3 ± 20.5 kg respectively, p = 0.73).

Recipient characteristics	Prograf®/Advagraf®	Envarsus®	p value
sample size N	56 (100%)	32 (100%)	
female N (%)	19 (33.9%)	13 (40.6%)	0.53
male N (%)	37 (66.1%)	19 (59.4%)	
age [years] M (SD)	55.4 (± 12.8)	59.3 (± 7.8)	0.08
weight [kg] MDN (IQR)	79.3 (± 20.5)	85 (± 25.5)	0.73
height [cm] M (SD)	170 (± 8.5)	171.8 (± 8)	0.34
BMI [kg/m2] MDN (IQR)	27.9 (± 7.2)	27.8 (± 7.4)	0.62
ethnicity N (%)			
Caucasian	52 (92.9%)	31 (96.9%)	
Turkish	1 (1.8%)	1 (3.1%)	
African	0 (0%)	0 (0%)	-
Asian	1 (1.8%)	0 (0%)	
Other	2 (3.6%)	0 (0%)	
smoking status N (%)			
non-smoker	30 (56.6%)	20 (62.5%)	
former smoker	12 (22.6%)	10 (31.3%)	
active smoker	10 (18.9%)	2 (6.3%)	-
unknown	1 (1.9%)	0 (0%)	

Table 1: Demographic and baseline characteristics of KTRs

BMI: body mass index; N: number.

Donor characteristics	Prograf®/Advagraf®	Envarsus®	p value
sample size N (%)	56 (100%)	32 (100%)	
female N (%)	26 (46.4%)	13 (40.6%)	0.60
male N (%)	30 (53.6%)	19 (59.4%)	
age [years] M (SD)	61 (±20)	55 (±20)	0.32
weight [kg] MDN (IQR)	75 (±19)	84 (±11)	0.00*
height [cm] M (SD)	170 (±18)	178 (±15)	0.16
BMI [kg/m2] MDN (IQR)	26.1 (±5.7)	26.3 (±4.3)	0.08
HLA mismatch N (%)			
0	1 (1.8%)	1 (3.2%)	
1	3 (5.4%)	0 (0%)	
2	4 (7.1%)	2 (6.5%)	
3	14 (25%)	10 (32.3%)	-
4	24 (42.9%)	12 (38.7%)	
5	10 (17.9%)	5 (16.1%)	
6	0 (0%)	1 (3.2%)	
Expanded-criteria donor (ECD)			
yes N (%)	33 (58.9%)	16 (50%)	0.42
no N (%)	23 (41.1%)	16 (50%)	
Donor after cardiac death (DCD)			
yes N (%)	2 (3.6%)	2 (6.5%)	0.61
no N (%)	54 (96.4)	29 (93.5%)	
Live donation			
N (%)	0	0	
Pediatric donor			
N (%)	0	0	

Table 2: Donor baseline and demographic characteristics

Donors of KTRs receiving Envarsus® had a significantly higher weight (84 ± 11 vs. 75 ± 19, p = 0.00), which corresponded accordingly to their KTRs showing a higher weight than KTRs in the Prograf®/Advagraf® group.

The data are presented as mean (M), standard deviation (SD), median (MDN) and interquartile range (IQR), absolute numbers (N) and relative percentages within group (%). Mann-Whitney U test was applied to non-parametric data and student´s t-test to parametric data. Chi-square test was used for the analysis of categorical data. Significance level $p < 0.05$.

N: number; BMI: Body Mass Index; HLA: Human Leukocyte Antigen; ECD: expanded-criteria donor; DCD: donor after cardiac death.

3.1.2 Past medical history

Components of medical history including underlying renal disease, need of pretransplant dialysis, dialysis duration and other relevant comorbidities of kidney-transplant recipients were consistent across both treatment groups.

	Prograf®/Advagraf®	Envarsus®	p value
Pretransplant dialysis			
yes N (%) no N (%)	55 (98.2%) 1 (1.8%)	29 (90.6%) 3 (9.4%)	0.14
Haemodialysis			
yes N (%) no N (%)	48 (85.7%) 8 (14.3%)	23 (71.9%) 9 (28.1%)	0.11
Peritoneal dialysis			
yes N (%) no N (%)	7 (12.5%) 49 (87.5%)	6 (18.8%) 26 (81.3%)	0.54
Dialysis duration			
Months MDN ± IQR	28 ± 23	29.5 ± 37	0.90
Diabetic nephropathy			
yes N (%) no N (%)	9 (16.1%) 47 (83.9%)	6 (18.8%) 26 (81.3%)	0.75
Hypertensive nephropathy			
yes N (%) no N (%)	4 (7.1%) 52 (92.9%)	4 (12.5%) 28 (87.5%)	0.46
Glomerulonephritis			
yes N (%) no N (%)	10 (17.9%) 46 (82.1%)	4 (12.5%) 28 (87.5%)	0.51
Hereditary polycystic kidney disease			
yes N (%) no N (%)	8 (14.3%) 48 (85.7%)	8 (25%) 24 (75%)	0.21
Other kidney diseases			
yes N (%) no N (%)	25 (44.6%) 31 (55.4%)	11 (34.4%) 21 (65.6%)	0.35
Allergies and intolerances			
yes N (%) no N (%)	16 (29.6%) 38 (70.4%)	10 (31.3%) 22 (68.8%)	0.87
Diabetes mellitus			
Type I	0 (0%)	0 (0%)	-
Type II			
yes N (%) no N (%)	9 (16.1%) 47 (83.9%)	7 (21.9%) 25 (78.1%)	0.50

Table 3: Relevant past medical history of KTRs

Data are presented as median (MDN), interquartile range (IQR), absolute numbers (N) and relative percentages (%) within treatment group. Chi-square test was used for the analysis of categorical data while Mann-Whitney U test was applied to non-parametric data, significance level $p < 0.05$.

3.1.3 Concomitant immunosuppressive therapy

The Prograf®/Advagraf® treatment group consisted of 35 patients exclusively on Prograf® and 1 patient exclusively on Advagraf®, while 20 patients eventually experienced a switch between the two formulations over the follow-up period. On the other hand, 32 patients received exclusively Envarsus® as part of the immunosuppressive regimen.

Immunosuppression	**Prograf®/Advagraf®** N = 56	**Envarsus®** N = 32	**p value**
Induction regimen			
Glucocorticoids	56 (100%)	32 (100%)	1.00
Basiliximab	48 (86%)	31 (97%)	0.15
Anti-thymocyte globulin (ATG)	8 (14%)	1 (3%)	0.15
Mycophenolate mofetil	55 (98%)	32 (100%)	0.34
Azathioprine	1 (1.8%)	0 (0%)	1.00
Prograf®	35 (63%)	-	-
Advagraf®	1 (2%)	-	-
Prograf® or Advagraf® (switch)	20 (36%)	-	-
Envarsus®	-	32 (100%)	-
Maintenance (month 12)			
Glucocorticoids	49 (88%)	28 (88%)	1.00
Mycophenolate mofetil	48 (86%)	28 (88%)	0.64
Azathioprine	1 (2%)	0 (0%)	1.00
Prograf®	35 (63%)	-	-
Advagraf®	1 (2%)	-	-
Prograf® or Advagraf® (switch)	20 (36%)	-	-
Envarsus®	-	32 (100%)	-

Table 4: Immunosuppressive therapy in KTRs

Assessment of immunosuppressive comedication of kidney transplant recipients at predefined study-visits did not show significant difference between groups. The data analysis includes patients who were lost to follow-up, which is reflected in a lower patient number, e.g., under glucocorticoids at 12 months post-transplantation.

Data are presented as absolute numbers (N) and relative percentages within group (%). Chi-square test was used for data analysis, significance level $p < 0.05$.

3.2 Blood-pressure among KTRs

The median blood pressure over the course of three study-visits did not differ between groups while treatment of high blood-pressure remained consistent across both groups. A trend towards lower diastolic blood pressure in the Envarsus® treatment group was noticed at 48-weeks post-transplantation.

Blood pressure	**Prograf®/Advagraf®**	**Envarsus®**	**p value**
1-week post-transplant			
systolic [mmHg] MDN(IQR)	140 (20)	140 (20)	0.13
diastolic [mmHg] MDN(IQR)	80 (14)	80 (8)	0.21
8 weeks post-transplant			
systolic [mmHg] MDN(IQR)	130 (20)	134.5 (19)	0.70
diastolic [mmHg] MDN(IQR)	80 (14)	80 (15)	0.75
48 weeks post-transplant			
systolic [mmHg] MDN(IQR)	130 (11)	130 (16)	0.27
diastolic [mmHg] MDN(IQR)	80 (10)	80 (8)	0.06

Table 5: Blood pressure of KTRs at predefined study-visits

MDN: median; IQR: interquartile range; KTX: kidney transplantation.

Mann-Whitney U test was applied to non-parametric data and student´s t-test to parametric data, significance level $p < 0.05$.

3.3 Kidney allograft function

Median serum creatinine level was significantly lower in the treatment group with Envarsus® compared to Prograf®/Advagraf® at 8 weeks (1.35 ± 0.51 and 1.57 ± 0.78 respectively; p = 0.02) and 48 weeks after transplantation (1.36 ± 0.55 and 1.58 ± 0.67 respectively; p = 0.046).

No significant differences between groups were detected regarding estimated glomerular filtration rate. However, a trend towards higher mean estimated glomerular filtration rate was noticed with Envarsus® at 8 weeks (54.93 ± 17.05 vs. 47.63 ± 17.83, p = 0.07) and 48 weeks after transplantation (55.53 ± 16.38 vs. 47.76 ± 17.15, p = 0.06).

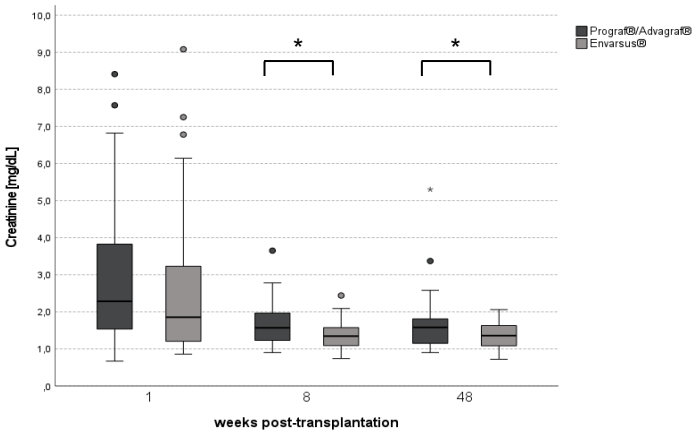
Kidney graft function	**Prograf®/Advagraf®**	**Envarsus®**	**p value**
1-week post-transplant			
eGFR [ml/min/1,73 m2] MDN(±IQR)	27.84 (±33.06)	35.94 (±42.39)	0.33
Creatinine [mg/dL] MDN(±IQR)	2.29 (±2.41)	1.86 (±2.16)	0.21
8 weeks post-transplant			
eGFR [ml/min/1,73 m2] M (±SD)	47.63 (±17.83)	54.93 (±17.05)	0.07
Creatinine [mg/dL] MDN(±IQR)	1.57 (±0.78)	1.35 (±0.51)	0.02*
48 weeks post-transplant			
eGFR [ml/min/1,73 m2] M (±SD)	47.76 (±17.15)	55.53 (±16.38)	0.06
Creatinine [mg/dL] MDN(±IQR)	1.58 (±0.67)	1.36 (±0.55)	0.05

Table 6: Kidney graft function at predefined study-visits

Data are displayed as mean (M), standard deviation (SD), median (MDN) and interquartile range (IQR). KTX: kidney transplantation; eGFR: estimated glomerular filtration rate.

Mann-Whitney U test was applied to non-parametric data and student´s t-test to parametric data, significance level $p < 0.05$.

a)



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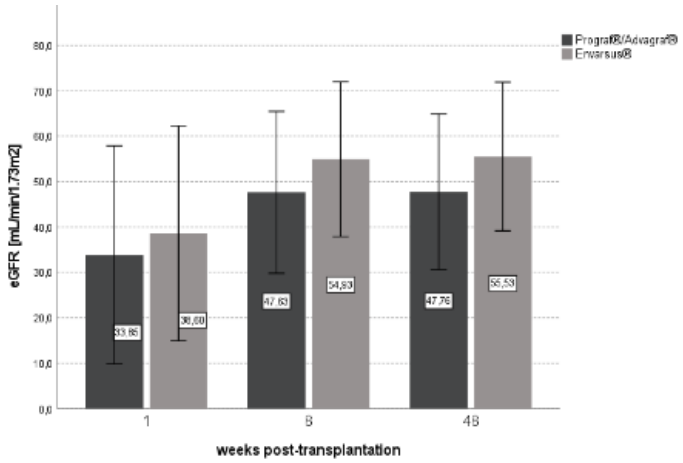


Figure 6: Graphical display of kidney graft function

a) Box plot of measured creatinine levels at predefined study-visits **b)** Clustered bar chart of mean estimated glomerular filtration rate at predefined study-visits. Error bars represent standard deviation (SD ± 1). eGFR: estimated glomerular filtration rate.

Mann-Whitney U test was applied to non-parametric data and student´s t-test to parametric data, significance level $p < 0.05$, indicated by (*).

3.4 Acute graft rejection

All acute rejections were biopsy-proven acute rejections (BPAR). Indication biopsies resulted in 3 borderline rejections, 2 were humoral rejections and 12 were classified according to the latest BANFF classification system. Out of 18 acute rejections, 13 occurred in the Prograf®/Advagraf® treatment group and 5 occurred in the Envarsus® group.

	Prograf®/Advagraf®	**Envarsus®**	**p value**
Kidney graft rejection			
N (%)	13 (23.2%)	5 (15.6%)	0.40
BANFF classification N (%)			
Borderline rejection	1 (7.7%)	2 (40%)	
BANFF IA	0 (0%)	0 (0%)	
BANFF IB	2 (15.4%)	0 (0%)	
BANFF IIA	6 (46.2%)	2 (40%)	
BANFF IIB	1 (7.7%)	1 (20%)	
BANFF III	1 (7.7%)	0 (0%)	
Humoral rejection			
N (%)	2 (15.4%)	0 (0%)	1
Total N (%)	13 (100%)	5 (100%)	

Table 7: Summary of acute graft rejections sorted by treatment group

Data are presented as absolute and relative frequencies. Chi-square test was used to analyse data, significance level $p < 0.05$. No significant difference between groups was detected. N: number; BANFF: Banff Classification System.

3.4.1 Incidence of acute graft rejection

The rejection-free survival for 12 months post-transplantation was compared between Envarsus® (3 incidents) and Prograf®/Advagraf® (12 incidents) treatment groups using log-rank analysis. Borderline rejections were not included in the analysis.

The incidence of acute graft rejection in the Envarsus® group was lower but the difference between groups was not statistically significant (Hazard ratio 0.5; CI 95% 0.2-1.4; log-rank p = 0.16).

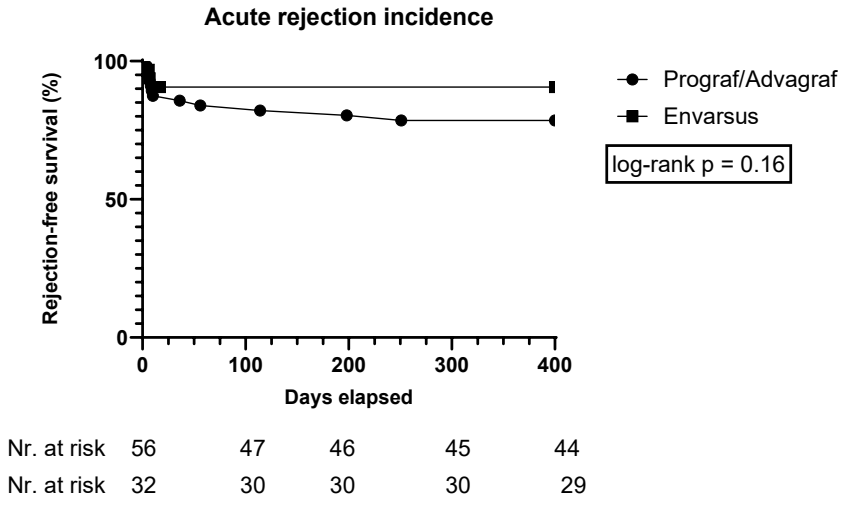


Figure 7: Kaplan-Meier survival curve for acute graft rejection

3.5 BK polyoma- and cytomegalovirus infections

Positive cytomegalovirus viremia was defined as > 10^2 copies/mL of a PCR (polymerase chain reaction) test, while BK polyomavirus viremia was determined by any positive value of the PCR test.

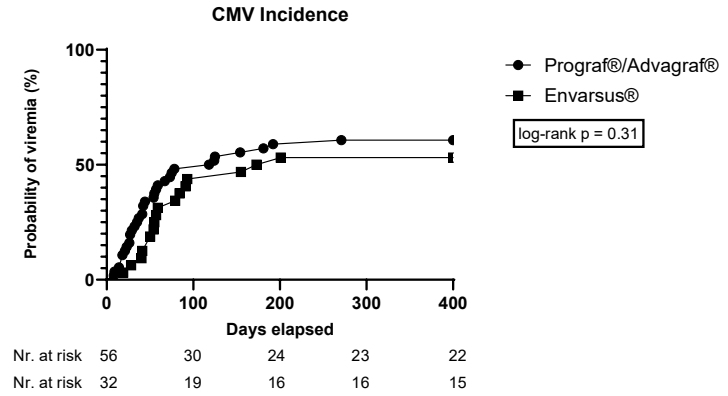
	Prograf®/Advagraf®	Envarsus®	p value
BK polyomavirus infection			
N (%)	10 (17.9%)	9 (28.1%)	0.26
CMV infection			
N (%)	34 (60.7%)	17 (53.1%)	0.49
CMV serostatus			
Donor + /Recipient -			
N (%)	15 (26.8%)	5 (15.6%)	
Donor + /Recipient +			
N (%)	24 (42.9%)	8 (25%)	
Donor - /Recipient +			
N (%)	11 (19.6%)	9 (28.1%)	
Donor - /Recipient -			
N (%)	5 (8.9%)	8 (25%)	
Donor unknown /Recipient +			
N (%)	1 (1.8%)	2 (6.3%)	

Table 8: Summary of virus infections sorted by treatment group

Data are presented as absolute and relative frequencies. Chi-square test was used to analyse data, significance level $p < 0.05$. No significant difference between groups was detected. N: number; CMV: cytomegalovirus.

3.5.1 Incidence of BK polyoma- and cytomegalovirus viremia

a)



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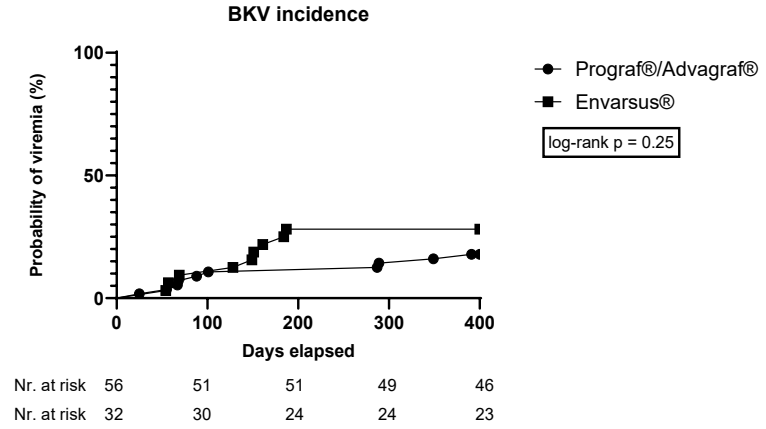


Figure 8: Kaplan-Meier survival curve for CMV and BKV viremia

a) Survival curves representing cytomegalovirus viremia and **b)** BK-polyomavirus viremia in KTRs for each treatment group.

Incidence of BKV viremia was higher in the Envarsus® group. A log-rank analysis of the survival curves did not show a statistically significant difference between groups. CMV: cytomegalovirus; BKV: BK polyomavirus.

3.6 Concentration-to-dose ratio and metabolizer status

Concentration-to-dose ratio (C/D ratio, CDR) was calculated as the ratio of tacrolimus trough level (Tac C0) to the respective tacrolimus total daily dose (Tac dose) at the end of two months and twelve months after kidney transplantation. (46) The analysis showed a significant increase of C/D ratio from two months to twelve months after transplantation within each treatment group (Prograf®/Advagraf® MDN ± IQR: 1.46 ± 1.16 vs. 2.27 ± 1.71, $p < 0.01$; Envarsus® M ± SD 1.67 ± 0.96 vs. 2.80 ± 1.65, $p = 0.01$). Wilcoxon signed-rank test was used for Prograf®/Advagraf® group, while one sample student´s t-test was used for Envarsus® group. The Median C/D ratio between Prograf®/Advagraf® and Envarsus® group at 2 months (1.45 ± 1.2 vs. 1.73 ± 0.8, $p = 0.71$) and 12 months (2.27 ±1.7 vs. 2.49 ± 2.5, $p = 0.38$) post-transplantation did not differ.

Metabolizer status was determined based on concentration-to-dose ratio (ng/mL*mg) as follows: <1.05 fast metabolizer (FM); 1.05-1.54 intermediate metabolizer (IM); ≥ 1.55 slow metabolizer (SM).

	Study visit	Prograf®/Advagraf® N=56	Envarsus® N = 32	p value
Tac dose [mg/d] M ± SD	2 months	5.24 ± 3.0	6.43 ± 3.7	0.21
	12 months	3.21 ± 1.5	3.13 ± 2.1	0.35
Tac C0 [ng/mL] M ± SD	2 months	7.41 ± 2.5	9.01 ± 2.9	0.05
	12 months	6.37 ± 2.0	6.17 ± 1.3	0.59
C/D ratio [ng/mL*mg] MDN ± IQR	2 months	1.45 ± 1.2	1.73 ± 0.8	0.71
	12 months	2.27 ±1.7	2.49 ± 2.5	0.38
Metabolizer type	2 months	FM: 8 (23.5%) IM: 12 (35.3%) SM: 14 (41.2%)	FM: 4 (23.5%) IM: 2 (11.8%) SM: 11 (64.7%)	-
	12 months	FM: 5 (10.6%) IM: 8 (17%) SM: 34 (72.3%)	FM: 4 (14.3%) IM: 4 (14.3%) SM: 20 (71.4%)	-

Table 9: Clinical parameters in therapeutic drug monitoring

Tacrolimus daily dose, trough concentrations, concentration-to-dose ratio, and metabolizer status were defined at 2 months and 12 months after kidney transplantation according to treatment group. Tac: tacrolimus; Tac C0: tacrolimus trough concentration; C/D ratio: concentration-to-dose ratio; FM: fast metabolizer; IM: intermediate metabolizer; SM: slow metabolizer.

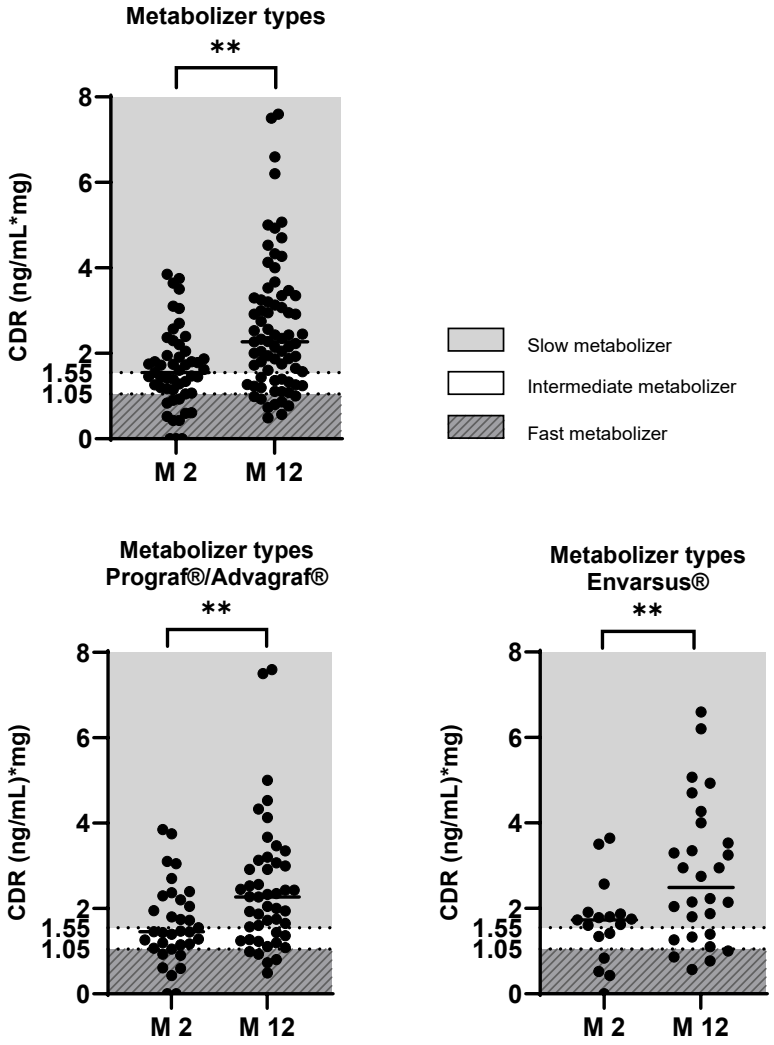


Figure 9: Scatterplot of concentration-to-dose ratio

The graphic shows the distribution of metabolizer types at the end of 2 months and 12 months post-transplantation. C/D ratio increased significantly from month 2 to month 12 post-transplantation within each treatment group, resulting in more slow metabolizers over time. C/D ratio: concentration-to-dose ratio; M: month, ** indicates $p < 0.01$.

3.6.1 Influence of metabolizer type on outcome

Cox proportional hazard models were used to assess the influence of metabolizer type on acute rejection rate and risk of developing cytomegalovirus and BK polyomavirus viremia for 12 months post-transplantation.

The metabolizer status was determined based on C/D ratio at two months post-transplantation.

Fast metabolizers were associated with significantly higher hazard ratio for acute rejection 4.5 (CI 95% 1.4 – 14.4; p = 0.01). The analysis showed a significantly higher risk increase in fast metabolizers within Prograf®/Advagraf® group for acute rejection (HR 5.0, CI 95% 1.3 – 20.3, p = 0.02). The hazard ratio for Envarsus® fast metabolizers was not significantly different than for slow and intermediate metabolizers (HR 2.72; CI 95% 0.1 – 28.5; p = 0.45). This interpretation is further limited due to small sample size.

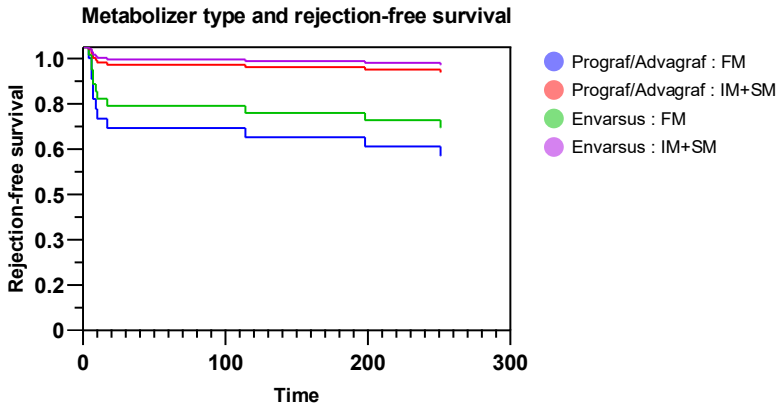
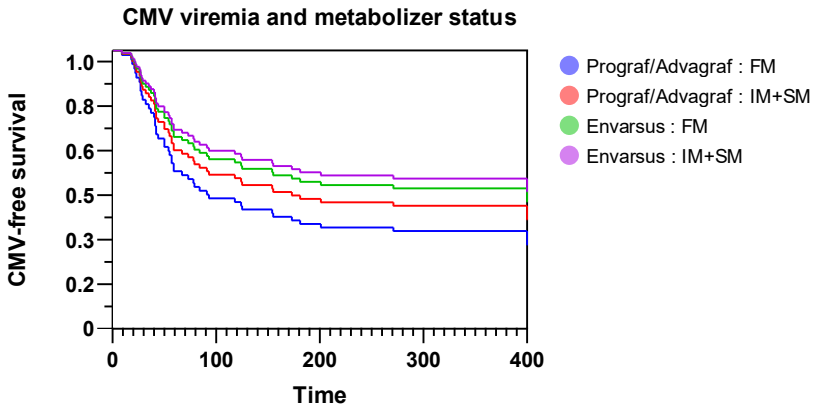


Figure 10: Cox proportional hazard model for graft rejection

Rejection-free survival curves were generated and stratified regarding metabolizer status for both treatment groups.

Time represents days since KTX. CMV: cytomegalovirus; BKV: BK polyomavirus; SM: slow metabolizer; IM: intermediate metabolizer; FM: fast metabolizer.

a)



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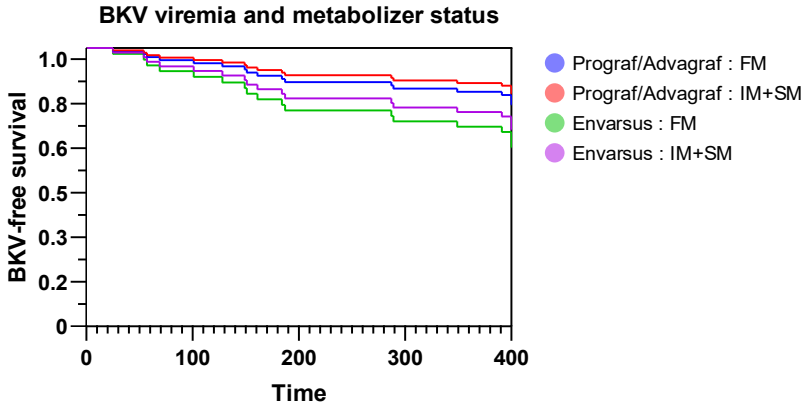

Figure 11: Cox proportional hazard model for viremia

The influence of metabolizer status on incidence of **a)** CMV viremia and **b)** BKV viremia was assessed for 12 months since kidney transplantation. Fast metabolizers were under higher risk for CMV viremia with a hazard ratio of 1.3 (CI 95% 0.6 – 2.4, p = 0.47) and for BKV viremia with a hazard ratio of 1.1 (CI 95% 0.3 – 3.2, p = 0.82) than slow and intermediate metabolizers regardless of treatment group, although statistically not significant.

Time represents days since KTX.CMV: cytomegalovirus; BKV: BK polyomavirus; SM: slow metabolizer; IM: intermediate metabolizer; FM: fast metabolizer.

Group	HR	95% CI	p-value
Envarsus®: Prograf®/Advagraf®			
Acute rejection	0.5	0.2 – 1.4	0.16
BKV	1.7	0.7 – 4.3	0.25
CMV	0.7	0.4 – 1.3	0.31
FM vs. IM+SM			
Acute rejection	4.5	1.4 – 14.4	0.01*
BKV	1.1	0.3 – 3.2	0.82
CMV	1.3	0.6 – 2.4	0.47
Prograf®/Advagraf®: FM (C/D ratio < 1.05)			
Acute rejection	5.0	1.3 – 20.3	0.02*
BKV	1.0	0.1 – 4.1	0.99
CMV	1.4	0.6 – 2.9	0.48
Envarsus®: FM (C/D ratio < 1.05)			
Acute rejection	2.72	0.1 – 28.5	0.45
BKV	1.4	0.2 – 5.7	0.71
CMV	1.1	0.2 – 3.3	0.93

Table 10: Hazard ratios for acute rejection, BKV and CMV viremia

The incidence of acute rejection and CMV viremia did not differ regarding treatment group. Higher risk for BKV viremia was noticed with Envarsus® (HR 1.7, CI 95% 0.7 – 4.3, p = 0.25).

Fast metabolizers, irrespective of treatment group, showed a significantly higher risk for acute rejection than slow metabolizers. Fast metabolizers in the Prograf®/Advagraf® group showed a significantly higher risk for acute rejection. HR: hazard ratio; CI: confidence interval; C/D ratio: concentration-to-dose ratio; FM: fast metabolizer; BKV: BK polyomavirus; CMV: cytomegalovirus.

(*) indicates significance level p < 0.05.

3.7 Tacrolimus trough levels, IPV and TTR

Furthermore, we analyzed the following interval parameters for Envarsus® and Prograf®/Advagraf® treatment groups.

Mean tacrolimus trough levels (C0) were calculated in 4-week intervals over the first three months post-transplantation and compared between patients receiving Envarsus® and Prograf®/Advagraf® using a student's t-test. A trend towards higher mean tacrolimus trough levels was noticed with Envarsus® at 9 – 12 weeks after transplantation (8.8 ± 2.2 vs. 7.7 ± 2.3, p = 0.05).

Intrapatient coefficient of variation (IPV%) was calculated as the ratio of standard deviation to tacrolimus trough level expressed in percentage.
The mean intrapatient coefficient of variation (IPV%) was then calculated in 4-week intervals over the first three months post-transplantation to assess the variability of tacrolimus trough levels within group.
The mean IPV% ± SD was compared between patients receiving Envarsus® and Prograf®/Advagraf® using a student's t-test; no significant differences were detected.

Time in target range (TTR%; ng/mL) is expressed as percentage of days when tacrolimus trough concentrations fall within the defined range: 7-10 ng/mL for the first 8 weeks post-transplantation and 6-9 ng/mL starting at 9 to 12 weeks post-transplantation.
Time in target range was calculated in 4-week intervals over the first three months post-transplantation of measured tacrolimus trough levels following linear interpolation after Rosendaal. (66)
The median TTR% ± IQR was compared between patients receiving Prograf®/Advagraf® and Envarsus® using a Mann-Whitney U test. There was a significant difference between groups regarding time in target range at 5 – 8 weeks post-transplantation (31.9 (±50) vs. 57.7 (±63.5) respectively, p = 0.03).

	Time posttransplant	Prograf®/Advagraf® N=56	Envarsus® N=32	p value
Tac trough-level [ng/mL] M ± SD	1 - 4 weeks	8 (±1.5)	8 (±1.3)	1
	5 - 8 weeks	8 (±2.5)	7.9 (±1.5)	0.86
	9 - 12 weeks	7.7 (±2.3)	8.8 (±2.2)	0.05
IPV [%] M ± SD	1 - 4 weeks	44.2 (±20.8)	40.4 (±13.7)	0.35
	5 - 8 weeks	26.1 (±18)	20.5(±13)	0.14
	9 - 12 weeks	24.1 (±22.5)	24.8 (±16.8)	0.93
TTR [%] MDN ± IQR	1 - 4 weeks	39.3 (±34.8)	37.5 (±17.9)	0.36
	5 - 8 weeks	31.9 (±50)	57.7 (±63.5)	0.03*
	9 - 12 weeks	42.9 (±100)	57.1 (±55.5)	0.66

Table 11: Interval parameters for the first three months post-transplantation

Tac: tacrolimus; IPV: intra-patient variability; TTR: time in target range; M: mean; SD: standard deviation; MDN: median; IQR: interquartile range.

Mann-Whitney U test was applied to non-parametric data and student´s t-test to parametric data, significance level $p < 0.05$.

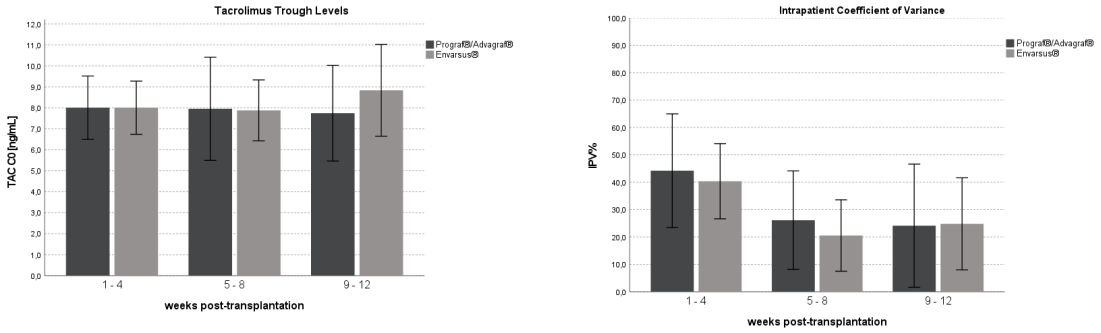

Figure 12: Graphical display of trough concentrations and IPV

Clustered bar chart of **a)** tacrolimus trough levels and **b)** tacrolimus intra-patient variability (percentage). Graphs represent the mean of parameters in 4-week intervals during the first three months post-transplantation. Error bars represent standard deviation (SD ± 1).

Tac C0: tacrolimus trough level; IPV: intra-patient variability.

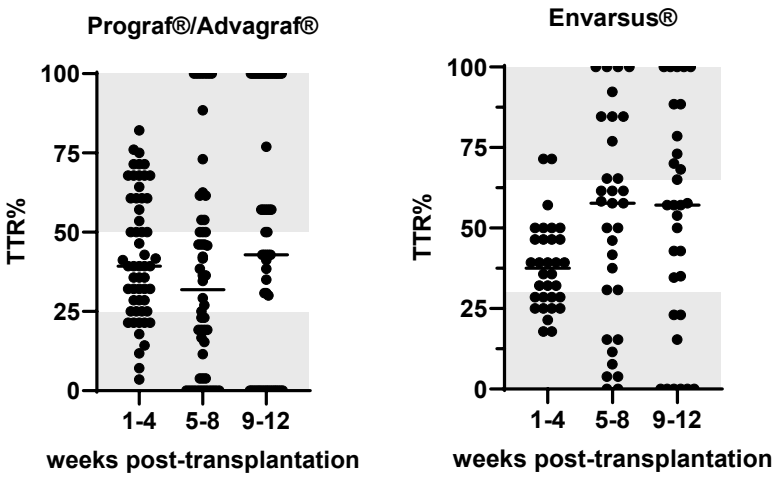

Figure 13: Scatterplot of time in target range

TTR of patients receiving Prograf®/Advagraf® and Envarsus® over 4-week intervals during the first three months following kidney transplantation. TTR: time in target range.

4 Discussion

We conducted an analysis for a subgroup of patients, who were part of a bigger prospective single-centre observational trial. The primary goal was to compare kidney function, the rate of acute rejection, and the incidence of BKV and CMV infections between KTRs receiving treatment with novel once-daily extended-release tacrolimus Envarsus® (LCP-Tac, LCPT) and KTRs receiving the former formulations Prograf® (IR-Tac) and Advagraf® (ER-Tac). Further objective of this thesis was to evaluate the impact of different formulations on the fluctuation of tacrolimus blood concentration levels and the scale of exposure to tacrolimus.

4.1 Graft function, graft rejection and virus infections

In our kidney transplant cohort, we found significantly higher eGFR and lower serum creatinine levels in patients treated with *de novo* Envarsus® at month 2 and month 12 post-transplantation compared to patients on Prograf®/Advagraf®. These data are contrary to large, randomized controlled trials comparing Envarsus® with Prograf®, who only found beneficial effects of Envarsus® in subgroups namely older and black patients. (67). Overall, Envarsus® has especially shown to be beneficial due to its pharmacokinetic properties in patients with a fast metabolizer status (68), which also explains the benefit of the black patients, who carry allelic variants for fast metabolizer status in much higher frequencies compared to the white population. (69) Accordingly, Thölking and colleagues managed to slow down kidney function deterioration in the subgroup of tacrolimus fast metabolizers by switching them early from Prograf® to Envarsus®. (52) In our small cohort, we also detected improved outcomes in patients with fast metabolizer status treated with *de novo* Envarsus® compared to Prograf®/Advagraf® as outined below.

The differences in eGFR outcomes between the two groups might be attributable to the difference we see in acute graft rejections since de novo Envarsus®-treated patients experienced less acute rejections within the first year after transplantation. Still, significance was not reached, and the data need to be carefully interpreted due to low n-numbers in our cohort.

We found no statistically significant difference in incidence rates for BK polyomavirus and cytomegalovirus viremia for both treatment groups, consistent to studies comparing Envarsus® to Prograf® in de novo and stable KTRs. (54,55,67) However, we noticed a slightly higher, but non-significant risk of BKV viremia in KTRs treated solely with Envarsus®, which somehow points to the fact that patients treated with de novo Envarsus® might be more effectively immunosuppressed.

4.2 Tac daily dose and metabolizer status

Slightly higher mean Tac daily doses with Envarsus® than with Prograf® or Advagraf® to reach target trough concentration levels were needed over the first two months post-transplantation, showing no significant difference between groups. Previous studies have shown higher initial daily dose requirement with Envarsus® than with Prograf®, but significantly lower daily dose requirements with Envarsus® over time. (54,55,67)

Since tacrolimus nephrotoxicity cannot be excluded even when trough concentrations fall within the target range, Thölking et al. suggested using the concentration-to-dose ratio (C/D ratio) as a tool to predict tacrolimus metabolism and help identify patients at higher risk for overexposure and impairment of kidney function. (46) Earlier studies used the C/D ratio determined at 3 months post-transplantation to evaluate the impact that tacrolimus metabolism rate has on outcomes such as graft rejection and virus infections. (70–73) We defined metabolizer status based on C/D ratio determined as early as 2 months post-transplantation and could demonstrate a significant increase in C/D ratio from month 2 to month 12 post-transplantation in both treatment groups. The latter was noticed in a shift to more slow metabolizers over time.

Previously, conversion from Prograf® to Envarsus® has led to increased bioavailability and a higher C/D ratio. (52) In our study, 64.7% of KTRs treated with Envarsus® were slow metabolizers at the end of 2 months since transplantation, while the Prograf®/Advagraf® treatment group had only 41.2% slow metabolizers at the same timepoint. We could not prove if this difference was significant due to the small sample size in each treatment group. However, it seems that the switch to a slow metabolizer status happens earlier with Envarsus®. This can be especially

beneficial for KTRs exhibiting faster metabolism for tacrolimus because conversion from Prograf® to Envarsus® treatment maintained higher glomerular filtration rates in fast metabolizers. (52,74,75)

Patients with a C/D ratio lower than 1.05, meaning they metabolise tacrolimus faster, exhibited a faster reduction of kidney function, higher risk for calcineurin-inhibitor nephrotoxicity and higher rates of BKV viremia when compared to patients with a higher C/D ratio. (74,75) In our analysis fast metabolizers overall showed a significantly higher risk for acute rejection than intermediate and slow metabolizers. Fast metabolizers also showed a higher risk to develop BKV and CMV viremia than intermediate and slow metabolizers, although this result was statistically not significant. When comparing fast to slow and intermediate metabolizers within each treatment group, we noticed a trend toward less acute graft rejections and a higher incidence for BKV viremia with Envarsus®. We suggest that this the result of a stronger immunosuppression with Envarsus®.

4.3. Trough concentrations, IPV and TTR

The difficulties in adjusting tacrolimus blood concentrations are well known to transplant clinicians. High fluctuations of tacrolimus blood concentrations have been associated with adverse allograft outcomes such as late acute rejection and graft failure. (70) Even though target IPV values are unclear, a mean IPV higher than 30% represents the suggested threshold by many studies because it has been linked to poorer outcomes including kidney function impairment, acute rejection, graft loss, development of de novo donor specific antibodies and progression of histological lesions. (76) To determine the intra-patient variability (IPV) of tacrolimus concentrations, we used repeated measures of trough blood concentrations during the first three months after transplantation. Our analysis showed a higher mean IPV than the cut-off mentioned above in the first month post-transplantation, 44.2% in the Prograf®/Advagraf® group and 40.4% in the Envarsus® group, showing no statistically significant difference between treatment groups. In the second and third month post-transplantation the mean IPV in each treatment group was noticeably lower when compared to the first month but still remained as high as 26%.

Therefore, we could not prove that one tacrolimus formulation was better than the other in terms of trough concentration variability.

Additionally, we assessed the time in target range (TTR) of tacrolimus trough concentrations, a parameter which has formerly been associated with patient and graft survival. Living donor KTRs receiving tacrolimus with a TTR higher than 78% showed better graft survival and a lower risk for acute rejection and infection. (77) Furthermore, a low TTR has been associated with higher risk for *de novo* DSA, acute rejection, and graft loss. (78) In our study, a median TTR of 57.1% was reached with Envarsus® at two months post-transplantation, which was significantly higher than the median TTR of 42.9% in the Prograf®/Advagraf® group. However, both treatment groups showed median TTR values noticeably lower than 78% over the three-month period since transplantation.

4.4 Limitations

The gold standard for pharmacological studies are randomized-controlled trials. Our study design deviated from this standard since randomization and blinding of study participants were not performed.

Moreover, the relatively small sample size, especially in the Envarsus® treatment group, may have reduced the precision of our estimates and affected the power of our study. This became even more evident when evaluating the impact of metabolizer status on our endpoints acute rejection rate and incidence of virus infections. A few patients were lost to follow-up or were relocated in smaller centres for their routine checks. This was partially due to COVID-19 epidemic to prevent hospital overload. However, this reflects real life clinical circumstances in regard to scheduling and organisation. Our analytical results could only be applied to the white population, since over 90% of our patients were of Caucasian ethnicity. Diagnosis of acute graft rejection was based on histopathological results of kidney biopsies, which were performed only in cases following indication. Thus, clinically unapparent graft changes possibly went unnoticed and might have caused bias in evaluation of rejection rates.

Regarding TTR, more than three missing values were estimated by linear interpolation which may have not represented the true exposure to tacrolimus.

4.5. Conclusion

Overall, our data add to the consisting evidence that *de novo* Envarsus® is a safe alternative in kidney transplant recipients compared to Prograf®/Advagraf®. Especially, patients with high metabolizer status might benefit from this tacrolimus formulation. The observation of trend wise lower rejection rate and higher BK polyomavirus viremia incidence may reflect a stronger immunosuppressive effect despite targeting similar trough levels with *de novo* Envarsus®. Further biological effect analysis is needed to identify markers potentially guiding immunosuppression.

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