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DISSERTATION

Conversion of liver transplant recipients from twice-daily to novel once-daily Tacrolimus: a 2-year prospective study on adherence, safety, and efficacy.

Umstellung nach Lebertransplantation von zwei- zu einmal täglicher Gabe von Tacrolimus: 2-Jahres-Verlaufsbeobachtung zur Adhärenz, Sicherheit, und Wirksamkeit.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

AE	Adverse event
AUC	Area under the curve
ALT	Alanine aminotransferase
AP	Alkalic phosphatase
AST	Aspartate aminotransferase
avg.	average
BAASIS©	Basel Assessment of Immunosuppressant Adherence Medication Scale
BMI	Body Mass Index
CDK	Chronic kidney disease
C/D	Concentration/dose ratio = $\frac{Cmin}{TDD}$
CI	Confidence interval
C_{min}	24-hour minimum serum concentration, trough level
C_{max}	24-hour maximum serum concentration
CNI	Calcineurin inhibitor
COV	Coefficient of variation
COMMIT	Clinical Checklist on Managing Modifiable Risk in Transplantation
CYP	Cytochrome P450
dnDSA	de novo donor-specific antibody
eGFR	Estimated glomerular filtration rate
ER-Tac	Extended release, once-daily Tacrolimus, Advagraf®
EMA	European Medicines Agency
et al.	et alii – complete list of authors under literature cited
FDA	Food and Drug Administration
Fluctuation	(%) dosing interval fluctuation relative to avg. concentration = $100 * \frac{(Cmax-Cmin)}{Cavg}$
GGT	Gamma-glutamyltransferase
HbA1C	Hemoglobin A1C
HCV-RNA	Hepatitis C virus ribonucleic acid
IL-2	Interleukin 2

IPV	Intra-patient variability of consecutive Tacrolimus measurements, SD or COV
IR-Tac	Immediate release, twice-daily Tacrolimus, Prograf®
kg	kilogram
LCP-Tac	Novel once-daily extended-release Tacrolimus, Envarsus®
m	meter
min	minimum
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for end-stage liver disease
MMF	Mycophenolate mofetil
ml	milliliter
mg	milligram
NASH	non-alcoholic steatohepatitis
ng	nanogram
NFAT	Nuclear factor of activated T-cells
OR	Odds ratio
PPS	Per protocol set
PRES	Posterior reversible encephalopathy syndrome
QoL	Quality of life
QUEST	Quality of life in essential tremor
RC	Relative change
SAE	Serious adverse event
SIRS	Systemic inflammatory response syndrome
SD	Standard deviation
Swing	(%) dosing interval fluctuation relative to min. concentration $= 100 * \frac{(Cmax-Cmin)}{Cmin}$
TAG	Triglyceride
TDD	Total daily dosage
TDM	Therapeutic drug monitoring
T_{max}	Time to maximum whole blood concentration
UK	United Kingdom
US	United States of America

VAS	Visual Analogue Scale
vs	versus
WHO	World Health Organization
y	year

Abstract

Background – After a liver transplant, patients need to regularly take immunosuppressive drugs to prevent organ rejection. However, 15-40% experience difficulties adhering to their regimen. This increases their risk for graft loss, retransplantation, and death. The primary objective of this study was to explore whether simplifying the immunosuppressive regimen of stable liver transplant patients from conventional twice-daily (immediate-release Tacrolimus, IR-Tac, Prograf®) to novel once-daily Tacrolimus (novel extended-release Tacrolimus, LCP-Tac, Envarsus®) would improve adherence. In pharmacokinetic studies, LCP-Tac demonstrated lower dose requirements (-30%) and peak serum concentrations (-30%) compared to IR-Tac. The secondary objective was to determine whether these properties would translate into a reduction in typical side effects including nephrotoxicity, neurotoxicity, and diabetogenicity without an increase in rejection rates.

Methods – We conducted a two-year, single arm, prospective conversion trial in 165 liver transplant recipients. At baseline, we converted patients from IR-Tac to LCP-Tac and conducted follow-up examinations at ten time points. We measured adherence using the Basel Assessment of Immunosuppressant Adherence (BAASIS©) questionnaire before conversion (t = 0) and at months 6, 12, 18, and 24. Any adverse event or changes to the medication regimen were noted. At each time point, we collected comprehensive laboratory values including liver, renal and metabolic panels. We compared the intra-patient variability (IPV) of Tacrolimus serum levels during the study period with the variability observed prior to conversion as a potential surrogate parameter for adherence.

Results - A total of 161 patients received the study drug. Overall adherence on the BAASIS© increased from 50% at baseline with the twice-daily regimen to 80% at the time of completion under once-daily Tacrolimus ($p < 0.001$). After two years, patient and graft survival were 97%. No episodes of organ rejection occurred. 5 (3%) patients died and 22 (14%) withdrew from the study, mostly due to non-serious adverse events. There were no changes in graft, renal, or metabolic function. The intra-patient variability (IPV) of Tacrolimus serum levels did not change after conversion to LCP-Tac. Out of 51 patients who experienced tremor at study entry, 20 (40%) reported partial or complete symptom remission after conversion.

Conclusion – The results of the study presented here implicate that the use of LCP-Tac might improve immunosuppressant adherence in liver transplant recipients as compared to conventional IR-Tac, while providing comparable safety and efficacy for the prevention of

allograft rejection. Larger, randomized studies should examine potential positive long-term effects on patient and graft survival. In addition, patients who experience tremor with the IR-Tac formula might benefit from conversion to LCP-Tac.

Hintergrund - Nach einer Lebertransplantation müssen Patienten regelmäßig Immunsuppressiva einnehmen, um eine Abstoßung des Organs zu verhindern. 15-40% haben jedoch Schwierigkeiten, dem Therapieplan zu folgen. Dies erhöht das Risiko für Transplantatverlust, Re-Transplantation und Tod. Das primäre Ziel dieser Studie war zu untersuchen, ob die Umstellung von Patienten mit stabilem Verlauf nach Lebertransplantation von einer zweimal täglichen (immediate release Tacrolimus, IR-Tac, Prograf®) auf eine einmal tägliche Tacrolimus Formel (novel once-daily Tacrolimus, LCP-Tac, Envarsus®) die Adhärenz verbessert. In früheren Studien zeigte LCP-Tac niedrigere Dosisanforderungen (-30%) und Serum-Spitzenspiegel (-30%) als IR-Tac. Das sekundäre Ziel war zu untersuchen, ob diese Eigenschaften zu einer Minderung typischer Nebenwirkungen wie Nephro-, Neurotoxizität, und Diabetogenität führen, ohne das Abstoßungsrisiko zu erhöhen.

Methoden - Wir führten eine zweijährige, einarmige, prospektive Konversionsstudie mit 165 Patienten nach Lebertransplantation durch. Zu Studienbeginn wurden die Patienten von IR- auf LCP-Tac umgestellt und an zehn Zeitpunkten untersucht. Die Adhärenz wurde mithilfe des BAASIS©-Fragebogens (Basel Assessment of Immunosuppressant Adherence) vor Umstellung (t = 0) und den Monaten 6, 12, 18 und 24 bestimmt. Unerwünschte Ereignisse und Änderungen des Medikationsschemas wurden dokumentiert. Zudem bestimmten wir umfassende Laborwerte einschließlich Leber-, Nieren-, und Stoffwechselparameter. Als Surrogat-Parameter für die Adhärenz verglichen wir die Intra-Patienten-Variabilität (IPV) der Tacrolimus-Serumspiegel unter LCP-Tac mit der Variabilität vor Umstellung unter IR-Tac.

Ergebnisse - 161 Patienten erhielten das Studienmedikament. Die Adhärenz (erhoben mit dem BAASIS©-Fragebogen) verbesserte sich von 50% unter einer zweimal täglichen, auf 80% mit einer einmal täglichen Gabe von Tacrolimus zum Studienende ($p < 0.001$). Nach zwei Jahren betrug das Patienten- und Transplantatüberleben 97%. Es traten keine Abstoßungen auf.
5 (3%) Patienten starben und 22 (14%) schieden aus der Studie aus, primär infolge nicht schwerwiegender Ereignisse. Transplantat-, Nieren- und Stoffwechselfunktion waren stabil. Die IPV der Tacrolimus-Serumspiegel zeigte keine signifikante Änderung unter LCP-Tac. 20 von 51 (40%) Patienten, bei denen unter IR-Tac ein Tremor auftrat, berichteten von einer Besserung oder Remission der Symptome nach Umstellung.

Schlussfolgerung – Die Ergebnisse der vorliegenden Studie sprechen dafür, dass sich die Medikamentenadhärenz bei Patienten nach Lebertransplantation durch Umstellung der

Immunsuppression von IR-Tac auf LCP-Tac bei vergleichbarer Sicherheit und Effektivität verbessert. In größeren, kontrollierten Studien sollten mögliche positive Langzeiteffekte auf das Transplantat- und Patientenüberleben untersucht werden. Zudem könnten Patienten, bei denen unter IR-Tac Tremor auftritt, von einer Umstellung auf LCP-Tac profitieren.

1. Introduction

After a liver transplant, patients need to regularly take immunosuppressive drugs to prevent organ rejection. Twice-daily Tacrolimus (immediate-release Tacrolimus, IR-Tac, Prograf®) has been considered the gold standard since the 1990s due to its high efficacy. However, 15-40% of liver transplant patients experience difficulties sticking with their regimen: they delay intake, skip doses, or even take self-administered "drug holidays". This increases their risk for graft loss, retransplantation, and death (1–8).

One approach to improve patients' adherence is the use of once-daily Tacrolimus formulations. Such formulations provide controlled drug release over 24 hours, characterized by lower peak concentrations (C_{max}) and longer time to peak (T_{max}) than traditional immediate release dosage forms. The first once-daily Tacrolimus formulation available (extended-release Tacrolimus, Advagraf®, ER-Tac) has to date failed to replace IR-Tac as the standard of care, over recent years, due to evidence for increased rejection rates and dose requirements as well as overall higher mortality in female patients (9–13).

In 2014, the European Medicines Agency (EMA) approved a second once-daily Tacrolimus formulation (novel once-daily Tacrolimus, LCP-Tac, Envarsus®) for the prevention of rejection in kidney and liver transplant recipients. LCP-Tac uses a so-called "particle size reduction technology" that allows for controlled and delayed release of Tacrolimus along the entire gastrointestinal tract. In a pharmacokinetic head-to-head study, LCP-Tac showed a more constant drug delivery profile as compared to both IR-Tac and ER-Tac which led to lower dose requirements (-30% and -40%, respectively) and lower maximum concentrations (C_{max} -30%) to achieve comparable trough levels (14, 15).

The different pharmacokinetic profile of LCP-Tac might lead to an improved safety and tolerability profile since Tacrolimus toxicity correlates with peak concentrations (C_{max}) and high drug exposure AUC (area under the curve). Some of the most common side effects include tremor, chronic kidney disease and metabolic syndrome – reducing both life expectancy and quality of life (QoL), and thus remaining a significant challenge for transplant physicians and their patients. The flatter pharmacokinetic profile of LCP-Tac might also allow for the targeting of lower, less toxic Tacrolimus whole blood concentrations than currently used (16).

We hypothesized that conversion of stable liver transplant patients from conventional twice-daily Tacrolimus (IR-Tac) to novel once-daily Tacrolimus (LCP-Tac): (i) improves adherence, (ii) effectively prevents organ rejection, and (iii) reduces typical side effects such as nephrotoxicity, neurotoxicity, and metabolic disturbances.

To test these hypotheses, we conducted a pivotal 24-month prospective trial on adherence, efficacy, and safety in 165 liver transplant recipients after conversion from IR-Tac to LCP-Tac.

1.1 Tacrolimus

1.1.1 Background: The development of effective immunosuppressive drugs

Over the past sixty years, liver transplantation has evolved from an experimental approach in animal models to an effective, lifesaving treatment for patients with end-stage liver disease. Today, more than thirty thousand liver transplants are performed around the world each year. While surgical advances made transplantation technically feasible as early as the 1960s, it was the pharmaceutical innovation in the field of immunosuppression during the 1980s and 1990s that allowed for its recent success story (17, 18).

In 1963, Thomas Starzl performed the first ever liver transplant at the University of Colorado (US). The patient, a three-year-old boy suffering from biliary atresia, died during the procedure from uncontrollable bleeding. During the years that followed, more patients survived the surgery, but until 1979, less than one out of three patients lived past the first year. The major challenge faced by transplant physicians and patients at the time was – and remains to this day - organ rejection (19).

When immune cells encounter foreign tissue such as a transplant, they initiate an immune cascade, which is comparable to the body's defence mechanism against bacteria. In transplantation, this immune reaction is primarily orchestrated by T-lymphocytes, as demonstrated by universal graft acceptance in T-cell deficient animal models (20).

The rejection process starts when both host and donor antigen-presenting cells present donor antigens and activate host T-cells. Progressive recruitment and attack mechanisms of immune cells culminate in the destruction and loss of the new organ. Thus, any immunosuppressive drug that effectively prevents rejection must reduce T-cell activity (21, 22).

Until 1979, most of the 170 liver transplants worldwide were performed by Starzl and his team at the University of Colorado and Roy Calne at the University of Cambridge. Their research efforts were focused on trying to find an effective medical treatment for organ rejection. From the late 1960s onwards, they used a triple immunosuppressive regimen that comprised Azathioprine, Prednisone, and Anti-Lymphocyte Serum. This regimen performed well in early animal models but failed to protect human patients from organ rejection at acceptable rates, as less than 25% survived the first year. As a result, many universities terminated their transplant programs (19).

During the 1970s, researchers at the Swiss Sandoz AG immunological laboratory lead by Stähelin made a revolutionary discovery. They isolated a novel compound from soil samples:

Cyclosporine. Routine assays revealed that Cyclosporine was highly immunosuppressive without the cytotoxic activity of chemotherapy, and it became the prototype of a novel family of immunosuppressive drugs: Calcineurin inhibitors (CNIs) (23).

Drugs of this class target Calcineurin phosphatase, a key mediator in Interleukin-2 activation and T-cell proliferation. In 1979, Roy Calne published the results of a breakthrough trial in The Lancet. He used Cyclosporin A instead of the aforementioned triple regimen as the primary immunosuppressant in 32 kidney-, 2 pancreas-, and 2 liver recipients: in response, one-year survival increased to around 70%. It was only at this point that solid organ transplantation became an accepted medical treatment. In the following years, expansion of transplantation programs accelerated: in 1984, Starzl and his team (now at the University of Pittsburgh) performed 166 liver transplants in a single year – about as many as had been done in the entire world between 1963 and 1979 (17, 24).

Only a few years later, in 1994, transplantation medicine entered its modern era when the Food and Drug Administration (FDA) approved a novel CNI: Tacrolimus. Though Tacrolimus and Cyclosporin A are chemically unrelated, both drugs target Calcineurin phosphatase and thus share their principal side effects of nephrotoxicity, neurotoxicity, and diabetogenicity. However, Tacrolimus proved even more effective for the prevention of rejection than Cyclosporine: one-year survival rates after liver transplantation further increased, from 70% to 85%. Patients also felt better, since somatic complications of Cyclosporine such as hypertrichosis and gingival hyperplasia did not occur during Tacrolimus treatment (16, 25).

Since then, twice-daily immediate-release Tacrolimus (IR-Tac) has become the gold standard after solid organ transplantation, with more than 90% of patients receiving the drug at the time of discharge. But as short-term survival increased over the last two decades, long-term challenges of Tacrolimus therapy have emerged (26, 27).

All immunosuppressive drugs inevitably increase the risk for malignant and infectious disease, depending on the length and intensity of treatment. This also applies to transplant patients, who need to take immunosuppressants for the rest of their lives after surgery. However, in addition to the general side effects of chronic immunosuppression, patients receiving CNIs exhibit a heterogenous and cumulative daily dose-dependent toxicity profile dominated by high rates of nephrotoxicity and diabetogenicity: one out of three patients suffers from chronic kidney disease after ten years of CNI treatment, and one out of two develops a metabolic syndrome, which significantly reduces their survival and QoL (28–32).

CNI toxicity remains the main challenge in long-term transplant care. Consequently, there have been attempts to replace this class of drugs with novel antibodies which also target T-cell activation, such as Basiliximab – an IL-2 receptor antagonist. But to date, no drug with comparable potency and less toxicity is available. In response to this situation, the transplant community has shifted its focus to studying the pharmacological determinants of CNI toxicity and potential strategies to improve outcomes, including novel dosing regimens and drug formulations – which might be interpreted as the fine-tuning of the current standard of care (33–36).

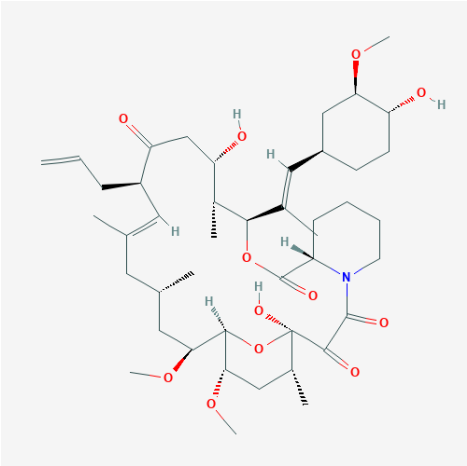
When physicians initiate perioperative Tacrolimus treatment in organ transplantation, toxicity often manifests acutely with a rise in serum creatinine or glucose levels. In early clinical trials, researchers noticed that they could lessen or even reverse toxicity by reducing the total daily drug dose (TTD) and the corresponding AUC. Subsequent studies showed that the incidence of Tacrolimus side effects not only correlates closely with the AUC, but also depends on 24-hour peak concentrations (C_{max}). Modern immunosuppression protocols try to minimize CNI toxicity by reducing the TDD, a strategy known as "CNI-sparing regimens". However, as lower blood levels are targeted, optimal adherence becomes crucial to avoid rejection - missing a dose could lead to a rapid fall of serum concentrations below efficacy levels, with detrimental consequences (37–41).

Researchers hope that the use of the novel once-daily formulation LCP-Tac could improve long term outcomes after transplantation in comparison to IR-Tac via three main mechanisms. Firstly, once-daily dosing could help to achieve the essential compliance that would allow for the safe targeting of lower drug exposure (AUC) than is currently used. This effect may in turn reduce the life limiting effects of CNI toxicity. Secondly, 24-hour pharmacokinetic evaluations show that Tacrolimus concentrations fluctuate significantly less within one day (C_{max} - C_{min}), with LCP-Tac at identical AUC compared to both IR-Tac and ER-Tac; so far, no available studies have examined whether this reduction in fluctuation could allow physicians to use lower Tacrolimus target levels after transplantation without causing an increase in rejection rates when using LCP-Tac – which may also lessen side effects. Thirdly, high peak concentrations (C_{max}) correlate with Tacrolimus nephro- and neurotoxicity as well as diabetogenicity. Since LCP-Tac exhibits up to 30% lower C_{max} than other available formulations, its use as a primary immunosuppressant might even reduce Tacrolimus side effects at identical AUC and adherence rates as compared to current IR-Tac based protocols.

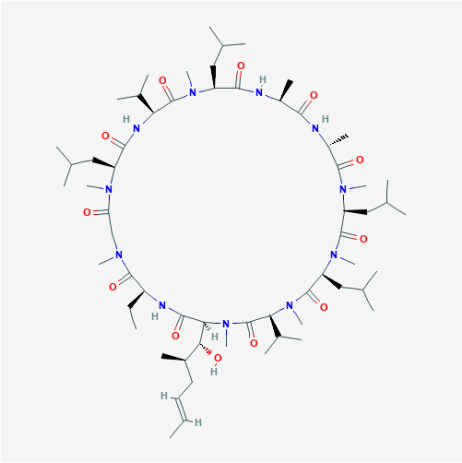
1.1.2 Pharmacology

Both Tacrolimus and Cyclosporin belong to the family of Calcineurin inhibitors (CNIs), a widely used class of immunosuppressant drugs. Clinicians mostly prescribe CNIs for the prevention of allograft rejection after organ transplantation. Topical formulations are also used to treat autoimmune mediated forms of dermatitis and keratoconjunctivitis sicca (42).

Although Tacrolimus and Cyclosporine exhibit similar efficacy and side effects, they are chemically unrelated. The polypeptide Cyclosporine comprises eleven amino acids and is produced by the fungus Tolypocladium inflatum. Tacrolimus is a macrolide lactone, which exhibits structural similarity to Erythromycin derivatives. Tacrolimus is produced by the actinobacteria Streptomyces tsukubaensis (43).



Tacrolimus: $C_{44}H_{69}NO_{12}$



Cyclosporine A: $C_{62}H_{111}N_{11}O_{12}$

Figure 1: 2D Chemical structure depiction. *Reproduced with permission by the National Center for Biotechnology Information, PubChem, U.S. National Library of Medicine Bethesda, Maryland, US (44, 45).*

Both drugs bind to different cytoplasmic target proteins (Tacrolimus: the FK506 binding protein; Cyclosporine: the immunophilin cyclophilin), each regulating the enzyme activity of calcineurin phosphatase. This enzyme modulates translocation of several transcription factors involved in T-cell receptor signaling, including nuclear factor of activated T-cells (NFAT). By blocking the calcineurin phosphatase pathway, Tacrolimus and Cyclosporine reduce T-cell activation and proliferation – which are essential for the immune response against the graft during rejection. A Cochrane meta-analysis of 16 randomized controlled trials showed that one-year mortality (RR 0.85, 95% CI 0.73 to 0.99), graft loss (RR 0.73, 95% CI 0.61 to 0.86), acute rejection (RR 0.81, 95% CI 0.75 to 0.88), and steroid-resistant rejection (RR 0.54, 95% CI 0.47 to 0.74) in the first year are significantly reduced in liver transplant patients treated with Tacrolimus compared to

Cyclosporine. Given the greater efficacy of Tacrolimus for the prevention of rejection, it remains the mainstay immunosuppressant after solid organ transplantation today – although the exact mechanism of its superior efficacy remains unknown (25, 46–51).

While intravenous formulations of Tacrolimus are available, oral application is preferred due to its lower risk of toxicity and practicability after discharge. After a liver transplant, IR-Tac is normally started at 0.1 to 0.2 mg/kg/day, split into a morning and evening dose. Clinical application involves daily monitoring of serum trough concentrations (C_{min}) during post-transplant hospitalization. The trough concentrations equal 24-hour minimum levels, as Tacrolimus is metabolized more rapidly during the night, and are measured before application of the morning dose. If Tacrolimus is used without additional induction agents, the levels should not fall outside a range between 5-15 ng/ml during early weeks, and 3-8 ng/ml after the first year. Regular monitoring of blood concentrations ensures efficacy and helps to minimize exposure dependent toxicity, since oral Tacrolimus bioavailability varies widely between patients, ranging from around 5 to 90% (mean ~25%) (52, 53).

This wide range stems from limited enteric drug absorption due to poor solubility in water as well as genetic differences in enteric and hepatic drug-metabolizing enzymes. Early metabolism occurs in the mucosa of the upper gastrointestinal tract. Both Tacrolimus and Cyclosporine are then transformed in the liver by Cytochrome P450 3A (CYP3A4) and ultimately excreted into the bile. Peak concentrations (C_{max}) occur two to three and five hours after application with immediate release and extended-release Tacrolimus dosage forms, respectively. Substances that affect CYP3A metabolism can alter trough levels, such as calcium channel blockers or antifungals. Other factors that can affect blood concentrations include intake of high-fat foods, age, infections, ethnicity, hepatic and renal function, or gastrointestinal disease. For Tacrolimus, the half-life ranges between 5-16 hours in blood plasma and steady state is reached after 3-7 days. In serum samples, around 99% of Tacrolimus is bound to erythrocytes and albumin (54–57).

During the early weeks after transplantation, clinicians gradually decrease the frequency of Tacrolimus concentration measurements. After six months, levels may be measured every four to twelve weeks. Tight monitoring is resumed when concomitant medications, Tacrolimus dosage, or formulations are altered until concentrations are stable again. This is particularly important

when the prescribed medications comprise potent inhibitors or inducers of CYP3A4, for example, macrolide antibiotics (53, 58–60).

1.1.3 Safety and Tolerability

The principal direct side effects of CNIs include nephrotoxicity, neurotoxicity, and diabetogenicity. The corresponding disease burden adds to the inherent indirect side effects of chronic immunosuppression: long-term therapy increases the risk for malignant and infectious disease, which together make up 40% of the causes of death after liver transplantation (61, 62).

Renal dysfunction due to CNI therapy remains the most important contributor to all-cause mortality after solid organ transplantation. Tacrolimus causes renal vasoconstriction, leading to reduced filtration and progressive destruction of nephrons. Kidney failure can occur in two forms: acute renal injury with an increase in plasma creatinine – usually reversible with dose reduction - and chronic kidney disease (CDK). CDK occurs in around 20% of patients after five years of Tacrolimus treatment – some liver transplant survivors require dialysis or a kidney allograft (28, 63–66).

The progressive renal failure is exacerbated by the diabetogenic and hypertensive effects of Tacrolimus treatment. CNIs can decrease peripheral insulin sensitivity and increase blood pressure through renal vasoconstriction. In around 50% of liver transplant recipients, this spiral leads to a metabolic syndrome, including diabetes, hypertension, dyslipidemia, and obesity. Patients with metabolic syndrome are not only at increased risk for cardiovascular death, but the associated hypertensive and hyperglycemic damage can act synergistically to injure the kidneys. Patients may respond to dose reductions or require antihypertensive and diabetic drugs (30, 31, 67–71).

Another major problem is neurotoxicity, which is typically more severe with intravenous application. Expression of Calcineurin Phosphatase can be found in many tissues beyond immune cells, including diverse areas of the brain and the peripheral nervous system. Severe adverse effects such as seizures or posterior reversible encephalopathy syndrome (PRES) can rarely occur during the early post-transplant period, although improvements occur rapidly as dosage is reduced. Patients with PRES can present with variable clinical manifestations such as seizures, headaches, or abnormal vision. Imaging reveals typical subcortical edema without signs of infarction. More frequent, non-serious neurological symptoms occur in 30-60% of patients,

who experience tremor, insomnia, restlessness and agitation, or paresthesia. Postural hand tremor is the most common neurological side effect, affecting around one out of four patients treated with Tacrolimus. While these effects do not contribute to increased mortality, they can impede patients' QoL and may reduce medication compliance (38, 72–76).

In addition to its diabetogenic effects, Tacrolimus can cause a variety of other metabolic disturbances including hyperuricemia, hyperkalemia, and hypomagnesemia. Some patients also experience gastrointestinal disorders such as diarrhea or vomiting, which require careful monitoring to ensure adequate Tacrolimus blood concentrations (53).

The most important predictor of Tacrolimus toxicity is 24-hour drug exposure (AUC). In clinical studies, serum trough levels (C_{min}) closely correlate with the AUC ($R > 90\%$). The C_{min} corresponds to the serum Tacrolimus concentration measured before application of the morning dose and is used as a relative measure of the AUC. In the mid-1990s, researchers at Fujisawa USA Inc. (now Astellas Pharma Inc.) demonstrated that there is a linear relationship between the Tacrolimus C_{min} and renal injury in 824 liver and kidney transplant recipients. In their study, an increase in the C_{min} of 1 ng/ml was associated with a 1% increase in the incidence of a rise in creatinine, the correlate for renal function used by the researchers ($p <= 0.01$). Similarly, Böttiger et al. showed that the incidence of Tacrolimus adverse events increases linearly until a C_{min} of around 20 ng/ml and rises exponentially thereafter. The relationship between high Tacrolimus exposure and the occurrence of side effects has also been demonstrated in rat models, where intracerebral Tacrolimus concentrations highly correlate with neurotoxic events (36, 37, 39, 40, 77).

In clinical practice, Tacrolimus side effects closely respond to dose reductions and a corresponding fall in blood concentrations. These observations led physicians to try to minimize Tacrolimus toxicity by reducing the TDD. Tacrolimus treatment requires regular monitoring of the C_{min} to manage dosage. This practice is known as therapeutic drug monitoring (TDM) and is part of the standard practice recommended for long-term management of liver transplant recipients by American and European guidelines. Since the risk of rejection increases disproportionately when concentrations fall below 3 ng/ml, most transplant centers use a C_{min} target of between 3-8 ng/ml one year after surgery (16, 26, 69, 78).

To refine C_{min}-guided Tacrolimus therapy, a German research group led by Thölking recently proposed an additional pharmacokinetic predictor that could help clinicians avoid Tacrolimus toxicity - the concentration/dose (C/D) ratio. The C/D ratio equals the C_{min} divided by the TDD. It can be used to gauge a patient's Tacrolimus metabolism: a low ratio indicates that the patient requires a high TDD to reach adequate C_{min} values – so called fast Tacrolimus metabolizers – and vice versa. In their first study, Thölking split a sample of 248 consecutive renal transplant recipients into three groups based on the C/D ratio: slow, intermediate, and fast metabolizers. They showed that fast Tacrolimus metabolizers (low C/D ratios) exhibit significantly worse renal function based on the estimated glomerular filtration rate (eGFR) at months 1,2,3,6,12 and 24 ($p = 0.004$) compared to slow metabolizers. In the second study, the same group split a sample of 115 consecutive liver transplant recipients into fast and slow Tacrolimus metabolizers based on the median C/D ratio - again, fast metabolizers exhibited significantly worse eGFR than slow metabolizers at months 6,12 and 36 ($p = 0.018$). In a third study of 55 renal transplant recipients, the researchers showed that fast Tacrolimus metabolizers exhibit significantly higher C_{max} at identical C_{min} and AUC values than slow metabolizers. Analysis of fast Tacrolimus metabolizers showed that they not only display significantly elevated C_{max}, but also higher rates of CNI nephrotoxicity – which the researchers defined as significantly higher rates of isometric vacuolization of tubular epithelial cells, as compared to slow metabolizers. Isometric vacuolization is a widely used metric for renal injury caused by CNI therapy, and was assessed by independent, blinded pathologists in this study. Based on their observations, they concluded that a high C_{max} is associated with increased rates of renal injury at identical 24-hour drug exposure. Likewise, in an early study in 2001, Ishibashi et al. showed that the diabetogenic effects of Tacrolimus significantly depend on elevated C_{max} in rat models. Similarly, clinical studies that involve repeated measurements of Tacrolimus concentrations suggest that CNI neurotoxicity is also exacerbated in patients who exhibit high peak levels (36, 41, 74, 79, 80).

In summary, Tacrolimus causes variable side effects including nephrotoxicity, neurotoxicity and diabetogenicity as well as other metabolic and gastrointestinal disturbances. The severity of these effects depends on total drug exposure (AUC), which can be estimated by its surrogate C_{min}. High peak concentrations exacerbate renal injury, in particular in fast Tacrolimus metabolizers, as indicated by a low C/D ratio, and are associated with neurologic and diabetogenic complications.

In pharmacokinetic head-to-head evaluations, LCP-Tac led to 30% lower C_{max} when compared to IR-Tac at comparable C_{min} and AUC values. Given the association between the C_{max} and the occurrence of Tacrolimus nephrotoxic, neurotoxic and diabetogenic side effects, conversion from IR-Tac to LCP-Tac may thus improve CNI tolerability and safety. The distinct pharmacokinetic profile of LCP-Tac in comparison to IR-Tac may also allow for an absolute reduction in daily Tacrolimus dosage in excess of the recommended conversion ratio and a corresponding lower target C_{min} without causing an increase in rejection rates (14).

1.1.4 Available formulations

Currently, three oral Tacrolimus formulations are available: twice-daily immediate-release Tacrolimus (Prograf®, IR-Tac), extended release once-daily Tacrolimus (Advagraf®, ER-Tac), and novel extended-release once-daily Tacrolimus (Envarsus®, LCP-Tac). Although all three release the same drug, they are not interchangeable due to different pharmacokinetic properties (Figure 2).

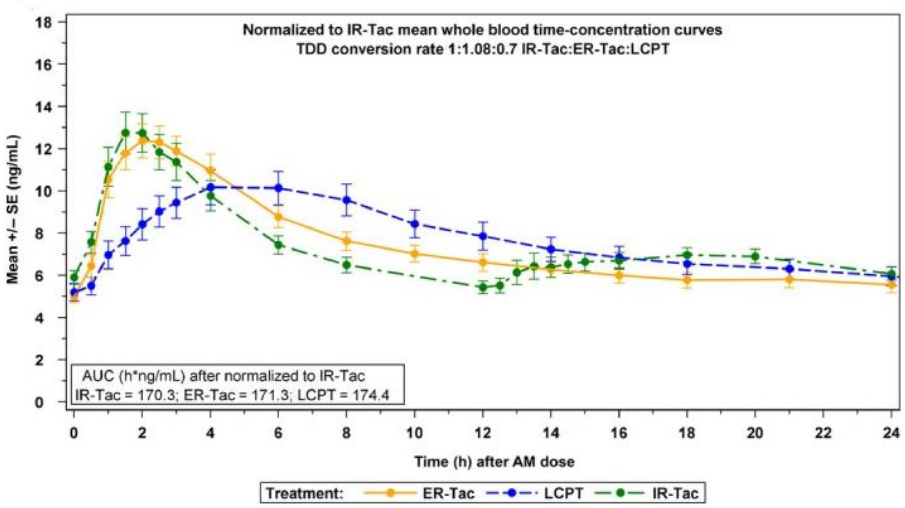


Figure 2: Exposure (AUC) - normalized mean whole blood concentrations of Tacrolimus based on mg conversion factors of 1: 1.08 : 0.70 – IR-Tac: ER-Tac : LCP-Tac. AUC, area under the curve; SE, standard error of the mean; TDD, total daily dose – reproduced with permission from the American Journal of Transplantation and Tremblay et al. (14).

IR-Tac has been the mainstay immunosuppression for the prevention of organ rejection since the 1990s. IR-Tac is classified as an immediate release agent - pharmacokinetic profiles of IR-Tac show supratherapeutic peak levels one to four hours following application as well as a high peak to trough variability (C_{max}-C_{min}). Patients must take the medication every twelve hours to ensure

efficacy and manage each dose in accordance with food intake, as they need to take IR-Tac one to three hours before a meal (69).

Modified release dosage forms use techniques that delay drug absorption with the aim of reducing dosing frequency or side effects. ER-Tac was the first once-daily Tacrolimus tablet approved for the prevention of kidney and liver transplant rejection in 2007. It uses an ethylcellulose matrix and hypromellose gel layer to avoid rapid drug uptake along the gastrointestinal tract. Like LCP-Tac, the formula was developed to improve adherence through reduced dosing, but ER-Tac exhibited no significantly lower peak concentrations compared to IR-Tac (13, 81).

Early clinical trials advocated its use. In a study published by Beckebaum et al. in 2011, researchers switched 119 stable liver transplant recipients from IR-Tac to ER-Tac. After conversion, overall adherence as measured with the Basel assessment of immunosuppressant adherence (BAASIS©) questionnaire improved from 40% at baseline to 70% after twelve months (82).

In addition, an industry-sponsored retrospective evaluation of European registry data by Adam et al. (2015) and a follow up study by Adam et al. (2019) suggest that patients who receive ER-Tac benefit from improved long-term patient (3y: 88% vs. 82%, $p = 0.07$; and 4y: 85% vs. 80%, $p = 0.017$) and graft survival (3y: 88% vs. 80%, $p = 0.01$; and 4y: 83% vs. 77%, $p = 0.001$) compared to patients treated with IR-Tac at years three and four after surgery, respectively. However, several drawbacks make interpretation of their results difficult. Firstly, around 90% of patients in the registry data used by the researchers received IR-Tac, while only 10% received ER-Tac. An undisclosed number of the10% receiving ER-Tac were actively enrolled in clinical trials and most likely received tight and extensive monitoring. Secondly, physicians who decided to treat patients with ER-Tac outside of a clinical trial were likely haven choosen those patients because they considered them to be at different baseline risk for rejection events compared to those who were maintained on IR-Tac. In fact, Adam et al. (2015) report that the patients who received ER-Tac were more likely to receive additional MMF (93.6 vs. 65.8%, respectively; $p < 0.0001$), but less likely to require corticosteroid induction therapy (58.5 vs. 94.7%; $p < 0.0001$), indicating that there were baseline risk differences between the two patient groups. Thirdly, the authors do not restrict their analysis to patients who received either IR-Tac or ER-Tac from the first month after surgery up to the end of the observation period (years 3 and 4, respectively). In the study from 2015, patients were allocated to either of the two groups based

on their immunosuppression at month 1, regardless of whether they were switched to a different regimen afterwards. In the second study (2019), the maintenance immunosuppression at the last patient follow-up was used to classify patients. The authors tried to eliminate bias using propensity-score matching based on recipient (age, model for end-stage liver disease (MELD) score, viral hepatitis status, ascites before transplantation), donor (age, cause of death) and graft (ischemic time, graft preservation solution) characteristics as well as early additional immunosuppression (e.g. steroids, MMF, Basiliximab) in both studies, but they used a different set of variables for matching.

The possible bias due to unobserved variables (e.g. the improved patient care in clinical trials with ER-Tac, the decision of the treating physician to switch a particular patient to the ER-Tac formula due to her unique clinical and psychological characteristics, the ambiguity regarding the classification of IR and ER-Tac treatment length in both studies, as well as methodological problems such as model dependence in propensity-score matching) makes interpretation of the result difficult. So far, no randomized controlled trial (RCT) has demonstrated improved survival in patients treated with ER-Tac when compared with IR-Tac. In fact, the largest publicly available RCT showed worse one-year patient and graft survival rates with ER-Tac (-1.6% and -0.3% compared to IR-Tac), while both the 2015 (+1% and 2%) and 2019 (+2% and +2%) propensity-score matched analysis showed superior one-year patient and graft survival rates with ER-Tac (12, 13, 83–86).

Ultimately, ER-Tac has failed to replace IR-Tac as the standard of care for the prevention of organ rejection over the last decade for two major reasons. Firstly, in adult transplant patients, the AUC and C_{min} were between 5-15% lower for ER-Tac when compared with IR-Tac at the recommended 1:1 conversion ratio. Consequently, several RCTs provided evidence for higher rejection rates under ER-Tac therapy. And secondly, a multicenter, randomized, two-arm study in 475 primary liver transplant recipients revealed a three times higher mortality rate in female patients (18.4%) than in male ones (6.8%). As a result, ER-Tac is not approved for use in liver transplantation in the United States and is used only seldomly with selected patients in Europe (12, 13, 87).

LCP-Tac is a novel once-daily extended-release Tacrolimus formulation. According to the manufacturer's drug information, it uses a so-called "melt-dose technology", which enhances absorption and bioavailability by reducing drug particle size down to single molecules. In a phase II trial, Gaber et al. demonstrated safe and effective use of LCP-Tac as well as a lower

C_{max} (-30%, p<0.001), C_{max}/C_{min} ratio (p<0.001), percent fluctuation[a] (p<0.001) and swing[b] (p<0.001), as well as a 30% reduction of the TDD (p = 0.02) of Tacrolimus in a group of 47 kidney transplant recipients when compared with IR-Tac (27, 88, 87).

Alloway et al. conducted a second pharmacokinetic study in 57 stable liver transplant recipients which supports these findings. Their data showed that patients treated with LCP-Tac exhibit significantly lower C_{max} (-30%, p < 0.001), fluctuation (p < 0.001) and swing percentages (p < 0.001), as well as a reduction of TDD of 30%, despite comparable efficacy and safety to IR-Tac (89).

Data from the ASCTCOFF trial, a pharmacokinetic phase 3b head-to-head study between all three available Tacrolimus formulations in renal transplant recipients again confirmed that LCP-Tac has significantly lower maximum concentrations (-30%), longer time to peak (T_{max}), and less peak to trough variability (both fluctuation and swing) than IR- and ER-Tac. In addition, LCP-Tac allowed for a 30% dose reduction while providing comparable Tacrolimus drug exposure as measured by the AUC and C_{min} (14).

So far, it remains unclear whether the reduced dosing and different pharmacokinetics of LCP-Tac may improve adherence or side effects in transplant recipients when compared with IR-Tac, the current standard of care.

1.2 Non-adherence to immunosuppression after liver transplantation

Despite the need for consistent immunosuppression, between 15-40% of liver transplant recipients do not adhere to their agreed treatment, putting themselves at risk for poor outcomes and increasing health care costs (8).

When patients skip doses and concentrations fall, immune cells resume attacking the graft. Even smaller deviations from the treatment plan such as delayed intake may have adverse effects. Tacrolimus is a narrow index drug, and irregular timing of application can lead to alternating phases of under-immunosuppression with increased risk for graft loss and over-immunosuppression with increased risk for toxicity.

Since adherence rates remain high during the first few months after transplantation, studies on the negative effects of non-adherence focus on the incidence of late acute rejection. Late acute

[a] Fluctuation (%) = $100 * \frac{(Cmax - Cmin)}{Cavg}$

[b] Swing (%) = $100 * \frac{(Cmax - Cmin)}{Cmin}$

rejection (LAR) is defined as any rejection episode occurring later than six months after transplantation. It affects between 7-23% of patients and increases treatment costs as well as the risk for retransplantation and all-cause mortality. In a recent analysis of 970 consecutive liver transplantations at the university hospital in Birmingham (UK), there was a significant difference in 10-year graft survival between patients with LAR (74%), no rejection (81%), and those with early acute rejection (85%, $p < 0.01$). The authors suggest that the good prognosis for patients with early rejection confirms prior evidence that early immune activation may be required for allograft tolerance. By contrast, 29 (30%) of the patients with LAR in their sample developed chronic rejection, of whom 15 (51%) died (90–94).

The main problem in studying the consequences of non-adherence in transplantation is the lack of a standardized tool and comparable methods across trials which objectively and accurately measure drug intake. In early studies, many researchers used unexplained subtherapeutic immunosuppressant levels after hospital discharge as an indicator for irregular intake. Since patients receive careful monitoring and adjustments to their medication regimen during the early postoperative days and weeks, the clinical teams were surprised to find out that some patients exhibited significantly lower serum levels compared to their hospital stay. The change in drug exposure could thus not be explained by constant factors such as CYP3A4 or efflux pump protein polymorphisms. In response, they openly confronted patients, albeit in a supportive manner, about their drug intake.

In 1990, Schweizer et al. published the first study on non-compliance in renal, heart and liver transplant recipients after having observed that they could revert many LAR episodes with only small amounts of corticosteroids. Between 15-18% of all patients openly admitted non-compliance, which led to increased rejection and mortality in 40-90% of these cases. They reported that 3 (23%) out of the 13 liver transplant recipients in the study required hospitalization to treat rejection secondary to non-adherence - two patients died, while the third patient continued to require treatment for recurring rejection episodes (1).

Two years later, Eytan Mor conducted a study of 375 consecutive liver transplants recipients at Baylor Medical Center Dallas, US. A total of 27 (8%) patients experienced LAR, and 35% of those patients openly acknowledged non-compliance with their immunosuppression. The researchers suspected even higher non-adherence rates as more than 50% of patients exhibited subtherapeutic immunosuppressive levels at the time of admission and they reversed rejection easily with corticosteroids in 80% of cases (2).

Both Schweizer et al. as well as Mor et al. suggested that the high rate of treatable LAR episodes indicates that many patients might not openly admit non-adherence and they resume medication intake prior to clinic visit (1, 2).

Berlakovich et al. (2000) used a similar approach. They studied the incidence of LAR episodes in 118 patients transplanted for alcoholic liver cirrhosis and likewise defined non-adherence as unexplained subtherapeutic immunosuppressant levels. Based on this definition, they reported a significant difference in LAR episodes between non-compliant (21%) and compliant (5%) patients ($p < 0.01$) (3).

A Scottish research group lead by O'Carroll (2006) used low appointment attendance rates as an estimator for poor adherence in a retrospective evaluation of 435 liver transplant recipients. They showed that low attendance rates are strongly associated with both subtherapeutic immunosuppressant levels and LAR episodes. Based on this relationship, they estimated that rejection due to non-adherence might cause one out of ten deaths and one out of three retransplantations (7).

Berquist et al. (2006) conducted a retrospective chart review to study the consequences of non-adherence in 97 adolescent liver transplant patients at Stanford University Medical Center. They showed that documented non-adherence reported by the patient, parent, or healthcare provider is significantly associated with LAR, re-transplantation, and death secondary to chronic rejection (4).

Since these studies do not employ a standardized approach to measure drug intake, it is difficult to compare the data or estimate an effect size for non-adherence on LAR, retransplantation, or mortality. While Mor et al. as well as Schweizer et al. openly asked patients with easily treatable rejection episodes about drug intake, Berlakovich et al. used subtherapeutic immunosuppressant levels as their sole measure of adherence. In the study led by O'Carroll, the researchers correlated low attendance rates with subtherapeutic immunosuppressant levels and rejection episodes. By contrast, Berquist et al. solely used notes about non-adherence in patients' charts to estimate drug intake.

It is evident that future researchers need comparable methods and tools to measure the effect of non-adherence to immunosuppression on transplant outcomes. Nevertheless, these studies

indicate that irregular drug intake may cause more than one out of three LAR episodes and retransplantations as well as one out of ten deaths after liver transplantation.

1.3 Intra-patient variability (IPV) of Tacrolimus serum levels and adherence

Many patients experience difficulties communicating their problems with drug intake when not specifically asked. Given the high cost of tracking compliance via surveys or electronic pill monitoring, some researchers have proposed a different approach over recent years - they use the intra-patient variability (IPV) of consecutive Tacrolimus levels as a proxy for adherence. In transplantation research, the IPV refers to the degree of fluctuation in Tacrolimus serum levels. It can be measured via two related metrics: the standard deviation (SD) or the coefficient of variation (COV - the standard deviation divided by the mean) of consecutive Tacrolimus measurements when dosage remains unchanged.

The problem with using the IPV as a metric of adherence is that a plethora of factors such as drug-drug or drug-food interactions, circadian rhythm, or gastrointestinal disorders also affect Tacrolimus variability. Nevertheless, an elevated IPV is increasingly recognized as an independent risk factor for poor outcomes after solid organ transplantation including rejection, graft survival, toxicity and death – and it may correlate with low adherence (95, 96).

Early studies that used IPV as the major outcome variable after transplantation were conducted in kidney transplant recipients: a Dutch study published in 2010 by Borra et al. showed that an elevated COV six to twelve months after transplantation is associated with graft loss and renal impairment. In their sample of 297 patients, those with an IPV above the median had a threefold relative risk (exponent B = 3.125, p = 0.003) of reaching the composite endpoint of allograft loss, biopsy-proven nephropathy, and doubling of plasma creatinine (as a surrogate for renal dysfunction) in a multivariate cox regression analysis (97).

In 2016, the same group used a larger sample of 808 kidney transplant patients to demonstrate the robustness of this relationship - they showed that an elevated COV is independently associated with the composite endpoint of graft failure, rejection, and renal impairment. However, they reported a smaller effect than before: patients with an elevated IPV had a 1.4-fold risk (hazard ratio: 1.42, 95% CI: 1.06–1.90; p = 0.019) for the composite endpoint in multivariate cox regression analysis, as compared to a threefold risk reported for the same endpoint in the study lead by Borra (98).

In a similar study in 356 renal transplant recipients by Sapir, researchers showed that every 1-unit increase in Tacrolimus SD is associated with a 1.3-fold risk increase (hazard ratio 1.27 ,

95% confidence interval 1.03-1.56) of reaching the composite endpoint of rejection, transplant glomerulopathy and graft loss (99).

Subsequent studies by Seibert et al. (2018) as well as Süsal et al. (2019) aimed to quantify a critical threshold that could help clinicians to identify patients at risk. In their studies, an elevated IPV was defined as a COV exceeding 30%. Using this threshold, they found that a high IPV (COV>30%) in Tacrolimus exposure during months zero to six and years one to three after kidney transplantation predicted acute rejection, graft failure, and death-censored graft survival in 1,472 ($p < 0.02$) and 6,638 ($p <0.001$) patients, respectively (100, 101).

Similar findings have since been reported for liver transplantation: in 2014, a study in 150 adults published by Supelana et al. showed that patients with biopsy-proven rejection had significantly higher SDs of consecutive Tacrolimus blood concentrations when compared with the rest of the cohort that exhibited no signs of rejection ($p < 0.01$) (102).

In 2018, De Bello et al. showed that a high IPV (OR = 3.07, 95% CI: 1.14 - 8.24, $p = 0.03$), defined as a COV exceeding 35% between discharge and 24 months after transplantation, as well as consistently low Tacrolimus levels (<5 ng/ml) were independent risk factors for biopsy-proven acute rejection in 116 adult liver transplant patients (103).

Eyal Shemesh et al. of Mount Sinai hospital (NY, US) conducted the only large scale, prospective, multisite study on the IPV available to date. They calculated the SD of Tacrolimus measurements in 379 pediatric liver transplant recipients during a two-year period and found that an elevated IPV predicted LAR across eight different study sites in the United States (mean SD with late acute rejection: 2.4 [SD 3.6], versus without rejection: 1.6 [SD 1.1]; $p = 0.026$). Results from pediatric populations cannot readily be transferred to adults, but the study adds to the body of evidence that the relationship between serum level variability and rejection holds across different transplant patients (104).

While Tacrolimus IPV is likely associated with adherence, it remains difficult to gauge what percentage of the fluctuation can be attributed to irregular drug intake. Schweizer et al. (1990), Mor et al. (1992), and O'Carroll et al. (2006) showed that self-reported non-adherence can explain subtherapeutic serum immunosuppressant levels in many patients. Two recent publications by Lieber (2013) and Leino (2019) aimed to identify whether non-adherence can also predict the Tacrolimus IPV (6, 96).

In the first study, published in 2013, Lieber et al. examined the relationship between IPV, self-reported adherence, and poor outcomes in liver transplant patients. They show that self-reported non-adherence noted in patient charts is associated with a 2.7-fold increase in the SD of Tacrolimus blood levels in a retrospective analysis of 122 adult liver transplant recipients ($p = 0.03$). Using a second sample of 544 patients, they found that an increased SD of Tacrolimus is independently associated with allograft loss ($p = 0.04$) (6).

The second study was conducted in 50 liver and renal transplant recipients and published by Leino et al. in 2019. The researchers aimed to establish baseline parameters of Tacrolimus IPV in patients who were instructed to maintain strict adherence to the study protocol throughout the study period of six weeks, defined as taking every dose within 30 minutes of planned intake. This resulted in a compliance rate of 99.9%, as measured by electronic pill counts and self-reported diaries after completion. Given this high rate, their results could help clinicians to monitor IPV for irregular values in the future (96).

Pharmacokinetic evaluations showed that the median COVs of daily Tacrolimus levels were 14.4% in liver transplant patients (n=25) and 16.8% in kidney transplant patients (n =25), which the authors attribute to the high adherence rate. The robustness of their findings is strengthened by the fact that study participants switched between three different generic formulations of IR-Tac every two weeks and post hoc analysis revealed a near-perfect mean interval between the evening and morning dose of 11.86 hours. However, while the median COVs in their study lie below the cutoff values proposed for risk stratification (30-40%), study subjects exhibited COVs exceeding 30% in about 20% out of the total 287 study weeks (~6 weeks * 50 patients), despite the high (99.9%) reported compliance rate (96).

These findings show that potentially hazardous variation (COV >30%) can occur even when drug intake is optimal, challenging the notion that the IPV is a valid measure of adherence. In a recent review by Kuypers (2019), the author summarizes known factors that affect Tacrolimus IPV (Figure 3) (95).

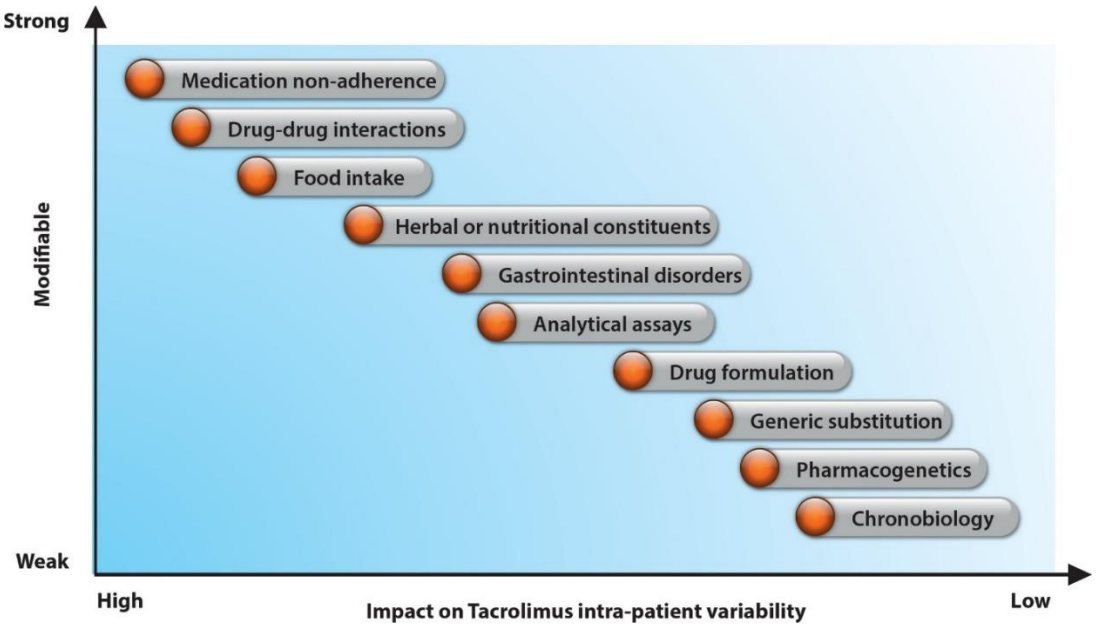


Figure 3: Variables that affect Tac IPV, ranked by their potential to be modified through clinical interventions (e.g., patient education on dosing and timing, avoiding certain types of food or herbal constituents, supervised switching to generic formulations) and their impact. The effect size of each variable on IPV has not been determined in comparative clinical trials but is based on individual studies (e.g. Leino et al.) and expert panels such as the "Guidance Report and Clinical Checklist by the Consensus on Managing Modifiable Risk in Transplantation (COMMIT) Group" (69, 96). Reproduced with permission from "Clinical pharmacology and therapeutics" (95).

Given this wide range of variables, Gustavsen et al. (2019) recently compared the IPV with two indirect measures of adherence in a prospective study of 295 kidney transplant recipients. In the first part of their analysis, the researchers tested whether a high IPV (COV > 30%) identifies the same patients as non-adherent when compared to a clinician's score or a standardized patient survey, the 'Basel Assessment of Immunosuppressant Adherence Medication Scale' (BAASIS©). When using Tacrolimus variability as a standalone tool, 12% of patients were identified as non-adherent after one year. For the clinician's score, the treating physicians were asked to classify their patients' adherence as excellent, suboptimal, or poor – anything but excellent was classified as non-adherent. This measure resulted in a non-compliance rate of 9%. The BAASIS© comprises four separate questions ("did you miss a single dose? – miss several doses? - delay intake? – reduce dosage by yourself?") about the previous four weeks. If patients reply yes to any of the items, they are classified as non-adherent – this was around 29% of patients in this study after twelve months. In total, 38% of the study participants were classified as incompliant on at least one of the three measures. This shows that there is only partial overlap between the definitions, raising the question of whether the instruments are valid (105).

Gustavsen et al. believe that the clinician's score may be the least useful measure because the frequency of the patient-physician contact decreases with time and a subjective assessment may be associated with limited reliability across different researchers (105).

Leino et al. have shown that large fluctuations in blood concentrations (elevated IPV) can occur despite timely drug intake due to a wide range of factors that affect Tacrolimus metabolism. Additionally, estimating compliance through measurement of drug levels is also prone to white coat adherence, which describes a situation when patients take their medication meticulously prior to their next clinic appointment. Conversely, patients might experience difficulties communicating problems with their drug intake but exhibit highly variable Tacrolimus concentrations.

In the second part of their analysis, Gustavsen et al. analyzed whether either of the three measures was associated with biopsy-proven rejection one year after surgery. Although the study period was too short to measure possible effects on LAR or retransplantation, non-adherence on the BAASIS© significantly increased the hazard rate of developing de novo donor-specific antibodies (dnDSAs). DnDSAs target donor antigens and can lead to graft dysfunction and loss in both kidney and liver transplant recipients. Moreover, a Dutch group led by Tielen showed that nonadherence on the BAASIS© was significantly associated with graft loss two years after surgery in 117 kidney transplant recipients (105–107).

The relative paucity of trials evaluating the effect of non-adherence on the BAASIS© on transplant outcomes compared to the number of studies evaluating the IPV is related to several factors. First, almost all studies on Tacrolimus IPV are retrospective. The analysis is inexpensive and only requires centers to have access to blood sample and outcome data (rejection rates, mortality). Second, the BAASIS© is relatively new tool, developed by the Leuven-Basel Research Group (LBARG) in the late 2000s, and has only recently gained attention in the transplant community - in 2017, an international expert panel of liver and kidney transplant researchers recommended the widespread implantation of the BAASIS© in clinical practice to increase the validity and reliability of adherence studies in transplantation (69, 108).

Both methods, namely questioning patients and measuring Tacrolimus variability have potential blind spots and require careful construction when used not only used as adherence measures, but as explanatory variables for poor outcomes. For this reason, Gustavsen et al. recommend the combined use of several tools such as the BAASIS© and Tac variability to study the

consequences of non-adherence or identify those at risk for poor outcomes due to incompliant behavior in clinical practice (105).

1.4 Available interventions to improve adherence

The problem of poor adherence prevails after transplantation and researchers have studied the topic across many disciplines. While patients take nearly every pill in acute treatment settings, only one in six follows the agreed treatment protocol in chronic therapy. This raises morbidity and mortality, and is estimated to induce costs of around $100 billion annually in the United States alone (109).

Like other conditions, adherence rates after liver transplantation also show a time-dependent trend - most patients follow immunosuppressant protocols tightly in the immediate post-transplant setting, but long-term non-adherence rates increase to 9% at six months and to 24% after three years (91).

While diverse factors such as lifestyle, socioeconomic status, demographic or psychosocial characteristics can influence adherence, the treatment related factors regimen complexity and side effects constitute the strongest predictors of correct drug intake (9, 109).

Today, it is widely accepted that twice-daily formulations are associated with inferior adherence rates compared to once-daily dosing. On average, each additional dose is associated with a 5-10% reduction in adherence rates (Figure 4). In this regard, physicians might contribute to non-adherence by prescribing complex drug regimens (9–11).

Figure 4: Adherence to medication and doses/day.

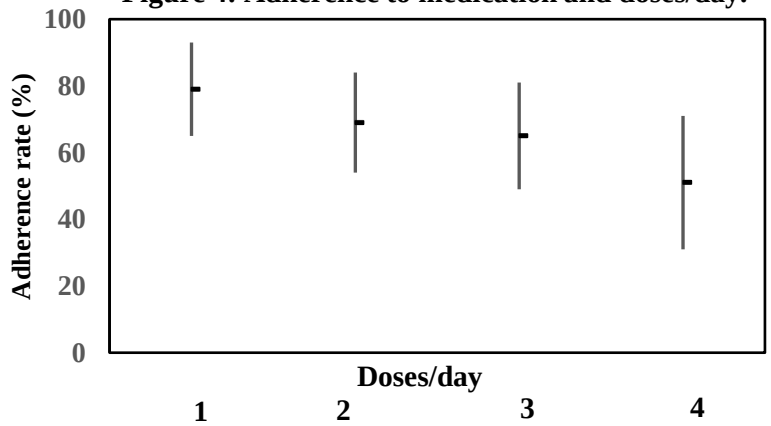


Figure 4: Medication adherence and frequency of daily doses. Vertical lines represent one standard deviation on either side of the mean adherence rate (horizontal bars). Data from Osterberg (109) and Claxton (110).

Over recent decades, researchers have tested many novel interventions such as motivational interviewing, reminders, or cash bonuses for improving adherence. But a large meta-analysis by Kripalani et al. (2007) showed that the only consistent method, and the one with the largest effect size, is the reduction of doses per day. For example, the reduction of daily doses of antihypertensive drugs or statins leads to improved adherence as well as reduced blood pressure or serum lipids (9).

Side effects constitute the second major determinant. Estimates for adherence with oral chemotherapy are as low as 16% (111). Patients know of the necessity to take the medication, but many experience intolerable side effects. In solid organ transplantation, Tacrolimus replaced Cyclosporine A not only due to higher efficacy in preventing rejection, but also because it does not cause somatic growth such as gingiva hyperplasia or hirsutism (25).

In 2012, Morales et al. published results from a survey on the barriers to adherence of more than nine thousand liver and kidney transplant recipients in Spain. Their analysis revealed that patients indeed perceived treatment related factors including frequent dosing and adverse effects as the most important barriers for compliance and significant impediments to their quality of life. The authors concluded that simplification of regimens and targeting of lower Tacrolimus levels could improve taking behavior and QoL as well as reduce side effects (112).

1.5 Study objectives

Non-adherence to treatment and toxic side effects of Tacrolimus therapy are compromising long-term outcomes after liver transplantation with an increased risk for graft loss and all-cause mortality as well as reduced QoL. Based on the currently available evidence, we hypothesized that conversion of stable liver transplant patients from IR-Tac to LCP-Tac would (i) improve adherence to immunosuppression as assessed by the BAASIS© questionnaire, and (ii) reduce typical side effects such as nephrotoxicity, neurotoxicity, and diabetogenicity, (iii) without causing an increase in rejection rates.

Thus, the primary objective of this study was to determine adherence, safety, and efficacy after conversion of the immunosuppressive medication from IR-Tac to LCP-Tac in stable liver transplant recipients. To test the hypotheses, we conducted a 24-month observational conversion study as part of our routine patient care after liver transplantation at our high-volume transplant center.

2. Materials and Methods

2.1 Participants

Inclusion criteria included female and male patients (aged >18 years) who had received a primary liver transplant from living or deceased donor at the Charité Berlin Virchow hospital >3 months prior to enrollment, and had agreed to follow the study protocol. Patients treated with an IR-Tac regimen as maintenance immunosuppression were recruited during regular outpatient clinic visits at the transplant center.

Exclusion criteria included any dose adjustments within six weeks prior to conversion of the primary immunosuppression, any rejection episodes within six months prior to conversion (based on the 2016 Banff-classification), HCV-RNA positive patients, patients with unstable disease defined as any acute disease (any disease requiring medical intervention), malignancy, or laboratories outside the reference range used at the center (107).

2.2 Study design

This was a single-center, two-year, single-arm, prospective phase IV-trial in 165 stable liver transplant recipients on adherence, efficacy, and safety after conversion from conventional twice-daily Tacrolimus (IR-Tac) to novel once-daily Tacrolimus (LCP-Tac).

The study period was 24 months for each patient and included ten study points: pre-conversion (baseline), weeks 1, 2, 3, and 4, and months 3, 6, 12, 18 and 24 after conversion. The study was conducted at the Chirurgische Klinik Campus Charité Mitte / Campus Virchow transplantation center in accordance with the declaration of Helsinki by the World Medical Association and approved by the internal institutional review board (EA2/027/16). All patients enrolled were part of the routine liver transplant follow-up care program at the center. Written informed consent was obtained from each patient prior to enrollment. Enrollment occurred between December 2015 and February 2018.

At baseline, patients were converted from IR-Tac to LCP-Tac based on a 1:0.7 mg ratio as recommended by the manufacturer. Necessary adjustments were made to achieve target Tacrolimus trough levels of 3-8 ng/ml. As part of the Calcineurin inhibitor-sparing regimen used at the center, downward dose adjustments toward the lower target range were conducted during subsequent months when no laboratory or clinical signs of rejection were identified. Concomitant immunosuppression was unaltered. If patients were excluded or withdrew from the study, they were reconverted to IR-Tac.

Laboratory and clinical parameters collected at each time point included liver (aspartate aminotransferase - AST, alanine aminotransferase - ALT, gamma-glutamyl transferase - GGT, alkaline phosphatase - AP, bilirubin, albumin) and renal (creatinine, estimated glomerular filtration rate - eGFR, urea) chemistry, electrolytes (sodium, potassium, chloride, calcium), blood count (hemoglobin, leukocytes, thrombocytes), glucose, lipid profile (total cholesterol, high density lipoprotein – HDL, low density lipoprotein – LDL, Triglycerides - TAG) and blood pressure, as well as Tacrolimus trough levels and dosage. Other variables collected at baseline and from month three onward included Magnesium, Phosphate, and HbA1C. Measurements were obtained through the Labor Berlin – Charité Vivantes GmbH.

At baseline and months 6, 12, 18, and 24, patients additionally underwent a standard physical examination that included evaluation of HEENT (head, eyes, ears, nose, and throat), the respiratory and cardiovascular system (auscultation), abdomen, extremities, and a standard neurological exam (mental status, cranial nerves, reflexes, sensory and motor system, gait). The

examination was conducted by physicians at the Charité transplantation facilities. Any adverse event or changes in the physical exam were noted and documented in the study records.

Physicians then conducted the 'Basel assessment of Immunosuppressant Adherence Medication Scale' (BAASIS©) survey with each patient and recorded the results on paper. The BAASIS© consists of four separate questions, namely: over the past four weeks "did you miss a single dose?" (a), "did you miss several doses?" (b), "did you delay intake?" (c), and "did you reduce dosage by yourself?" (d). It also includes a visual analog scale (VAS), on which patients can rate their intake from 0 (no dose taken) to 100 (each dose taken at the correct time ± 2 hours) to 110 (more doses taken than prescribed). A patient is classified as overall non-adherent on the BAASIS©, if they answer yes to any of the four items. The VAS is a supplementary item which does not count in the overall score. The digital transformation was reviewed by two independent investigators and the results were recorded in an electronic database, together with laboratory values and physical exam notes. Researchers who compiled interviews did not participate in data input.

The initial study design was powered to detect a 10-percentage change in overall adherence on the BAASIS© over two years. During the study, two additional analyses were carried out to supplement the ongoing data collection. The first supplementary analysis compared the Tacrolimus IPV throughout the study period (LCP-Tac) with the IPV during IR-Tac treatment as a surrogate parameter for adherence. The goal of this analysis was to see whether a potential change in adherence on the BAASIS© after conversion would be matched by a relative change in IPV. The second additional analysis was to compare the renal function (creatinine, eGFR) during the study period (LCP-Tac) with the renal function during IR-Tac treatment, and to compare the renal function of fast (< median C/D ratio) and slow Tacrolimus metabolizers (> = median C/D ratio) after conversion. The objective of this analysis was to identify potential trends in renal function as well as to see whether fast metabolizers would benefit disproportionately from conversion due to the lower C_{max} and higher bioavailability of LCP-Tac.

The IPV analysis was carried out as follows: to calculate the IPV (SD and COV) for the study period (LCP-Tac), Tacrolimus concentrations from months 3, 6, 12, 18, and 24 after conversion were used. Early measurements (weeks one to four) were excluded since many patients underwent frequent dose adjustments during this period of the study. LCP-Tac can take up to two weeks to reach steady state, and this variation would have confounded the analysis. To calculate the IPV for the IR-Tac period, only patients who received their transplant more than or

equal to 2.5 years prior to conversion were included in the analysis. This allowed for a minimum of six months between transplantation and the first value used for the IR-Tac IPV analysis (-24 months before conversion), since high disease and immunological activity early after surgery bias Tacrolimus fluctuations. For patients who matched this criterion, the Tacrolimus dosage and concentrations were retrieved from physical files. The ideal time point for each measurement (months -3, -6, - 12, -18, and -24 prior to conversion) was calculated for each patient based on their date of conversion to IR-Tac, and the closest values available in the files were recorded.

For the comparison of renal function between the study and the IR-Tac periods, creatinine values were retrieved at the same time points as used for the IPV analysis. The GFR was estimated using the Cockroft-Gault formula. For the classification of Tacrolimus fast and slow metabolizers, the C/D ratio was calculated at baseline before conversion, using the concentration and dosage values under IR-Tac treatment (113).

2.3 Variables

The objective of the study was to investigate changes in adherence, safety, and efficacy in stable liver transplant patients after conversion from twice-daily IR-Tac to novel once-daily LCP-Tac. The following endpoints were defined to assess the potential ability of the drug to affect outcomes.

Primary endpoint:

The primary patient-reported (PRO) endpoint was the percentage change in overall adherence on the 'Basel Assessment of Immunosuppressant Adherence Medication Scale' (BAASIS©) survey from baseline to month 24.

Secondary endpoints*:*

Adherence: the secondary adherence endpoint was change in Tacrolimus IPV (COV, SD) after conversion from IR-TAC to LCP-Tac.

Safety: the secondary safety endpoints included the incidence of adverse events (AEs) and discontinuation due to adverse events including serious adverse events (SAEs), side effects including changes in laboratory values or abnormal findings during physical examination including liver enzymes (AST, ALT, GGT, AP, bilirubin, albumin), kidney function (creatinine, eGFR, urea) including the effect of Tacrolimus metabolism (fast vs. slow) on renal parameters, lipid (TAG, LDL, HDL, total cholesterol) and cardiovascular profile (presence of hypertension,

use of antihypertensive medication), metabolic function (glucose, Hba1C, presence of diabetes, use of diabetic medication), as well as hematological changes (leukocytes, thrombocytes, Hb), and signs of abnormal neurological function during examination. The safety profile also included dose requirements and Tacrolimus trough levels at each follow-up.

Efficacy: the secondary efficacy endpoint was the event rate of treatment failure, defined as biopsy-proven acute rejection (BPAR) based on the Banff criteria, allograft loss, or death throughout the study period.

2.4 Statistical methods

Non-safety data were analyzed for the per-protocol set (PPS), including all patients who completed the entire study period of 24 months. Descriptive statistics were used to describe baseline characteristics, Tacrolimus dosing and trough levels, laboratory values, and adverse events (AEs). Continuous variables are presented as mean values with standard deviations. Categorical variables are stated as frequencies and corresponding percentages. Patient and graft survival were estimated using the Kaplan-Meier method.

Initial sample size was calculated using McNemar's test for dependent samples. Based on an expected difference of 10% on the overall adherence score on the BAASIS© and a dropout rate of 10%, a sample size of 165 patients was required to have 80% power to detect a difference between LCP-Tac and IR-Tac using a two-sided significance level of 0.05. Changes in adherence on the BAASIS© were further sub-characterized according to patient's age, regimen complexity (total number of pills per day, number of non-Tacrolimus doses per day, adjunct immunosuppression), gender, and primary indication for liver transplantation (e.g. acute liver failure, hepatocellular carcinoma).

Continuous data distribution was assessed for normality using the Shapiro-Wilk test statistic and visual examination. When the null hypothesis of normality was rejected, data were log transformed and retested. Comparison between normally distributed data was conducted using a t-test for dependent and independent samples for longitudinal and cross-sectional comparison. Not normally distributed variables were analyzed using the Wilcoxon signed-rank or Friedman test. When cross-sectional analysis was required, the Mann-Whitney U test was applied.

Longitudinal categorical data was analyzed by means means of the McNemars or Cochrane's Q tests. The Chi-square test was used for cross-sectional comparison. All statistical analysis was

performed using Microsoft Excel and IBM SPSS Statistics software Version 26 (SPSS Inc, Chicago ILL).

3. Results

3.1 Study population

3.1.1 Participants

We recruited 165 patients between October 2016 and March 2018. Of those, 161 (98%) received the study drug and 134 (83%) completed the 24-month follow-up period (Figure 1).

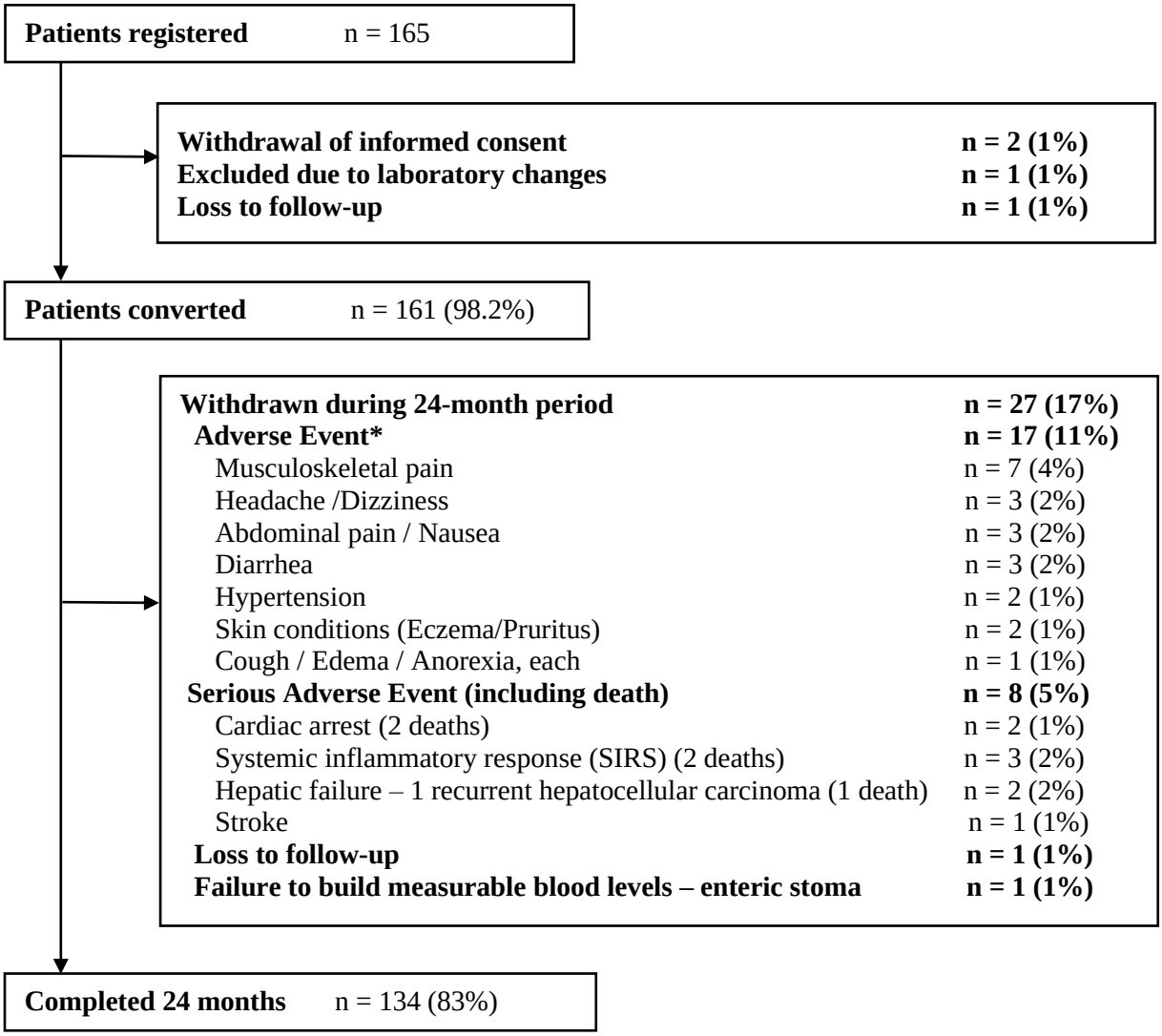


**Figure 5: Patient disposition; ***17 patients withdrew due to a total of 23 AEs - six patients reported two AEs at the time of withdrawal, e.g. diarrhea and musculoskeletal pain.

Four patients withdrew before, and twenty-seven after conversion to LCP-Tac. The reasons for withdrawal are outlined in Figure 5. Most dropouts were caused by non-serious adverse events (17/31, 54%) or non-disease related (6/31, 20%) reasons, such as withdrawal of informed consent or loss to follow-up. In one instance, we excluded a participant with enteric stoma as he failed to reach minimal Tacrolimus blood levels – likely to be because of insufficient absorptive surface of the intestine than required by the prolonged release formula.

Table 1: Patient participation at each stage, total patients registered n = 165.											
Stage	**t = 0**	**Week 1**	**Week 2**	**Week 3**	**Month 1**	**Month 3**	**Month 6**	**Month 12**	**Month 18**	**Month 24**	∑
Withdrawal due to											
Adverse Event (AE)	-	6	2	1	2	1	1	2	2	0	17
Serious AE	-	-	-	-	-	3	-	2	-	3	8
Other	4	1	-	-	-	-	-	-	-	1	6
Total	4	7	2	1	2	4	1	4	2	4	
Cumulative Dropouts	**4**	**11**	**13**	**14**	**16**	**20**	**21**	**25**	**27**	**31**	
% registered	**2%**	**7%**	**8%**	**8%**	**10%**	**12%**	**13%**	**15%**	**16%**	**19%**	
Completed stage	161	154	152	151	149	145	144	140	138	134	

Most patients who dropped out due to non-serious adverse events (n =17) suffered either from musculoskeletal pain (7/17, 41%), gastrointestinal symptoms (nausea/diarrhea; 6/17, 35%), neurological symptoms (headache/dizziness; 3/17, 28%), or a combination of them. Out of the 27 adverse events that lead to withdrawal, two out of three (16/27, 65%) occurred within four weeks after conversion to LCP-Tac, and none of them were classified as serious. By comparison, the onset of serious adverse events (8/27, 30%) – including the death of five patients - was distributed more evenly across the timeline: three occurred within the first six months, two between months six and twelve, and three between months 18 to 24 (Table 1).

The AE profile that patients experienced who dropped out differed from the AEs reported by the entire study population throughout the study period. For example, two of the most frequent adverse events (>10% of the 161 converted patients) were upper respiratory tract infections (n= 28, 17%) and fatigue (n = 18, 11%). However, no patient withdrew due to either of those AEs. A detailed classification of adverse events is presented in the safety section (3.4.2).

Dropouts were reconverted back to IR-Tac, except for a single case in which a patient received ER-Tac during hospitalization. In this case, hospitalization did not occur at the Charité hospital and the treating physicians prescribed ER-Tac at their own discretion.

3.1.2 Baseline characteristics

At study entry, patients were, on average, 55 years old (range: 21-85y), overweight (Body Mass Index[3], BMI >25), and 7.5 years post-transplant (range: 0.3 – 28y). Dropouts were slightly older than completers (p = 0.672), more likely to be female (p = 0.301), and about one year longer post-transplant (p = 0.415), but these differences did not reach statistical significance (Table 2). Most of the cohort was of Caucasian origin (96%).

The most common indications for liver transplantation were alcoholic liver disease (20%), hepatocellular carcinoma (20%), and autoimmune hepatitis (19%). More dropouts suffered from non-alcoholic steatohepatitis (NASH) at the time of transplantation (10% vs 1%), but the total number of patients with NASH was only five – and the differences across indications were not statistically significant (p = 0.360).

Across the cohort, one in two patients suffered from arterial hypertension, and one in four patients experienced dyslipidemia or diabetes. By comparison, dropouts had higher rates of hypertension (55% vs. 54%), dyslipidemia (32% vs. 22%), and diabetes (29% vs. 19%), but the corresponding p-values did not exceed the predefined threshold of 5%.

Participants took, on average, six different medications per day, ranging from one to 16 different pills. The number of antihypertensive drugs ranged from one to four, and about one in six patients received lipid-lowering drugs, either statins or fibrates. 15% of completers and 23% of dropouts required insulin or oral antidiabetic medication (p = 0.299).

More than half of the participants (n = 64, 59%) received a second or third immunosuppressant in addition to Tacrolimus: extended regimens included mycophenolate mofetil (MMF, n = 59), everolimus (n = 19), and prednisone (n = 13). Dropouts took fewer additional immunosuppressants (p = 0.041) and were more likely to receive a Tacrolimus-mono immunosuppressive regimen than completers (65% vs. 45%) were – but the difference across regimens did not reach statistical significance (p = 0.282).

3.2. Pharmacokinetic parameters

Early pharmacokinetic evaluations suggested that Tacrolimus dosage could be reduced by 30% to obtain comparable trough levels when converting patients from IR-Tac to LCP-Tac.

[3] Body Mass Index (BMI) = $\frac{weight\ (kg)}{height\ (m)^2}$; <18.5 (underweight), 18.5 - 24.9 (normal), overweight (>25), obesity grade I (30 – 34.9), II (35 – 39.9), and III (>40); as defined by the World Health Organization - WHO (114).

Table 2: Descriptive characteristics of the study group at baseline, means ± SD or N (%).			
Characteristic	**Completers**	**Dropouts**	**P-value**
N	134	31	
Female	64 (48%)	18 (58%)	0.301
Age at study entry, years	55 ± 13	56 ± 13	0.672
Time since transplantation, months	91 ± 85	101 ± 87	0.415
Median (Range)	58 (4-336)	62 (5-312)	
BMI	26 ± 5	25 ± 5	0.551
Classification			0.299
<18.5	5 (4%)	2 (6%)	
18.5-25	53 (40%)	14 (45%)	
25-30	47 (35%)	9 (29%)	
>30	30 (22%)	6 (19%)	
Ethnic background			0.275
Caucasian	129 (96%)	31 (100%)	
Other	5 (4%)	0 (0%)	
Primary indication			0.360
Autoimmune hepatitis	27 (20%)	3 (10%)	
Hepatocellular carcinoma	26 (19%)	6 (19%)	
Alcoholic liver disease	25 (19%)	7 (23%)	
Acute liver failure	14 (10%)	2 (6%)	
Chronic viral hepatitis C	9 (7%)	3 (10%)	
Cryptogenic cirrhosis	8 (6%)	2 (6%)	
Combined viral hepatitis B/D	8 (6%)	1 (3%)	
Liver cysts	4 (3%)	0 (0%)	
Nonalcoholic steatohepatitis	2 (1%)	3 (10%)	
Wilson's disease	2 (1%)	0 (0%)	
Other	9 (7%)	4 (13%)	
Arterial hypertension	73 (54%)	17 (55%)	0.971
Number of antihypertensive drugs			0.968
1	43 (32%)	10 (32%)	
2	22 (16%)	6 (20%)	
3	6 (4%)	1 (3%)	
4	1 (1%)	0 (0%)	
Dyslipidemia	29 (22%)	10 (32%)	0.210
Statins/Fibrates	17 (13%)	5 (16%)	0.611
Diabetes	25 (19%)	9 (29%)	0.198
Insulin/oral antidiabetics	20 (15%)	7 (23%)	0.299
Tacrolimus-based immunosuppression			
Additional immunosuppression			0.041*
Plus Mcyophenolate mofetil	52 (39%)	7 (23%)	
Plus Prednisone	11 (8%)	2 (6%)	
Plus Everolimus	17 (13%)	2 (6%)	
Regimen[a]			0.282
Tacrolimus monotherapy	60 (45%)	20 (65%)	
Tacrolimus double therapy	68 (51%)	11 (35%)	
Tacrolimus triple therapy	6 (4%)	0 (0%)	

*p< 0.05, Pearson's chi-square, Mann-Whitney, or t-test, as appropriate.

[a]Tacrolimus + no (mono), one (double), or two (triple) additional agents - MMF, Prednisone or Everolimus

Thus, the mean daily dose was reduced from 3.5 mg at study entry to 2.4 mg during the first week – which equals a relative decrease by 32% (Table 3).

On day seven after conversion from IR-Tac to LCP-Tac, mean Tacrolimus serum concentrations were 5.5 ng/ml, representing a 3% increase as compared to the 5.4 ng/ml at baseline, despite the dose reduction. The greater bioavailability of LCP-Tac was also reflected in a concomitant jump in the concentration/dose by 65% during the same period ($p<0.001$).

Over the course of the subsequent two years, further dose reductions were made as part of the CNI-sparing regimen used at the center, aiming at the lower range of the 3-8 ng/ml long-term target. At each stage during the study, downward and upward dose adjustments were made in approximately 25% and 5% of patients, respectively.

At month 24, the mean daily Tacrolimus intake was 1.6 mg, totaling a reduction of 51% compared to baseline. As we reduced dosage more than 30% after the first week, mean Tacrolimus levels also started to decrease. At month 24, blood concentrations were 24% lower than at baseline (5.4 vs. 4.1 ng/ml). During the same period, the mean concentration/dose ratio increased by 83% from 1.7 to 3.1 ng/ml per mg/day.

Table 3: Tacrolimus pharmacokinetic parameters at baseline and after conversion to LCP-Tac, mean ± SD or n (%). Includes all available data.

	t = 0 n = 162	**Week 1 n = 143**	**Month 1 n = 135**	**Month 3 n = 141**	**Month 6 n = 143**	**Month 12 n = 140**	**Month 18 n = 138**	**Month 24 n = 134**
Tacrolimus dose (mg/day)	3.5 ± 1.6	2.4 ± 1.2†	2.2 ± 1.1†	2.1 ± 1.1*	2.0 ± 1.0*	1.8 ± 1.0†	1.7 ± 1.0*	1.6 ± 0.9*
Tacrolimus blood concentration (ng/ml)	5.4 ± 2.1	5.5 ± 2,4	4.9 ± 2.0	5.0 ± 2.1	4.7 ± 2.0	4.6 ± 2.1	4.3 ± 1.7	4.1 ± 1.9
Concentration/dose ratio (ng/ml per mg/day)	1.7 ± 1.0	2.8 ± 1.7†	2.7 ± 1.6	3.0 ± 1.8	3.0 ± 1.9	3.1 ± 2.0*	3.1 ± 1.7	3.1 ± 1.7
% change in dosage to previous visit	-	-32 %	-8%	-5%	-4%	-9%	-4%	-6%
% change in blood concentrations to previous visit	-	+3%	-11%	+2%	-6%	-2%	-7%	-5%
% change in concentration/dosage to previous visit	-	+65%	-4%	+11%	0%	+3%	+0%	+0%
Nr. patients with dose decrease (%)	-	136 (95%)	43 (26%)	30 (22%)	49 (36%)	34 (24%)	30 (22%)	16 (12%)
Nr. patients with dose increase (%)	-	4 (3%)	6 (4%)	4 (3%)	6 (4%)	6 (4%)	7 (5%)	4 (3%)
Nr. Patients with no dose change (%)	-	3 (2%)	86 (52%)	101 (75%)	82 (60%)	99 (71%)	101 (73%)	109 (84%)

*$p<0.05$, †$p<0.001$, compared to previous visit, Student's t-test.

3.3 Primary endpoint: Patient-reported outcomes (PROs) - BAASIS©

3.3.1 Adherence to immunosuppression

Over the course of treatment, LCP-Tac was associated with significantly improved overall adherence on the BAASIS© compared to IR-Tac (Table 4). On study entry, only 51% (n = 68) of patients adhered to their treatment protocol based on IR-Tac: 15% (n =20) experienced difficulties taking every dose and 43% (n = 57) had taken their medication with a minimum delay of two hours at least once over the past four weeks.

After conversion to LCP-Tac, the rate of overall adherent patients on the BAASIS© increased significantly from 51% to 80% over 24 months, which equals a relative increase of 60%. At the end of the study period, only 2% (n = 3) reported having missed any dose of Tacrolimus, and 20% took their medication with delay – a significant improvement in adherence on both measures. We also observed that the number of patients who reported perfect adherence (VAS = 100) on the visual analogue scale improved by 80%.

Table 4: Basel Assessment of Immunosuppressant Adherence Medication Scale (BAASIS©) – patients who completed 24 month period (n = 134), N (%) or mean ± SD.

Item	Baseline	Month 6	Month 12	Month 24
Dose not taken	20 (15%)	8 (6%)	5 (4%)	3 (2%)*
Consecutive doses not taken	1 (1%)	0 -	1 (1%)	0 -
Dose taken with >2h delay	57 (43%)	41 (32%)	33 (25%)	26 (20%)*
Dose reduced	0 -	0 -	35 (1%)	0 -
Overall non-adherence	**66 (49%)**	**43 (33%)**	**35 (26%)**	**26 (20%)***
Visual Analogue Scale				
Scale (0-100)	93 ± 11	97 ± 9	98 ± 6	98* ± 4
Frequency optimal adherence [a]	59 (44%)	85 (67%)	98 (72%)	102 (78%)*

[a] Number of patients who reported 100% adherence on the Visual Analogue Scale, *p < 0.05, McNemars's test

3.3.2 Impact of gender, age, and primary indication for liver transplantation on adherence

Women reported higher overall adherence rates than men, both at baseline (55% vs. 47%, p = 0.242) and month 24 (84% vs. 77%, p = 0.201). However, both genders showed a highly significant (p < 0.001) increase in self-reported adherence by 30% on the BAASIS© (Figure 6A).

Young (<59 years) and elderly patients reported comparable non-adherence rates at study entry (54% vs. 44%, p = 0.232). But at month 24, the younger group reported significantly higher rates of non-adherence than the elderly did (27% vs. 11%, p = 0.030) (Figure 6B).

There was no significant difference in adherence between patients who had received a transplant due to alcohol induced liver cirrhosis and other indications (Figure 6C).

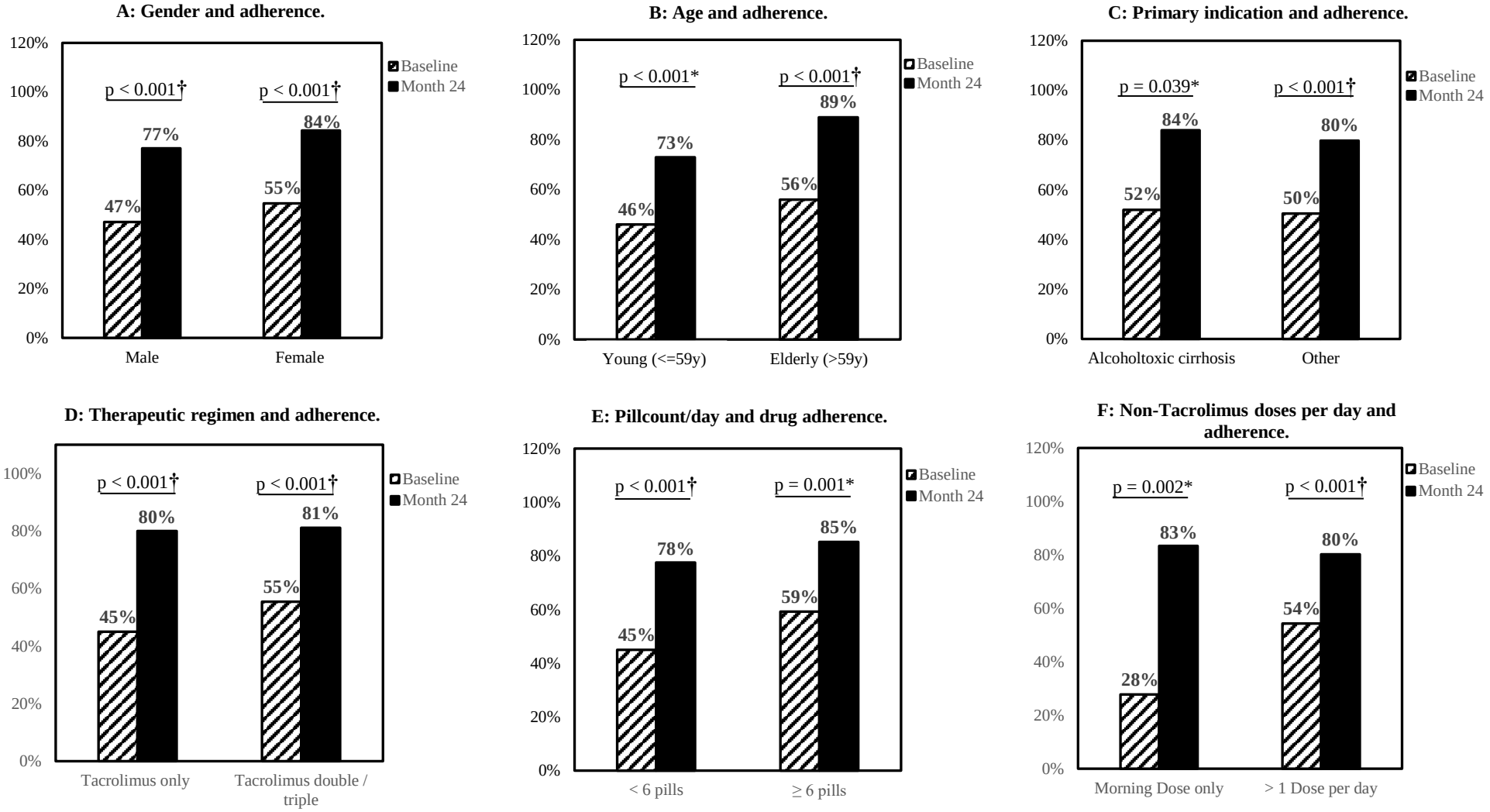
3.3.3 Impact of therapeutic complexity on adherence

The mean number of daily medications were similar at baseline for non-adherent (5.5/day) vs. adherent (6.2/day) patients (p = 0.140). At month 24, this difference remained non-significant (5.4/day vs. 6.0/day, p = 0.347). Likewise, the mean number of medication doses other than Tacrolimus per day did not differ between adherent and non-adherent patients at baseline (2.0 vs. 1.9, p = 0.392) or at month 24 (2.0 vs. 2.0, p = 0.795). Notably, the improvement in adherence was larger for patients with less than 6 pills per day (+33%) than it was for patients with more medications (26%, Figure 6E), but the difference between the groups did not reach statistical significance at baseline (delta 14%, p = 0.195) or month 24 (delta 7%, p = 0.529).

Patients with simple (only Tacrolimus) and complex (at least one additional immunosuppressive drug) regimens both reported significantly improved drug intake at month 24 (Figure 6D). The group with simple regimens reported 10% lower adherence at study entry than the complex group (45% vs. 55%., p = 0.153), and exhibited a larger increase in adherence by 35% versus 26% in the complex group by the time of completion (80% vs. 81%, p = 0.523) (Figure 6D).

The largest improvement in adherence (RC = 2.9) by any subgroup under examination was reported by patients who took two Tacrolimus doses per day at study entry when using IR-Tac, but only a single morning dose of all other medications (e.g. antihypertensive drugs). Before conversion, this subset of patients reported 29% adherence. After switching from IR-Tac to LCP-Tac, this rate increased to 83% (delta 54%, p = 0.002) at month 24, as these patients would now have to take any medication solely during their morning routine.

Figure 6: Evolution of overall adherence (BAASIS©) split by gender (A), age (B), primary indication (C), and therapeutic complexity (D-F), N =134.



* p =< 0.05, †p< 0.001, McNemar's test.

3.4 Secondary endpoints: Clinical outcomes

3.4.1 Adherence: Changes in Tacrolimus IPV

Over the course of treatment with LCP-Tac, neither the median coefficient of variation nor the standard deviation of consecutive Tacrolimus blood levels changed significantly when compared with the variation during the two years prior to conversion under IR-Tac (Table 5, Figures 7,8).

Table 5: Effect of conversion to LCP-Tac on intra-patient variability of Tacrolimus levels, n = 85.

	IR-Tac n = 85		**LCP-Tac** n = 85		
	Median	Range	Median	Range	p
Coefficient of variation C_0/Dose	21%	4% – 85%	22%	4% – 70%	0.57
Standard deviation C_0	1.1	0.2 – 5.0	1.2	0.2 – 3.6	0.89

Wilcoxon signed-rank or t-test, as appropriate.

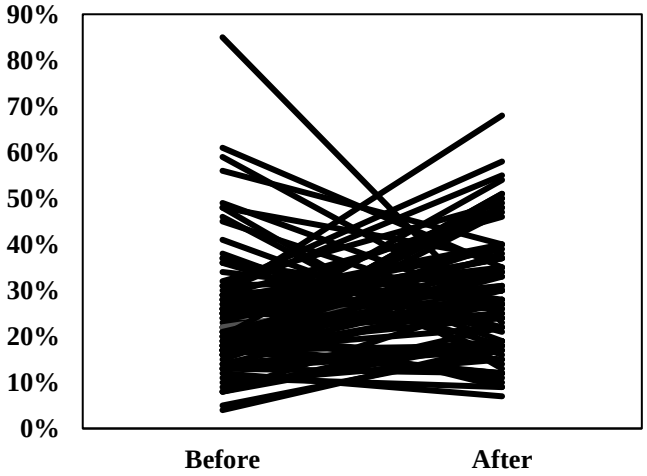


Figure 7: Coefficient of variation of dose-adjusted consecutive whole blood levels before and after conversion from IR-Tac to LCP-Tac, n = 85.

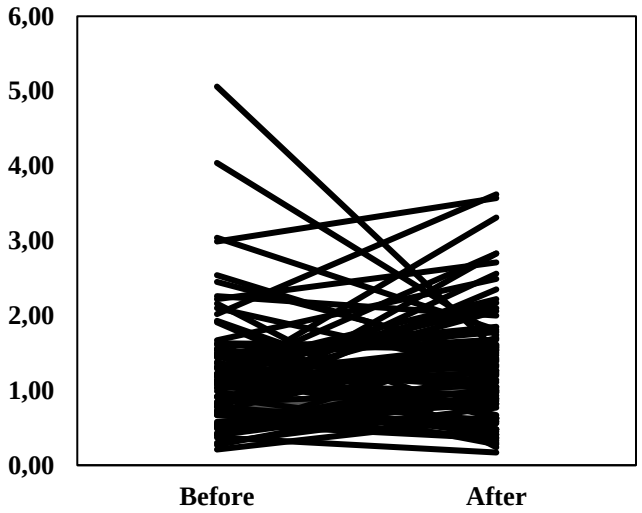


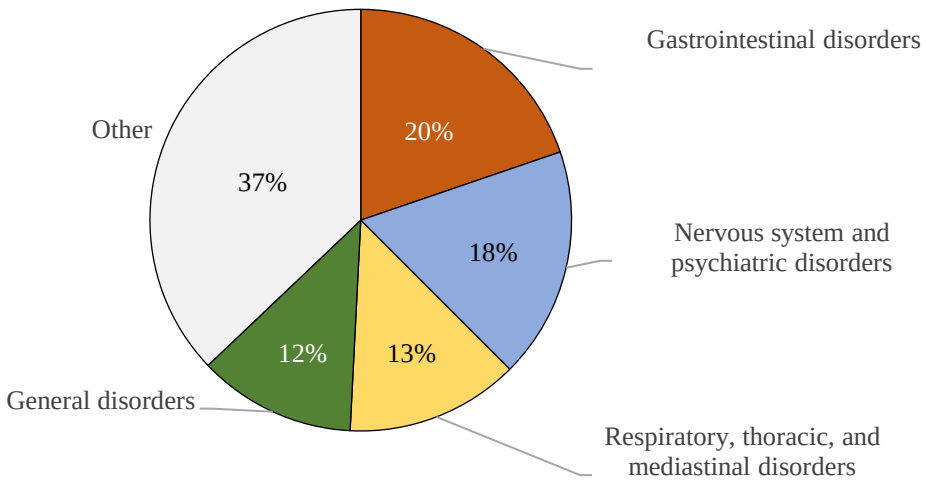
Figure 8: Standard deviation of consecutive whole blood levels before and after conversion from IR-Tac to LCP-Tac, n = 85.

3.4.2 Safety: Adverse events and side effects

3.4.2.1 Adverse events

Over the course of treatment with LCP-Tac, a total of n = 248 non-serious adverse events occurred, including those which led to withdrawal. Based on the MedDRA (Medical Dictionary for Regulatory Activities) classification, the most frequently affected system organ classes were gastrointestinal disorders, the nervous system and psychiatric disorders, as well as respiratory, thoracic, and mediastinal disorders (Figure 9).

Figure 9: Adverse events by system organ class[a], fraction of total non-serious adverse events, N = 248.



[a]based on MedDRA (Medical Dictionary for Regulatory Activities) system organ class.

Patients reported four very common (>10%) distinct events throughout the study: upper respiratory tract infections, headaches, diarrhea, and fatigue (Table 4). Less frequent events included pruritus (9%), (self-reported) weight gain (9%), abdominal pain (8%), and musculoskeletal pain (7%). Notably, while musculoskeletal pain was only the 8th most common adverse event, it was the most frequently cited reason for withdrawal.

Of those fifteen patients who reported pruritus, eight had been diagnosed with concomitant diseases associated with this symptom: six were previously diagnosed with ischemic type biliary lesions (ITBL), one patient required treatment for uremia due to kidney failure, and one participant suffered from atopic dermatitis at study entry.

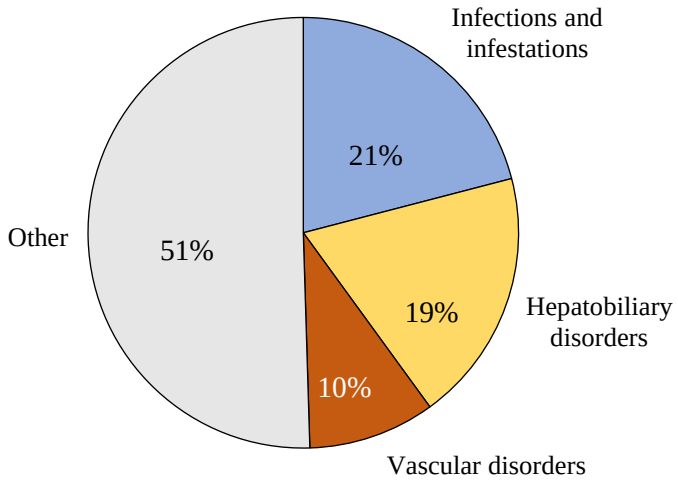
Table 6: Very common (>= 10%) and common (1-10%) adverse events during the study period, N (% of patients). MedDRA (Medical Dictionary for Regulatory Activities) preferred terms.

Adverse events	Frequency
Respiratory, thoracic and mediastinal disorders	
Viral upper respiratory tract infection	28 (17%)
Cough	5 (3%)
Nervous system and psychiatric disorders	
Headache	25 (16%)
Dizziness	6 (4%)
Paresthesia	3 (2%)
Tremor	4 (2%)
Restlessness/Agitation	4 (2%)
Insomnia	2 (1%)
Gastrointestinal disorders	
Diarrhea	18 (11%)
Abdominal pain	13 (8%)
Nausea and vomiting symptoms	11 (7%)
Gastroenteritis	4 (2%)
Acid reflux (esophageal)	3 (2%)
General disorders	
Fatigue	18 (11%)
Asthenia	4 (2%)
Dry mouth	3 (2%)
Pyrexia	3 (2%)
Hyperhidrosis	2 (1%)
Skin and subcutaneous disorders	
Pruritus	15 (9%)
Eczema	8 (5%)
Basal cell carcinoma	3 (2%)
Metabolism and nutrition disorders	
Weight gain	14 (9%)
Edema, peripheral	3 (2%)
Weight loss	3 (2%)
Renal and urinary disorders	
Urinary tract infection	8 (5%)
Infections and infestations	
Herpes zoster	2 (1%)
Vascular disorders	
Hypertension worsened	10 (6%)
Musculoskeletal and connective tissue disorders	
Bone pain/Arthralgia	12 (7%)
Muscle cramps	3 (2%)
Investigations	
Hepatic enzyme increased	5 (3%)
Proteinuria	2 (1%)
Cardiac disorders	
Palpitations	2 (1%)
Ear and labyrinth disorders	
Tinnitus	2 (1%)

3.4.2.2 Non-fatal serious adverse events

In 51 cases, patients required hospitalization or extensive treatment for non-fatal serious adverse events (Table 7). The most frequently affected system organ classes were infections and infestations, hepatobiliary disorders, and vascular disorders (Figure 10).

Figure 10: Non-fatal serious adverse events by system organ class[a], fraction of total non-fatal serious adverse events, N = 51.



[a]based on MedDRA (Medical Dictionary for Regulatory Activities) system organ class.

Around 20% (n = 13) of 51 cases were caused by severe infections, including hospitalization for reactivation of viral infections and pneumonia. In five cases – excluding the case of fatal hepatic failure due to a recurrent tumor - malignant disease was diagnosed during the study period, namely: two cases of lung adenocarcinoma, two cases of recurrent hepatocellular carcinoma, and a single case of post-transplant lymphoproliferative disorder (PTLD).

Table 7: Serious adverse events: includes all events which led to death, hospitalization, or disability throughout the study period, N (% of patients). MedDRA (Medical Dictionary for Regulatory Activities) preferred terms.

Serious Adverse Events	Frequency
Death	
Cardiac Arrest	2 (1%)
SIRS (Systemic inflammatory response syndrome)	2 (1%)
Hepatic failure – recurrent hepatocellular carcinoma	1 (1%)
Non-fatal serious adverse events	
Infections and infestations	
Abscess	3 (2%)
Pneumonia	3 (2%)
Urosepsis	2 (1%)
Hepatitis B/ Epstein-Barr reactivation	2 (1%)
Herpes zoster	1 (1%)
Hepatobiliary disorders	
Bile duct stenosis	3 (2%)
Recurrent Hepatocellular carcinoma	2 (1%)
Cholangitis	2 (1%)
Hepatic failure	1 (1%)
Graft dysfunction	1 (1%)
Hepatomegaly	1 (1%)
Vascular disorders	
Thrombosis	3 (2%)
Pulmonary embolism	2 (1%)
Renal and urinary disorders	
Acute renal failure	2 (1%)
Urinary Calculi	2 (1%)
Respiratory, thoracic and mediastinal disorders	
Lung adenocarcinoma	2 (1%)
Pleural effusion	2 (1%)
Gastrointestinal disorders	
Gastrointestinal hemorrhage (gastric, small intestine)	2 (1%)
Diarrhea	2 (1%)
Nervous system disorders	
Stroke	1 (1%)
Transient ischemic attack	1 (1%)
Psychiatric disorders	
Hallucinations	1 (1%)
Paranoid schizophrenia	1 (1%)
Other	
Amyloidosis	1 (1%)
Anemia requiring transfusion	1 (1%)
Coronary artery disease	1 (1%)
Hyponatremia	1 (1%)
Post-transplant lymphoproliferative disorder (PTLD)	1 (1%)
Retinal detachment	1 (1%)
Rheumatoid arthritis	1 (1%)
Toxic epidermal necrolysis	1 (1%)

3.4.2.3 Evolution of clinical and laboratory parameters – electrolytes, hepatic, renal, hematologic, metabolic, and cardiovascular function

During the two-year treatment with LCP-Tac, graft (hepatic), renal, hematologic, metabolic (including electrolytes), and cardiovascular function, as assessed by laboratory and clinical parameters, remained stable (Table 8). While some of these parameters showed statistically significant changes, the relative differences did not exceed 4% across any measure.

Electrolytes

Plasma electrolytes remained steady throughout the study, except for a statistically significant change in Potassium (-4% decrease to baseline, $p < 0.001$) and Magnesium levels (+3% to baseline, $p = 0.002$) at month 24 (Table 8). However, these changes did not translate into imbalances that required clinical intervention. A single case of hyponatremia was successfully treated, and the patient remained in the study.

When we split the sample by median potassium levels (4.4 mmol/l) at study entry, we found that the decrease was only significant for patients who already showed elevated levels at baseline (Figure 11E).

Similarly, we found that the significant increase in Magnesium on a population level was driven by a subset of patients with low levels at study entry (Figure 11J).

Hepatic function

Assessment of the liver parenchyma enzymes (AST, ALT) and the biliary tree enzymes (GGT, AP) showed stable graft function and we did not observe significant deviations on a population level across these parameters. In subgroup analysis split by median AST entry levels, patients with elevated parameters saw a subsequent decrease and vice versa; however, these differences – while statistically significant - were equivalent to an absolute change of only one unit per liter in each subgroup (Table 8, Figure 11).

Measures of hepatic excretion (Bilirubin) and protein biosynthesis (Albumin) increased significantly ($p = 0.001$, $p = 0.027$) between baseline and month 12, and month 24, respectively (Table 8). However, these differences diminished over the two-year period: at month 24, there was a non-significant increase in Bilirubin of 4% and only a slight change in Albumin (+1%).

Renal function

Neither the estimated glomerular filtration rate (eGFR, 67ml vs. 67ml) nor plasma creatinine (1.14mg/dl vs. 1.17mg/dl) changed significantly between baseline and month 24 (Table 8).

During the same period, Urea decreased from 43 to 40 mg/dl ($p = 0.028$). We split the sample at median baseline values (Urea = 34; Figure 11K), and found that patients with low levels (29 U/l) at entry saw only a small increase from 29 to 32 ($p = 0.048$) units, but patients with elevated values at baseline (51U/l) exhibited a relatively larger decrease to 44 units per liter ($p < 0.001$).

Hematologic function

Hematologic function was steady (Table 8), but we observed a significant increase in Hemoglobin (+3%, $p = 0.038$) from baseline (13.3 g/dl) until completion of year two (13.7 g/dl). In one single case, a patient required treatment due to severe anemia but remained on the study protocol. Subgroup analysis revealed that patients with low Hemoglobin levels at entry saw a significant increase over the study period (12.2 vs 12.8 g/dl; $p < 0.001$), while patients with high levels (14.8 g/dl) at entry had a smaller, and non-significant decrease to 14.5g/dl ($p = 0.859$) (Figure 11).

Metabolic and cardiovascular parameters

Both oral glucose and HbA1c levels increased by about 3% between baseline and month 24, but this change was only significant for HbA1c ($p = 0.857$ vs. $p < 0.001$). When we split the whole sample by median HbA1C values, the upward trend was significant in both the group with low and with elevated parameters at baseline ($p < 0.001$, $p = 0.013$). The absolute change on a population level over the two-year period was 0.2% from 5.4 at baseline to 5.6% at the time of completion.

Therapeutic regimens were adjusted twice due to diabetic status: one patient showed elevated HbA1c (>6.5%) at baseline and required metformin plus insulin by the end of the study; glucose levels improved significantly in another patient and the insulin dose was reduced.

Except for a small but significant increase in HDL (+4%, $p < 0.001$), the lipid profile was steady for the entire cohort. This increase in HDL remained significant in subgroup analysis, for both patients with elevated and low levels at study entry.

Systolic and diastolic blood pressure decreased by one ($p = 0.581$) and four mmHg ($p < 0.001$) on a population level, respectively. We prescribed new antihypertensive drugs in three patients and stopped medication in one.

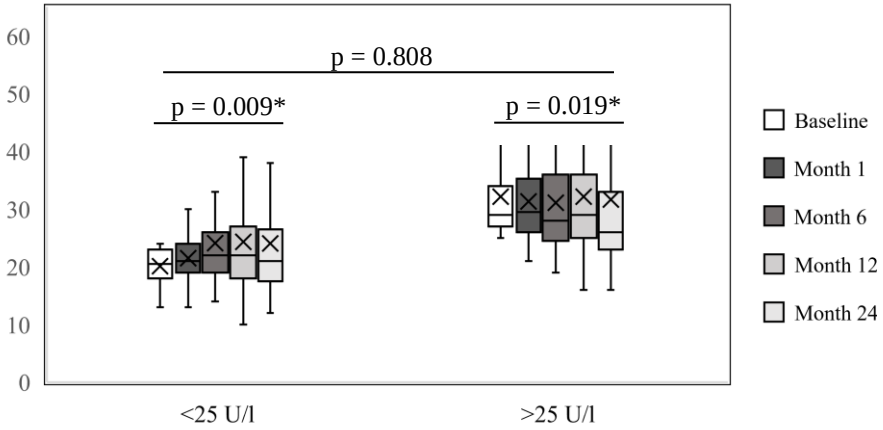
Table 8: Clinical and laboratory parameters at baseline, 12, and 24 months after conversion to LCP-Tac, median (range) or mean ± standard deviation. P-values calculated pairwise versus baseline. Includes all patients who completed the entire study period (n=134).

Parameter. reference range	Baseline		Month 12		P – value	Month 24		P - value
Electrolytes								
Potassium, 3.5 – 4.5 mmol/l	4.4	(3.4 – 5.9)	4.3	(3.0 – 6.0)	0.075	4.2	(3.1 – 6.0)	<0.001†
Calcium, 2.2 – 2.6 mmol/l	2.3	(2.1 – 2.6)	2.3	(2.0 – 2.7)	0.203	2.3	(2.1 – 2.6)	0.634
Magnesium, 0.66 – 0.99 mmol/l	0.8	(0.5 – 1.2)	0.8	(0.6 – 1.7)	<0.001†	0.8	(0.6 – 1.2)	0.002*
Phosphate, 0.87 – 1.45 mmol/l	1.0	(0.5 – 1.5)	1.0	(0.6 – 2.1)	0.859	1.0	(0.6 – 2.1)	0.868
Hepatic								
AST, <50 U/l	25	(0 – 88)	25	(0 – 90)	0.155	24	(0 – 175)	0.808
ALT, <41 U/l	21	(0 – 164)	21	(0 - 209)	0.745	21	(0 - 113)	0.988
AP, 40-130 U/l	78	(12 – 457)	83	(1 - 652)	0.647	79	(1 - 638)	0.381
GGT, 8-61 U/l	24	(1 - 380)	25	(0 – 453)	0.985	23	(0 – 299)	0.785
Bilirubin, <1.20 mg/dl	0.4	(0.2 – 2.5)	0.5	(0.2 – 2.6)	0.002*	0.5	(0.2 – 1.7)	0.220
Albumin, 35 - 52 g/l	42	± 3	43	± 4	0.456	42	± 5	0.027*
Renal								
Creatinine, 0.70-1.20 mg/dl	1.0	(0.5 – 4.7)	1.0	(0.5 – 5.5)	0.220	1.0	(0.5 – 6.4)	0.815
eGFR, ml/min/1.73m^2	69	(3.6 – 90)	68	(15 - 90)	0.464	69.5	(15 - 90)	0.661
Urea, 17- 48 mg/dl	39	(56 – 159)	34	(15 - 112)	0.029*	37	(13 - 177)	0.028*
Hematologic								
Hemoglobin, 12.5 -17.2 g/dl	13.3	± 1.9	13.6	± 1.9	0.002*	13.5	± 1.9	0.038*
Leukocytes, 3.9 - 10.5 /nl	6.4	(1.6 – 16.5)	6.1	(2.1 - 23.8)	0.100	6.1	(2.0 – 18.5)	0.523
Thrombocytes, 150-370 /nl	199	(49 – 542)	200	(51 - 679)	0.534	203	(53 - 849)	0.057
Metabolic & Cardiovascular								
HbA1c, %	5.4	(4.1 – 13.5)	5.4	(3.5 – 9.9)	0.063*	5.6	(3.3 – 11.0)	<0.001†
Fasting plasma glucose, mg/dl	100	(47 – 370)	97	(69 – 372)	0.100	99	(55 - 387)	0.857
Triglycerides, <200 mg/dl	104	(39 – 408)	105	(1 - 442)	0.294	102	(1 - 376)	0.131
Total Cholesterol, <200 mg/dl	181	± 38	182	± 38	0.221	179	± 37	0.960
HDL, >35 mg/dl	57	(21 – 103)	61	(26 - 106)	<0.001†	58	(12 – 104)	0.001*
LDL, <130 mg/dl	113	± 34	112	± 31	0.127	112	± 34	0.928
Systolic blood pressure, <140 mmHg	139	± 19	139	± 20	0.689	138	± 19	0.591
Diastolic blood pressure, <90 mmHg	84	± 13	81	± 12	0.017	79	± 13	<0.001†
Weight, kg	77	± 16	76	± 16	0.343	77	± 17	0.147

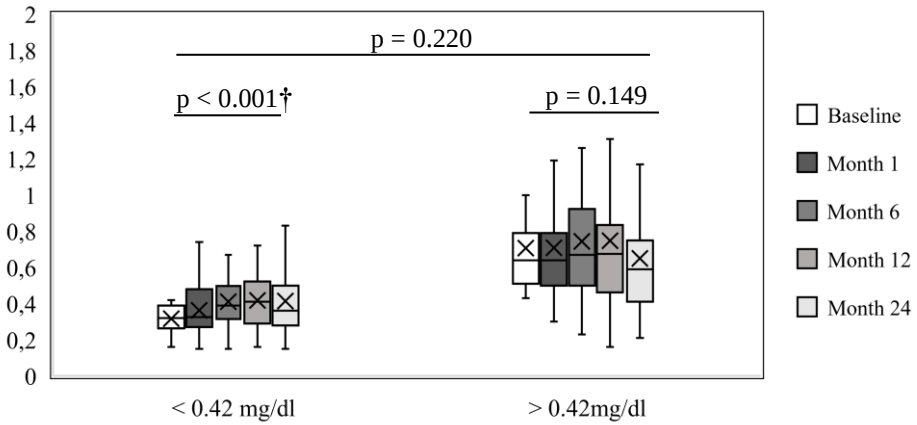
*p< 0.05, †p<0.001, compared to baseline, Wilcoxon signed-rank or t-test, as appropriate.

Figure 11: Detailed evolution of hepatic (A-C), renal (D-F), metabolic (F-K), and hematologic (L) parameters. Samples are split by median values. P-values refer to delta between values at baseline and month 24 within subgroups (low bars) and across the entire sample (high bar).

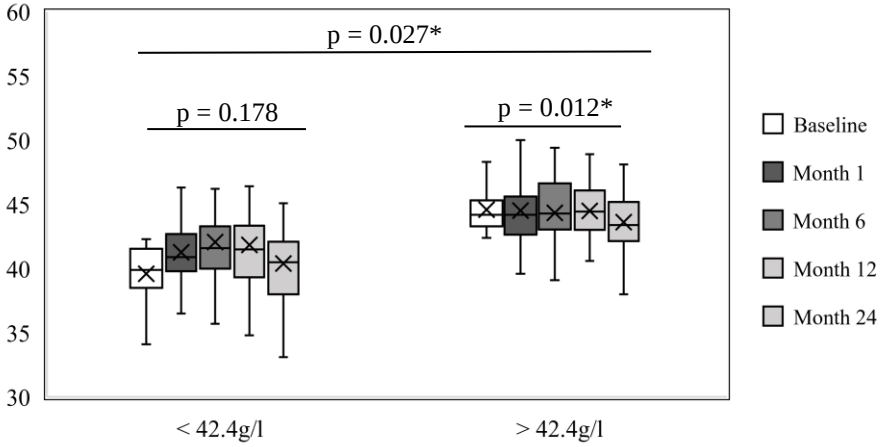
A: Alanine-Aspartate-Transferase, median t_0 = 25 U/l; patients with low (<=25 U/l) and high (>25 U/l) levels at baseline, N = 116.



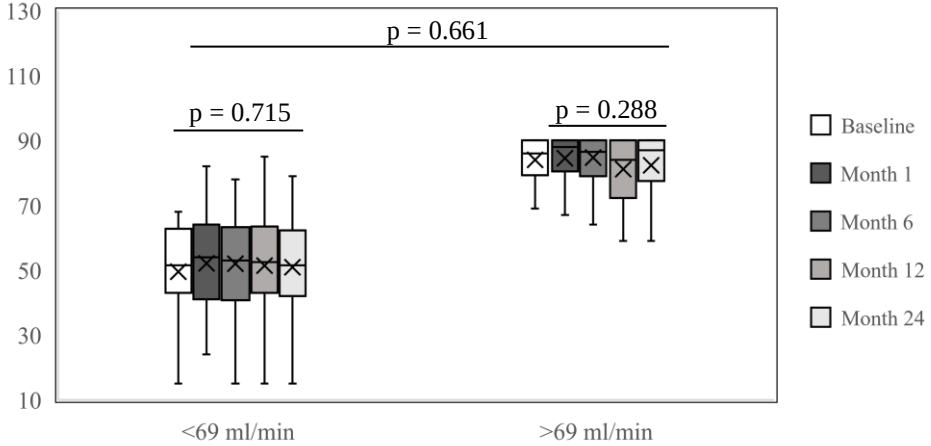
B: Bilirubin, median t_0 = 0.42 mg/dl; patients with low (<=0.42 mg/dl) and high (> 0.42mg/dl) levels at baseline, N = 116.



C: Albumin, median t_0 = 42.4 g/l; patients with low (< 42.4 g/l) and high (>= 42.4 g/l) levels at baseline, N = 93.



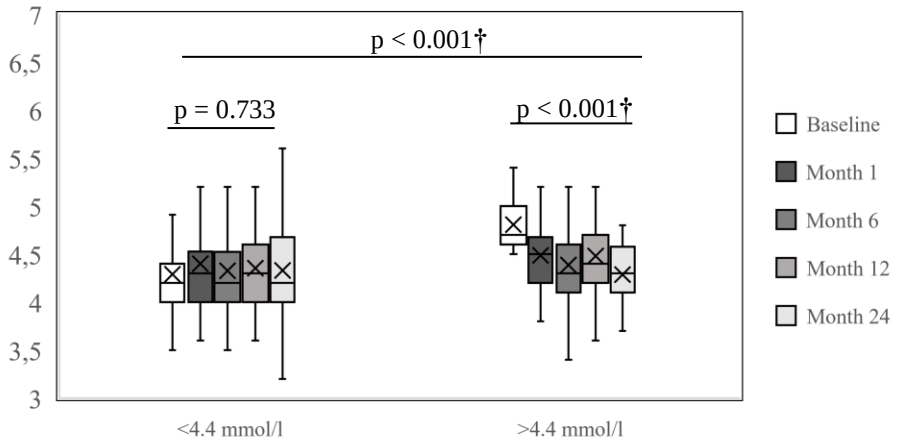
D: Glomerular filtration rate (GFR), median t_0 = 69 ml/min/1.73m$^{2/}$; patients with low (<=69ml/min) and high (>69ml/min) levels at baseline, N = 117.



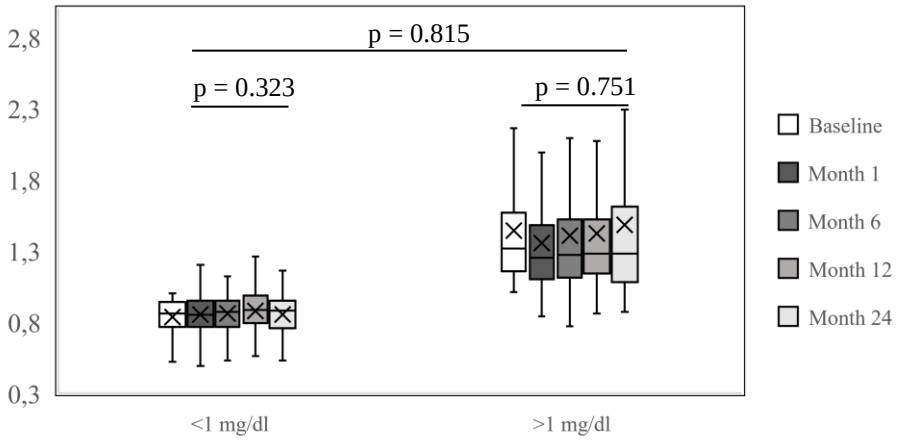
*p< 0.05, †p<0.001, Wilcoxon signed-rank or t-test, as appropriate.

Figure 11: (continued).

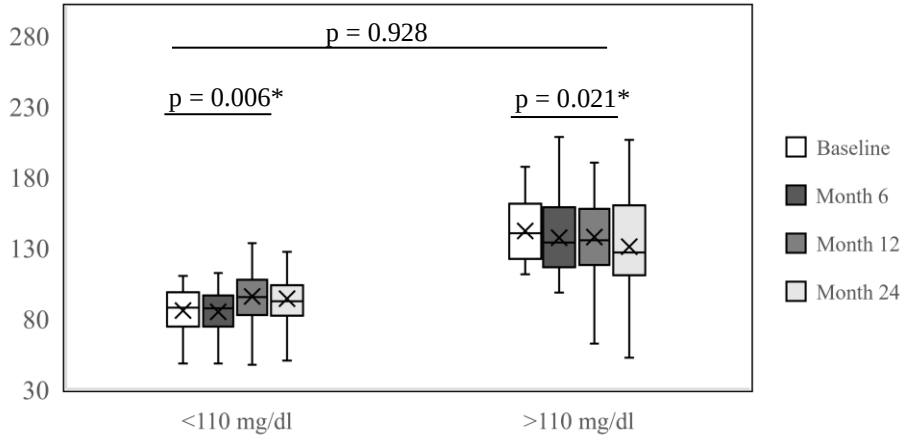
E: Potassium, median t_0 = 4.4 mmol/l, patients with low (<4.4) and high (>=4.4) levels at baseline, N = 118.



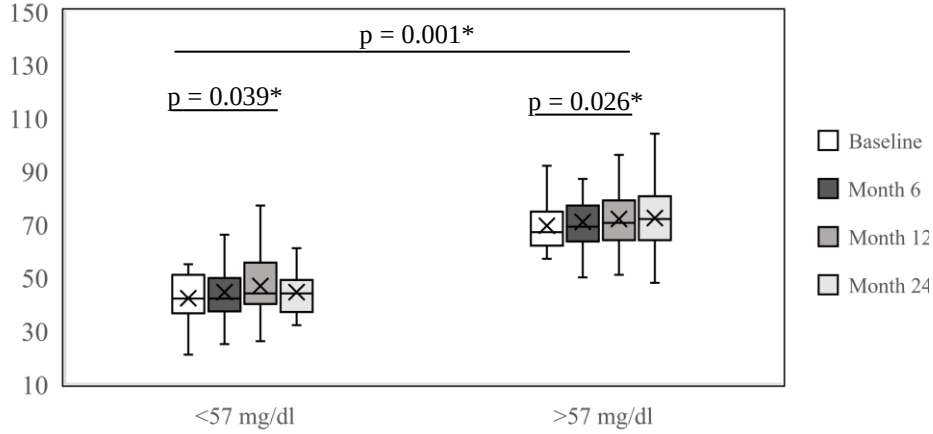
F: Creatinine, median t_0 = 1 mg/dl; patients with low (<=1 mg/dl) and high (>1 mg/dl) levels at baseline, N = 117.



G: LDL, median t_0 = 110 mg/dl, patients with low (<=110 mg/dl) and high (>110 mg/dl) levels at baseline, N = 96.

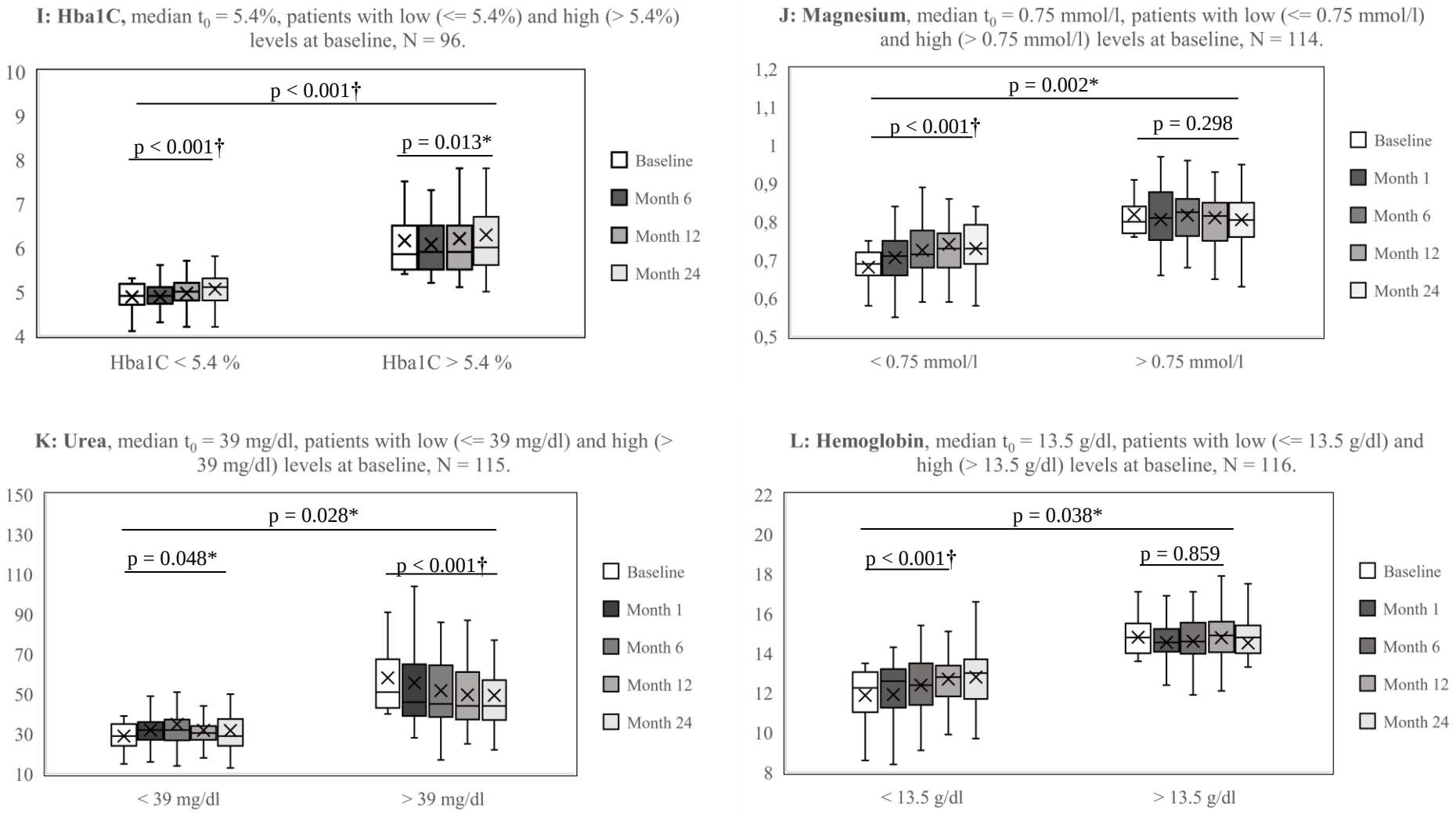


H: HDL, median t_0 = 57 mg/dl, patients with low (<=57 mg/dl) and high (>57 mg/dl) levels at baseline, N = 96.



*p< 0.05, †p<0.001, Wilcoxon signed-rank or t-test, as appropriate.

Figure 11: (continued).



*p< 0.05, †p<0.001, Wilcoxon signed-rank or t-test, as appropriate.

3.4.2.4 Neurologic function – changes in tremor rates

Although the study was not powered to investigate changes in tremor rates, we noticed a pattern of improvement when analyzing the data. Out of the 134 patients who completed the study, 51 (38%) experienced drug induced tremor at study entry. The symptoms were not classified by a qualified movement disorder neurologist, however Tacrolimus neurotoxicity typically manifests with postural hand tremor. At the time of completion, 20 (39%) of those patients reported improvements in tremor: eleven (22%) patients reported a noticeable decrease and tremor ceased entirely in nine (18%) patients. Two patients experienced new onset tremor and symptoms worsened in two, as reported by the patients (Figure 12).

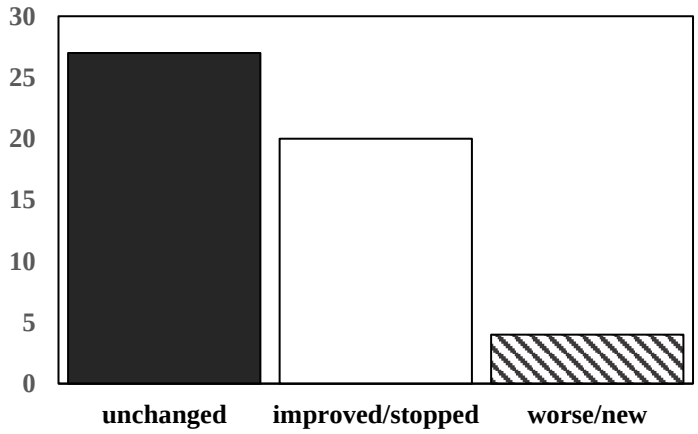


Figure 12: Changes in tremor at month 24, patients who completed entire study period, n = 134.

3.4.3 Efficacy: Patient and graft survival

Over the course of treatment with LCP-Tac, the two-year patient and graft survival were 97%. Five patients died throughout the study period. No episodes of rejection or allograft loss occurred, despite the dose reduction.

Two patients died of cardiac arrest at 60 and 632 days after conversion from IR-Tac to LCP-Tac, respectively. Both suffered from comorbidities at baseline, including diabetes, hypercholesterolemia, and hypertension. One patient had received a coronary artery stent two years prior to enrollment and died of sudden cardiac arrest. The second patient required surgery due to diabetic foot syndrome; two days after the intervention, he suffered from a myocardial infarction and intravenous line infection leading to death.

One patient died of systemic inflammatory response syndrome (SIRS) due to pneumonia after 64 days. This patient suffered from obesity, hypertension, and chronic kidney disease at the time of

admission. In another case, a previously treated hepatocellular carcinoma recurred in a patient during the first week and led to liver failure and death on day 182.

One patient died from SIRS on day 568 caused by mesenteric ischemia. In this case, laboratory results showed pancytopenia; the treating physicians assumed that the reduced cell count was likely caused by mycophenolate mofetil toxicity, the concomitant immunosuppression that had been part of the patient's regimen before study entry.

We note that one patient required treatment for acute liver failure after 18 months, who had received ER-Tac during hospitalization. The transplant center declined a retransplantion because the patient suffered from severe alcohol abuse. We classified this case as serious adverse event leading to withdrawal because the person did not receive LCP-Tac at the time of death but point out that the patient died within weeks of admission.

3.5 Other Analysis

3.5.1 Pharmacokinetics and renal function in slow and fast Tacrolimus metabolizers after conversion to LCP-Tac

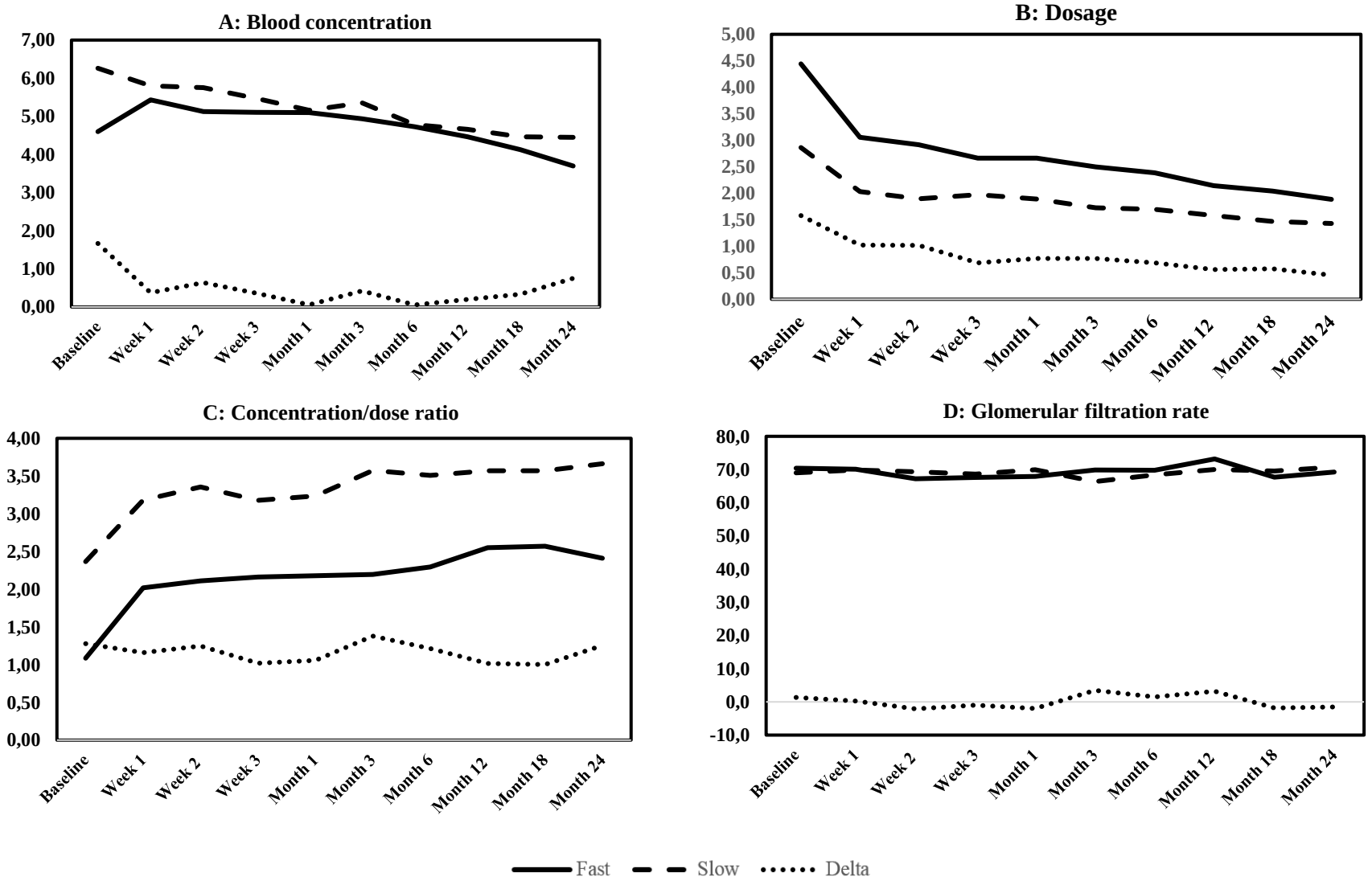
Prior research suggested that patients who require a higher dosage to reach minimal blood levels, so-called fast metabolizers, might be at higher risk for renal injury. We categorized patients into fast (C/D<1.5) and slow (C/D > 1.5) Tacrolimus metabolizers using the median C/D before conversion to LCP-Tac and analyzed blood levels, dosage, C/D ratio, and eGFR over the study period (Figure 13).

After conversion, the higher bioavailability of LCP-Tac led to a jump in the concentration/dose curve for both the fast (1.1 to 2.0 ng/ml per mg/day) and slow (2.4 to 3.2 ng/ml per mg/day) metabolizer groups – which equals an absolute difference of 1.3 ng/ml at baseline and 1.2 ng/ml after conversion. The difference trended around this value and reached 1.3 ng/ml at month 24 again (Figure 6), thus not indicating a disproportionately higher bioavailability of LCP-Tac in fast metabolizers when compared with slow metabolizers.

Renal function appeared to be stable for both groups, resembling the neutral to positive trend observed for the entire cohort described above. At baseline, fast metabolizers had an average eGFR of 70. This value increased to 73 at month 12 ($p = 0.196$) but fell to 71 after two years ($p = 0.241$). During the same period, slow metabolizers exhibited an increase from 69 to 70 ($p = 0.142$) and 71 ($p = 0.342$) after one and two years, respectively.

Throughout the two-year period, Tacrolimus dosage could be reduced safely for both groups while remaining within the target range of 3-7 ng/ml (Figure 13).

Figure 13: Influence of metabolic group on Tacrolimus pharmacokinetics (A-C) and renal function (D), N =124. Groups split by median C/D ratio at baseline into fast (C/D <1.5) and slow (C/D>1.5) metabolizers. Delta denotes difference between groups.



3.5.2 Renal function two years before and 24 months after conversion from IR-Tac to LCP-Tac

For the same subsample of 85 out of the 134 participants that we used to study changes in the Tacrolimus IPV, data on renal function two years prior to conversion to LCP-Tac were collected. In this group of 85 patients, mean creatinine remained stable at around 1.0 mg/dl when comparing the two years before and after conversion (Figure 14). The estimated glomerular filtration rate changed slightly, but not significantly, decreasing from 70 ml/min 24 months prior to conversion to 67 ml/min at baseline (p = 0.084); subsequently, renal function recovered slightly to 69 ml/min after two years of treatment with LCP-Tac (p = 0.672, Figure 15).

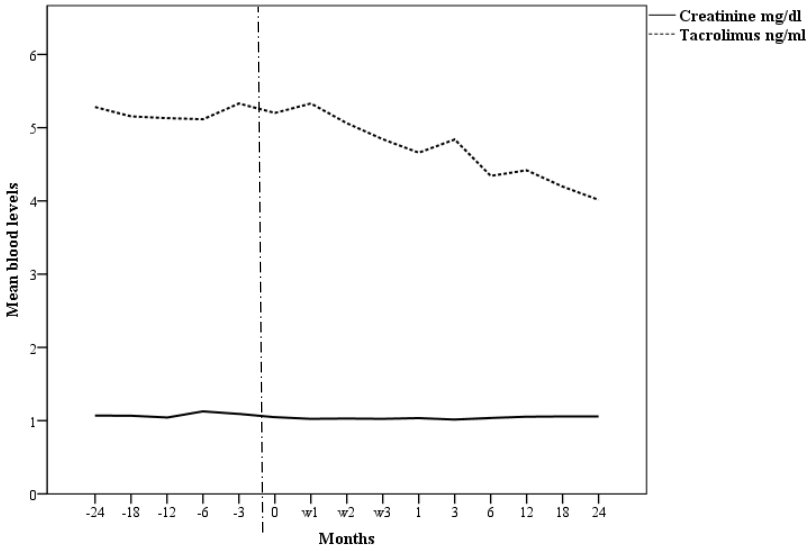


Figure 14: Creatinine and Tacrolimus levels 24 months before and after conversion from IR-Tac to LCP-Tac, n =85.

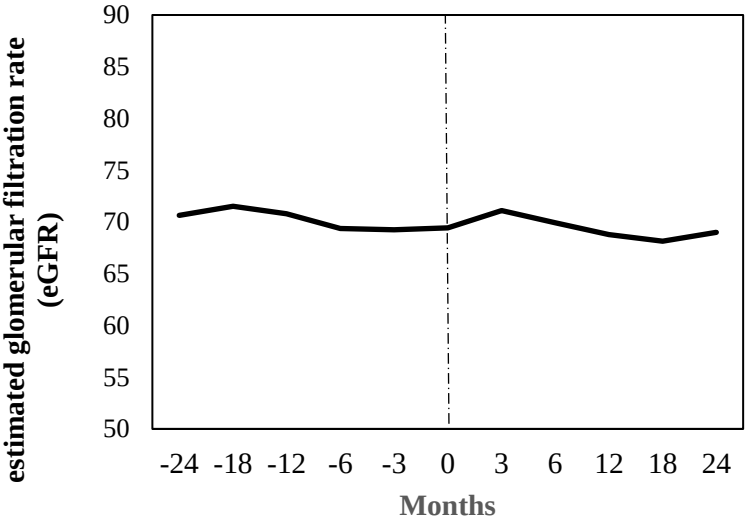


Figure 15: Estimated glomerular filtration rate (eGFR) in ml/min, 24 months prior and after conversion from IR-Tac to LCP-Tac, n = 85.

4. Discussion

4.1 Key findings

In this investigator-initiated study we are reporting data from a single-arm, two-year trial on immunosuppressant adherence, safety, and efficacy in 161 liver transplant recipients after conversion from twice-daily (IR-Tac) to novel once-daily (LCP-Tac) Tacrolimus. As our main result, we found that the medication adherence - as measured with the Basel assessment of Immunosuppressant Adherence (BAASIS©) scale - improved from 50% to 80% over the 24-month study period. This effect was mainly driven by there being fewer patients who skipped doses (2% vs. 15%) or delayed intake (25% vs. 43%) at the time of completion compared to baseline.

As a surrogate parameter for drug intake, we compared the intra-patient variability (IPV) of Tacrolimus drug levels under the standard IR-Tac regimen during the two-year period before conversion with the IPV observed throughout the 24-month study period, during which patients received LCP-Tac. We found no change in the Tacrolimus IPV after conversion to LCP-Tac.

The patient and graft survival rates indicate that LCP-Tac protects stable liver transplant patients from organ rejection with comparable efficacy to the preceding IR-Tac treatment. At the time of enrollment, patients were on average 7.5 years post-transplant. Initial conversion from the IR-Tac to the LCP-Tac regimen was conducted on a 1 to 0.7 mg basis (-30%) to reach comparable trough levels. Over the entire study period, the mean daily dose was reduced by 50%. Although the average Tacrolimus serum concentrations correspondingly fell by 20%, we observed no signs of rejection according to the Banff criteria. Five patients died due to cardiac arrest (n=2), SIRS (n=2), and recurrent hepatocellular carcinoma. The death rate is comparable with data from prior trials with similar observation periods and patient characteristics.

The laboratory and clinical parameters of renal, metabolic, and cardiovascular function remained stable throughout the study period. We found no significant difference between the renal parameters 24 months before conversion from IR-Tac to LCP-Tac as compared to both baseline and the time of completion. Thölking et al. proposed the C/D ratio as a tool to identify fast Tacrolimus metabolizers, who are at increased risk for kidney injury as they exhibit exacerbated peak concentrations compared to slow metabolizers. We used the C/D ratio to classify patients at baseline, and neither report a significant difference in renal function between slow and fast Tacrolimus metabolizers before, or 24 months after conversion to LCP-Tac. Fast metabolizers

also did not gain disproportionate bioavailability of Tacrolimus from conversion to LCP-Tac when compared to slow metabolizers.

A total of 17 out of 161 (11%) patients were reconverted to IR-Tac due to adverse events – half of them within the first month. Common reasons for withdrawal were musculoskeletal pain, gastrointestinal complaints, and neurological symptoms. Other frequent adverse events included infections, eczema, and pruritus – although more than 50% of patients with pruritus suffered from associated conditions such as ischemic type biliary lesions or kidney failure. In three cases, patients were reconverted due to non-fatal serious adverse events including hepatic failure, SIRS, and stroke. The overall adverse event profile is in line with previous studies on patients receiving IR- or LCP-Tac.

Unexpectedly, we noticed that 40% of patients who experienced tremor at baseline under IR-Tac reported a noticeable decrease or complete cessation after conversion to LCP-Tac. Patients who suffer from postural tremor under conventional immediate-release Tacrolimus could thus benefit substantially from conversion.

To our knowledge, this is the first study exploring changes in adherence, clinical safety, and efficacy parameters, as well as Tacrolimus variability in liver transplant recipients after conversion from IR-Tac to LCP-Tac. Future studies will show whether treatment with LCP-Tac reduces retransplantation and mortality rates in liver transplant patients compared to IR-Tac.

4.2 Context

4.2.1 Adherence: The BAASIS©

Non-adherent transplant recipients risk the loss of their graft and inflate treatment costs. Over recent decades, researchers across different medical disciplines have tested myriad interventions to help patients with drug intake - but reducing drug regimen complexity constitutes the only measure that consistently improves adherence (9–11, 115–123).

In our study, we halved the daily Tacrolimus tablet intake of liver transplant patients from twice daily to once daily. This improved overall adherence to immunosuppression on the BAASIS© from 50 to 80%. Fewer patients skipped doses (15% vs. 2%) and one in two stopped delaying drug intake (43% vs. 20%), independently of gender, primary indication, or the number of concomitant medications per day. Patients who took only a morning dose of any medication after conversion reported the largest advance in adherence (+55%, 2.9-fold increase to baseline). In line with this observation, patients with Tacrolimus as a mono immunosuppressant regimen

reported a larger increase in adherence after conversion to LCP-Tac than those who received a second or third immunosuppressive drug (35% vs. 26%). Thus, the relative changes in adherence were the largest when patients had a substantial reduction in daily dosing after conversion.

We also found that patients younger than 59 years old showed less improvement in adherence (27% vs. 33%) and significantly less overall compliance to immunosuppression than the elderly group at month 24 (73% vs. 89%). This result is in line with the findings of prior studies which demonstrated that liver transplant patients are less compliant with their medication during young adulthood. The transition from adolescence to adulthood is characterized by complex developmental tasks, and this can undermine a person's ability to take responsibility for their medication schedule. A study in 121 patients with Rheumatoid Arthritis also showed that middle-aged patients (30-55 years) and those with busy lifestyles were at increased risk for non-adherence as they tried to not forget drug intake throughout their daily activities, while the elderly structured their daily routines around medication intake. No comparable studies have been published in transplant recipients, but the same principles may apply to this patient group. Thus, the delta between the two age groups in this study may be driven by differences in psychosocial development and the magnitude of daily responsibilities (124–127).

Although we found that adherence rates were larger for elderly patients than for the young, both groups reported dramatic improvements in compliance after conversion to the once-daily Tacrolimus regimen (33% and 27%, respectively). Prior studies have shown that transplant patients treated with IR-Tac are less adherent to their drug schedule during evenings, when their daily activities are less predictable. These patients often cite forgetfulness and different priorities as major reasons for poor adherence. Thus, the large effect on medication adherence after conversion from twice-daily to once-daily Tacrolimus across the study population may be explained by a substantial decrease in the probability that psychological barriers and the activities of daily living impede with the correct intake (112, 128).

In 2011, Beckebaum et al. showed that conversion from IR-Tac to ER-Tac improved adherence by around 36% on the BAASIS© questionnaire over a twelve-month period in 125 liver transplant recipients. We found that conversion to LCP-Tac yields a similar effect size (+30%) and reduces self-reported tremor – which has not been reported for ER-Tac (82).

Possible positive long-term clinical consequences of the improved drug intake depend on the relationship between self-reported non-adherence on the BAASIS© survey and hard end points such as organ rejection and graft loss. One major drawback of our study is the limited validity of

the survey to predict clinical outcomes. The Leuven-Basel research group developed the BAASIS© during the late 2000s to conceptualize and quantify adherence to immunosuppression in transplant patients. The survey has gained international acceptance over the last decade and is recommend by international transplantation experts such as the "Guidance Report and Clinical Checklist by the Consensus on Managing Modifiable Risk in Transplantation" (2017) COMMIT group. Studies by Gustavsen et al. (2019) and Tielen et al. (2014) have shown that non-adherence on the BAASIS© is associated with the development of dnDSAs and graft loss one and two years after kidney transplantation, respectively. However, to date, no study links non-adherence on the BAASIS© to clinical endpoints in liver transplantation (69, 105, 106, 129).

Regarding the results of the study presented here, the reduction of patients who regularly skip doses (15% at baseline vs. 2% at the time of completion) may prove the most beneficial. Patients who skip a dose of Tacrolimus may be at increased risk for graft loss compared to patients who only delay drug intake, as they experience a sustained window of under-immunosuppression. In a prospective study on late consequences of non-adherence in 146 kidney transplant recipients published by Vlamnick et al. in 2004, patients who regularly skipped immunosuppression during the previous year had a 3.2 times higher risk of late acute rejection ($p<0.05$), but researchers used no standardized interviewing technique. It is possible that the dose-skipping item on the BAASIS© will be able to identify such patients in long-term clinical studies in the future. (130).

In summary, this is the first study to demonstrate that conversion from conventional twice-daily (IR-Tac) to novel once-daily Tacrolimus (LCP-Tac) improves overall adherence on the BAASIS© survey. Long-term, controlled studies are required to determine whether the improved drug intake with LCP-Tac translates into improved patient and graft survival compared to IR-Tac.

4.2.2 The Intra-patient variability (IPV) of Tacrolimus serum levels and adherence

Over recent years, an increasing number of researcher have suggested that the intra-patient variability (IPV) of Tacrolimus levels may be a valid tool to measure drug adherence in transplant patients. According to this view, patients who comply with their immunosuppression regimen would exhibit lower IPVs as compared to patients who report difficulties taking their medication correctly. Based on this assumption, interventions that increase drug compliance should decrease the IPV (96, 131).

However, we found that the Tacrolimus IPV did not change significantly after conversion from IR-Tac to LCP-Tac, despite an improvement in overall adherence on the BAASIS© survey by

30%. Similarly, Shuker et al. (2015) report that the IPV remained stable in 247 renal transplant patients converted from IR-Tac to ER-Tac, despite Beckebaum et al. (2011) having demonstrated that conversion from IR-Tac to ER-Tac improved adherence by 36% on the BAASIS© (82, 132).

Glander et al. (2018) recently compared the IPV of all three available Tacrolimus formulations in 80 kidney transplant recipients over one year. In their sample, the differences in the COV of Tacrolimus levels between LCP-Tac (n=18; COV=32%), ER-Tac (n=33; COV=28%), and IR-Tac (n=20; COV=29%) were not statistically significant (133).

This leads us to the conclusion that there is no evidence that switching patients between the three available formulations will affect the Tacrolimus IPV. In addition, the IPV and the BAASIS© seem to measure different phenomena. Several mechanisms could explain these findings.

Firstly, the IPV and the BAASIS© measure different time periods. Since the half-life of Tacrolimus lies at around twelve hours, the IPV is only sensitive to the drug exposure during the previous 2-4 days. The BAASIS© captures non-adherence throughout the past month and may thus be more sensitive to irregular medication intake than the IPV.

Secondly, patients who take every dose correctly might still exhibit different IPVs when switched between the three Tacrolimus formulations. So far, baseline IPV values in adherent patients have only been studied with twice-daily formulations (96).

Thirdly, the IPV may generally be a poor model for adherence, as other variables such as co-medications, food intake, or delayed blood withdrawal during outpatient clinic visits may explain most of the variation. To date, no studies have validated the IPV as a measure for non-adherence. Future studies should compare the IPV at different time periods after transplantation; since adherence decreases with time, the IPV should increase during the first three years, even when excluding the early months after surgery due to high disease activity (91).

In summary, we found that the Tacrolimus IPV remained stable after conversion from IR-Tac to LCP-Tac. This finding contradicts the hypothesis that the improved adherence on the BAASIS© would translate into a reduction in the IPV. Possible explanations include different time periods between the two instruments, differences in the relationship between adherence and the IPV of each Tacrolimus formulation, as well as factors that influence Tacrolimus absorption, such as drug-drug interactions, food intake, or gastrointestinal disorders (95).

4.2.3 Safety and Tolerability

While Tacrolimus provides effective protection from rejection, its toxic side effects decrease patients' quality of life and survival. In clinical practice, physicians try to mitigate Tacrolimus toxicity by targeting minimal blood concentrations (38, 112, 134).

In addition to the AUC, the C_{max} is an important pharmacokinetic predictor of Tacrolimus side effects including neurological events, renal toxicity, and diabetogenicity. Thus, one potential benefit of the LCP-Tac formula is a 30% lower C_{max} at identical AUC compared to IR-Tac. Thus, we hypothesized that conversion could lead to a reduction in typical side effects (30, 41, 67, 68, 135).

4.2.3.1 Adverse events

Non-serious adverse events

Tacrolimus causes a wide range of adverse reactions other than renal dysfunction, tremor, or diabetes mellitus. More than 20% of de novo kidney recipients treated with LCP-Tac experienced typical events such as diarrhea, anemia, urinary tract infections, or hypertension during the first year after transplantation in early phase III trials. However, researchers used higher induction doses during these trials as compared to our study, and patients generally received high maintenance doses for the first year – which are in turn associated with the incidence of adverse events (136).

Patients in this study reported five very common (>10%) adverse reactions: upper respiratory tract infections, headache, diarrhea, abdominal pain or nausea, and fatigue. The difference between our results and those provided in the phase III trials likely stems from the described difference in dosing regimens and periods since transplantation in our study compared to de novo recipients. The incidence of adverse events is highest during the first year when induction dosage is high. In our study, subjects who suffered from side effects such as post-transplant diabetes or hypertension already received adequate treatment at the time of conversion.

When we compare our data with other conversion studies, we can provide a clearer overview of adverse events patients may experience when switching to LCP-Tac. In our study, patients experienced very similar adverse events to those reported by Alloway et al. in their study of 44 liver transplant patients converted to LCP-Tac over one year. The mean time since liver transplantation at the time of enrollment in their study was 5 - vs. 8 years in our cohort - and both study populations were converted on a 1 to 0.7 mg basis from IR to LCP-Tac (89).

In either cohort, more than five percent of patients suffered from fatigue, upper respiratory tract infections, tremor, headache, diarrhea, pruritus, and musculoskeletal pain. By comparison, we report slightly higher rates of nausea, eczema, weight gain, and worsening of hypertension – and lower rates of pyrexia, insomnia, and peripheral edema. Around 10% of patients dropped out due to non-serious adverse events in Alloway's study, compared to 11% in our trial.

In general, the profile of adverse events in our study resembled data from trials in long-term transplant survivors receiving IR-Tac. Thus, it may be concluded that treatment with LCP-Tac seems to be associated with similar safety and tolerability as IR-Tac (82, 132).

Non-fatal serious adverse events

Non-fatal serious adverse events included any event which led to hospitalization or disability throughout the study period. We recorded 51 events. Frequently affected organ classes were infectious, hepatobiliary, and vascular disorders.

More than 30% of the non-fatal serious adverse events we recorded were typical for patients with chronic immunosuppression, including infectious or malignant disease. Surgical complications such as bile duct stenosis constitute typical problems after transplant surgery. Other recorded instances of severe disease, such as rheumatoid arthritis, thrombosis, or retinal detachment were isolated and scattered across the study period (137).

A single patient suffered from toxic epidermal necrolysis. In toxic epidermal necrolysis, cytotoxic CD8+ T-cells mediate generalized lysis of the epidermis. Its etiology remains unclear, but disease onset is associated with intake of antibiotics, anticonvulsants, and nonsteroidal anti-inflammatory drugs. Calcineurin-inhibitors, including Cyclosporine and Tacrolimus, constitute effective treatments for toxic epidermal necrolysis due to their ability to reduce T-cell activity. This evidence suggests that LCP-Tac may not have been causal for this episode (138, 139).

The key to resolving severe side effects of Tacrolimus such as increased risk for infectious and malignant disease is the induction of allograft tolerance in the recipient or the eradication of graft immunogenicity, which would allow for complete cessation of immunosuppression. Today, only minimization of Tacrolimus exposure has the potential to alleviate the associated disease burden. Except for the single episode of TEN, the profile of the recorded serious adverse events was comparable to the known side effect profile of Tacrolimus (12, 16, 53, 140).

4.2.3.2 Renal, other metabolic, and cardiovascular function

Renal function

Chronic kidney disease remains a major burden of Tacrolimus therapy and reduces the life expectancy of patients after solid organ transplantation. We hypothesized that the lower C_{max} of LCP-Tac could potentially lead to a gradual recovery of renal function. However, over the two-year study period, treatment with LCP-Tac was associated with stable kidney parameters as measured by the eGFR, creatine, urea, and potassium as compared to baseline. This observation did not change when we split the cohort into patients by median renal function at baseline, or into fast and slow Tacrolimus metabolizers (141).

Finally, we conducted a retrospective chart review on study subjects' creatinine and eGFR rate during the two years before and after conversion. Again, renal function exhibited no significant change compared to baseline either during the two years prior or after conversion to LCP-Tac: the eGFR decreased from 70 ml/min to 67 ml/min during IR-Tac treatment at baseline and slightly increased to 69 ml/min after two years under LCP-Tac. Neither trend was statistically significant. Based on our study, we thus cannot conclude that LCP-Tac recovers renal function after conversion from IR-Tac at a significant rate.

Recently, Einsiedel (2020) published results from an investigator-initiated trial in 120 liver transplant patients. Participants were either maintained on conventional IR-Tac or switched to LCP-Tac for one year. The researchers did not randomize patients, and the reasons for allocation to either study arm were not reported. The eGFR of patients in the LCP-Tac group improved during the study period (65.3 ml/min at conversion vs. 70.9ml/min at month 12; $p < 0.001$). By contrast, patients who were maintained on IR-Tac exhibited deteriorating renal function after one year (70.6 ml/min at baseline vs. 66.3 ml/min at month 12; $p < 0.001$). However, the difference between the two groups was not statistically significant at any point during the study. In addition, mean whole blood Tacrolimus levels in the LCP-Tac arm fell by 27% ($p < 0.001$), while the change in the IR-Tac arm was not significant. Thus, it cannot be concluded whether the recovery in renal function was either due to the proposed benefit of the new formulation – the reduced C_{max} - or due to the decrease in the C_{min} and the corresponding AUC. A significant reduction in blood levels in the IR-Tac arm might have recovered renal function at the same rate. This seems plausible given that the relationship between renal function and Tacrolimus levels approximates a linear function. In addition, the lack of randomization and the pre-existing GFR trends before conversion make it difficult to interpret the data. Einsiedel et al. argue that the improved

bioavailability may have caused the improvement. However, the different bioavailability of LCP-Tac compared to IR-Tac merely reflects improved absorption along the gastrointestinal tract, but 24-hour exposure is identical when the same target levels are used. Thus, the assumption that this change has any clinical consequences at all is questionable (40, 142).

Data on renal function in randomized head-to-head studies between IR-Tac and LCP-Tac stems from early phase III trials. Bunnapradist et al. (2013) randomly assigned 326 stable kidney transplant patients to either IR-Tac or LCP-Tac and could not demonstrate any difference in renal function after twelve months between the two groups. Likewise, Budde et al. (2014) randomized 543 de novo kidney transplant recipients to the two formulations, and also reported no significant difference in renal function after one year. However, the researchers in both studies used different conversion ratios between IR-Tac and LCP-Tac (1 to 0.8) for maintenance immunosuppression, and Budde et al. used higher induction doses than recommended by the manufacturer today, which may have concealed a renoprotective effect. Thus, the hypothesis that LCP-Tac improves renal function when compared to IR-Tac must be investigated in randomized controlled studies with identical dosing regimens to those currently used (143, 144).

Additionally to the described evidence, Alloway et al. (2014) showed that renal function remained stable one year after conversion of 44 stable liver transplant patients from conventional IR-Tac to LCP-Tac using a 1:0.7 ratio – although the researchers did not report exact figures (89).

Since our findings clearly support the results of the previous studies, we conclude that no quality evidence suggests that LCP-Tac protects renal function at a higher rate than IR-Tac when converted on a 1:0.7 or 1:0.8 mg basis.

Other metabolic and cardiovascular function

Next to renal toxicity, a typical side effect of Tacrolimus therapy is diabetogenicity. The issue as wo which release properties or pharmacokinetics lead to this effect in humans remains unresolved. In animal models, Tacrolimus induces insulin resistance and increases the glucose absorption in the jejunum. During the first month after a kidney transplant, 50% of patients suffer from post-transplant diabetes mellitus. The prevalence decreases to 13% after one year, when patients require a lower dosage of Tacrolimus (30, 67, 68).

However, chronic insulin resistance associated with Tacrolimus can lead to an increase in circulation of fatty acids promoting dyslipidemia, hypertension, and obesity – the metabolic

syndrome. Up to 50% of patients suffer from metabolic syndrome one year after transplantation, which decreases graft and patient survival. Liver transplant patients with metabolic syndrome are at four-fold risk for cardiovascular events (30, 31, 71).

During early applications of Tacrolimus at the University of Pittsburgh during the 1990s, researchers noticed that consecutive dose reductions recovered previously lost glucose sensitivity and normalized blood pressure. Since then, dose reduction has become an important approach to reducing Tacrolimus side effects (16, 70).

We wanted to investigate whether treatment with LCP-Tac could improve metabolic disturbances and cardiovascular function in liver transplant patients. However, we found no clinically relevant changes in parameters of glucose sensitivity, lipid profile, or blood pressure over the course of treatment. The HbA1c (+0.2%), high-density lipoprotein (+1mg/dl) and diastolic blood pressure (-3mmHg) exhibited statistically significant changes over the 24-month period on a population level. However, laboratory changes throughout the study period only translated into clinically meaningful differences in six cases: we prescribed insulin in one patient (Hba1c >6.5% at baseline) and stopped insulin treatment in another; three patients required new antihypertensive medication and we stopped medication in one.

In the above-mentioned large phase III RCT in 543 kidney transplant recipients by Budde et al. (2014), researchers showed that Triglycerides were, on average, 20 mg/dl lower in the LCP-Tac group than in the IR-Tac group ($p = 0.058$) after twelve months. Nevertheless, the differences between subjects receiving LCP-Tac and IR-Tac regarding HDL (3 mg/dl), LDL (2 mg/dl), total cholesterol (1 mg/dl), and the incidence of hypertension (0.6%) were negligible in their study. Similarly, Bunnapradist et al. showed no differences in metabolic and cardiovascular profile between both formulations (143, 144).

Based on these findings, we cannot support the hypothesis that metabolic disturbances associated with Tacrolimus will differ between patients treated with LCP-Tac and IR-Tac.

4.2.3.3 Neurological function

Between 30-50% of patients taking Tacrolimus experience postural hand tremor, which significantly impacts their quality of life. In animal models, whole blood Tacrolimus levels correlate closely with intracerebral concentrations and neurotoxic events. Thus, Tacrolimus peak concentrations are likely to cause these symptoms. Since LCP-Tac exhibits significantly lower

peak concentrations when compared to IR-Tac, we hypothesized that conversion could potentially reduce Tacrolimus-induced tremor (38, 39, 50).

In a study published in 2015, Langone et al. found that switching kidney transplant patients from IR-Tac to LCP-Tac resulted in a clinically and statistically significant change in tremor. Researchers converted 38 patients on a 1 to 0.7 mg basis and trough levels remained stable over the study period of two weeks. In their study, a blinded movement disorder neurologist assessed tremor using the Fahn-Tolosa-Marin (FTM) scale and an accelerometer before and after conversion to LCP-Tac. They also questioned patients using the QUEST (quality of life in essential tremor) and Patient Global Impression of Change surveys. The authors suggest that Tacrolimus neurotoxicity is more sensitive to a reduction in peak concentrations than renal or metabolic side effects, since trough levels remained stable (38).

In our study, around two in five patients who experienced tremor at study entry reported either symptom improvement or complete cessation after conversion to LCP-Tac. However, we decreased the TDD by 50% over the entire study period of two years as part of our Calcineurin inhibitor-sparing regimen, which led to lower C_{min} and AUC at the time of completion. Thus, the observation of lower tremor rates under LCP-Tac at identical trough levels to those described by Langone et al. needs to be confirmed in future studies, so as to be able to conclude that the change in tremor rates stems from a reduction in the C_{max} instead of the AUC.

As of December 2020, RCTs specifically powered to detect changes in neurological function and other typical Tacrolimus side effects between IR-Tac and LCP-Tac are enrolling patients at the Medical University of South Carolina (planned study size: 40 patients) and Mayo Clinic, Rochester, US (planned study size: 240 patients). It remains important to see whether Langone's and our findings will be confirmed with high quality evidence from these RCTs (145, 146).

4.2.3.4 Summary: Adverse Events and Side Effects

In summary, publicly available data including the present study suggest that the differences in the renal, metabolic, and cardiovascular profile between patients treated with IR-Tac or LCP-Tac are clinically negligible and not significant.

We found weak evidence for a reduction in tremor in patients after conversion to LCP-Tac. Randomized, controlled trials designed to test this specific hypothesis are currently under way in the United States (145, 146).

Regarding future treatment plans, LCP-Tac may allow for more effective targeting of minimal C_{min} values with the goal of preserving renal function – but this, again, remains to be proven in a high quality, randomized study.

4.2.4 Efficacy: Patient and graft survival

The core parameter of efficacy for immunosuppressive drugs is the event rate of biopsy-proven rejection and graft loss. LCP-Tac uses a prolonged release formula with the goal of improving adherence and reducing side effects. While both measures can improve patients' QoL, they remain surrogate parameters that complement hard clinical endpoints such as rejection, graft loss, and death.

When ER-Tac, the first once-daily Tacrolimus formulation was introduced, phase II and III trials showed comparable efficacy and safety parameters for the prevention of organ rejection when compared to IR-Tac. However, subsequent studies that were conducted after the market introduction of ER-Tac revealed that the actual drug exposure at the recommended conversion ratio was significantly lower, leading to increased rejection rates. In addition, female patients exhibited a three-fold increased death rate compared to male patients. This example illustrates that post-marketing monitoring, such as this study, remain crucial in order to avoid medication errors once federal agencies approve a new drug (12, 13).

In 2015, Veloxis Pharmaceuticals Inc. filed for FDA approval of LCP-Tac providing data from two major RCTs in kidney transplant recipients. The first study, published by Budde et al. in 2014, was an RCT in 543 de novo kidney transplant recipients. LCP-Tac showed comparable biopsy-proven rejection rates to IR-Tac (13.1 % vs 13.5%, $p = 0.900$). The second study, published by Bunnapradist et al. in 2013, was a randomized controlled non-inferiority trial in 326 stable renal transplant recipients converted from IR-Tac to LCP-Tac. Both study arms exhibited similar rejection rates (2.5% vs. 0.6%, $p = 0.371$) (89, 143, 144).

However, as described above, both studies used a 1:0.8 mg conversion ratio, in contrast to the 1:0.7 ratio recommended today. Given the difference in conversion ratios between the only two available RCTs and our study, as well as the knowledge of post-marketing dose adjustments with ER-Tac, we lacked evidence to indicate that LCP-Tac would prove adequately potent at the recommend conversion ratio across a different organ class such as the liver. A single, small-scale study by Alloway et al. (2014) provided early data from a phase II conversion trial of 44 liver transplant patients. The researchers used a 1:0.7 conversion ratio and recorded one case of rejection over a 52-week observation period (89).

In our study, we found no signs of rejection across a three times larger study population, despite a 50% dose reduction and a study period which was twice as long when compared to Alloway. Another argument for the efficacy of LCP-Tac for the prevention of rejection can be made when considering our cohort exhibited lower mean Tacrolimus concentrations (5.4 ng/ml) at study entry than both the liver transplant recipients in the study led by Alloway (8.2 ng/ml) or the kidney transplant recipients in the study led by Bunnapradist (6.1 ng/ml). Nevertheless, the median time since transplantation of our population was 55 months compared to 32 in the study led by Alloway. Since the risk of rejection decreases with time, this could explain why we did not observe any.

In summary, growing evidence including this trial suggests that LCP-Tac is as effective for the prevention of solid organ rejection as IR-Tac at the recommended conversion ratio of 1 to 0.7 mg. The formulation could also prove highly beneficial as part of Calcineurin inhibitor-sparing regimens when physicians try to target lower C_{min} and AUCs, which is necessary in order to reduce Tacrolimus toxicity.

4.3 Strengths and Limitations

Several points support the validity and reliability of the results of this study in contributing to the discussion of Tacrolimus based immunosuppression protocols after solid organ transplantation.

We believe that the study design has allowed us to draw an informative picture of what clinicians can expect when using LCP-Tac in liver transplant patients. The setting within the routine transplant aftercare program is likely to be more representative when compared to previously published phase II and III trials, which are typically tightly monitored. This increases the robustness of the results.

One of the limitations of adherence research in transplantation is the lack of standardized techniques to capture incompliant drug intake by transplant patients. In addition, no clinically meaningful difference for adherence rates such as the minimum required doses per month to prevent rejection has been described. In response, since 2016, international transplant experts have started to recommend the use of the BAASIS© in clinical trials and routine practice. Their declared long-term goal remains to determine the exact relationship between non-adherence and graft loss, as well as to allow for standardized comparisons between interventions, organ classes, and transplant centers. In this regard, this study contributes to a growing body of standardized adherence research in organ transplantation that uses the BAASIS© instrument.

In addition, the length of the study increased the likelihood that additional safety concerns or positive effects of the new formulation might have been captured and decreased the probability that patients gave false positive answers in the questionnaire.

At the time of the planning of this study, LCP-Tac had only recently been approved. We did not implement standardized assessments of typical Tacrolimus side effects such as postural tremor because we wanted to provide an exploratory investigation of the drug in a typical clinical setting. Nevertheless, our study provides evidence that patients with tremor could benefit from conversion to LCP-Tac.

However, our study suffers from three major limitations. Firstly, we enrolled all patients in the LCP-Tac group and did not randomize patients to either LCP-Tac or IR-Tac. Without a randomized control group, only limited evidence can be provided for potential treatment effects.

Secondly, we reduced dosage by more than the 30% conversion ratio to minimize CNI exposure. While it is standard practice to reduce TDD in long-term transplant aftercare to protect patients from Tacrolimus side effects, this limits our ability to conclude whether the reduction in tremor

originated from lower peak exposure or lower trough levels. Thus, we cannot refute the hypothesis that comparable improvements in tremor might be obtained if the dosage was reduced by 20% in patients maintained on an IR-Tac regimen.

The third limitation of this study is the limited validation of the BAASIS© survey for hard clinical endpoints such as retransplantation and death in liver transplantation. To date, only the studies by Gustavsen et al. (2019) and Tielen et al. (2014) have linked replies on the survey to deteriorating graft function and rejection in kidney transplant recipients (105, 106).

4.4 Outlook

This study shows that conversion of stable liver transplant patients from IR-Tac to LCP-Tac improves adherence on the BAASIS© and may reduce Tacrolimus-induced tremor in up to 40% of patients. Up to now, no data suggest that LCP-Tac can improve patient or graft survival. Since neither the renal nor metabolic functions appear to change significantly under LCP-Tac therapy when compared to IR-Tac, advances in transplant outcomes may require novel immunosuppressants with fewer toxic side effects.

Currently, Swiss Novartis AG is investigating Iscalimab, a novel anti-CD-40-antibody, as the primary immunosuppressant in de novo kidney transplant recipients. Since Iscalimab specifically targets the activation of T-cells, it may reduce adaptive immunity against the graft without causing non-specific side effects of Tacrolimus in the gut, brain, or kidneys (147–151).

Within the next decade, ongoing trials will challenge whether Tacrolimus is to remain the gold standard for the prevention of organ rejection, and if so, which formulation will provide efficacy with limited side effects. From a non-pharmacological perspective, some transplant centers have posted results from sleeve gastrectomies in liver transplant patients to combat the effects of obesity – an approach that, albeit risky, might mitigate the long term risks for transplant patients associated with insulin resistance (152).

4.5 Implications of this study

The results presented here can help with the optimization and individualization of Tacrolimus based immunosuppression protocols after liver transplantation. In addition, this study contributes to a growing registry of standardized adherence research using the BAASIS© instrument. However, new treatment strategies for the prevention of organ rejection, for example targeted therapies against activated lymphocytes, are needed to avoid Tacrolimus toxicity.

While one-year survival after liver transplantation has improved substantially over the last few decades, long-term mortality remains high. In the UK, patients who survive the first twelve months die ten years younger than an age-matched cohort – an effect that widens for younger patients and can partly be attributed to Calcineurin inhibitor toxicity (62).

Since the viability of novel immunosuppressive drugs such as Iscalimab remains vague, and even more so the realization of inducing graft tolerance in patients, the transplant community is focused on optimizing existing therapies. Because donor organs are increasingly scarce, this this has become an even greater necessity. In 2019, more than nine thousand patients waited for a transplant in Germany; as the population ages and deaths from accidents become rarer, both the quality and quantity of organs have declined. This is exacerbated by the fact that the willingness to register as an organ donator has decreased substantially in Germany in response to the uncovering of manipulation of the organ transplant allocation system by transplant surgeons.

With the introduction of LCP-Tac, transplant physicians can offer their patients a novel option which may reduce side effects and improve QOL. However, whether or not LCP-Tac becomes the new standard of care in transplantation depends on its cost effectiveness, that is the cost of the intervention relative to an appropriate measure of its effect (153).

High quality data including our study suggest that LCP-Tac provides comparable efficacy for the prevention of organ rejection to the current standard of care, IR-Tac. In Germany, annual treatment costs for LCP-Tac (7,800€) lie 20% below those for IR-Tac and ER-Tac (each 9,800€) due to the lower dose requirements. Since generic immediate-release Tacrolimus formulations are currently priced at approximately 80% of IR-Tac, treating a single patient with LCP-Tac requires very similar annual expenditure to the treatment with generic IR-Tac. So far, only weak evidence has suggested that LCP-Tac can reduce tremor or that it may preserve renal function at a superior rate than IR-Tac (133).

Thus, compared to the two innovator Tacrolimus formulations IR- and ER-Tac, LCP-Tac might provide a more cost-effective solution in Germany, as clinical studies have provided evidence for comparable efficacy with potentially fewer side effects and improved QoL at approximately 20% less cost.

4.6 Conclusion

This study demonstrates that novel once-daily Tacrolimus (LCP-Tac) improves adherence after conversion from conventional twice daily Tacrolimus (IR-Tac), while providing comparable efficacy for the prevention of organ rejection.

We also found evidence that supports the notion LCP-Tac can reduce tremor and preserve renal function as part of low dose regimens.

From a clinical perspective, suitable candidates for conversion to LCP-Tac include patients with simple regimens (no evening dose of concomitant medications) with the aim of facilitating adherence or those experiencing tremor under an IR-Tac based immunosuppression protocol.

The field of adherence research thus offers wide perspectives to further improve drug intake within the available treatment options.

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Appendix

Eidesstattliche Versicherung

„Ich, Marius Jonathan Ibach, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: ‚Conversion of liver transplant recipients from twice-daily to novel once-daily Tacrolimus: a 2-year prospective study on adherence, safety, and efficacy. / Umstellung nach Lebertransplantation von zwei- zu einmal täglicher Gabe von Tacrolimus: 2-Jahres-Verlaufsbeobachtung zur Adhärenz, Sicherheit, und Wirksamkeit.‘ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum Unterschrift

<u>Anteilserklärung an etwaigen erfolgten Publikationen</u>

Marius Jonathan Ibach hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1:

Maurer MM, **Ibach M**, Plewe J, Winter A, Ritschl P, Globke B, Öllinger R, Lurje G, Schöning W, Pratschke J, Eurich D. Reducing the Pill Burden: Immunosuppressant Adherence and Safety after Conversion from a Twice-Daily (IR-Tac) to a Novel Once-Daily (LCP-Tac) Tacrolimus Formulation in 161 Liver Transplant Patients. Biomedicines. 2022 Feb;10(2):272.

Beitrag im Einzelnen:

1. Die Tabellen 1,2,3,4,5, und 6 sowie die Abbildungen 2 und 3 sind aus meiner statistischen und qualitativen Auswertung entstanden und wurden von mir erstellt.
2. Von mir wurde die IPV-Analyse als Sekundärparameter für die Adhärenz konzeptualisiert, relevante Daten gesammelt, und ausgewertet.

3. Ich habe die Kategorisierung der UAW mittels MedDRA konzeptualisiert und bei der Analyse die Veränderungen in den neurologischen Nebenwirkungen festgestellt.
4. Ergebnisdarstellung anhand der STROBE Guidelines und Editing/Writing Manuskript.

_____

Unterschrift, Datum und Stempel des/der erstbetreuenden Hochschullehrers/in

_____

Unterschrift des Doktoranden/der Doktorandin

Lebenslauf

"Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht."

Publikationsliste

1. Maurer MM, **Ibach M**, Plewe J, Winter A, Ritschl P, Globke B, Öllinger R, Lurje G, Schöning W, Pratschke J, Eurich D. Reducing the Pill Burden: Immunosuppressant Adherence and Safety after Conversion from a Twice-Daily (IR-Tac) to a Novel Once-Daily (LCP-Tac) Tacrolimus Formulation in 161 Liver Transplant Patients. Biomedicines. 2022 Feb;10(2):272. DOI: 10.3390/biomedicines10020272

2. **Ibach, M**; Eurich, D; Dobrindt, E; Lurje, G; Schöning, W; Öllinger, R; Pratschke, J; Globke, B. (2021): Orthotopic Liver Transplantation for Budd-Chiari Syndrome: Observations from a 30-Year Liver Transplant Program. In: *Medicina* 57 (8). DOI: 10.3390/medicina57080821.

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Bescheinigung Statistik

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Bescheinigung

Hiermit bescheinige ich, dass Herr *Marius Jonathan Ibach* innerhalb der Service Unit Biometrie des Instituts für Biometrie und klinische Epidemiologie (iBikE) bei mir eine statistische Beratung zu einem Promotionsvorhaben wahrgenommen hat. Folgende Beratungstermine wurden wahrgenommen:

- Termin 1: *04.12.2019*
- Termin 2: *16.12.2020*

Folgende wesentliche Ratschläge hinsichtlich einer sinnvollen Auswertung und Interpretation der Daten wurden während der Beratung erteilt:

- *Visuelles Prüfen von Normalverteilungsannahmen*
- *abhängig und unabhängige statistische Testverfahren z.B. t-test, Wilcoxon-signed rank test, Chi-square test, Friedman's test*
- *Interpretation und Präsentation der p-Werte*

Diese Bescheinigung garantiert nicht die richtige Umsetzung der in der Beratung gemachten Vorschläge, die korrekte Durchführung der empfohlenen statistischen Verfahren und die richtige Darstellung und Interpretation der Ergebnisse. Die Verantwortung hierfür obliegt allein dem Promovierenden. Das Institut für Biometrie und klinische Epidemiologie übernimmt hierfür keine Haftung.

Datum: 04.01.2021 Name des Beraters/ der Beraterin: Pimrapat Gebert

Unterschrift Beraterin, Institutsstempel

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