Final Report:

"Prospective randomized trial to compare a twice daily to a once daily administration of the Tacrolimus in lung transplanted patients"

Investigator Initiated Trial (IIT)
of the Department of Respiratory Medicine,
Hannover Medical School, Germany

Principal Investigator: Jens Gottlieb, M.D.

Financially supported by Astellas Pharma GmbH

Protocol number: DE-09-RG-53
EudraCT number: 2009-011324-60
Version 1.2, 7th May 2009
Amendment 4, 30th September 2010
Final Report August 2012

Study Title

Prospective randomized trial to compare a twice daily to a once daily administration of the Tacrolimus in lung transplanted patients

Test drug/investigational product
Tacrolimus (Prograf®) Tacrolimus Modified Release Formulation (Advagraf®)

indication studied
Indicated conversion from CyA to Tac after lung transplantation (single, double or heart/lung) at least 12 months after transplantation

Study design
open, randomized, prospective

Sponsor
Medizinische Hochschule Hannover, represented by Michael Born
Carl-Neuberg-Str. 1, 30625 Hannover
Tel.: +49 511 532 6514, Email: born.michael@mh-hannover.de

Protocol number
DE-09-RG-53

development phase of study
phase 3

study initiation date
31.07.2009

Date of early study termination
02.07.2012

Principal investigator
Klinik für Pneumologie, Medizinische Hochschule Hannover,
Carl-Neuberg-Str. 1, 30625 Hannover; Tel. 0511/532-3560

name of company/sponsor signatory (the person responsible for the study report within the company/sponsor)
PD Dr. med. Jens Gottlieb, Tel. 0511/532-3560, Fax. 0511/532-8094

statement indicating whether the study was performed in compliance with Good Clinical Practices (GCP), including the archiving of essential documents
The Sponsor (KS-MHH assigned by MHH) of this study is responsible according to ICH GCP guidelines for assuring proper study conduct as regards protocol adherence and validity of the data recorded on the CRF´s.
Data Quality Control (QC) will be performed by the appropriate departments of the MHH.
Quality Assurance (QA), in form of protocol, ICF, CRF, report, in-house and on-site audits, will be performed by the QA Unit of the MHH.
According to § 4 AMG the Sponsor (KS-MHH) will take responsibility for the inducement, organization and financing of the clinical trial. Sponsor and Investigator assure that the clinical trial will be conducted in accordance with the established laws and instructions according to ICH-GCP-Guidelines (1996), declaration of Helsinki (1996) as well as the directives of the AMG and the GCP-V (2004). The Investigators accept the requirements by signing the study protocol.

date of the report
August 2012

Synopsis

Name of Sponsor/Company: Medizinische Hochschule Hannover, represented by Michael Born Carl-Neuberg-Str. 1, 30625 Hannover Tel.: +49 511 532 6514, Email: born.michael@mh-hannover.de
Name of Finished Product: 1. Advagraf ® 2. Prograf ®
Name of Active Ingredient: 1. Tacrolimus 2. Tacrolimus modified release formulation
Title of Study: Prospective randomized trial to compare a twice daily to a once daily administration of the Tacrolimus in lung transplanted patients
Investigators: Dr. med. Jens Gottlieb, Claudia de Wall, Dr. Christoph Duesberg, Dr. Christine Knuth, Dr. Jessika Rademacher, Dr. Hendrik Suhling, Dr. Katrin Meyer, Dr. Mark Greer
Study centre(s): Medizinische Hochschule Hannover, Klinik für Pneumologie Carl-Neuberg-Straße 1, 30625 Hannover
Publication (reference): Not jet
Studied period (years): 31.07.2009-01.06.2012
Phase of development: **3**
Objectives: The primary objective of this study is to • Improvement of adherence as measured by Tacrolimus trough level below the target level and dispensing of less than 50% of the prescribed doses in the last three days measured electronically before this subtherapeutic drug monitoring The secondary objectives of this study are: • Deterioration of graft function as defined as more than 20% decline in maximum FEV1 before and at month 12 after conversion • Number of drug holidays (intake of less than 50% of prescribed doses in 24 hours) measured electronically • Evaluation of renal function in pts converted from CyA to Tac in combination with MMF and steroids as assessed by serum creatinine, creatinine clearance (Cockgroft Gault), MDRD and Cystatin C before and at month 1, 3, 6 and 12 after conversion • Evaluation of cardiovascular risk factors (hypertension, hyperlipidaemia, hypertriglyceridemia, diabetes mellitus) • Incidence of CMV infections and other infections • Efficacy: Incidence of acute rejection episodes, graft and patient survival • Safety: Incidence of adverse events • Comparison of MPA-mini-AUC under Tacrolimus once and twice daily administration
Methodology: Patients after single, double or heart/lung transplantation will be randomized 12 hours after the last CyA administration to an individually titrated treatment scheme based on either Prograf® (two daily doses of Tacrolimus) or Advagraf® (one daily dose of Tacrolimus).
Number of patients (planned and analysed): Planned 48, because of early termination only 25
Diagnosis and main criteria for inclusion: Patients (≥18 and ≤ 70 years) ≥ 3 months after single, double or heart/lung transplantation. · Pts ≥ 3 months after single lung, double lung or heart/lung transplantation and

· Pts treated with Cyclosporine, steroids and MMF and
· Pts ≥18 and ≤ 70 years and
· Pts with one oft he following

- Pts with recurrent acute rejections (RAR)
  (Pts with concomitant stable and non-advanced BOS are eligible)
  two or more acute rejections in last 3 months (first 4 weeks post Tx excluded)
- Pts with steroid-resistant or ongoing acute rejections (OAR)
- Pts with CyA associated side effects

Test product, dose and mode of administration, batch number:
Tacrolimus (Prograf®)
Active ingredient: tacrolimus
Twice daily
Tacrolimus Modified Release Formulation (Advagraf®)
Active ingredient: tacrolimus
Once daily

The initial dose of Tacrolimus for both groups will be calculated by the last CyA dosing (initial tacrolimus dose = last ciclosporin dose divided by 50). The trough level of Tacrolimus will be aimed at 8-12 ng/ml. In case of drug toxicities target drug levels may be individually be lowered to 5-8 ng/ml.

Duration of treatment:
12 months

Reference therapy, dose and mode of administration, batch number
Active ingredient: mycophenolate mofetil
MMF target dose is 2000 mg/d, in pts. with cytopenia or GI-intolerance it should be reduced by 25% after switch from CyA to Tac and if Tac target trough levels are achieved.

Criteria for evaluation:

Efficacy:

Compliance will be measured by two ways: firstly, by electronic measurement of study drug dispensing and secondly subtherapeutic drug levels.

Criteria for Continuation of Treatment

- Renal function
- Immunosuppressant therapy barrier scale (ITBS)
- Immunosuppressant therapy adherence instrument (ITAS)
- Pulmonary function
- Test Drug Concentration

Safety:

The most relevant safety parameters assessed within the study are measurement of renal function, incidence of adverse events, absolute change in serum lipids (cholesterol, HDL, LDL, triglycerides) and incidence of diabetes mellitus.

Statistical methods:

The primary aim of this clinical trial is to reject the null hypothesis that the mean of the proportions of too low Tacrolimus trough levels caused by non-compliance from patients that take Prograf® is equal to the mean of the proportions of patients that receive Advagraf®.

The standard deviations in each group are assumed to be equal. A two-sided t-test for two independent groups will be used to assess the hypothesis and the null-hypothesis will be rejected if the respective P-value is less than 0,05. The percentage of noncompliant observations per patient will be transformed with an arcsin transformation before applying the t-test in the primary analysis. The respective 95%-CI for the difference in means will be back-transformed for presentation of results.

Summary – Conclusions

Efficacy Results: Adherence in both groups was excellent**.** Regarding adherence in taking and timing of drugs both drugs were equally effective. Drug holidays with subtherapeutic drug levels occurred exclusively in group A (advagraf). A trend towards more patients in group A experiencing subtherapeutic drug levels was observed. Limited by the small number of patients in both groups due to early termination of the trial, no significant differences were noted in terms of acutre rejection, new

onset of BOS, infections (incl CMV).

Safety Results: As expected in a cohort of lung transplant recipients adverse events were frequent. Most frequent AEs were of infectious origin and most infections arose from the respiratory system. Even 20 serious adverse events occurred in 25 recipients but were related to hospilizations only. No death or life-threatening conditions were noted. Kidney functions as measured by various methods of GFR estimation revealed no significant differences between pstioents treted with once-daily versus twice daily tacrolimus. A trend was noted towards higher drug exposure to mycophenolat acid, ut this analysis was limited by the small number of patients due to early termination of the trial. For the same reasons, no consequences concerning cardiovascular consequences (incl. new onset of diabetes) could be drawn.

Conclusion: Adherence in both groups was excellent**.** Regarding adherence in taking and timing of drugs both drugs were equally effective. Drug holidays with subtherapeutic drug levels occurred exclusively in group A (advagraf). A trend towards more patients in group A (advagraf) experiencing subtherapeutic drug levels was observed. Limited by the small number of patients in both groups due to early termination of the trial, no significant differences were noted in terms of acute rejection, new onset of BOS, infections (incl CMV).
As expected in a cohort of lung transplant recipients adverse events were frequent. Most frequent AEs were of infectious origin and most infections arose from the respiratory system. Even 20 serious adverse events occurred in 25 recipients but were related to hospilizations only. Patients treated with advagraf experienced by trend more adverse events (11.3 AEs per patient vs. 6.2 per patient). No death or life-threatening conditions were noted. Kidney functions as measured by various methods of GFR estimation revealed no significant differences between patients treated with once-daily versus twice daily tacrolimus. A trend was noted towards higher drug exposure to co-medication with mycophenolat acid, ut this analysis was limited by the small number of patients due to early termination of the trial. For the same reasons, no consequences concerning cardiovascular consequences (incl. new onset of diabetes) could be drawn.
In the light of other existing data in liver transplantation once-daily tacrolimus seem to be an efficient and safe drug in solid organ transplantation. Superiority in clinically meaningsful endpoints should be confirmed in future prospective studies.

Date of report
August 2012

TABLE OF CONTENTS

1. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviations will be explained in text when mentioned for the first time.

2. ETHICS

2.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

Ethik-Kommission der Medizinischen Hochschule Hannover

Carl-Neuberg-Straße 1

30625 Hannover

2.2 Ethical Conduct of the Study

Nr. 5281M mono

2.3 Patient Information and Consent

Normaly the patient obtained the patient information on the day of enrolment. Only three patient received it earlier. Pat. 7 received the ICF one day before enrolment, patient 8 three days before enrolment and patient 24 five days before enrolment.

3. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Study Monitoring and Auditing

Principal Investigator:	PD Dr. med. Jens Gottlieb
Address:	Medizinische Hochschule Hannover,
	Department of Respiratory Medicine,
	Carl-Neuberg Str. 1
	30265 Hannover
Phone-Nr.:	**0511-532-3560**
Fax-Nr.:	**0511-532-8532**
Email:	Gottlieb.Jens@mh-hannover.de

Contact Person at	
Astellas Pharma GmbH:	Katharina Margreiter
	Head Clinical Project Management Academic Research
	Medical Department
Address:	Georg-Brauchle-Ring 64-66 / Campus E
	80992 München
Phone-Nr.:	**089-4544-1786**
Fax-Nr.:	**089-4544 -5786**
Email :	Katharina.Margreiter@de.astellas.com

The Sponsor (KS-MHH assigned by MHH) of this study is responsible according to ICH GCP guidelines for assuring proper study conduct as regards protocol adherence and validity of the data recorded on the CRF´s.
Data Quality Control (QC) will be performed by the appropriate departments of the MHH.
Quality Assurance (QA), in form of protocol, ICF, CRF, report, in-house and on-site audits, will be performed by the QA Unit of the MHH.

Sponsor responsibilities

It is an Investigator Initiated Trial by the Department of Respiratory Medicine of the Hannover Medical School. All organizational issues will be done by the department. The Hannover Medical School will take the Sponsor role and will assign the KS-MHH for reviewing and assuring that the clinical study obeys AMG and GCP regulatories.
According to § 4 AMG the Sponsor (KS-MHH) will take responsibility for the inducement, organization and financing of the clinical trial. Sponsor and Investigator assure that the clinical trial will be conducted in accordance with the established laws and instructions according to ICH-GCP-Guidelines (1996), declaration of Helsinki (1996) as well as the directives of the AMG and the GCP-V (2004). The Investigators accept the requirements by signing the study protocol.

4. INTRODUCTION

Prevalence data of non-compliance in solid organ transplantations fluctuate is reported in up to 39% of transplant recipients (z. B. for lung transplantations 13 – 22%; Kugler et al.). Non-compliance with immunosuppressive therapy is associated with an increased risk of late-acute rejections and the development of chronic transplant dysfunction. Chronic transplant dysfunction (bronchiolitis obliterans syndrome, BOS) is the second most causing for organ failure after the first year following lung transplantation and often leads to re-transplantation or death. Preventative procedures for improving the compliance are simplification of the dose of the immunosuppressants (a once daily dose instead of a twice daily dose), the prescription of an immunosuppressant with less side-effects and to raise the patient´s awareness for having the greatest responsibility for the efficacy of his therapy. Prospective studies and metaanalysis revealed that the probability for a good compliance can be more than doubled at once daily administration in comparison to twice daily and the best predictor for a good compliance is an easy therapy. For this reason we want to investigate the extent of profit for our lung transplant patients receiving once daily basis immunosuppression in comparison to those who receive twice daily dose. Hypothesis: Patients of the once daily administration group of the immunsupressive

medication will have a better compliance compared to the twice daily group (as measured by the endpoints variability and medication abstraction from the electronic devices)

5. STUDY OBJECTIVES

Aim of this study is to demonstrate that the prescription of an extended release formulation of Tacrolimus that needs to be given once daily, only, can impact on compliance and immunosuppressive protection.

The primary objective of this study is to

- Improvement of adherence as measured by Tacrolimus trough level below the target level and dispensing of less than 50% of the prescribed doses in the last three days measured electronically before this subtherapeutic drug monitoring

The secondary objectives of this study are:

- Deterioration of graft function as defined as more than 20% decline in maximum FEV1 before and at month 12 after conversion
- Number of drug holidays (intake of less than 50% of prescribed doses in 24 hours) measured electronically
- Evaluation of renal function in pts converted from CyA to Tac in combination with MMF and steroids as assessed by serum creatinine, creatinine clearance (Cockgroft Gault), MDRD and Cystatin C before and at month 1, 3, 6 and 12 after conversion
- Evaluation of cardiovascular risk factors (hypertension, hyperlipidaemia, hypertriglyceridemia, diabetes mellitus)
- Incidence of CMV infections and other infections
- Efficacy: Incidence of acute rejection episodes, graft and patient survival
- Safety: Incidence of adverse events
- Comparison of MPA-mini-AUC under Tacrolimus once and twice daily administration

6. INVESTIGATIONAL PLAN

6.1 Overall Study Design and Plan-Description

Aim of this study is to demonstrate that the prescription of an extended release formulation of Tacrolimus that needs to be given once daily, only, can impact on compliance and immunosuppressive protection.

Patients after single, double or heart/lung transplantation will be randomized 12 hours after the last CyA administration to an individually titrated treatment scheme based on either Prograf® (two daily doses of Tacrolimus) or Advagraf® (one daily dose of Tacrolimus).

Per patient the proportion of subtherapeutic Tacrolimus trough levels caused by non-adherence of lung and heart transplanted patients that are treated with Prograf® (two daily doses of Tacrolimus) and Advagraf® (one daily dose of Tacrolimus) will be measured and treatment groups will be compared to reject the null-hypothesis that the use of an extended release formulation does not impact on patient compliance.
A trough level is said to be caused by non-adherent, if an individual has a Tacrolimus trough level below the target of 8 ng/ml (or any individual defined target range) and if more than 50% of the required drugs-tablets in the last three days were not appropriately taken from the automatic dispenser.

Unavoidably this is an open study. The prospective randomized trial is planned with duration of 24 months (12 months recruitment time and 12 months follow-up).
The study will be performed in patients between 18 and 70 years with single lung or double lung or heart/lung transplantation that was at least 3 months ago.

6.2 Discussion of Study Design, including the Choice of Control Groups

Unavoidably this is an open study. The prospective randomized trial was planned to specifically answer the question of better adherence. A duration of 12 months of follow-up seemed to be appropriate. Lower than therapeutic trough levels may occur in transplant reciuepients due to frequent interactions with othe medications. Therefore, the primary endpoint of drug holidy plus sub therapeutic trough levels was chosen. Adherence was measured by self-rating and MEMS-device.

6.3 Selection of Study Population

Patients (≥18 and ≤ 70 years) ≥ 3 months after single, double or heart/lung transplantation.

6.3.1 Inclusion criteria

- Pts ≥ 3 months after single lung, double lung or heart/lung transplantation and
- Pts treated with Cyclosporine, steroids and MMF and
- Pts ≥18 and ≤ 70 years and
- Pts with one oft he following
- Pts with recurrent acute rejections (RAR)
  (Pts with concomitant stable and non-advanced BOS are eligible)
  two or more acute rejections in last 3 months (first 4 weeks post Tx excluded) defined by
    - transbronchial biopsy ≥A1 according to ISHLT or

- decline of FEV_1 > 10 % baseline after exclusion of infection, airway complication, effusion etc. and improvement to steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) = FEV1 improvement > 10% compared to the last measurement before AR treatment

- Pts with steroid-resistant or ongoing acute rejections (OAR) defined by
  - transbronchial biopsy ≥A1 at least 4 weeks following steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) or
  - no FEV_1 improvement (< 5% baseline) at least 14 days following ACR steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) after exclusion of infection, airway complication, effusion etc. or
- Pts with CyA associated side effects (e.g. hyperlipidaemia, hypertriglyceridemia, hypertension, hirsutism, gingival hyperplasia)

6.3.2 Exclusion criteria

- Pregnant or breast feeding women
- Pts who are not using a double-barrier method of birth control
- Pts with systemic infections
- Pts with severe diarrhea, vomiting, active ulcer
- Pts with severe liver disease or liver cirrhosis
- Pts with m-Tor inhibitors
- Pts with hypersensitivity to Tacrolimus, other macrolides or other tablet ingredients

6.3.3 Removal of patients from therapy or assessment

Patient developing intolerable side effects of tacrolimus will be discontinued from study medication.

6.4 Treatments

6.4.1 Treatments administered

Tacrolimus (FK506)

Tacrolimus is a compound produced as a fermentation product of Streptomyces tsukubaensis. It is a macrolide lactone with a potent immunosuppressive activity5. At the molecular level, the effects of tacrolimus appear to be mediated by binding to a cytosolic protein (FKBP12). The FKBP12-tacrolimus complex binds to and inhibits calcineurin, leading to an inhibition of T-cell signal transduction pathways, thereby preventing transcription of a discrete set of lymphokine genes. The drug suppresses the formation of lymphokines (such as interleukin-2, -3 and γ-interferon) and

the expression of the interleukin-2 receptor. Thus, the drug suppresses T-cell activation and T-helper-cell-dependent B-cell proliferation. In particular, tacrolimus inhibits the formation of cytotoxic lymphocytes, which are mainly responsible for graft rejection. These effects are similar to those of cyclosporine, but tacrolimus has a ten to 100 fold higher potency than cyclosporine on a molecular basis11. The compound bears no structural relationship to cyclosporine.

In comparative trials of clinical organ transplantation, tacrolimus has been proven to be superior to cyclosporine in the prevention of acute rejection in liver, lung, heart and kidney transplants. Switch from tacrolimus to cyclosporine is an accepted indication in LTx transplantation in case of recurrent and refractory acute cellular rejection (ACR) and in some cases of BOS pats. switched to Tacrolimus respond by improvement of graft function (Sararhudi et al). In RCT Tac shows lower incidence of ACR (Hachem et al. 2007).

Tacrolimus has been on the market for more than ten years under the trade name Prograf® (Prograft® in Belgium, Luxembourg and the Netherlands) and is one of the two cornerstone immunosuppressants following organ transplantation. A life-long maintenance therapy with an immunosuppressive agent is necessary to prevent transplant rejection.

The marketed formulations of tacrolimus (Prograf®) are approved in the European Union (except Latvia, Lithuania, Estonia and Malta) for both adult and paediatric use for the prevention of transplant rejection in liver, kidney and heart allograft recipients and for the treatment of allograft rejection resistant to treatment with other immunosuppressive medicinal products.

Tacrolimus Modified Release Formulation (Advagraf®)

Prograf® capsules require twice-daily dosing. Advagraf® has been developed to provide once-daily dosing with a similar safety and efficacy profile as the current twice-daily formulation. Systemic exposure to tacrolimus i.e. area under the concentration-time curve over the dosage-time interval (AUCτ) has been found to be a significant explanatory variable of efficacy and safety. Therefore, the target goals for the Advagraf® formulation to be therapeutically equivalent to Prograf® were to achieve the AUC of tacrolimus to be within the bioequivalence criteria relative to twice daily dosing with Prograf®. If systemic exposure is equivalent, therapeutic equivalence between the two formulations can also be assumed. Additional criteria for the purpose of therapeutic monitoring were good correlation of trough concentration to AUC (similar to that obtained for Prograf®) and the same target trough concentration range as Prograf®.

Transplant subjects often receive immunosuppressive regimens consisting of multiple medications; thus, a formulation that could be taken once daily is considered to be of benefit to the subject. Advagraf®, the first calcineurin inhibitor formulated to enable once daily administration has the potential to improve subject compliance. Poor compliance has been shown to be one of the factors associated with late graft loss1,2. In a prospective cohort study of 278

adult recipients of cadaveric donor renal transplants, Weng et al. (2005)3 demonstrated a statistically significant association for adherence to medication regimen with once daily dosing versus twice daily dosing. It is expected that Advagraf® may help to improve compliance with dosing - as no evening dose is required – therefore decreasing the risk of late graft rejection and loss, and having less interference with the daily life activities of the subject.

The clinical development program for Advagraf® to date includes twelve Phase I studies (in healthy volunteers), eight Phase II studies and three completed Phase III study (all in transplant recipients). The Phase I studies performed in healthy volunteers (N=242) compared the biopharmaceutics of tacrolimus for Advagraf® and Prograf®. The Phase II studies (N=475 Advagraf® subjects) performed in transplant subjects compared the parameters of systemic exposure to tacrolimus from Advagraf® administered once daily to Prograf® administered twice daily.

Further details can be found in the current version of the Advagraf® Summary of Product Characteristics which contains comprehensive information on tacrolimus.

The formulations of tacrolimus modified formulation (Advagraf®) are approved and registered in the European Union (Advagraf® is not yet commercially available in all European countries).

12 hours after the last CyA administration the patients will be randomized to Tacrolimus twice or once daily.

The initial dose of Tacrolimus will be calculated by the last CyA dosing (initial tacrolimus dose = last ciclosporin dose divided by 50).

6.4.2 Identity of investigational product(s)

The oral formulation of Prograf® is available as 0.5 mg, 1 mg, 5 mg hard capsules and the intravenous formulation as 5 mg/ml concentrate for solution for infusion. Advagraf® is available in the same capsule strengths as Prograf® (0.5mg, 1mg and 5mg)

6.4.3 Method of assigning patients to treatment groups

Patients assign to treatment group after a randomisationlist

6.4.4 Selection of doses in the study

The trough level of Tacrolimus will be aimed at 8-12 ng/ml. In case of drug toxicities target drug levels may be individually be lowered to 5-8 ng/ml

6.4.5 Selection and timing of dose for each patient

Prograf®: Administer orally every 12 hours in the morning and in the evening

Advagraf®: Administer orally every 24 hours in the morning

Owing to a food effect, Prograf® and Advagraf® Capsules are taken one hour before or at least two to three hours after a meal

6.4.6 Prior and concomitant therapy

Mycophenolate Mofetil

Mycophenolate Mofetil (MMF) is an inhibitor of the de novo purine synthesis with apparent selectivity for B and T lymphocytes17 and has been developed as a replacement for Azathioprine for use in conjunction with cyclosporine. Phase III studies demonstrate that MMF is superior to both placebo and azathioprine when used in combination with cyclosporine and steroids. Mycophenolate mofetil has been approved in Europe and the USA for the prophylaxis of organ rejection in kidney allograft recipients when used in combination with cyclosporine and steroids.

The combination of tacrolimus and MMF has been evaluated in a dose ranging study comparing tacrolimus / steroids, tacrolimus / 1 g MMF per day / steroids and tacrolimus / 2 g MMF per day / steroids in 232 subjects. The combination of tacrolimus with 1 g and 2 g MMF showed a significant reduction in the incidence of first acute and steroid-resistant rejection episodes in comparison to the control arm with no MMF. No significant difference in the incidence of acute rejections was observed between the 1 g and 2 g MMF groups. All three treatment arms had a comparable safety profile, although diarrhoea and leucopenia - known to be more frequently observed with the use of MMF - were most pronounced in the 2 g MMF arm. It was concluded that the combination of tacrolimus, 1 g MMF, and steroids is a safe and effective regimen for rejection prophylaxis following lung transplantation.

In a US multicenter dose comparison study of MMF in combination with tacrolimus the control arm received a tacrolimus-azathioprine-steroid triple regimen. The 2 g/d dose of MMF did show superior efficacy over control in terms of acute rejection frequency. This study is, however, difficult to relate to the European situation because (i) the majority of subjects were not caucasian, (ii) the organ allocation system in the US is different to that in Europe (resulting in a different mismatch profile), and (iii) all subjects received antibody induction.

In a more recent study comparing three different immunosuppressive regimens, 223 kidney transplanted subjects were randomized to receive either a tacrolimus-MMF-steroids, tacrolimus-azathioprine-steroids or cyclosporine-MMF-steroids based regimen. Study results show a similar incidence of acute rejection, subject and graft survival for the three different treatments schedules. The combination of tacrolimus-MMF (2 g/d) demonstrated, nevertheless, its superiority in terms of incidence of steroid resistant rejection at one year. Study results were confirmed at two years.

A pilot study conducted in Spain has also proved the efficacy of a tacrolimus-MMF-steroids based regimen in the treatment of renal transplanted recipients receiving grafts from older donors.

The mean age of subjects was 65.8 years while donors' mean age was 63.3 years. A total of 35 subjects was treated with tacrolimus 0.1 mg/kg/d, MMF 2 g/d and steroids 0.5 mg/kg/d. Subjects and graft survival were 94 % and 88 % at one and two years respectively. No cases of graft loss other than the patients who died were reported.

Steroids:

All patients will be on steroids during the duration of the study.

6.4.7 Treatment compliance

Compliance will be measured by two ways: firstly, by electronic measurement of study drug dispensing and secondly subtherapeutic drug levels.

ProMate (Helping Hand)

The 'ProMate' is a device that records each point in time whenever a blister is inserted in it. The 'ProMate' will be dispensed at visit 1. Each device can be identified by a unique device number. This device number can be found on the device and on the device box. The device number has to be recorded in the CRF.

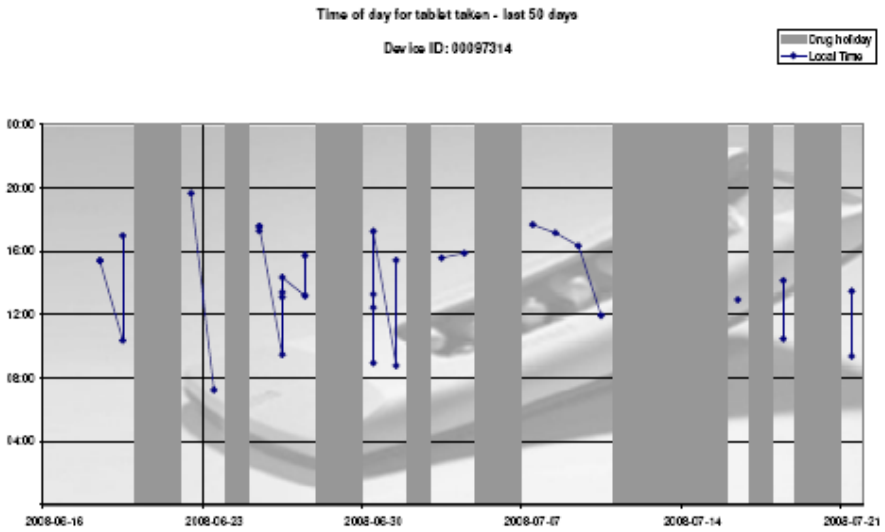
The patient needs to be thoroughly instructed on how to use the 'ProMate'. The patient needs to be instructed to pull out the blister at each dosing time point, remove the capsule/s needed to be swallowed and insert the blister thereafter immediately. If the patient does not re-insert the blister after taking the capsule within 10 seconds, there will be a brief beeping alarm. At visit 2, 3, 4 and 5 the device will be checked (i.e. if there was an alarm indicating that the battery failed) and the patient should be instructed about the use of the device again if needed. Whenever a blister is empty, the patient shall insert a new blister into the 'ProMate'. If a new blister is not at hand immediately, the patient should insert the empty blister into the 'ProMate' after the intake of the last capsule. The patient should then insert the new blister at the time of the next planned dose, i.e. the next morning.

The device comes with a preinstalled adapter card. With this adapter card, the device can be used for the 0.5 mg or 1 mg capsules. In case, the patient exclusively takes 5 mg capsules, the adapter card needs to be removed as the 5 mg blisters are wider than the 0.5 mg and 1 mg blisters. In case, there is a dose change, the adapter card possibly needs to be (re)-inserted or removed depending on the dose change. At visit 5, the device will be returned to the investigator. At that visit, the investigator shall pull out and insert the blister once to set a last time stamp. This is needed to be able to identify if the battery failed or the patient did not use the device. The device is equipped with a sound alarm function in case the battery is empty (continuous beep sound when the blister is inserted in the device). In this case, the patient should return the device at the next planned visit. Three different aspects of compliance will be assessed with the 'ProMate', adapted from van Wijngaerden et al. and Deschamps et al. [9, 10]: • Timing compliance 'ProMate': Number of

correct dosing intervals, i.e. time between capsule intake / number of observed days * 100; correct dosing interval is defined as an interval between 22 and 26 hours • Taking compliance 'ProMate': number of blister card removals during observational period / number of prescribed doses * 100 • Drug holidays 'ProMate': number of the periods during the observational period with two or more missed consecutive doses Additionally, a longitudinal description of compliance in terms of execution of the dosing regimen and persistence to the prescribed treatment will be presented.

In case of malfunction of the ProMate device the compliance and drug holiday will be not rated.

Example (screen shot):



6.5 Efficacy and Safety Variables

6.5.1 Efficacy and safety measurements assessed and flow chart

Diagnosis and Grading of Acute Rejection Episodes

If clinical and/or laboratory signs indicate the occurrence of a rejection episode a transbronchial biopsy (TBB) will be performed. In Pts unable to undergo TBB acute rejection is defined clinically as reversion of symtoms and/or improvement of FEV1 of at least 10% compared to the last recorded value before rejection treatment, test be performed.

The biopsy should be performed prior to the initiation of any anti-rejection therapy and as soon as possible after the onset of clinical/laboratory signs indicative of possible rejection. The histological evaluation of the biopsy will be performed by the local histopathologist following the ISHLT criteria.

Classification of Acute Rejection Episodes

Spontaneously Resolving Acute Rejection:

A spontaneously resolving rejection is defined as a rejection episode which has not been treated with new or increased corticosteroid medication, antibodies or any other medication and which has resolved, irrespective of any tacrolimus or MMF dose changes.

Corticosteroid Sensitive Acute Rejection:

A corticosteroid sensitive acute rejection is defined as a rejection episode treated with new or increased corticosteroid medication only and which has resolved, irrespective of any tacrolimus or MMF dose changes.

- recurrent acute rejections (RAR)
- two or more acute rejections in 3 months defined by
    - transbronchial biopsy ≥A1 according to ISHLT or
    - decline of FEV1 > 10 % baseline after exclusion of infection, airway complication, effusion etc. and improvement to steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) = FEV1 improvement > 10% compared to the last measurement before AR treatment
- steroid-resistant or ongoing acute rejections (OAR) defined by
    - transbronchial biopsy ≥A1 at least 4 weeks following steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) or
    - no FEV1 improvement (< 5% baseline) at least 14 days following ACR steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) after exclusion of infection, airway complication, effusion etc.

Time to First Acute Rejection:

Time to first acute rejection episode is defined as the number of days from transplantation (Day 0) to the first clinical, laboratory or histological signs that are considered to be related to the first acute rejection episode.

Graft Loss

Graft loss is defined as: re-transplantation, or death.

The date of graft loss is the earliest date of any of these events.

Assessment of Renal Dysfunction

Renal dysfunction will be defined as GFR < 30 mL/min/1.73m2 (MDRD formula), renal replacement therapy or need for kidney transplantation

Renal function

Renal function will be assessed by GFR using MDRD formula and Cystatin C after Larson.

Renal Function will also be assessed by Creatinine Clearance using Cockcroft and Gault formula.

Safety Assessment

The most relevant safety parameters assessed within the study are measurement of renal function, incidence of adverse events, absolute change in serum lipids (cholesterol, HDL, LDL, triglycerides) and incidence of diabetes mellitus.

Vital Signs

Vital signs are to be assessed at every scheduled study visit. Weight will be measured according to the hospital's routine procedure. Blood pressure should be measured after five minutes of rest.

Adverse Events

Adverse Events, including clinically significant laboratory abnormalities, will be assessed by the investigator and will be recorded in the CRF as described in section 5.6.

Laboratory Assessments

Routine laboratory assessments will be performed at every scheduled study visit at the local laboratory at each study site.

Blood samples should be taken in the morning after a fasting period of at least six hours, preferably before study drug administration. Each local laboratory must provide a current and approved list of reference ranges, including units for each parameter.

The laboratory values taken for Inclusion/Exclusion Criteria at Visit 1 must not be older than 48 hours at the time of reperfusion.

The following parameters are to be determined on each patient visit:

Haemoglobin, WBC, thrombocytes, LDH serum creatinine, urine stix, sodium, potassium, liver enzymes, GFR (MDRD), Chol, TG, glucose, HbA1c, Cyst. C.

Other assessments

Immunosuppressant therapy barrier scale (ITBS)

Immunosuppressant therapy barrier scale (ITBS) was developed to assess transplant patients' perceived barriers to IST adherence and was completed by 222 transplant patients who lived in Georgia, USA. A renal transplant population subset was used to test the ITBS reliability and validity. The ITBS subscales correlated negatively with a self-reported measure of IST adherence, IST serum concentrations and IST pharmacy refill adherence rate ($P<0.01$). The 'uncontrollable barrier' subscale was positively correlated to kidney graft rejection ($P<0.01$), thus demonstrating the ITBS's validity. Males and older patients reported more adherence barriers ($P<0.05$).
The ITBS is contained in the "Non-compliance" questionnaire.

Immunosuppressant therapy adherence instrument (ITAS)

The Immunosuppressant therapy adherence instrument (ITAS) is a five-item scale was developed that asked 122 respondents to indicate how often they were nonadherent to immunosuppressant therapy (IST) given a particular circumstance. The four-item scale, adherence measured by IS RRARs, and "target" IS serum concentrations had positive correlations ($p < 0.01$). Item scores were shown to be negatively related to rejection occurrence and increased SCr ($p < 0.05$). The immunosuppressant therapy adherence scale is the first published, valid and reliable instrument that measures recipients IST adherence. A german translation is validated as well.

Pulmonary function:

Spirometry according to ATS standards will be performed recording forced exspiration volume in 1s [FEV1] and the inspiratory vital capacity and maximal expiratory flow at 25-75% VC. Total lung capacity, residual volume will be recorded by bodyphlethysmography Diffusion capacity, Capillary blood gas analysis will measure pO2, pCO2, Hb, Hb-CO. BOS staging will be performed according to the International Society of Heart and Lung Transplantation system at the first and the last visit. Baseline (or best) FEV1 will be defined as the average of the two highest measurements obtained at least 3 weeks apart during postoperative course.

FLOWCHART

Data to be recorded	Visit 1 Randomization	Visit 2 Month3 (+/- 1 week)	Visit 3 Month 6 (+/- 1 week)	Visit 4 Month 9 (+/- 1 week)	Visit 5 Month 12 (+/- 1 week)
Informed consent for data collection	x				
Demographic data	x				
Concomitant diseases	x				
Transplantation history [1]	x				
Rejection episode history since (last) Tx	x				
Vital sign, weight	x	x	x	x	x
Laboratory assessments [2]	x	x	x	x	x
Pulmonary function test	x	x	x	x	x
BOS-staging	x				x
MPA-AUC	x	x			
Compliance parameters:					
ITAS questionnaire	x	x	x	x	x
Compliance VAS	x	x	x	x	x
ITBS questionnaire	x	x	x	x	x
Investigator and nurse compliance assessment	x	x	x	x	x
Tacrolimus trough levels (all available)	x				
Explain and dispend “Helping Hand”	x				
Check “Helping Hand”		x	x	x	x
“Helping Hand” measurements					
Return “Helping Hand”					x
End of study data [3]					x
On an ongoing basis:					
Tacrolimus daily dose					
Immunosuppressive medication					
Rejection episodes					
Adverse drug reactions					

[1] Recipient data: number and type of lung TX, BOS status, date of (last) transplantation, primary reason for transplantation, ABO blood type, viral status (CMV), need to dialysis, donor status: CMV

[2] Haemoglobin, WBC, thrombocytes, LDH, serum creatinine, urine stix, sodium, potassium, liver enzymes, GFR (MDRD), Chol, TG, glucose, HbA1c, Cyst. C

[3] Overall efficacy and tolerability, graft survival, re-lung-Tx, patient survival, withdrawal: at visit 4 5 or in case of early termination at the time of termination

6.5.2 Primary efficacy variable

Lower than therapeutic trough levels may occur in transplant reciuepients due to frequent interactions with othe medications. Therefore, the primary endpoint of drug holidy plus sub therapeutic trough levels was chosen. Adherence was measured by self-rating and MEMS-device.

6.5.3 Drug concentration measurements

Tacrolimus whole blood trough levels are routinely monitored locally using EMIT or HPLC-MS/MS analysis. Up to 2 ml blood are required per sample, the amount may vary according to analysis method. The blood samples should be taken in the morning before administration of tacrolimus. Tubes and tubing made of PVC must not be used. The whole blood trough levels should be assessed two to three times per week during hospitalization, at each outpatient visit and whenever clinically indicated.

6.6 Data Quality Assurance

Quality Assurance at Department of Respiratory Medicine

Histopathology of allograft rejection and clinical staging of BOS will be performed according the current criteria established by the International Society of Heart and Lung Transplantation. Spirometry was performed according to ATS/ERS guidelines (19). Clinical acute rejection was defined as any biopsy > grade 1 or clinically as a reversible deterioration of graft function responding to steroid pulse therapy (15 mg/ kg methylprednisolone for three days, maximal 1000 mg/d) after ruling out other conditions.

BAL will be performed according to ATS standard recommendations.

Study Monitoring and Auditing

The Sponsor (KS-MHH assigned by MHH) of this study is responsible according to ICH GCP guidelines for assuring proper study conduct as regards protocol adherence and validity of the data recorded on the CRF´s.

Data Quality Control (QC) will be performed by the appropriate departments of the MHH.

Quality Assurance (QA), in form of protocol, ICF, CRF, report, in-house and on-site audits, will be performed by the QA Unit of the MHH.

6.7 Statistical Methods Planned in the Protocol and Determination of Sample Size

6.7.1 Statistical and analytical plans

Within the standard procedures of a lung transplanted patient, the patient generally has 40 to 50 visits to the hospital ambulance (or a general practitioner). On a routine basis Tacrolimus trough levels will be measured at each visit. The proportion of the Tacrolimus trough levels below the norm-level of 8 where in addition more than 50% of prescribed Prograf® or Advagraf® doses

have not been taken appropriately during the last three days before measurement of the trough level according to the automatic dispenser will be determined for each study participant.

The primary aim of this clinical trial is to reject the null hypothesis that the mean of the proportions of too low Tacrolimus trough levels caused by non-compliance from patients that take Prograf® is equal to the mean of the proportions of patients that receive Advagraf®.

The standard deviations in each group are assumed to be equal. A two-sided t-test for two independent groups will be used to assess the hypothesis and the null-hypothesis will be rejected if the respective P-value is less than 0,05. The percentage of noncompliant observations per patient will be transformed with an arcsin transformation before applying the t-test in the primary analysis. The respective 95%-CI for the difference in means will be back-transformed for presentation of results.

6.7.2 Determination of sample size

Within 12 months a maximum number of 50 patients will be available at Medizinische Hochschule Hannover.

Sample size calculation is based on 44 lung transplanted patients that came for check-up to the ambulance between 1.7.2005 and 21.12.2006. For these patients ten trough levels were available. The average number of Tacrolimus trough levels below the norm-level of 8 was 33,4. The corresponding standard deviation 26,9. No information on how this numbers are reduced by incorporating compliance control by electronic measurement are available.

Three different scenarios are given below, under which circumstances the study can be successful. For each scenario we assume a two-sided type I error of 5%. The sample size per group is given for a power of 80% to detect a difference between group means with the two-sided t-test for two independent groups.

	Scenario		
	1	2	3
mean Prograf® group	20	25	30
mean Advagraf® group	10	12,5	15
common standard deviation	12	15	18
n per group	24	24	24

Thus with the supposed to be available sample size of 24 patients in each group the study will have 80% power to detect a reduction in mean proportion of too low trough levels caused by non-

compliance from 20% to ten (or 25% to 12,5% or 30% to 15%) assuming that the common standard deviation is 12% (or corresponding 15% or 18%) with a 0,05 two-sided significance level

6.8 Changes in the Conduct of the Study or Planned Analyses

Amendment 1 (10.08.2009)

The Inclusion criteria "Pts > 1 year after single, double or heart/lung transplantation" changed in "Pts> 3 months after single, double or heart/lung transplantation"

Approved by the ethics committee on 14.08.2009

Amendment 2 (01.12.2009)

The device „Helping Hand" will not be returned at visit 4, it will be returned at visit 5.

Approved by the ethics committee on 16.12.09

Amendment 3 (05.07.2010)

Patients with advanced BOS (level 3) should not included in the study.

Approved by the ethics committee on 23.07.2010

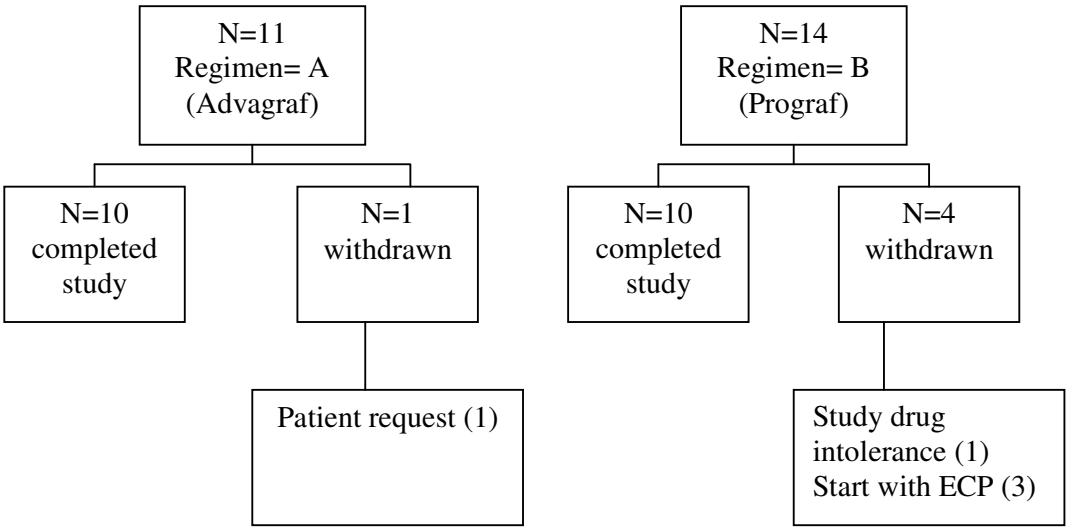
Amendment 4 (30.09.2010)

The ciclosporin target level should be under 250 ng/ml before the conversion to tacrolimus. After the conversion the target level should be controlled till a level < 200 ng/ml.

Approved by the ethics committee on 19.10.2010

7. STUDY PATIENTS

7.1 Disposition of Patients



7.2 Protocol Deviations

A single (# 22) was included 2 months after transplantation.

8. EFFICACY EVALUATION

8.1 Data Sets Analysed

All patients intended to treat (ITT) were analyzed.

8.2 Demographic and Other Baseline Characteristics

age at study beginning, years	46 (22 -62)
gender female, n (%)	8 (32)
FEV1% best at inclusion	80 (± 53)
time after transplantation, days	590 (59 – 2756)
follow-up in study, days	362 (15-413)
concomitant immunsuppression	
glucocorticoids , n (%)	25 (100)
MMF	25 (100)

8.3 Measurements of Treatment Compliance

Parameter from helping hand measurement

Advagraf:

Taking Compliance: 98% Timing Compliance: 94%

Prograf:

Taking Compliance: 97% Timing Compliance: 93%

Drugholidays (dispensing of less than 50% of the presscribed doses in 24 hours):

Advagraf: 92 drug holidays (9 patients) – 9 events in 6 patients (54%) related to a low tacrolimus through level measured at the latest three days after the forgotten taking.

Prograf 8 drug holidays (4 patients) – no relation to an low tacrolimus through level (0%).

During the study period, 753 tacrolimus through level have been determinded. 19% were subtherapeutic (Advagraf: 23%, Prograf: 15%).

8.4 Efficacy Results and Tabulations of Individual Patient Data

8.4.1 Analysis of efficacy

Improvement of adherence:

See 13.2.4

Deterioration of graft function (group A – advagraf, group B – prograf):

1 patient in group A and 3 pts in group B deteriorated to FEV1 <80% baseline.

patient	group	Best-FEV1 before conversion	Visit 1	visit 5
1	A	3370	1740 ⇒ 53%	2270 ⇒ 85%
2	B	2540	1810 ⇒ 72%	1790 ⇒ 71%
3	A	2750	2210 ⇒ 80%	2710 ⇒ 99%
4	B	2220	2070 ⇒ 93%	2290 ⇒ 103%
5	A	3800	2480 ⇒ 67%	3040 ⇒ 82%
9	A	2760	2040 ⇒ 75%	1730 ⇒ 63%
10	B	3260	3260 ⇒ 100%	2440 ⇒ 75%
11	B	1710	1710 ⇒ 100%	2450 ⇒ 143%
12	B	3010	2730 ⇒ 91%	3070 ⇒ 101%
13	A	1970	1770 ⇒ 91%	1900 ⇒ 97%
14	A	3480	3460 ⇒ 99%	3130 ⇒ 90%
16	A	4150	3100 ⇒ 83%	4420 ⇒ 107%
17	B	3750	3200 ⇒ 88%	3540 ⇒ 97%
18	A	2580	2310 ⇒ 90%	2100 ⇒ 81%
20	B	2050	1370 ⇒ 72%	1290 ⇒ 68%
21	B	3370	2520 ⇒ 75%	3110 ⇒ 90%
22	B	1960	1430 ⇒ 73%	2340 ⇒ 119%
23	B	2500	2210 ⇒ 88%	2680 ⇒ 107%
24	A	2990	2240 ⇒ 75%	3270 ⇒ 109%

25	A	3480	3420 ⇒ 98%	3350 ⇒ 96%

Number of drug holidays (intake of less than 50% of prescribed doses in 24 hours) measured electronically:
See 8.4.3

Evaluation of renal function in pts converted from CyA to TAC in combination with MMF and steroids (group A – advagraf, group B – prograf):

Visit 1	group	Kreatinin	Cockgroft Gault	GFR MDRD	Cystatin c
1	A	58	145	139	93
2	B	108	80	64	43
3	A	73	101	121	75
4	B	162	54	32	37
5	A	104	83	53	52
9	A	70	82	90	/
10	B	118	72	60	61
11	B	258	21	18	19
12	B	100	94	74	79
13	A	196	23	24	25
14	A	123	75	58	45
16	A	110	104	68	51
17	B	94	83	90	68
18	A	119	83	59	36
20	B	161	50	34	31
21	B	101	73	70	57
22	B	93	102	81	/
23	B	74	105	91	/
24	A	83	111	103	/
25	A	125	66	56	40
Visit 2					
1	A	124	74	57	87
2	B	111	74	62	54
3	A	126	61	64	63
4	B	134	60	39	43
5	A	131	72	40	46
9	A	72	81	87	78
10	B	102	92	71	81
11	B	280	18	16	19
12	B	139	66	51	54
13	A	228	19	20	21
14	A	303	29	21	21
16	A	158	74	45	52
17	B	122	66	67	31
18	A	150	65	45	31
20	B	212	39	25	23
21	B	132	58	51	/
22	B	116	79	63	/
23	B	95	79	67	79
24	A	106	84	78	63

25	A	107	76	66	58
Visit 3	group	Creatinine (umol/l)	Cockgroft Gault (ml/min)	GFR MDRD (ml/min/1,73m2)	Cystatin c (ml/min)
1	A	113	85	64	73
2	B	108	76	64	/
3	A	103	73	81	70
4	B	126	63	42	40
5	A	149	94	74	79
9	A	84	69	73	98
10	B	155	62	44	59
11	B	257	18	18	23
12	B	117	81	62	60
13	A	216	20	22	/
14	A	148	59	47	/
16	A	142	85	51	/
17	B	103	83	81	46
18	A	161	64	42	43
20	B	135	64	42	32
21	B	106	72	66	71
22	B	144	66	49	54
23	B	91	80	71	80
24	A	112	84	73	/
25	A	142	57	48	31
Visit 5					
1	A	122	77	58	84
2	B	136	63	49	57
3	A	111	71	74	/
4	B	185	37	27	26
5	A	179	37	28	28
9	A	87	64	70	63
10	B	146	76	47	57
11	B	388	10	11	18
12	B	152	59	45	44
13	A	308	13	24	13
14	A	178	51	38	35
16	A	122	99	60	67
17	B	95	89	88	56
18	A	151	68	45	43
20	B	123	74	46	50
21	B	156	50	42	42
22	B	87	110	88	61
23	B	135	55	45	74
24	A	106	86	77	64
25	A	113	78	62	51

Evaluation of cardiovascular risk factors:

0= none

1= improved

2= unchanged

3= deteriorated

patient	group	hypertension	hyperlipedemia	hypertriglyceridemia	diabetes mellitus
1	A	0	1	0	0
2	B	3	2	0	3
3	A	0	0	0	0
4	B	0	0	0	0
5	A	0	0	0	0
9	A	0	0	0	3
10	B	3	0	0	0
11	B	1	0	0	0
12	B	1	0	0	0
13	A	1	0	0	0
14	A	1	0	0	0
16	A	2	0	2	0
17	B	0	0	0	3
18	A	2	0	0	0
20	B	0	0	0	0
21	B	2	0	3	0
22	B	2	3	0	0
23	B	0	0	0	0
24	A	0	0	0	2
25	A	0	0	1	0

Incidence of CMV infections and other infections:
CMV-infections (defined by pp65-antigenemia $\geq$1/400.000 cells):
Pat. 10 (B): 2/400000
Pat. 18 (A): 41/400000
Pat. 22 (B): 205/400000
Pat. 25 (A): 45/400000

Infections with antibiotic treatment:
Pat. 9 (A)
Pat. 14 (A)
Pat. 17 (B)
Pat. 18 (A)
Pat. 21 (B)

Incidence of acute rejection episodes, graft and patient survival:
Treatment of acute rejection with urbason:
Pat. 2 (B): 11/2009
Pat. 10 (B): 9/2010
Pat. 13 (A): 02/2011
Pat. 16 (A): 12/2010
Pat. 22 (B): 11/2011

Graft survival:
BOS status
BOS 0 $\Rightarrow$ > 90%
BOS 0p $\Rightarrow$ 81 - 90%
BOS 1 $\Rightarrow$ 66 - 80%
BOS 2 $\Rightarrow$ 51 - 65%
BOS 3 $\Rightarrow$ < 50%

patient	group	Visit 1	Visit 5
1	A	2	0p
2	B	1	1
3	A	1	0p
4	B	0p	0p
5	A	1	0p
9	A	1	2
10	B	0	1
11	B	1	0
12	B	0p	0
13	A	0p	0p
14	A	0	0
16	A	1	0
17	B	0p	0
18	A	0p	1
20	B	1	1
21	B	0p	0p
22	B	2	0p
23	B	1	0
24	A	1	0p
25	A	0	0

(in purple: progress in BOS status)

Patient survival:
No patient died during the study

Incidence of adverse events:
See 9.2.4

Comparison of MPA-mini-AUC:

Visit 1	group	AUC (0-12h)	0 min	MMF 30 min	MMF 2h
1	A	47	6,6	5,3	4,9
2	B	41,5	0,9	12,7	4,9
3	A	n.d.	n.d.	n.d.	n.d.
4	B	47,1	3,3	9,5	7,9
5	A	47,3	2,1	16,7	5,8
9	A	28,5	0,7	8,1	3,2
10	B	55,5	6,7	7,5	7,9
11	B	23,2	0,5	5,9	2
12	B	48,9	1,1	18,7	7,1
13	A	33,9	2,5	7,2	3,6
14	A	30,2	0,7	3,7	6,6
16	A	31,8	1,2	1,2	8,1
17	B	27,7	1,1	3,1	4,3
18	A	53	1	7,6	15,8
20	B	n.d.	n.d.	n.d.	n.d.
21	B	n.d.	n.d.	n.d.	n.d.
22	B	21,8	1,1	1,2	3
23	B	31,9	1,7	8,2	3,3
24	A	22,5	0,9	0,8	3,9

25	A	29,8	9,1	3,1	29,8
Visit 2	group	AUC (0-12h)	0 min	MMF 30 min	MMF 2h
1	A	29,4	2,4	2,2	1,8
2	B	70	7,8	1,2	4,4
3	A	n.d.	n.d.	n.d.	n.d.
4	B	85,9	6,5	11,5	11,2
5	A	74,1	5,1	8,2	11,1
9	A	25,1	1,3	6,3	1,7
10	B	34,2	1,7	3,6	5,2
11	B	n.d.	n.d.	n.d.	n.d.
12	B	65,5	4,8	14,6	6,4
13	A	n.d.	n.d.	n.d.	n.d.
14	A	86,8	6,6	20,5	8,5
16	A	53,6	2,3	7,5	10,4
17	B	34,3	1,7	2,8	5,5
18	A	60,9	4,2	7,5	8,3
20	B	n.d.	n.d.	n.d.	n.d.
21	B	n.d.	n.d.	n.d.	n.d.
22	B	41,1	1,3	3	9,3
23	B	43,3	3,7	4	3,5
24	A	20,6	0,9	2,5	2,1
25	A	52,5	2,9	11,8	7

8.4.2 Statistical/analytical issues

The standard deviations in each group are assumed to be equal. A two-sided t-test for two independent groups was used to assess the hypothesis and the null-hypothesis will be rejected if the respective P-value is less than 0.05. The percentage of noncompliant observations per patient was transformed with an arcsin transformation before applying the t-test in the primary analysis. The respective 95%-CI for the difference in means will be back-transformed for presentation of results.

8.4.2.1 Adjustments for Covariates

No adjustment were made for covariates.

8.4.2.2 Handling of Dropouts or Missing Data

By two patients malfunction of the mems device so they were excluded from the analyses, three patient switch to ECP, one patient withdrew informed consent, one patient switch to ciclosporin because of hypersensitivity to Tacrolimus

8.4.2.3 Interim Analyses and Data Monitoring

No intermin analysis was performed. Data Quality Control (QC) was performed by the appropriate departments of the MHH. Source data verification was performed during monitoring of the supporting MHH structures.

8.4.2.4 Use of an "Efficacy Subset" of Patients

not applicable

8.4.2.5 Examination of Subgroups

Due to the small sample size, no subgroup analysis was performed

8.4.3 Tabulation of individual response data

patient	medication	Drug holidays	**Drug holidays in connection with subtherapeutic tacrolimus level**
1	Advagraf	9	**0**
2	Prograf	0	**0**
3	Advagraf	2	**1**
4	Prograf	1	**0**
5	Advagraf	3	**0**
9	Advagraf	0	**0**
10	Prograf	0	**0**
12	Prograf	0	**0**
13	Advagraf	15	**1**
14	Advagraf	38	**4**
15	Advagraf	5	**1**
17	Prograf	2	**0**
18	Advagraf	4	**1**
20	Prograf	0	**0**
21	Prograf	2	**0**
22	Prograf	3	**0**
23	Prograf	0	**0**
24	Advagraf	8	**1**
25	Advagraf	8	**0**

8.4.4 Drug dose, drug concentration, and relationships to response

Patient trough levels and drug dose were documented in CRF.

8.4.5 Drug-drug and drug-disease interactions

Interactions of tacrolimus with the other medication in lung transplant reciepients is frequent and complex. This issue was not assessed specifically.

8.4.6 By-patient displays

not applicable

8.4.7 Efficacy conclusions

Regarding adherence in taking and timing of drugs both drugs were equally effective. Drug holidays with subtherapeutic drug levels occurred exclusively in group A (advagraf). More patients in group A had subtherapeutic drug levels. Limited by the small number of patients in both group due to early termination of the trail, no significant differences were noted in terms of acute rejection, new onset of BOS, infections (incl CMV).

9. SAFETY EVALUATION

9.1 Extent of Exposure

Advagraf: 124 months

Prograf: 125 months

9.2 Adverse Events (AEs)

9.2.1 Brief summary of adverse events

207 AEs

Advagraf: 131 AEs

Summary: gastrointestinal symptoms 11, renal symptoms 13, infections 10, respiratory symptoms 35, cardiovascular symptoms 2, neurologic symptoms 6, other 48

Prograf: 93 AEs

Summary: gastrointestinal symptoms 11, renal symptoms 10, infections 17, respiratory symptoms 13, cardiovascular symptoms 1, neurologic symptoms 4, other 26

9.2.2 Display of adverse events

Refere to figure 1 under 14.3.1

9.2.3 Analysis of adverse events

An Adverse Event (AE) is defined as any untoward medical occurrence in a subject administered a study drug and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug. Abnormal laboratory findings will be rated as AE, if a clinical action is performed or the investigator defines these as clinical significant.

If a diagnosis is made from the sign and/or symptom, the diagnosis should be recorded in preference to the listing of individual signs and symptoms. If not, the investigator should record each sign and symptom as an individual AE.

An Adverse Reaction (AR) is defined as any prejudicial and unintended reaction to the study drug, independent from the dose. The classification as reaction will be done when a relation of the event to the study drug is at least considered as possible.

Criteria for Causal Relationship to the Study Drug

Adverse events that fall under either "Possible" or "Probable" should be defined as "adverse events whose relationship to the study drugs could not be ruled out."

Causal Relationship to the Study Drug	**Criteria for Causal Relationship**
Unassessable / Unclassifiable (1)	A report suggesting an adverse reaction which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.
Definitely not Conditional / Unclassified (2)	A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and/or in which other drugs, chemicals or underlying disease provide plausible explanations.
Unlikely (3)	A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.
Possible (4)	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Probable / Likely (5)	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on re-administration (rechallenge) or withdrawal (dechallenge).
High probable / Certain (6)	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary

Criteria for Defining the Severity of an Adverse Event

• Mild: No disruption of normal daily activities

• Moderate: Affect normal daily activities

• Severe: Inability to perform daily activities

• Life-threatening

9.2.4 Listing of adverse events by patient

23/25 (92%) of patients developed at least a single AE.

Pat. 1: gastrointestinal symptoms 5, renal symptoms 1, infections 1, respiratory symptoms 5, cardiovascular symptoms 1, other 4 = 17 AES

Pat. 2: gastrointestinal symptoms 1, infections 2, respiratory symptoms 2, cardiovascular symptoms 1, other 5 = 11 AES

Pat. 3: gastrointestinal symptoms 1, renal symptoms 2, respiratory symptoms 8, other 5 = 16 AEs

Pat. 4: gastrointestinal symptoms 2, infections 1, neurologic symptoms 1, other 1 = 5 AEs

Pat. 5: renal symptoms 3, respiratory symptoms 4, neurologic symptoms 1, other 2 = 10 AEs

Pat. 6: infections 1= 1 AE

Pat. 7: infections 1, cardiovascular symptoms 1 = 2 AEs

Pat. 9: gastrointestinal symptoms 2, renal symptoms 1, infections 1, respiratory symptoms 3, neurologic symptoms 3, other 9 = 19 AEs

Pat. 10: respiratory symptoms 3, neurologic symptoms 1, other 5 = 9 AEs

Pat. 11: gastrointestinal symptoms 3, renal symptoms 3, infections 4, respiratory symptoms 1 , neurologic symptoms 2, other 3 = 16 AES

Pat. 12: renal symptoms 1, other 3 = 4 AEs

Pat. 13: gastrointestinal symptoms 3, renal symptoms 2, infections 1, respiratory symptoms 1, neurologic symptoms 1, other 2 = 10 AEs

Pat. 14: gastrointestinal symptoms 1, renal symptoms 2, infections 2, respiratory symptoms 2, neurologic symptoms 1, other 4 = 12 AEs

Pat. 15: respiratory symptoms 5, other 2 = 7 AEs

Pat. 16: infections 1, respiratory symptoms 2 = 3 AEs

Pat. 17: renal symptoms 1, infections 2, respiratory symptoms 2, other 2 = 7 AEs

Pat. 18: renal symptoms 1, infections 1, respiratory symptoms 5, other 6 = 13

Pat. 20: gastrointestinal symptoms 2, renal symptoms 2, infections 4, respiratory symptoms 3, other 3 = 14 AEs

Pat. 21: gastrointestinal symptoms 2, renal symptoms 1, infections 1, respiratory symptoms 2, other 4= 10 AEs

Pat. 22: gastrointestinal symptoms 1,infections 2, respiratory symptoms 1, other 3 = 7 AEs
Pat. 23: gastrointestinal symptoms 1, renal symptoms 2, infections 1, respiratory symptoms 1, other 2 = 7 AEs
Pat. 24: gastrointestinal symptoms 1, renal symptoms 1, infections 2, respiratory symptoms 2, cardiovascular symptoms 1, other 2 = 9 AES
Pat. 25: infections 1, respiratory symptoms 1, other 13 = 15 AEs
Exactly description of AES in Appendix 16.2.7

9.3 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

9.3.1 Listing of deaths, other serious adverse events, and other significant adverse Events

Refere to 14.3.2

9.3.1.1 Deaths

No patient died during the study

9.3.1.2 Other Serious Adverse Events

20 SAEs (all hospitalisations) occurred during the study period. The main causes for hospitalization were: 29 % infection, 19 % respiratory causes, 14 % renal causes. 12 SAEs developed in group A, 8 in group B.

9.3.2 Analysis and discussion of deaths, other serious adverse events, and other significant adverse events.

Definitions
A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:
Results in death
Is life threatening (an event in which the subject is at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe)
Results in persistent or significant disability/incapacity
Results in congenital anomaly, or birth defect
Requires inpatient hospitalization or leads to prolongation of hospitalization (hospitalization for treatment/observation/examination caused by AE is to be considered as serious)
Other medically significant events

A suspicious case of an unexpected serious adverse reaction will be defined as Suspected Unexpected Serious Adverse Reaction (SUSAR). A serious adverse reaction is unexpected when

it is not reported in the appropriate base document such as Investigators Broschure (IB), Investigational Medicinal Product Dossier (IMPD) or summary of product characteristics (Fachinformation, SMPC).

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above (i.e. a medically significant event). Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

9.4 Clinical Laboratory Evaluation

9.4.1 Listing of individual laboratory measurements by patient (16.2.8) and each abnormal laboratory value (14.3.4)

Refere to 16.2.8 and 14.3.4

9.4.2 Evaluation of each laboratory parameter

9.4.2.1 Laboratory Values Over Time

mediane (interquartile):

	Visit 1	Visit 5
Creatinine (umol/l)	108 (88-137)	136 (112-173) Advagraf: 122 (110-178) Prograf: 141 (116-163)
creatinine clearance after cockgroft gault (ml/min)	80 (55-98)	66 (50-77) Advagraf: 70 (48-79) Prograf: 61 (47-79)
GFR after MDRD (ml/min/1,73m2)	60 (48-90)	47 (39-68) Advagraf: 59 (36-71) Prograf: 46 (38-59)
GFR after Cystatin C (ml/min)	48 (38-66)	51 (35-63) Advagraf: 51 (32-66) Prograf: 53 (38-58)

	Visit 1	Visit 2
AUC (mg*h/l)	28,50 (4,95-45,15)	Advagraf: 24,55 (5,50-47,80) Prograf; 31,70 (3,60-43,30)
MPA-CO (mg/l)	3,10 (1,5-6,5)	Advagraf: 2,70 (1,60-8,20) Prograf: 3,30 (1,30-5,20)
MPA-CO (mg/l) nach 30 Minuten	12,25 (6,18-31,3)	Advagraf: 14,25 (7,15-30,3) Prograf: 11,10 (5,20-32,47)

MPA-CO (mg/l) nach 2 Stunden	5,10 (2,2-7,45)	Advagraf: 4,25 (1,9-6,7) Prograf: 5,50 (2,30-7,90)

9.4.2.2 Individual Patient Changes

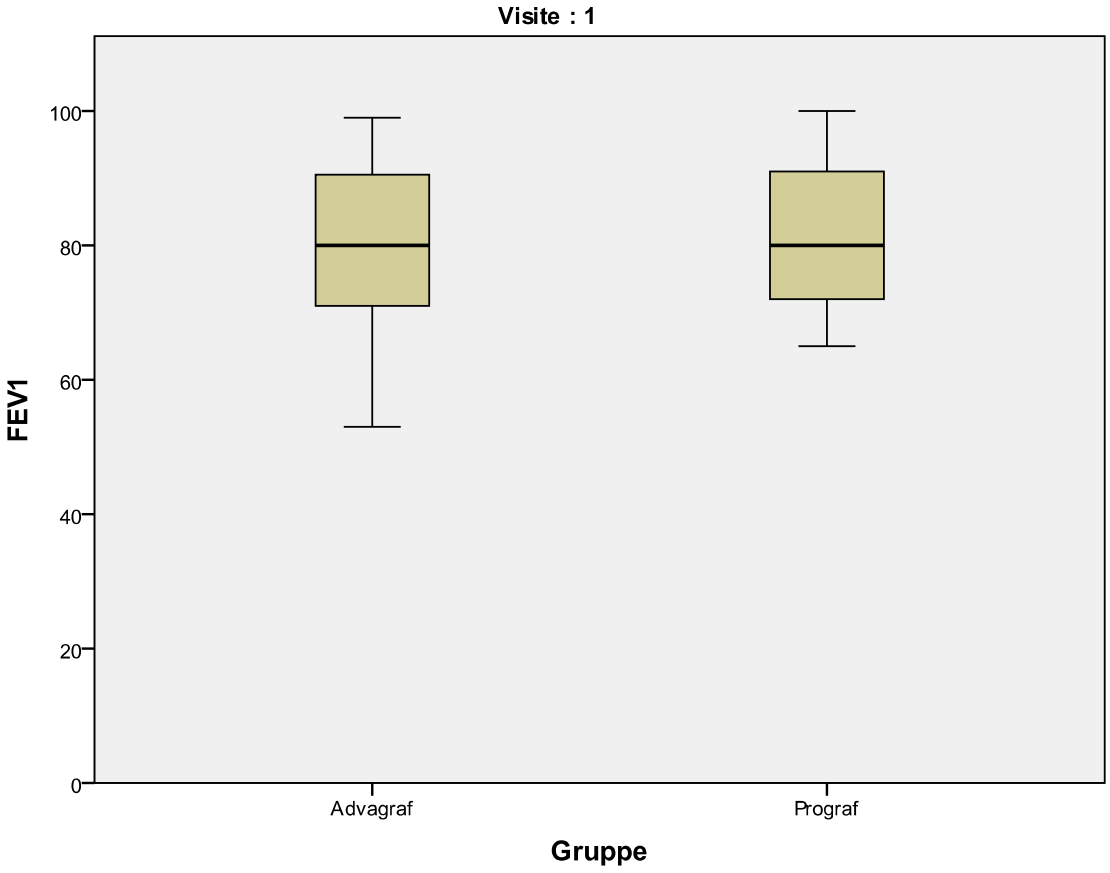
Due to the small sample size, no subgroup analysis was performed

9.4.2.3 Individual Clinically Significant Abnormalities

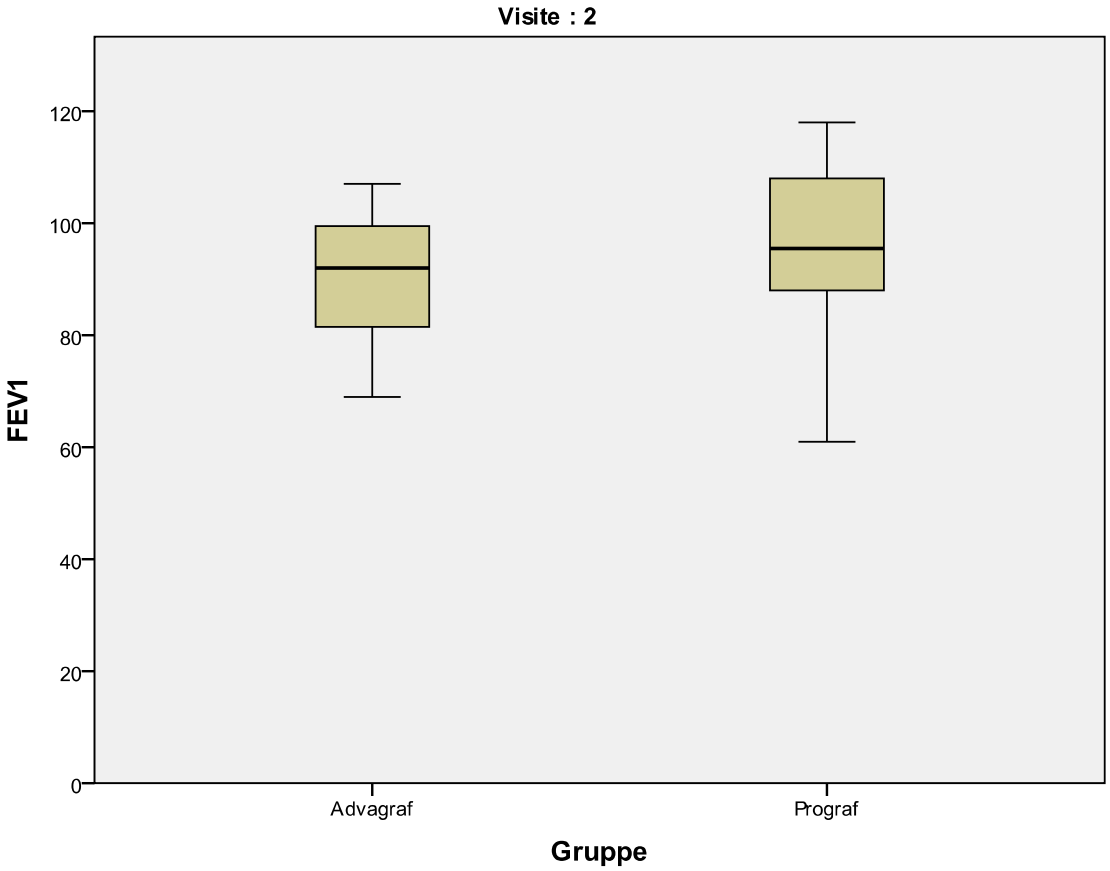
not applicable.

9.5 Vital Signs, Physical Findings, and Other Observations Related to Safety

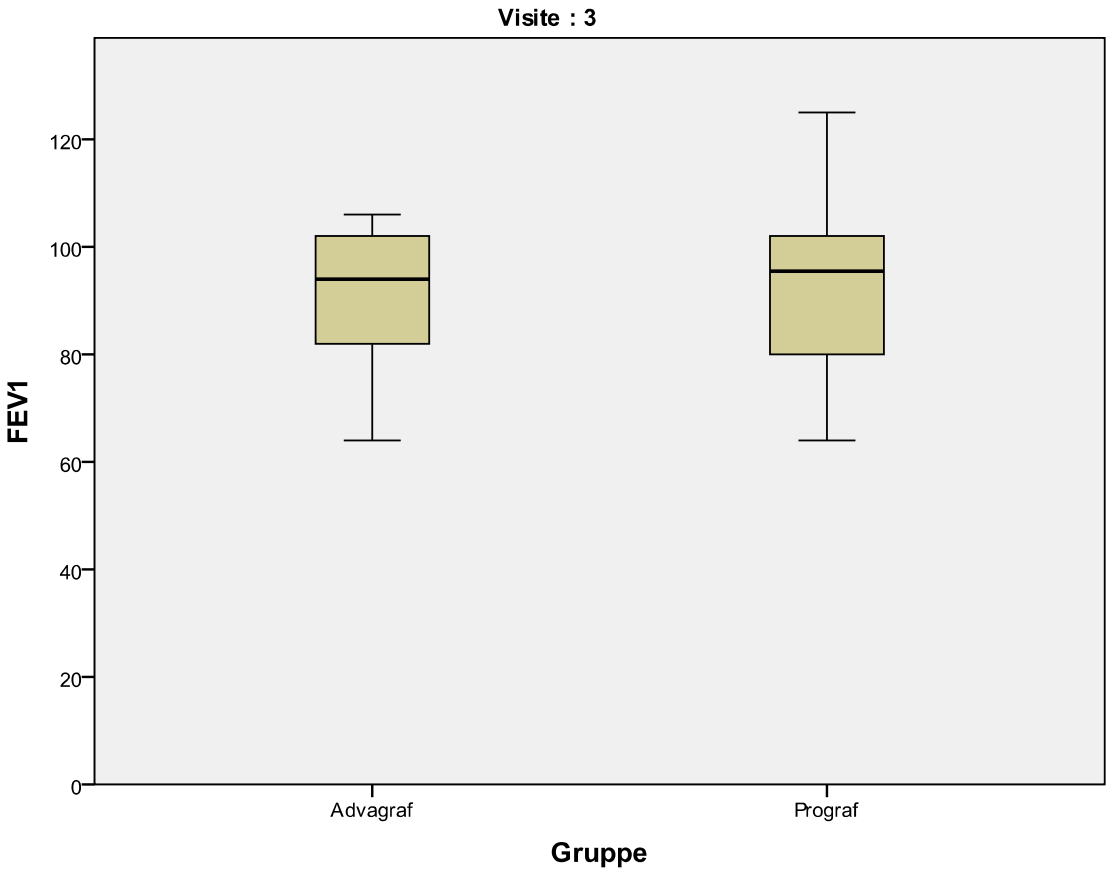
FEV1 in percent from the best-FEV1 before study including.



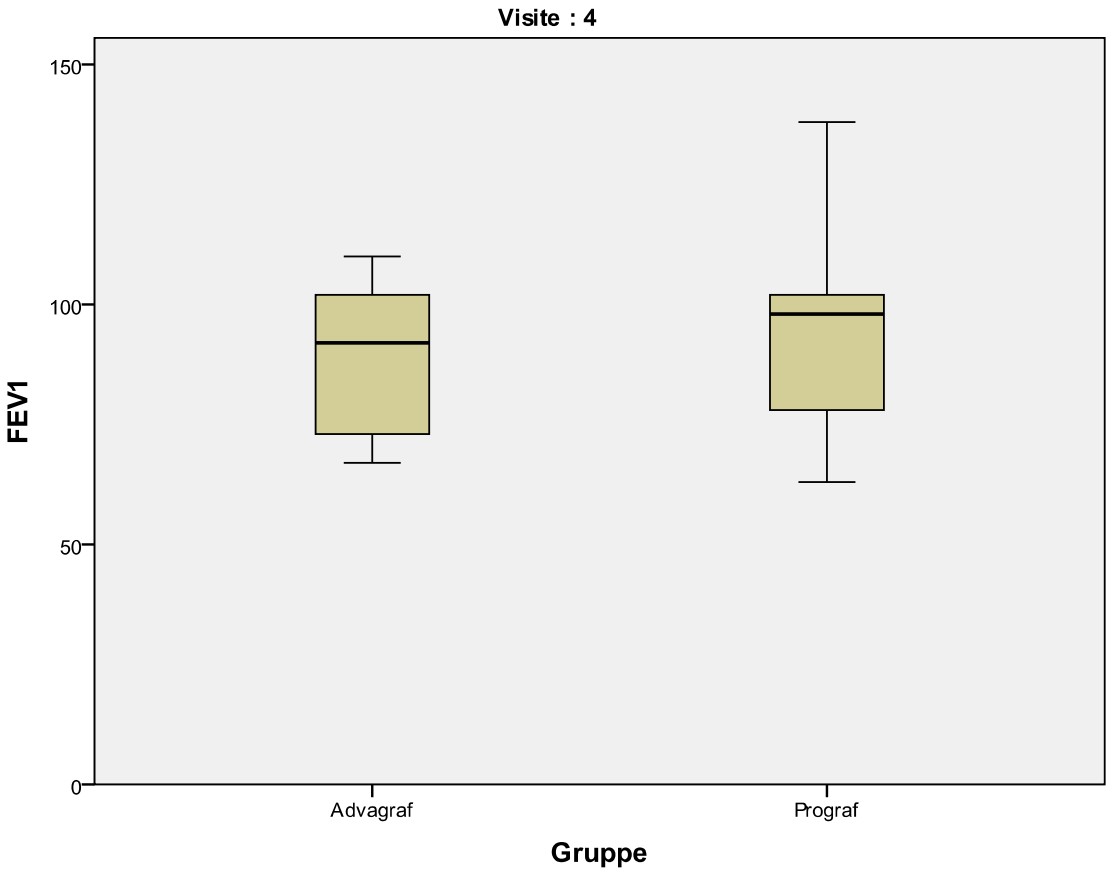
Visit 1:
Advagraf: 80% (67-91)
Prograf: 80 % (72-91,5)



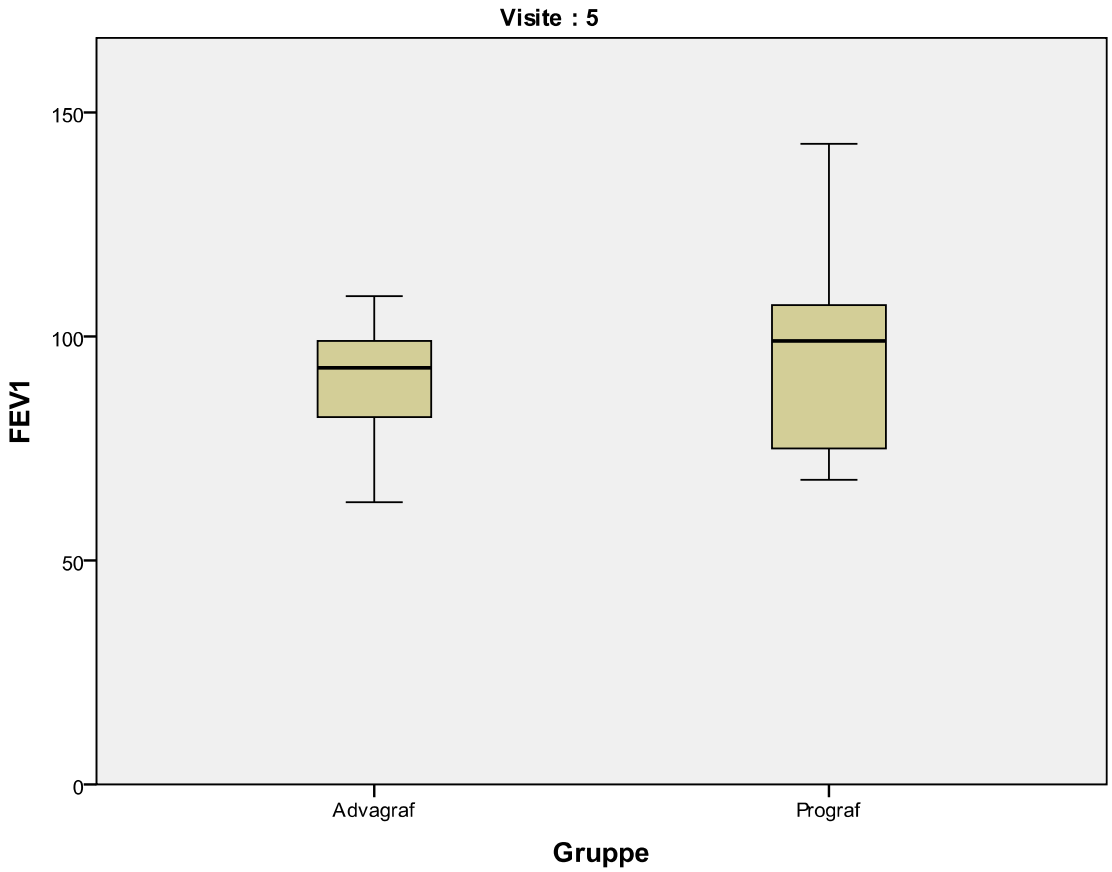
Visit 2:
Advagraf: 92% (81-100)
Prograf: 95,5 % (84,75-110,50)



Visit 3:
Advagraf: 94% (80-102)
Prograf: 95,5 % (77,75-107,25)



Visit 4:
Advagraf: 92% (71,50-102,25)
Prograf: 98% (76,50-106,25)



Visit 5:
Advagraf: 93% (81,75-101)
Prograf: 99% (74-110)

9.6 Safety Conclusions

As expected in a cohort of lung transplant recipients adverse events were frequent. Most frequent AEs were of infectious origin and most infections arose from the respiratory system. Even 20 serious adverse events occurred in 25 recipients but were related to hospilizations only. No death or life-threatening conditions were noted. Kidney functions as measured by various methods of GFR estimation revealed no significant differences between pstioents treted with once-daily versus twice daily tacrolimus. A trend was noted towards higher drug exposure to mycophenolat acid, ut this analysis was limited by the small number of patients due to early termination of the trial. For the same reasons, no consequences concerning cardiovascular consequences (incl. new onset of diabetes) could be drawn.

10. DISCUSSION AND OVERALL CONCLUSIONS

Efficacy results are summarized in section 8.1 to 8.4. Adherence in both groups was excellent**.** Regarding adherence in taking and timing of drugs both drugs were equally effective. Drug

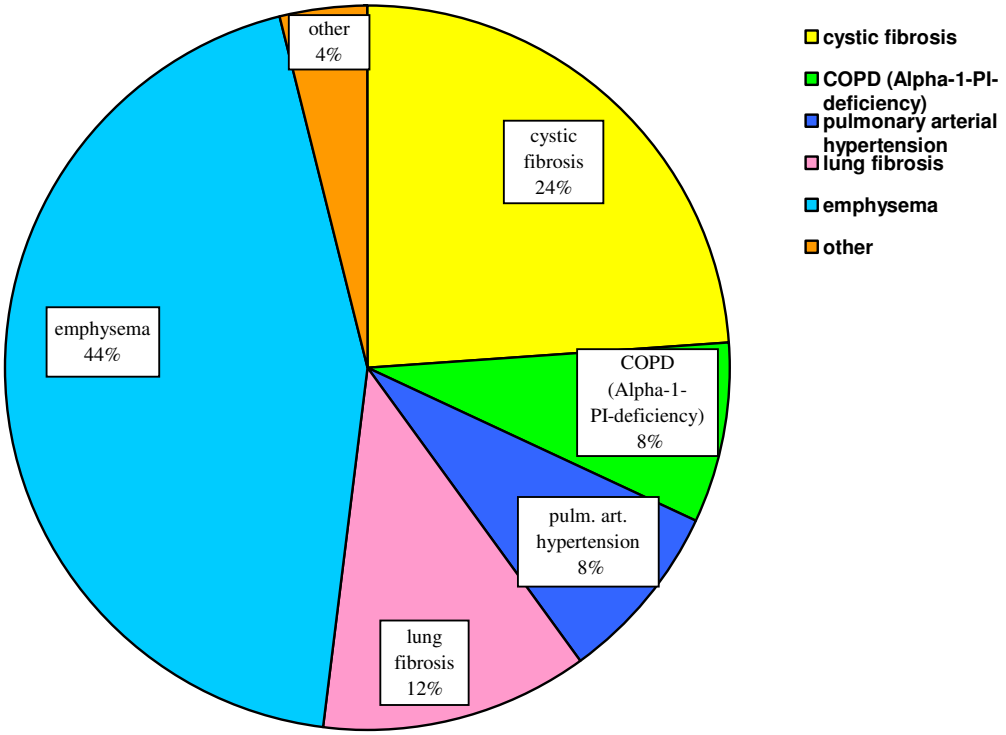
holidays with subtherapeutic drug levels occurred exclusively in group A (advagraf). A trend towards more patients in group A (advagraf) experiencing subtherapeutic drug levels was observed. Limited by the small number of patients in both groups due to early termination of the trial, no significant differences were noted in terms of acutre rejection, new onset of BOS, infections (incl CMV).

Safety results are summarized in section 9.1 to 9.4. As expected in a cohort of lung transplant recipients adverse events were frequent. Most frequent AEs were of infectious origin and most infections arose from the respiratory system. Even 20 serious adverse events occurred in 25 recipients but were related to hospilizations only. Patients treated with advagraf experienced by trend more adverse events (11.3 AEs per patient vs. 6.2 per patient). No death or life-threatening conditions were noted. Kidney functions as measured by various methods of GFR estimation revealed no significant differences between patients treated with once-daily versus twice daily tacrolimus. A trend was noted towards higher drug exposure to co-medication with mycophenolat acid, ut this analysis was limited by the small number of patients due to early termination of the trial. For the same reasons, no consequences concerning cardiovascular consequences (incl. new onset of diabetes) could be drawn.

In the light of other existing data in liver transplantation once-daily tacrolimus seem to be an efficient and safe drug in solid organ transplantation. Superiority in clinically meaningsful endpoints should be confirmed in future prospective studies.

11. TABLES, FIGURES AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT

11.1 Demographic Data



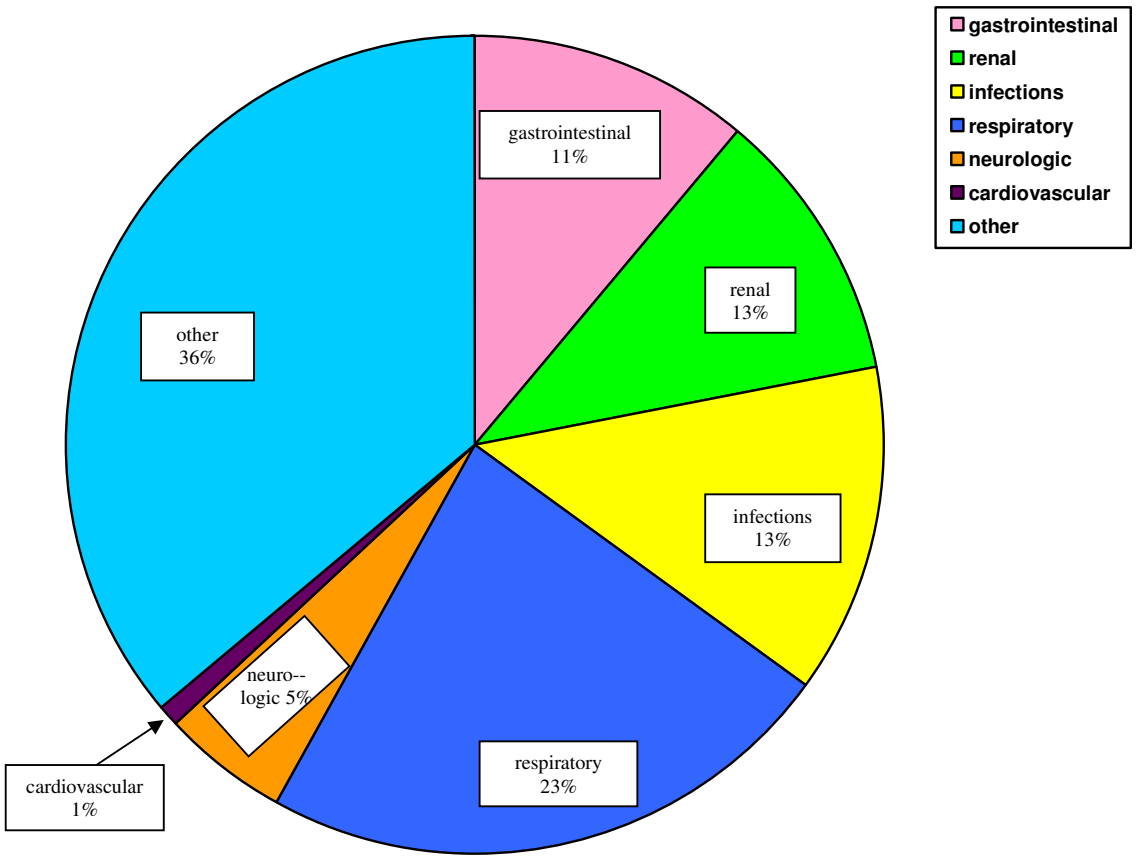
11.2 Efficacy Data

Not applicable.

11.3 Safety Data

Not applicable.

11.3.1 Displays of adverse events



11.3.2 Listings of deaths, other serious and significant adverse events

SAE No.	Rand. No.	Initials	Onset date	Description of SAE	Actions taken	Outcome	Relationship to study drug
1	1	U-B	10.10.2009	Hospitalisation due to nausea, vomiting, headache	5	1	4
2	9	R-W	5.2.2010	Rhinovirus, infection with pneumonia	5+6	1	2
3	11	AGF	26.3.2010	Thrombotic microangiopathy	2	3	2
4	11	AGF	24.6.2011	Hyperkalemia	1	3	3
5	2	H-S	11.11.2009	suspected reversible neutrophil graft dysfunction	5	1	3
6	5	E-J	14.9.2010	Hospitalisation due to symptoms of vertigo, recurrent diarrhoea, emisis and downfall	6	1	4
7	5	E-J	12.10.2010	acute kidney failure	5+6	3	2
8	10	AMM	27.8.2010	suspicion of an acute rejection	5	1	3
9	11	AGF	8.11.2010	hospitalisation due to acute diarrhoea	6	1	4
10	13	ANF	28.10.2010	hospitalisation due to acute diarrhoea because of gastroenteritis	6	3	4
11	13	ANF	1.2.2011	hospitalisation due to suspicion of pneumonia / suspicon of an acute rejection	5+6	1	3
12	14	ADM	27.10.2010	Hospitalisation due to deterioration in renal function and anaemia unknown origin	6	1	2
13	15	AOF	30.9.2010	hospitalisation due to CMV infection	6	3	3
14	15	AOF	23.11.2010	hospitalisation due to CMV infection or rejection reaction	6	4	4
15	18	ABM	4.11.2010	bronchopulmonary infection	6	1	3

16	22	ABM	12.5.2011	hospitalisation due to CMV infection	6	1	3
17	20	AMF	23.3.2011	hospitalisation due to renal biopsy	6	3	3
18	14	ADM	26.7.2011	hospitalisation due to contusion of head	6	1	4
19	25	AGM	13.10.2011	hospitalisation die to thrombosis of the right leg	6		4
20	23	AZF	1.12.2011	hospitalisation diue to infection with moraxella catarrhalis	6	1	2

Actions taken:

1. No action taken
2. Trial drug dosage adjusted/temporarily interrupted
3. Trial drug permanently discontinued due to this adv. ev.
4. Non-drug therapy given
5. Concomitant medication taken
6. Hospitalisation/prolonged hospitalisation

Outcome:

1. Completely recovered
2. Recovered with sequelae
3. Condition improving
4. Condition still present and unchanged
5. Condition deteriorated
6. Death

Relationship to study drug

1. highly probable
2. probable
3. possible
4. unlikely
5. definitely not
6. not assessable

11.3.3 Abnormal laboratory value listing (each patient)

patient	group	date	laboratory measurement	value	normal range
1	A	31.7.2009	leukocytes (Tsd/ul)	22,6	4,4-11,3
1	A	31.7.2009	LDH (ul/l)	470	till 248
1	A	18.9.2009	leukocytes (Tsd/ul)	14,3	4,4-11,3
2	B	2.3.2010	leukocytes (Tsd/ul)	2,6	4,4-11,3
3	A	14.8.2009	LDH (ul/l)	412	till 248
9	A	19.2.2010	urea nitrogen (mmol/l)	10,2	3-3-6,7
9	A	19.2.2010	cystatin c (mg/l)	0,99	0,53-0,95
9	A	19.2.2010	leukocytes (Tsd/ul)	16,6	4,4-11,30
9	A	16.3.2010	uric acid (umol/l)	403	140-340
9	A	30.4.2010	Iron (umol/l)	6	11-25

10	B	7.12.2010	Iron (umol/l)	4	14-27
11	B	19.4.2010	Elevated beta-HCG u/l	6	till 5
11	B	24.6.2010	potassium (mmol/l)	7,8	3,6-5,4
13	A	17.8.2010	elevated value of HbA1c (%)	6,9	4,8-5,9
14	A	26.10.2010	haemoglobin (g/dl)	9,5	13,8-17,5
15	A	20.10.2010	GOT (u/l)	81	till 31
15	A	20.10.2010	GPT (u/l)	60	till 34
15	A	20.10.2010	GGT (u/l)	1064	till 38
15	A	20.10.2010	AP (u/l)	344	35-104
17	B	12.1.2011	glucose (mmol/l)	12	3,9-5,5
18	A	27.10.2010	glucose (mmol/l)	8,8	3,9-5,5
20	B	3.12.2010	GGT (u/l)	165	till 38
21	B	1.3.2011	uric acid (umol/l)	452	200-420
22	B	18.2.2011	magnesium (mmol/l)	0,61	0,75-1,10
23	B	2.1.2012	uric acid (umol/l)	438	140,34
23	B	15.3.2012	creatine (umol/l)	135	45,48
23	B	30.6.2011	Iron (umol/l)	85	45,81
24	A	31.1.2012	uric acid (umol/l)	567	200,42
24	A	9.5.2011	potassium (mmol/l)	5,5	3,6-54
25	A	2.2.2012	CRP (mg/l)	32	till 8
25	A	12.4.2012	triglyceride (mmol/l)	424	0,62-3,93

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13. APPENDICES

13.1 Study Information.

Prospektiv randomisierte Studie zum Vergleich einer 2x täglichen mit einer 1x täglichem Gabe der Basisimmunsuppression bei Patienten nach Lungentransplantation
EudraCT Nummer: 2009-011324-60
Protocol Nummer: DE-09-RG-53

Patienteninformation

Sehr geehrte Patientin, sehr geehrter Patient,

schwankende Spiegel der Immunsuppression nach Lungentransplantation stellen ein erhöhtes Risiko für akute Abstoßungen und der Entwicklung eines chronischen Transplantatversagens dar. Für stabile Blutspiegel der Medikamente ist die verlässliche Einnahme der Medikamente Grundvoraussetzung. Eingeschränkte Mitarbeit des Patienten (mangelnde Therapieadhärenz) bezüglich Einnahmezeitpunkt und -dosis kann eine Ursache für dieses Problem sein. Das chronische Transplantatversagen nach Lungentransplantation , das als Bronchiolitis obliterans Syndrom (kurz BOS) bezeichnet wird, stellt auch heute noch den entscheidenden, das Langzeitüberleben nach Lungen- oder Herz-Lungentransplantation beeinflussenden Faktor dar. Etwa die Hälfte aller Patienten ist 5 Jahre nach der Transplantation an der chronischen Abstoßung erkrankt. BOS ist die zweithäufigste Ursache für den Organ-Verlust jenseits der ersten 3 Monate und führt bei den vitalen Organen zu Re-Transplantationen oder zum Tod.
Maßnahmen durch die die Patientenmitarbeit (Adhärenz) er Patienten verbessert werden können, sind z.B. die Vereinfachung der Dosierung der Immunsuppression (z.B. statt einer 2x täglichen eine 1x tägliche Gabe), die Verordnung von Immunsuppressiva mit weniger Nebenwirkungen und die Verstärkung des Bewusstseins beim Patienten, dass er selbst die größte Verantwortung für die Wirksamkeit seiner Therapie trägt.

Ziel Der klinischen Prüfung

Eine prospektive Studie mit Nierentransplantierten zeigte, dass die Dosierungsfrequenz einen unabhängigen Risikofaktor für mangelhafte Therapietreue bei der Einnahme von Immunsuppressiva darstellt und mit einer Einmalgabe die Wahrscheinlichkeit für eine gute Therapietreue gegenüber einer Zweimalgabe mehr als verdoppelt werden kann (Weng et al.). Eine Analyse verschiedener Studien kam

zu dem Ergebnis, dass die beste Erfolgsaussicht für eine gute Therapietreue ein möglichst einfaches Medikamentenregime ist (Laederach-Hofmann et al.).

Aus diesem Grund wollen wir bei unseren Lungentransplantierten Patienten untersuchen, in welchem Ausmaß der Patient von einer 1x täglichen Gabe des Immunsuppressivum Tacrolimus im Vergleich zu einer 2x täglichen Gabe profitiert. Tacrolimus ist ein zugelassenes Medikament nach Organtransplantation, wenn wiederholte Abstoßungen unter anderen Immunsuppressiva (üblicherweise Ciclosporin) auftreten. Tacrolimus ist als zweimal täglich einzunehmendes Medikament (Prograf) oder einmal täglich einzunehmendes Medikament (Advagraf) verfügbar.

Ablauf

Patienten nach Einzel-, Doppel- oder Herz/Lungentransplantation, welche bisher mit Ciclosporin in Kombination mit Everolimus oder MMF und Steroiden behandelt werden, sollen eingeschlossen werden, wenn Probleme mir der Transplantatfunktion auftreten (Wiederholte akute oder chronische Abstoßungen). Mithilfe einer zufälligen Aufteilung werden sie entweder dem Prograf-Arm (2x tägliche Gabe) oder dem Advagraf-Arm (1x tägliche Gabe) zugewiesen.

Sie werden bereits routinemäßig auf akute Abstoßungsreaktionen, Transplantatfunktion (FEV1), Auftreten von BOS und Infektionen untersucht. Um die Wirksamkeit und Verträglichkeit des Medikamentes zu prüfen, wird Ihnen wie bisher zu den routinemäßigen Nachsorgeterminen Blut abgenommen.

Anhand dieser Blutproben werden die Funktion der Lunge und das Vorliegen von Infektionen sowie Ihr allgemeiner Gesundheitszustand überwacht. Über die Standarduntersuchung nach Transplantation hinaus sind keine zusätzlichen Untersuchungen notwendig. Die Beobachtungsdauer im Rahmen dieser Studie beträgt 1 Jahr.

Nutzen und risiken

Die allgemein am häufigsten unter immunsuppressiver Therapie beobachteten Nebenwirkungen umfassen Nierenschäden und Komplikationen am Nervensystem, Störungen des Zuckerstoffwechsels, Magen-Darm Störungen, Bluthochdruck sowie Infektionsneigung.

Im Allgemeinen ist das Nebenwirkungsprofil einer immunsuppressiven Behandlung mit Tacrolimus vergleichbar mit dem einer konventionellen Therapie mit Ciclosporin. Lediglich Nervenstörung wie Zittern, Durchfall und Diabetes können, wie im Rahmen verschiedener Studien festgestellt wurde, unter Tacrolimus etwas häufiger auftreten. Diese Erscheinungen sind jedoch nach Dosisreduktion im Langzeitverlauf meist rückläufig. Andererseits wurden CMV-Infektionen, Bluthochdruck, Fettstoffwechselstörungen, vermehrter Haarwuchs und Zahnfleischwucherung weniger häufig bzw. kaum beobachtet. Ihr behandelnder Arzt wird Sie bei jedem Untersuchungstermin im Hinblick auf eventuelle Nebenwirkungen befragen und untersuchen, um mögliche Probleme zu erkennen und sie entsprechend zu behandeln. Unter Tacrolimus werden weniger akute Abstoßungen beobachtet.

Für alle teilnehmenden Patienten ist eine Versicherung bei der Allianz Versicherungs- AG, 10900 Berlin, Telefax: 01802/400102, (Probanden-Jahresvertrag Nr. GHA 30/0446/5302393/490) abgeschlossen, deren Höchstleistungssatz pro Patient 500.000€ beträgt.

FREIWILLIGKEIT DER TEILNAHME

Die Teilnahme an der Studie ist freiwillig. Sie können die Teilnahme ablehnen oder jederzeit während der Studie Ihr Einverständnis ohne Angabe von Gründen zurückziehen, ohne dass Ihnen dadurch Nachteile in der Behandlung oder der Beziehung zu Ihrem Arzt entstehen. Ihr Arzt wird Sie im Verlauf der Studie über neue Erkenntnisse informieren, die Ihre Therapie bereichern könnten.

Voraussetzung zur Teilnahme ist, dass Sie Ihr Einverständnis schriftlich auf der beigefügten Einverständniserklärung erklären. Bitte lesen Sie diese Informationen aufmerksam durch und stellen Sie alle Fragen, die Sie zu der Studie haben, Ihrem Prüfarzt.

Verwendung Ihrer Daten

Die im Rahmen dieser Studie erhobenen Daten werden zum Zweck der wissenschaftlichen Auswertung aufgezeichnet und anonymisiert (d. h. ohne Namensnennung) weiterverarbeitet.

Nur die mit der Klinischen Prüfung vom Prüfarzt oder vom Sponsor beauftragten Personen sowie autorisierte Personen der Gesundheits- und Zulassungsbehörden haben im Rahmen der entsprechenden gesetzlichen Vorschriften Zugang zu Ihren persönlichen Daten. Diese Personen unterliegen der Schweigepflicht und sind zur Beachtung des Datenschutzes verpflichtet.

Im Falle der Veröffentlichung der Studienergebnisse bleibt die Vertraulichkeit Ihrer persönlichen Daten ebenfalls gewährleistet. Die Beachtung des Bundesdatenschutzgesetzes ist in vollem Umfang sichergestellt.

Kontaktperson

Sollten Sie Fragen zu der Studie haben, wenden Sie sich bitte an die zuständigen Prüfärzte:

Dr. med. Jens Gottlieb, Tel. (0511) 532-4681

Dr. med. Thomas Fühner, Tel. (0511) 532-4681

Eine Kopie dieser Informationsschrift und der Einverständniserklärung wird Ihnen ausgehändigt.

EINVERSTÄNDNISERKLÄRUNG (ADVAGRAF-STUDIE)

Patientenname, Vorname: _____

Das Aufklärungsgespräch erfolgte am: _____

Ich habe die Patienteninformation gelesen und hatte Gelegenheit, Fragen zu stellen. Ich habe die ärztliche Aufklärung über die möglichen Vor- und Nachteile der Behandlung sowie über meine Rechte als Teilnehmer der Klinischen Prüfung verstanden.

Ich habe verstanden, dass ich meine Einwilligung jederzeit auch ohne Angabe von Gründen zurücknehmen kann, ohne dass mir dadurch Nachteile in meiner weiteren Behandlung entstehen.

Ich stimme der Teilnahme an dieser Studie zu.

Ort, Datum: _____

Unterschrift des Patienten: _____

Name des Prüfarztes: _____
(in Blockbuchstaben)

Ort, Datum: _____

Unterschrift des Prüfarztes: _____

Datenschutzerklärung

Prospektiv randomisierte Studie zum Vergleich einer 2x täglichen mit einer 1x täglichem Gabe der Basisimmunsuppression bei Patienten nach Lungentransplantation

Mir ist bekannt, dass bei dieser klinischen Prüfung personenbezogene Daten, insbesondere medizinische Befunde, über mich erhoben, gespeichert und ausgewertet werden sollen. Die Verwendung der Angaben über meine Gesundheit erfolgt nach gesetzlichen Bestimmungen und setzt vor der Teilnahme an der klinischen Prüfung folgende freiwillig abgegebene Einwilligungserklärung voraus, d.h. ohne die nachfolgende Einwilligung kann ich nicht an der klinischen Prüfung teilnehmen.

Einwilligungserklärung zum Datenschutz (Arzneimittelgesetz)

1) Ich erkläre mich damit einverstanden, dass im Rahmen dieser klinischen Prüfung erhobene Daten, insbesondere Angaben über meine Gesundheit, in Papierform und auf elektronischen Datenträgern im Rahmen meiner Visiten in der Lungentransplantationsnachsorgeambulanz der MHH aufgezeichnet werden. Soweit erforderlich, dürfen die erhobenen Daten pseudonymisiert (verschlüsselt) weitergegeben werden:

a) an den Sponsor (MHH) oder eine von diesem beauftragte Stelle zum Zwecke der wissenschaftlichen Auswertung.

b) im Falle eines Antrags auf Zulassung: an den Antragsteller und die für die Zulassung zuständige Behörde (z.B. Bundesinstitut für Arzneimittel und Medizinprodukte, Kurt-Georg-Kiesinger-Allee 3,53175 Bonn)

c) im Falle unerwünschter Ereignisse: an den Sponsor (MHH), die Firma Astellas Pharma GmbH, an die jeweils zuständige Ethik-Kommission und die zuständige Bundesoberbehörde Bundesinstitut für Arzneimittel und Medizinprodukte (Kurt-Georg-Kiesinger-Allee 3,53175 Bonn, Telefon: 0228/207-4318, Fax: 0228/207-4355, E-Mail: klinpruefung@bfarm.de), sowie von dieser an die Europäische Datenbank.

2) Außerdem erkläre ich mich damit einverstanden, dass autorisierte und zur Verschwiegenheit verpflichtete Beauftragte des Sponsors MHH sowie die zuständigen inländischen und ausländischen Überwachungsbehörden in meine beim Prüfarzt vorhandenen personenbezogenen Daten, insbesondere meine Gesundheitsdaten, Einsicht nehmen, soweit dies für die Überprüfung der ordnungsgemäßen Durchführung der Studie notwendig ist. Für diese Maßnahme entbinde ich den Prüfarzt von der ärztlichen Schweigepflicht.

3) Die Einwilligung zur Erhebung und Verarbeitung meiner personenbezogenen Daten, insbesondere der Angaben über meine Gesundheit, ist unwiderruflich. Ich bin bereits darüber aufgeklärt worden, dass ich jederzeit die Teilnahme an der klinischen Prüfung beenden kann. Im Fall eines solchen Widerrufs meiner Einwilligung, an der Studie teilzunehmen, erkläre ich mich damit einverstanden, dass die bis zu diesem Zeitpunkt gespeicherten Daten ohne Namensnennung weiterhin verwendet werden dürfen, soweit dies erforderlich ist, um

a) Wirkungen des zu prüfenden Arzneimittels festzustellen,

b) sicherzustellen, dass meine schutzwürdigen Interessen nicht beeinträchtigt werden,

c) der Pflicht zur Vorlage vollständiger Zulassungsunterlagen zu genügen.

4) Ich erkläre mich damit einverstanden, dass meine Daten nach Beendigung oder Abbruch der Prüfung mindestens zehn Jahre aufbewahrt werden, wie es die Vorschriften über die klinische Prüfung von Arzneimitteln bestimmen. Danach werden meine personenbezogenen Daten gelöscht, soweit nicht gesetzliche, satzungsmäßige oder vertragliche Aufbewahrungsfristen entgegenstehen.

5) Ich bin über folgende gesetzliche Regelung informiert: Falls ich meine Einwilligung, an der Studie teilzunehmen, widerrufe, müssen alle Stellen, die meine personenbezogenen Daten, insbesondere Gesundheitsdaten gespeichert haben, unverzüglich prüfen, inwieweit die gespeicherten Daten für die in Nr. 3 a) bis c) genannten Zwecke noch erforderlich sind. Nicht mehr benötigte Daten sind unverzüglich zu löschen.

Ort, Datum: _____

Unterschrift des Patienten: _____

Name des Prüfarztes: _____
(in Blockbuchstaben)

Ort, Datum: _____

Unterschrift des Prüfarztes: _____

13.1.1 Protocol and protocol amendments

Prospective randomized trial to compare a twice daily to a once daily administration of the Tacrolimus in lung transplanted patients

Investigator Initiated Trial (IIT)
of the Department of Respiratory Medicine,
Hannover Medical School, Germany

Principal Investigator: Jens Gottlieb, M.D.

Financially supported by Astellas Pharma GmbH

Protocol number: DE-09-RG-53

EudraCT number: 2009-011324-60

Version 1.2, 7th May 2009
Amendment 4, 30th September 2010

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I. SIGNATURES

1. SIGNATURE PAGE

Prospective randomized trial to compare a twice daily to a once daily administration of the tacrolimus in lung transplanted patients

1.1 PROTOCOL AUTHORS:

Signature:	
Isabelle Bodmann Study Coordinator	Date
Signature:	
Jens Gottlieb, M.D. Principal Investigator	Date

1.2 PROTOCOL APPROVED BY:

Signature:	
Isabelle Bodmann Study Coordinator	Date
Signature:	
Jens Gottlieb M.D. Principal Investigator	Date
Signature:	
Claudia De Wall Subinvestigator	Date
Signature:	
Dr. Bernd Eisele KS-MHH	Date
Signature:	
Michael Born Representative of MHH	Date

II. CONTACT DETAILS

Principal Investigator:	Dr. med. Jens Gottlieb
Address:	Medizinische Hochschule Hannover,
	Department of Respiratory Medicine,
	Carl-Neuberg Str. 1
	30265 Hannover
Phone-Nr.:	**0511-532-3560**
Fax-Nr.:	**0511-532-8532**
Email:	Gottlieb.Jens@mh-hannover.de

Study Coordinator:	Isabelle Bodmann
Address:	Medizinische Hochschule Hannover,
	Department of Respiratory Medicine,
	Carl-Neuberg Str. 1
	30265 Hannover
Phone-Nr.:	**0511-532-2667**
Fax-Nr.:	**0511-532-8532**
Email:	Bodmann.Isabelle@mh-hannover.de

Study Coordinator:	Ewa Piekarska
Address:	Medizinische Hochschule Hannover,
	Department of Respiratory Medicine,
	Carl-Neuberg Str. 1
	30265 Hannover
Phone-Nr.:	**0511-532-9863**
Fax-Nr.:	**0511-532-8532**
Email:	Piekarska.Ewa@mh-hannover.de

Contact Person at	
Astellas Pharma GmbH:	Katharina Margreiter
	Head Clinical Project Management Academic Research
	Medical Department
Address:	Georg-Brauchle-Ring 64-66 / Campus E
	80992 München
Phone-Nr.:	**089-4544-1786**

Fax-Nr.: **089-4544 -5786**

Email : Katharina.Margreiter@de.astellas.com

III. SYNOPSIS

Title:	Prospective randomized trial to compare a twice daily to a once daily administration of the basic immunosuppressive regimen in lung transplanted patients
Indication:	Indicated conversion from CyA to Tac after lung transplantation (single, double or heart/lung) at least 12 months after transplantation
Rationale for the study:	To determine the optimal adherence with Tacrolimus twice versus once daily administration
Study design:	Open, randomized, prospective
Number of patients:	48 patients (24/24)
Duration of study:	24 months (recruitment 12 months, follow-up 12 months)
Inclusion criteria:	Pts ≥ 3 months after single, double or heart/lung transplantation Pts treated with CyA, MMF and steroids Pts ≥ 18 ≤ 70 years Pts with recurrent acute rejections (Pts with concomitant stable and non-advanced BOS are eligible) Pts with ongoing or steroid-resistant acute rejections Pts with CyA associated side effects (e.g. hyperlipidaemia, hypertriglyceridemia, hypertension, hirsutism, gingival hyperplasia)
Exclusion criteria:	Pregnant or breast feeding women Women of child-bearing potential who are not practicing an acceptable method of birth control Pts with systemic infections Pts with severe diarrhea, vomiting, active ulcer Pts with severe liver disease or liver cirrhosis Pts with m-Tor inhibitors
Medication and dosage:	12 hours after the last CyA administration the patients will be randomized to Tacrolimus twice or once daily. The initial dose of Tacrolimus will be calculated by the last CyA dosing (divided by 50). The trough level of Tacrolimus will be aimed at 8-12 ng/ml. MMF should be reduced by a quarter to one third after the aimed Tac trough levels are achieved. Steroids will be given according to standard protocol.
Primary objective:	Improvement of adherence as measured by Tacrolimus trough level below the target level and dispensing of less than 50% of the prescribed

	doses in the last three days measured electronically before this subtherapeutic drug monitoring
Secondary objectives:	• Deterioration of graft function as defined as more than 20% decline in maximum FEV_1 before and at month 12 after conversion • Number of drug holidays (intake of less than 50% of prescribed doses in 24 hours) measured electronically • Evaluation of renal function in pts converted from CyA to Tac in combination with MMF and steroids as assessed by serum creatinine, creatinine clearance (Cockgroft Gault), MDRD and Cystatin C before and at month 1, 3, 6 and 12 after conversion • Evaluation of cardiovascular risk factors (hypertension, hyperlipidaemia, hypertriglyceridemia, diabetes mellitus) • Incidence of CMV infections and other infections • Efficacy: Incidence of acute rejection episodes, graft and patient survival • Safety: Incidence of adverse events • Comparison of MPA-mini-AUC under Tacrolimus once and twice daily administration
Time estimate:	First patient in: Q1 / 2009 Last patient out: Q1 / 2011 Final report: Q2 / 2011
Responsible Project Leader	Jens Gottlieb, M.D. Klinik für Pneumologie, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, 30625 Hannover; Tel. 0511/532-3560
Project Leader	Claudia de Wall Klinik für Pneumologie, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, 30625 Hannover; Tel. 0511/532-3531

IV. FLOWCHART

Data to be recorded	Visit 1 Randomization	Visit 2 Month3 (+/- 1 week)	Visit 3 Month 6 (+/- 1 week)	Visit 4 Month 9 (+/- 1 week)	Visit 5 Month 12 (+/- 1 week)
Informed consent for data collection	x				
Demographic data	x				
Concomitant diseases	x				
Transplantation history [1]	x				
Rejection episode history since (last) Tx	x				
Vital sign, weight	x	x	x	x	x
Laboratory assessments [2]	x	x	x	x	x
Pulmonary function test, ~~BOS-staging~~	x	x	x	x	x
BOS-staging	x				x
MPA-AUC	x	x			
Compliance parameters:					
ITAS questionnaire	x	x	x	x	x
Compliance VAS	x	x	x	x	x
ITBS questionnaire	x	x	x	x	x
Investigator and nurse compliance assessment	x	x	x	x	x
Tacrolimus trough levels (all available)	x	◄———	———	———	———►
Explain and dispend “Helping Hand”	x				
Check “Helping Hand”		x	x	x	x
“Helping Hand” measurements		◄———	———	———	———►
Return “Helping Hand”					x
End of study data [3]					x
On an ongoing basis:	◄———	———	———	———	———►
Tacrolimus daily dose	◄———	———	———	———	———►
Immunosuppressive medication	◄———	———	———	———	———►
~~Concomitant medication~~	◄———	———	———	———	———►
Rejection episodes	◄———	———	———	———	———►
Adverse drug reactions	◄———	———	———	———	———►

[4] Recipient data: number and type of lung TX, BOS status, date of (last) transplantation, primary reason for transplantation, ABO blood type, viral status (CMV, ~~EBV), ventilation prior to Tx~~, need to dialysis, donor status: CMV

[5] Haemoglobin, WBC, thrombocytes, LDH, serum creatinine, urine stix, sodium, potassium, liver enzymes, GFR (MDRD), Chol, TG, glucose, HbA1c, Cyst. C

[6] Overall efficacy and tolerability, ~~patient satisfaction~~, graft survival, re-lung-Tx, patient survival, withdrawal: at visit 4~~5~~ or in case of early termination at the time of termination

1 INTRODUCTION

Prevalence data of non-compliance in solid organ transplantations fluctuate is reported in up to 39% of transplant recipients (z. B. for lung transplantations 13 – 22%; Kugler et al.). Non-compliance with immunosuppressive therapy is associated with an increased risk of late-acute rejections and the development of chronic transplant dysfunction. Chronic transplant dysfunction (bronchiolitisobliterans- syndrome-BOS) is the second most causing for organ failure after the first year following lung transplantation and often leads to re-transplantation or death. Preventative procedures for improving the compliance are simplification of the dose of the immunosuppressants (a once daily dose instead of a twice daily dose), the prescription of an immunosuppressant with less side-effects and to raise the patient´s awareness for having the greatest responsibility for the efficacy of his therapy. Prospective studies and metaanalysis revealed that the probability for a good compliance can be more than doubled at once daily administration in comparison to twice daily and the best predictor for a good compliance is an easy therapy. For this reason we want to investigate the extent of profit for our lung transplant patients receiving once daily basis immunosuppression in comparison to those who receive twice daily dose. Hypothesis: Patients of the once daily administration group of the immunsupressive medication will have a better compliance compared to the twice daily group (as measured by the endpoints variability and medication abstraction from the electronic devices)

1.1 Background

Non-compliance is most probably the most important factor for late acute rejection episodes. These reactions may lead to chronic transplant/allograft injury or organ loss in lung-Tx patients. It could be demonstrated in a prospective trial that 21.2 % of patients who were identified to be non-compliant suffered from late acute rejection compared to only 8 % of patients who were considered to adhere to the immunosuppressive treatment regimen well.

Two meta-analyses confirm these results and the link between non-compliance and graft loss. According to Butler et al., non-compliance increases the risk for organ loss 7-fold and 36 % of organ losses were attributed to non-compliance. Similar data were presented by Denhaerynck et al. showing that 20 % of late acute rejections and about 17 % of graft losses were due to non-compliance. Due to the possible implications of non-compliance on patient's life, it is of utmost importance to control the regular drug intake and improve it, if needed. One way is to regularly educate the patient. These educational measures however may not always be sufficient to achieve adequate compliance. Another approach is to simplify the treatment regimen. It is known that the probability of non-compliance rises with the dosing frequency. Consequently, a simplified immunosuppressive treatment regimen may enhance compliance.

Advagraf is a new prolonged release formulation of tacrolimus. A once daily dosing is sufficient for maintaining therapeutic tacrolimus levels. Patients may benefit from this new dosage regimen and their compliance may be enhanced.

This study aims to gather first compliance data of patients treated with Advagraf after lung Tx. Different methods of compliance measurement will be used in this study to gain a picture of clinical reality as comprehensive as possible. These methods comprise patient questionnaires (ITAS, ITBS), an investigator assessment and the measurement of tacrolimus trough levels as well as the use of electronic compliance monitoring with a device.

1.1.1. Tacrolimus (FK506)

Tacrolimus is a compound produced as a fermentation product of Streptomyces tsukubaensis. It is a macrolide lactone with a potent immunosuppressive activity5. At the molecular level, the effects of tacrolimus appear to be mediated by binding to a cytosolic protein (FKBP12). The FKBP12-tacrolimus complex binds to and inhibits calcineurin, leading to an inhibition of T-cell signal transduction pathways, thereby preventing transcription of a discrete set of lymphokine genes. The drug suppresses the formation of lymphokines (such as interleukin-2, -3 and γ-interferon) and the expression of the interleukin-2 receptor. Thus, the drug suppresses T-cell activation and T-helper-cell-dependent B-cell proliferation. In particular, tacrolimus inhibits the formation of cytotoxic lymphocytes, which are mainly responsible for graft rejection. These effects are similar to those of cyclosporine, but tacrolimus has a ten to 100 fold higher potency than cyclosporine on a molecular basis11. The compound bears no structural relationship to cyclosporine.

In comparative trials of clinical organ transplantation, tacrolimus has been proven to be superior to cyclosporine in the prevention of acute rejection in liver, lung, heart and kidney transplants. Switch from tacrolimus to cyclosporine is an accepted indication in LTx transplantation in case of recurrent and refractory acute cellular rejection (ACR) and in some cases of BOS pats. switched to Tacrolimus respond by improvement of graft function (Sararhudi et al). In RCT Tac shows lower incidence of ACR (Hachem et al. 2007).

Tacrolimus has been on the market for more than ten years under the trade name Prograf® (Prograft® in Belgium, Luxembourg and the Netherlands) and is one of the two cornerstone immunosuppressants following organ transplantation. A life-long maintenance therapy with an immunosuppressive agent is necessary to prevent transplant rejection.

The marketed formulations of tacrolimus (Prograf®) are approved in the European Union (except Latvia, Lithuania, Estonia and Malta) for both adult and paediatric use for the prevention of transplant rejection in liver, kidney and heart allograft recipients and for the treatment of allograft rejection resistant to treatment with other immunosuppressive medicinal products. Currently the oral formulation of tacrolimus is available as 0.5 mg, 1 mg, 5 mg hard capsules and the intravenous formulation as 5 mg/mL concentrate for solution for infusion.

1.1.2 Tacrolimus Modified Release Formulation (Advagraf®)

Prograf® capsules require twice-daily dosing. Advagraf® has been developed to provide once-daily dosing with a similar safety and efficacy profile as the current twice-daily formulation. Systemic exposure to tacrolimus i.e. area under the concentration-time curve over the dosage-time interval (AUCτ) has been found to be a significant explanatory variable of efficacy and safety. Therefore, the target goals for the Advagraf® formulation to be therapeutically equivalent to Prograf® were to achieve the AUC of tacrolimus to be within the bioequivalence criteria relative to twice daily dosing with Prograf®. If systemic exposure is equivalent, therapeutic equivalence between the two formulations can also be assumed. Additional criteria for the purpose of therapeutic monitoring were good correlation of trough concentration to AUC (similar to that obtained for Prograf®) and the same target trough concentration range as Prograf®. Owing to a food effect, Prograf® capsules are taken one hour before or at least two to three hours after a meal which is an additional burden for the subject, especially concerning the evening dose, as it may interfere with daily life activities.

Transplant subjects often receive immunosuppressive regimens consisting of multiple medications; thus, a formulation that could be taken once daily is considered to be of benefit to the subject. Advagraf®, the first calcineurin inhibitor formulated to enable once daily administration, is available in the same capsule strengths as Prograf® (0.5mg, 1mg and 5mg) and has the potential to improve subject compliance. Poor compliance has been shown to be one of the factors associated with late graft loss1,2. In a prospective cohort study of 278 adult recipients of cadaveric donor renal transplants, Weng et al. (2005)3 demonstrated a statistically significant association for adherence to medication regimen with once daily dosing versus twice daily dosing. It is expected that Advagraf® may help to improve compliance with dosing - as no evening dose is required – therefore decreasing the risk of late graft rejection and loss, and having less interference with the daily life activities of the subject.

The clinical development program for Advagraf® to date includes twelve Phase I studies (in healthy volunteers), eight Phase II studies and three completed Phase III study (all in transplant recipients). The Phase I studies performed in healthy volunteers (N=242) compared the biopharmaceutics of tacrolimus for Advagraf® and Prograf®. The Phase II studies (N=475 Advagraf® subjects) performed in transplant subjects compared the parameters of systemic exposure to tacrolimus from Advagraf® administered once daily to Prograf® administered twice daily.

Further details can be found in the current version of the Advagraf® Summary of Product Characteristics which contains comprehensive information on tacrolimus.

The formulations of tacrolimus modified formulation (Advagraf®) are approved and registered in the European Union (Advagraf® is not yet commercially available in all European countries).

1.1.3 Tacrolimus in Combination with Mycophenolate Mofetil

Mycophenolate Mofetil (MMF) is an inhibitor of the *de novo* purine synthesis with apparent selectivity for B and T lymphocytes17 and has been developed as a replacement for Azathioprine for use in conjunction with cyclosporine. Phase III studies demonstrate that MMF is superior to both placebo and azathioprine when used in combination with cyclosporine and steroids. Mycophenolate mofetil has been approved in Europe and the USA for the prophylaxis of organ rejection in kidney allograft recipients when used in combination with cyclosporine and steroids.

The combination of tacrolimus and MMF has been evaluated in a dose ranging study comparing tacrolimus / steroids, tacrolimus / 1 g MMF per day / steroids and tacrolimus / 2 g MMF per day / steroids in 232 subjects. The combination of tacrolimus with 1 g and 2 g MMF showed a significant reduction in the incidence of first acute and steroid-resistant rejection episodes in comparison to the control arm with no MMF. No significant difference in the incidence of acute rejections was observed between the 1 g and 2 g MMF groups. All three treatment arms had a comparable safety profile, although diarrhoea and leucopenia - known to be more frequently observed with the use of MMF - were most pronounced in the 2 g MMF arm. It was concluded that the combination of tacrolimus, 1 g MMF, and steroids is a safe and effective regimen for rejection prophylaxis following lung transplantation.

In a US multicenter dose comparison study of MMF in combination with tacrolimus the control arm received a tacrolimus-azathioprine-steroid triple regimen. The 2 g/d dose of MMF did show superior efficacy over control in terms of acute rejection frequency. This study is, however, difficult to relate to the European situation because (i) the majority of subjects were not caucasian, (ii) the organ allocation system in the US is different to that in Europe (resulting in a different mismatch profile), and (iii) all subjects received antibody induction.

In a more recent study comparing three different immunosuppressive regimens, 223 kidney transplanted subjects were randomized to receive either a tacrolimus-MMF-steroids, tacrolimus-azathioprine-steroids or cyclosporine-MMF-steroids based regimen. Study results show a similar incidence of acute rejection, subject and graft survival for the three different treatments schedules. The combination of tacrolimus-MMF (2 g/d) demonstrated, nevertheless, its superiority in terms of incidence of steroid resistant rejection at one year. Study results were confirmed at two years.

A pilot study conducted in Spain has also proved the efficacy of a tacrolimus-MMF-steroids based regimen in the treatment of renal transplanted recipients receiving grafts from older donors. The mean age of subjects was 65.8 years while donors' mean age was 63.3 years. A total of 35 subjects was treated with tacrolimus 0.1 mg/kg/d, MMF 2 g/d and steroids 0.5 mg/kg/d. Subjects and graft survival were 94 % and 88 % at one and two years respectively. No cases of graft loss other than in subject exitus were reported.

For detailed information on MMF please refer to the respective SPC.

1.2 Summary of Key Safety Information for Study Drugs

For possible adverse drug reactions of tacrolimus please refer to the Summary of Product Characteristics (SPC) for Prograf® and Advagraf®.

1.3 Risk-Benefit Assessment

Tacrolimus (INN, Prograf®, FK506) is an established potent immunosuppressive agent for the prophylaxis of rejection in liver, kidney and heart allograft recipients and for the treatment of allograft rejection resistant to treatment with other immunosuppressive medicinal products. It is approved in Germany for use in use in lung transplant recipients with refractory episodes of acute rejection under conventional immunosuppression.

The clinical use of tacrolimus as a baseline immunosuppressive agent in solid organ transplantation has been confirmed world-wide since its initial marketing authorization in 1993.

The patient exposure is growing annually and the established efficacy and safety profile of tacrolimus is well defined.

Owing to a food effect, Prograf® capsules are taken one hour before or at least two to three hours after a meal which is an additional burden for the subject, especially concerning the evening dose, as it may interfere with daily life activities and thus worsen patient compliance. Poor intake adherence e has been shown to be one of the factors associated with late graft loss.

Adherence to medications was shown to be significantly better in a once daily than in a twice daily dosing regimen. Based on these findings it is expected that Advagraf® (once daily tacrolimus) may help to improve compliance with dosing – as no evening dose is required – therefore decreasing the risk of late graft rejection and loss, and having less interference with the daily life activities of the subject.

A combined tacrolimus/MMF regimen is viewed by many clinicians as the optimal best immunosuppressive regimen in solid organ transplantation and is highly effective in prevention of acute rejections as it was shown in clinical trials .

As described above, all data currently show similar safety and efficacy of Advagraf® and Prograf® (for up to two-year treatment duration), the risk of this clinical study is very low and the benefit to the individual subject of staying on Advagraf® is considered high.

2 STUDY OBJECTIVES, DESIGN AND VARIABLES

2.1 Study Objectives

Aim of this study is to demonstrate that the prescription of an extended release formulation of Tacrolimus that needs to be given once daily, only, can impact on compliance and immunosuppressive protection.

The primary objective of this study is to

- Improvement of adherence as measured by Tacrolimus trough level below the target level and dispensing of less than 50% of the prescribed doses in the last three days measured electronically before this subtherapeutic drug monitoring

The secondary objectives of this study are:

- Deterioration of graft function as defined as more than 20% decline in maximum FEV1 before and at month 12 after conversion
- Number of drug holidays (intake of less than 50% of prescribed doses in 24 hours) measured electronically
- Evaluation of renal function in pts converted from CyA to Tac in combination with MMF and steroids as assessed by serum creatinine, creatinine clearance (Cockgroft Gault), MDRD and Cystatin C before and at month 1, 3, 6 and 12 after conversion
- Evaluation of cardiovascular risk factors (hypertension, hyperlipidaemia, hypertriglyceridemia, diabetes mellitus)
- Incidence of CMV infections and other infections
- Efficacy: Incidence of acute rejection episodes, graft and patient survival
- Safety: Incidence of adverse events
- Comparison of MPA-mini-AUC under Tacrolimus once and twice daily administration

2.2 Study Design and Dose Rationale

Aim of this study is to demonstrate that the prescription of an extended release formulation of Tacrolimus that needs to be given once daily, only, can impact on compliance and immunosuppressive protection.

Patients after single, double or heart/lung transplantation will be randomized 12 hours after the last CyA administration to an individually titrated treatment scheme based on either Prograf® (two daily doses of Tacrolimus) or Advagraf® (one daily dose of Tacrolimus).

Per patient the proportion of subtherapeutic Tacrolimus trough levels caused by non-adherence of lung and heart transplanted patients that are treated with Prograf® (two daily doses of Tacrolimus) and

Advagraf® (one daily dose of Tacrolimus) will be measured and treatment groups will be compared to reject the null-hypothesis that the use of an extended release formulation does not impact on patient compliance.
A trough level is said to be caused by non-adherent, if an individual has a Tacrolimus trough level below the target of 8 ng/ml (or any individual defined target range) and if more than 50% of the required drugs-tablets in the last three days were not appropriately taken from the automatic dispenser.

Unavoidably this is an open study. The prospective randomized trial is planned with duration of 24 months (12 months recruitment time and 12 months follow-up).
The study will be performed in patients between 18 and 70 years with single lung or double lung or heart/lung transplantation that was at least 3 months ago.

Switch from Ciclosporin to Tacrolimus:

At least 12 hours after the last CyA administration the patients will be switched to Tacrolimus twice or once daily. CyA trough level before switching ~~must~~ should be below 250 ng/ml (ACMIA). CyA trough levels should be performed ~~every 24 h~~ after switch until CyA trough levels are <200 ng/ml. The initial dose of Tacrolimus will be calculated by the last CyA dosing (initial
tacrolimus dose = last ciclosporin dose divided by 50). The target trough level of Tacrolimus will be aimed at 8-12 ng/ml. During the first 14 days, Tacrolimus levels should be controlled at day 4, 8 and day 14. After Day 14 levels should be controlled by order of investigator with a maximum of 14 days interval.

MMF:

Target dose is 2.000 mg/d, reduced in pts. with cytopenia or GI-intolerance. MMF dose ~~should~~ may be reduced by 25% after conversion from CyA to Tac after Tac target trough levels are achieved.

Steroids:

~~Steroids will be given at a dose of 0.05-0.10 mg/k~~g. All patients will be on steroids during the duration of the study.

Flowchart:

Check-ups (study visits) for both groups the same

2.3 Dose Rationale:

The combination of tacrolimus and MMF has been evaluated in a dose ranging study comparing tacrolimus / steroids, tacrolimus / 1 g MMF per day / steroids and tacrolimus / 2 g MMF per day / steroids in 232 subjects. The combination of tacrolimus with 1 g and 2 g MMF showed a significant reduction in the incidence of first acute and steroid-resistant rejection episodes in comparison to the

control arm with no MMF. No significant difference in the incidence of acute rejections was observed between the 1 g and 2 g MMF groups. All three treatment arms had a comparable safety profile, although diarrhoea and leucopenia – known to be more frequently observed with the use of MMF – were most pronounced in the 2 g MMF arm. It was concluded that the combination of tacrolimus, 1 g MMF, and steroids is a safe and effective regimen for rejection prophylaxis following lung transplantation.

Target trough levels of tacrolimus are based on the traditional target trough levels or tacrolimus used in the last 20 years in the lung transplant program of Hannover Medical School. Conversion rates (1mg Tacrolimus ~ 50 mg Cyclosporine) are used as well for years in the clinical routine in our program.

2.4 Variables

Adherence measures as subtherapeutic drug levels and simultaneous missing drug dispensing are used as robust and reproducible endpoints measurable by electronic devices like ProMate.

3 STUDY POPULATION

3.1 Selection of Study Population

Patients (≥18 and ≤ 70 years) ≥ 3 months after single, double or heart/lung transplantation.

3.2 Inclusion Criteria

· Pts ≥ 3 months after single lung, double lung or heart/lung transplantation and

· Pts treated with Cyclosporine, steroids and MMF and

· Pts ≥18 and ≤ 70 years and

· Pts with one oft he following

- Pts with recurrent acute rejections (RAR)

  (Pts with concomitant stable and non-advanced BOS are eligible)

  two or more acute rejections in last 3 months (first 4 weeks post Tx excluded) defined by

  - transbronchial biopsy ≥A1 according to ISHLT **or**
  - decline of FEV_1 > 10 % baseline after exclusion of infection, airway complication, effusion etc. and improvement to steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) = FEV1 improvement > 10% compared to the last measurement before AR treatment

- Pts with steroid-resistant or ongoing acute rejections (OAR) defined by
  - transbronchial biopsy ≥A1 at least 4 weeks following steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) **or**
  - no FEV_1 improvement (< 5% baseline) at least 14 days following ACR steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) after exclusion of infection, airway complication, effusion etc. **or**

- Pts with CyA associated side effects (e.g. hyperlipidaemia, hypertriglyceridemia, hypertension, hirsutism, gingival hyperplasia)

3.3 Exclusion criteria:

· Pregnant or breast feeding women

· Pts who are not using a double-barrier method of birth control

· Pts with systemic infections

· Pts with severe diarrhea, vomiting, active ulcer

· Pts with severe liver disease or liver cirrhosis

· Pts with m-Tor inhibitors

· Pts with hypersensitivity to Tacrolimus, other macrolides or other tablet ingredients

3.4 Discontinuation Criteria for Individual Subjects

Patient developing intolerable side effects of tacrolimus will be discontinued from study medication.

4 STUDY DRUGS

4.1 Description of Study Drugs

4.1.1 Test Drug(s)

4.1.1.1 Tacrolimus (Prograf®)

Active ingredient: tacrolimus

Prograf® is available as hard gelatin capsules with 0.5 mg, 1 mg and 5 mg of tacrolimus. The other ingredients are lactose, hydroxypropyl methylcellulose 2910, ethylcellulose and magnesium stearate.

0.5 mg capsule Light yellow hard gelatin capsules, size No.5, with “0.5 mg” printed in red on capsule cap and “[f] 607" on the capsule body.
1 mg capsule Opaque white hard gelatin capsules, size No. 5, with "1 mg" printed in red on the capsule cap and “[f] 617" on the capsule body.
5 mg capsule Grayish-red hard gelatin capsules, size No. 4, with "5 mg" printed in white on the capsule cap and “[f] 657" on the capsule body.

For a complete characterization of tacrolimus please refer to the Summary of Product Characteristics (SPC).

4.1.1.2 Tacrolimus Modified Release Formulation (Advagraf®)

Active ingredient: tacrolimus

Advagraf® is available as hard gelatin capsules with 0.5 mg, 1 mg and 5 mg of tacrolimus. The other ingredients are lactose, hydroxypropyl methylcellulose 2910, ethylcellulose and magnesium stearate.
0.5 mg capsule Hard gelatin capsules consisting of light yellow caps and orange bodies, size No 5, with “[f] 0.5 mg” printed in red on the capsule cap and body.
1 mg capsule Hard gelatin capsules consisting of white caps and orange bodies, size No 4, with “[f] 1 mg” printed in red on the capsule cap and body.
5 mg capsule Hard gelatin capsules consisting of grayish-red caps and orange bodies, size No 0, with “[f] 5 mg” printed in red on the capsule cap and body.

For a complete characterization of tacrolimus modified release formulation, please refer to the Summary of Product Characteristics (SPC).

4.1.1.3 Mycophenolate Mofetil (Cellcept®/MMF)

Active ingredient: mycophenolate mofetil

250 mg capsule: Hard gelatine capsules consisting of blue caps and brown bodies, size No 1, with "Cellcept 250" printed in black on the blue cap and "Roche" on the brown body.

For a complete characterization of mycophenolate mofetil please refer to the information provided in the Summary of Product Characteristics (SPC).

4.1.2 Comparative Drug(s)

Not applicable

4.2 Study Drug Handling

Drug prescription will be performed by local physicians according to the instruction of the transplant center.

4.2.1 Storage Conditions for Study Drug

The study medication should be kept dry and stored according to the instructions printed on the label and the subjects should be instructed accordingly.

Once the aluminum pouch is opened the Advagraf® capsules in the blister strips are stable for 12 months when stored according to the storage conditions printed on the label.

Medication must not be used after the expiry date indicated on the respective labels.

4.3 Randomization

This is an open study. To ensure admission before allocation the randomization will be performed centrally.

Independent study documentary will randomize patients either to tacrolimus once daily or twice daily according to a computer generated randomization list in order of the inclusion date and time of eligble patients. Drug prescription will be performed by local physicians according to the instruction of the transplant center.

5 TREATMENTS AND EVALUATION

5.1 Dosing and Administration of Study Drugs and Other Medications

5.1.1 Dose/Dose Regimen and Administration Period

5.1.1.1 Dosing and Administration of Tacrolimus

12 hours after the last CyA administration the patients will be randomized to Tacrolimus twice or once daily.

The initial dose of Tacrolimus will be calculated by the last CyA dosing (initial tacrolimus dose = last ciclosporin dose divided by 50).

The trough level of Tacrolimus will be aimed at 8-12 ng/ml. In case of drug toxicities target drug levels may be individually be lowered to 5-8 ng/ml

5.1.1.2 Dosing and Administration of MMF

MMF target dose is 2000 mg/d, in pts. with cytopenia or GI-intolerance it should be reduced by 25% after switch from CyA to Tacand if Tac target trough levels are achieved.

5.1.1.3 Dosing and Administration of Corticosteroids

~~Steroids will be given at a dose of 0.05-0.10 mg/k~~g. All patients will be on steroids during the duration of the study.

5.1.1.4 Prohibited Concomitant Medication (Drugs and Therapies)

· Azathioprin
· Basiliximab, Daclizumab
· ALG/ATG
· Cyclophosphamid
· Methotrexat
· Vincristin
· Alemtuzumab (Campath)
· Leflunomid
· Sirolimus
· Everolimus

5.1.2 Treatment Compliance

Compliance will be measured by two ways: firstly, by electronic measurement of study drug dispensing and secondly subtherapeutic drug levels.

ProMate (Helping Hand)

The 'ProMate' is a device that records each point in time whenever a blister is inserted in it. The 'ProMate' will be dispensed at visit 1. Each device can be identified by a unique device number. This device number can be found on the device and on the device box. The device number has to be recorded in the CRF.

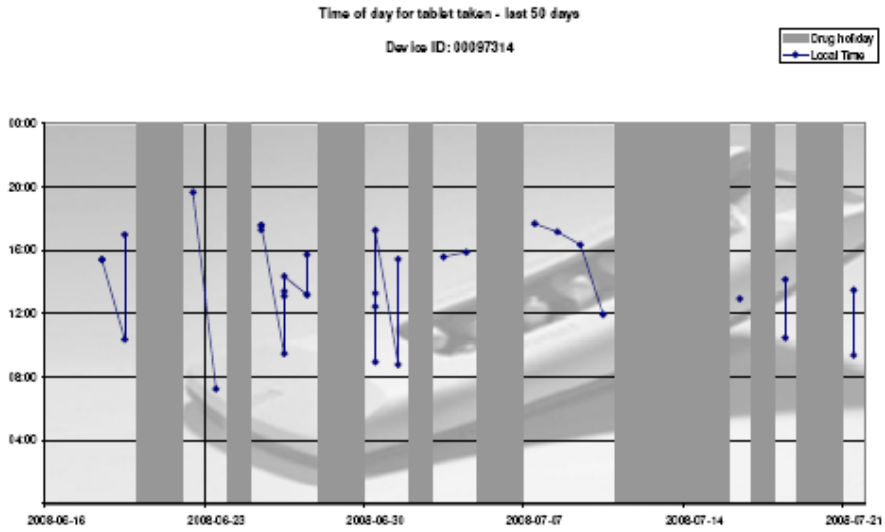
The patient needs to be thoroughly instructed on how to use the 'ProMate'. The patient needs to be instructed to pull out the blister at each dosing time point, remove the capsule/s needed to be swallowed and insert the blister thereafter immediately. If the patient does not re-insert the blister after taking the capsule within 10 seconds, there will be a brief beeping alarm. At visit 2, 3, 4 and 5 the device will be checked (i.e. if there was an alarm indicating that the battery failed) and the patient should be instructed about the use of the device again if needed. Whenever a blister is empty, the patient shall insert a new blister into the 'ProMate'. If a new blister is not at hand immediately, the patient should insert the empty blister into the 'ProMate' after the intake of the last capsule. The patient should then insert the new blister at the time of the next planned dose, i.e. the next morning.

The device comes with a preinstalled adapter card. With this adapter card, the device can be used for the 0.5 mg or 1 mg capsules. In case, the patient exclusively takes 5 mg capsules, the adapter card needs to be removed as the 5 mg blisters are wider than the 0.5 mg and 1 mg blisters. In case, there is a dose change, the adapter card possibly needs to be (re)-inserted or removed depending on the dose change. At visit 5, the device will be returned to the investigator. At that visit, the investigator shall pull out and insert the blister once to set a last time stamp. This is needed to be able to identify if the battery failed or the patient did not use the device. The device is equipped with a sound alarm function in case the battery is empty (continuous beep sound when the blister is inserted in the device). In this case, the patient should return the device at the next planned visit.

Three different aspects of compliance will be assessed with the 'ProMate', adapted from van Wijngaerden et al. and Deschamps et al. [9, 10]: • Timing compliance 'ProMate': Number of correct dosing intervals, i.e. time between capsule intake / number of observed days * 100; correct dosing interval is defined as an interval between 22 and 26 hours • Taking compliance 'ProMate': number of blister card removals during observational period / number of prescribed doses * 100 • Drug holidays 'ProMate': number of the periods during the observational period with two or more missed consecutive doses Additionally, a longitudinal description of compliance in terms of execution of the dosing regimen and persistence to the prescribed treatment will be presented.

In case of malfunction of the ProMate device the compliance and drug holiday will be not rated.

Example (screen shot):



5.1.3 Emergency Procedures and Management of Overdose

Experience with overdosage of tacrolimus is limited. Several cases of accidental overdosage have been reported; symptoms have included tremor, headache, nausea and vomiting, infections, urticaria, lethargy, increased blood urea nitrogen and elevated serum creatinine concentrations and increase in alanine aminotransferase levels.

No specific antidote to tacrolimus therapy is available. If overdosage occurs, general supportive measures and symptomatic treatment should be conducted.

Based on its high molecular weight, poor aqueous solubility and extensive erythrocyte and plasma protein binding, it is anticipated that tacrolimus will not be dialyzable. In isolated subjects with very high plasma levels, hemofiltration or-diafiltration has been effective in reducing toxic concentrations. In cases of oral intoxication, gastric lavage and/or the use of adsorbents (such as activated charcoal) may be helpful, if used shortly after intake.

Limited overdosage experience is available. Acute overdosages of up to 30 times the intended dose have been reported. Almost all cases have been asymptomatic and all patients recovered with no sequelae. Occasionally, acute overdosage has been followed by adverse reactions consistent with those listed in the ADVERSE REACTIONS section except in one case where transient urticaria and lethargy were observed. Based on the poor aqueous solubility and extensive erythrocyte and plasma protein binding, it is anticipated that tacrolimus is not dialyzable to any significant extent; there is no experience with charcoal hemoperfusion. The oral use of activated charcoal has been reported in treating acute overdoses, but experience has not been sufficient to warrant recommending its use. General supportive measures and treatment of specific symptoms should be followed in all cases of overdosage.

In acute oral and IV toxicity studies, mortalities were seen at or above the following doses: in adult rats, 52X the recommended human oral dose; in immature rats, 16X the recommended oral dose; and in adult rats, 16X the recommended human IV dose (all based on body surface area corrections).

5.1.4 Criteria for Continuation of Treatment

Once a subject has completed or been discontinued from the study, further immunosuppressive treatment is left to the discretion of the investigator.

5.1.5 Restrictions during the Study

Female subjects of childbearing potential must maintain double barrier method of birth control during the study and three months thereafter.

5.2 Demographics and Baseline Characteristics

Patients (≥18 and ≤ 70 years) ≥ 3 months after single lung, double lung or heart/lung transplantation.

5.3 Efficacy Assessment

<u>The primary objective of this study is to</u>

- Improvement of adherence as measured by Tacrolimus trough level below the target level and dispensing of less than 50% of the prescribed doses in the last three days measured electronically before this subtherapeutic drug monitoring

<u>The secondary objectives of this study are:</u>

- Deterioration of graft function as defined as more than 20% decline in maximum FEV1 before and at month 12 after conversion
- Number of drug holidays (intake of less than 50% of prescribed doses in 24 hours) measured electronically
- Evaluation of renal function in pts converted from CyA to Tac in combination with MMF and steroids as assessed by serum creatinine, creatinine clearance (Cockgroft Gault), MDRD and Cystatin C before and at month 1, 3, 6 and 12 after conversion
- Evaluation of cardiovascular risk factors (hypertension, hyperlipidaemia, hypertriglyceridemia, diabetes mellitus)
- Incidence of CMV infections and other infections
- Efficacy: Incidence of acute rejection episodes, graft and patient survival
- Safety: Incidence of adverse events
- Comparison of MPA-mini-AUC under Tacrolimus once and twice daily administration

5.3.1 Diagnosis and Grading of Acute Rejection Episodes

If clinical and/or laboratory signs indicate the occurrence of a rejection episode a transbronchial biopsy (TBB) will be performed. In Pts unable to undergo TBB acute rejection is defined clinically as reversion of symtoms and/or improvement of FEV1 of at least 10% compared to the last recorded value before rejection treatment, test be performed.

The biopsy should be performed prior to the initiation of any anti-rejection therapy and as soon as possible after the onset of clinical/laboratory signs indicative of possible rejection. The histological evaluation of the biopsy will be performed by the local histopathologist following the ISHLT criteria.

5.3.2 Classification of Acute Rejection Episodes

Spontaneously Resolving Acute Rejection:

A spontaneously resolving rejection is defined as a rejection episode which has not been treated with new or increased corticosteroid medication, antibodies or any other medication and which has resolved, irrespective of any tacrolimus or MMF dose changes.

Corticosteroid Sensitive Acute Rejection:

A corticosteroid sensitive acute rejection is defined as a rejection episode treated with new or increased corticosteroid medication only and which has resolved, irrespective of any tacrolimus or MMF dose changes.

- recurrent acute rejections (RAR)
- two or more acute rejections in 3 months defined by
    - transbronchial biopsy ≥A1 according to ISHLT or
    - decline of FEV1 > 10 % baseline after exclusion of infection, airway complication, effusion etc. and improvement to steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) = FEV1 improvement > 10% compared to the last measurement before AR treatment
- steroid-resistant or ongoing acute rejections (OAR) defined by
    - transbronchial biopsy ≥A1 at least 4 weeks following steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) or
    - no FEV1 improvement (< 5% baseline) at least 14 days following ACR steroid-pulse therapy (methylprednisolone 15 mg/kg for three days) after exclusion of infection, airway complication, effusion etc.

Time to First Acute Rejection:

Time to first acute rejection episode is defined as the number of days from transplantation (Day 0) to the first clinical, laboratory or histological signs that are considered to be related to the first acute rejection episode.

5.3.3 Graft Loss

Graft loss is defined as: re-transplantation, or death.

The date of graft loss is the earliest date of any of these events.

5.3.4 Assessment of Renal Dysfunction

Renal dysfunction will be defined as GFR < 30 mL/min/1.73m2 (MDRD formula), renal replacement therapy or need for kidney transplantation

5.3.5 Renal function

Renal function will be assessed by GFR using MDRD formula and Cystatin C after Larson.

Renal Function will also be assessed by Creatinine Clearance using Cockcroft and Gault formula.

5.4 Safety Assessment

The most relevant safety parameters assessed within the study are measurement of renal function, incidence of adverse events, absolute change in serum lipids (cholesterol, HDL, LDL, triglycerides) and incidence of diabetes mellitus.

5.4.1 Vital Signs

Vital signs are to be assessed at every scheduled study visit. Weight will be measured according to the hospital's routine procedure. Blood pressure should be measured after five minutes of rest.

5.4.2 Adverse Events

Adverse Events, including clinically significant laboratory abnormalities, will be assessed by the investigator and will be recorded in the CRF as described in section 5.6.

5.4.3 Laboratory Assessments

Routine laboratory assessments will be performed at every scheduled study visit at the local laboratory at each study site.

Blood samples should be taken in the morning after a fasting period of at least six hours, preferably before study drug administration.

Each local laboratory must provide a current and approved list of reference ranges, including units for each parameter.

The laboratory values taken for Inclusion/Exclusion Criteria at Visit 1 must not be older than 48 hours at the time of reperfusion.

The following parameters are to be determined on each patient visit:

Haemoglobin, WBC, thrombocytes, LDH serum creatinine, urine stix, sodium, potassium, liver enzymes, GFR (MDRD), Chol, TG, glucose, HbA1c, Cyst. C.

5.4.4 Other assessments

Immunosuppressant therapy barrier scale (ITBS)

Immunosuppressant therapy barrier scale (ITBS) was developed to assess transplant patients' perceived barriers to IST adherence and was completed by 222 transplant patients who lived in Georgia, USA. A renal transplant population subset was used to test the ITBS reliability and validity. The ITBS subscales correlated negatively with a self-reported measure of IST adherence, IST serum concentrations and IST pharmacy refill adherence rate ($P<0.01$). The 'uncontrollable barrier' subscale was positively correlated to kidney graft rejection ($P<0.01$), thus demonstrating the ITBS's validity. Males and older patients reported more adherence barriers ($P<0.05$).

The ITBS is contained in the "Non-compliance" questionnaire.

Immunosuppressant therapy adherence instrument (ITAS)

The Immunosuppressant therapy adherence instrument (ITAS) is a five-item scale was developed that asked 122 respondents to indicate how often they were nonadherent to immunosuppressant therapy (IST) given a particular circumstance. The four-item scale, adherence measured by IS RRARs, and "target" IS serum concentrations had positive correlations ($p < 0.01$). Item scores were shown to be negatively related to rejection occurrence and increased SCr ($p < 0.05$). The immunosuppressant therapy adherence scale is the first published, valid and reliable instrument that measures recipients IST adherence. A german translation is validated as well.

Pulmonary function:

Spirometry according to ATS standards will be performed recording forced exspiration volume in 1s [FEV1] and the inspiratory vital capacity and maximal expiratory flow at 25-75% VC. Total lung capacity, residual volume will be recorded by bodyphlethysmography Diffusion capacity, Capillary blood gas analysis will measure pO2, pCO2, Hb, Hb-CO. BOS staging will be performed according to the International Society of Heart and Lung Transplantation system at the first and the last visit. Baseline (or best) FEV1 will be defined as the average of the two highest measurements obtained at least 3 weeks apart during postoperative course.

5.6 Adverse Events and Other Safety Aspects

5.6.1 Definition of Adverse Events (AEs)

An Adverse Event (AE) is defined as any untoward medical occurrence in a subject administered a study drug and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug. Abnormal laboratory findings will be rated as AE, if a clinical action is performed or the investigator defines these as clinical significant.

If a diagnosis is made from the sign and/or symptom, the diagnosis should be recorded in preference to the listing of individual signs and symptoms. If not, the investigator should record each sign and symptom as an individual AE.

An Adverse Reaction (AR) is defined as any prejudicial and unintended reaction to the study drug, independent from the dose. The classification as reaction will be done when a relation of the event to the study drug is at least considered as possible.

An Unexpected Adverse Reaction (UAR) is a side effect whose modality or severity does not conform to the existing information concerning the study drug.

5.6.2 Definition of Serious Adverse Events (SAEs)

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

Results in death

Is life threatening (an event in which the subject is at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe)

Results in persistent or significant disability/incapacity

Results in congenital anomaly, or birth defect

Requires inpatient hospitalization or leads to prolongation of hospitalization (hospitalization for treatment/observation/examination caused by AE is to be considered as serious)

Other medically significant events

A suspicious case of an unexpected serious adverse reaction will be defined as Suspected Unexpected Serious Adverse Reaction (SUSAR). A serious adverse reaction is unexpected when it is not reported in the appropriate base document such as Investigators Broschure (IB), Investigational Medicinal Product Dossier (IMPD) or summary of product characteristics (Fachinformation, SMPC).

~~All rejections (refer to section 5.3.1) have to always be reported as SAE regardless of the compliance with the above mentioned seriousness criteria. These events will be provided to the DSMB for review.~~

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require

intervention to prevent one of the other outcomes listed in the definition above (i.e. a medically significant event). Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

If a subject becomes pregnant during treatment, this should be reported as if it were a SAE. Refer to **Section 5.6.7. Procedure in Case of Pregnancy**.

5.6.3 Criteria for Causal Relationship to the Study Drug

Adverse events that fall under either "Possible" or "Probable" should be defined as "adverse events whose relationship to the study drugs could not be ruled out."

Causal Relationship to the Study Drug	**Criteria for Causal Relationship**
Unassessable / Unclassifiable (1)	A report suggesting an adverse reaction which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.
Definitely not Conditional / Unclassified (2)	A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and/or in which other drugs, chemicals or underlying disease provide plausible explanations.
Unlikely (3)	A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.
Possible (4)	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Probable / Likely (5)	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on re-administration (rechallenge) or withdrawal (dechallenge).
High probable / Certain (6)	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which

	cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary

5.6.4 Criteria for Defining the Severity of an Adverse Event

- Mild: No disruption of normal daily activities
- Moderate: Affect normal daily activities
- Severe: Inability to perform daily activities
- Life-threatening

5.6.5 Obligations to produce records and to cooperate of the Investigator (§ 13 (1)-(6) GCP-V according to § 42 AMG)

SAEs

The Investigator has to inform the Sponsor (KS-MHH) immediately (within 24 hours) about the occurrence of a Serious Adverse Event (SAE). During SAE follow up a detailed written report should be submitted to the Sponsor. Additionally all SAE´s will be reported to Astellas Pharma and Institute for Clinical Pharmacology at the Hannover Medical School by the Investigators.

AEs

~~The Investigator should report to the Sponsor within three months about occurred Adverse Events (AEs) and their clinical diagnostic findings.~~ The current Adverse Event list will be reported to the Sponsor every 6 months by the Investigator.

5.6.6 Obligations to produce records and to cooperate of the Sponsor (§ 13 (1)-(6) GCP-V according to § 42 AMG)

AEs

The Sponsor has to document in detail all Adverse Events that were announced to him and to report those to the competent authority (BfArM) on demand.

SUSAR

The Sponsor has to report immediately, lately within 15 days after knowledge, about any Suspected Unexpected Serious Adverse Reaction (SUSAR) to the Ethics commission and to the competent authority (BfArM).

The Sponsor has to report immediately, lately within 7 days after knowledge and within maximal 8 more days all further relevant information about any Suspected Unexpected Serious Adverse Reaction

(SUSAR) that has resulted to death or has been life threatening to the Ethics commission and to the competent authority (BfArM).

Anew revision of the risk-benefit evaluation

The Sponsor has to report immediately, lately within 15 days after knowledge, about any incident that requires an anew revision of the risk-benefit evaluation to the Ethics commission and to the competent authority (BfArM). Including:

- Case report of expected serious adverse reactions with an unexpected outcome
- Increase of the occurrence of expected serious adverse reactions, which are not evaluated as clinically relevant
- Suspected Unexpected Serious Adverse Reactions, which occurred, after the participating patient has already completed the study
- Incidents in connection with the study conduction or the development of the study drug which possibly affect the safety of the participating patients

List of all Serious Adverse Reactions and Safety Reports

Annual or on demand during the conduction of the clinical trial the Sponsor has to report to the Ethics commission and to the competent authority (BfArM) a list with all occurred Suspected Unexpected Adverse Reactions and a report about the safety of the participating patients.

5.6.7 Follow-up to Adverse Events

All adverse occurring during the study are to be followed up until resolved or ~~judged to be no longer clinically significant, or until they become chronic to the extent that they can be fully characterized~~ maximum 30 days after the end of study.

If during adverse event follow-up, the case has progressed to the level of “SAE”, or if a new SAE, whose relationship to the study drug(s) could not be ruled out, is observed, the situation must be reported immediately by the investigator becoming aware of the information. The sponsor may request follow-up information for specific cases on an adhoc basis if more data is thought to be required for an adequate safety assessment.

5.6.8 Procedure in Case of Pregnancy

If a woman becomes pregnant during the study dosing period or within 28 days from the discontinuation of dosing, the investigator should report the information to the sponsor as if it was a SAE. The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated fertility date, pregnancy result and neonatal data etc., should be included in this information.
The investigator will follow the mother as well as the fetus concerned as if it is an SAE and report the outcome to the sponsor.
When the outcome of the pregnancy falls under the criteria for SAEs [e.g. spontaneous abortion, induced abortion, complications during pregnancy, stillbirth, death of newborn, congenital anomaly (including anomaly in a miscarried fetus)], aberration of the child during the first twelve months after birth, the investigator should respond in accordance with the report procedure for SAEs. Additional information regarding the outcome of a pregnancy (which is categorized as an SAE) is mentioned below.
Spontaneous abortion includes abortion and missed abortion.
Death of an infant within 1 month after birth should be reported as an SAE regardless of its relationship with the study drug.
If an infant dies more than 1 month after birth, it should be reported if a relationship between the death and intrauterine exposure to the study drug is judged at least as “possible” by the investigator,
In the case of a delivery of a living newborn, the “normality” of the infant is evaluated at birth.
“Normality” of the miscarried fetus is evaluated by visual examination unless test results which indicate a congenital anomaly are obtained prior to miscarriage.

Any pregnancy should be reported to the health authorities in the Annual Safety Report. If the report includes an SAE, this may require expedited reporting.
If it is estimated that it is a matter of a Suspected Unexpected Serious Adverse Reaction (SUSAR), this should be reported according to the regulatory guidelines.

5.6.9 Supply of New Information Affecting the Conduct of the Study

Any change of the study protocol or status will be reported to the internal review board and federal authorities (BfArM).

5.7 Test Drug Concentration

Tacrolimus whole blood trough levels are routinely monitored locally using EMIT or HPLC-MS/MS analysis. Up to 2 ml blood are required per sample, the amount may vary according to analysis method. The blood samples should be taken in the morning before administration of tacrolimus. Tubes and tubing made of PVC must not be used. The whole blood trough levels should be assessed two to three times per week during hospitalization, at each outpatient visit and whenever clinically indicated.

6 TERMINATION OF THE CLINICAL STUDY

The study will be terminated after last patient out.

7 STATISTICAL METHODOLOGY

Within the standard procedures of a lung transplanted patient, the patient generally has 40 to 50 visits to the hospital ambulance (or a general practitioner). On a routine basis Tacrolimus trough levels will be measured at each visit. The proportion of the Tacrolimus trough levels below the norm-level of 8 where in addition more than 50% of prescribed Prograf® or Advagraf® doses have not been taken appropriately during the last three days before measurement of the trough level according to the automatic dispenser will be determined for each study participant.

The primary aim of this clinical trial is to reject the null hypothesis that the mean of the proportions of too low Tacrolimus trough levels caused by non-compliance from patients that take Prograf® is equal to the mean of the proportions of patients that receive Advagraf®.

The standard deviations in each group are assumed to be equal. A two-sided t-test for two independent groups will be used to assess the hypothesis and the null-hypothesis will be rejected if the respective P-value is less than 0,05. The percentage of noncompliant observations per patient will be transformed with an arcsin transformation before applying the t-test in the primary analysis. The respective 95%-CI for the difference in means will be back-transformed for presentation of results.

Sample size calculation

Within 12 months a maximum number of 50 patients will be available at Medizinische Hochschule Hannover.

Sample size calculation is based on 44 lung transplanted patients that came for check-up to the ambulance between 1.7.2005 and 21.12.2006. For these patients ten trough levels were available. The average number of Tacrolimus trough levels below the norm-level of 8 was 33,4. The corresponding standard deviation 26,9. No information on how this numbers are reduced by incorporating compliance control by electronic measurement are available.

Three different scenarios are given below, under which circumstances the study can be successful. For each scenario we assume a two-sided type I error of 5%. The sample size per group is given for a power of 80% to detect a difference between group means with the two-sided t-test for two independent groups.

	Scenario		
	1	2	3
mean Prograf® group	20	25	30
mean Advagraf® group	10	12,5	15
common standard deviation	12	15	18
n per group	24	24	24

Thus with the supposed to be available sample size of 24 patients in each group the study will have 80% power to detect a reduction in mean proportion of too low trough levels caused by non-compliance from 20% to ten (or 25% to 12,5% or 30% to 15%) assuming that the common standard deviation is 12% (or corresponding 15% or 18%) with a 0,05 two-sided significance level.

8 OPERATIONAL AND ADMINISTRATIVE CONSIDERATIONS

8.1 Access to data and documents

As an investigator study no patient data will be reported to Astellas Phama. Astellas Pharma will receive a final study report.

8.2 Data management and collections

Data collected on each subject will be recorded on a case report form (CRF). The investigator is responsible for ensuring that all sections of each CRF are completed correctly, and that entries can be verified against source data. If certain data are not available or not applicable this will be indicated as such on the appropriate space on the CRF. Any errors should have a single line drawn through them and the correct data should be entered at the side with the investigator´s initials, the data and a short reason for the change. In order to facilitate further handling, CRFs should preferable be completed with a black ball-point. The study monitor will review the CRFs and check them for completeness.

Data of screening examination will first be kept in separate subject files (source data files) and will be entered into the Case Report Form (CRF) only if the subject is eligible for study participation and the data were verified by any investigator. Addenda of the CRF (i.e. clinical laboratory reports, ~~ECG printouts~~) should bear the study number, subject number, study day and time, and signature of the investigator.

Adverse events,~~ concomitant medication data~~ and clinical observations will be recorded on source data forms and will be transferred into the CRF after the investigator's assessment.

Other data of medical measurement without print-outs (time of study activities, i.e. time of blood urine sampling, administration of study medication) performed during the study will be entered directly into the Case report Form and will be handled as source data. All source data will be attached to the for this reason created “data source” folder. Clinical laboratory parameters will be provided in laboratory print-outs which are to be signed by the investigator. Comments on all clinically significant abnormal values should be given by the investigator on these print-outs.

9 QUALITY ASSURANCE

9.1 QA at Department of Respiratory Medicine

Histopathology of allograft rejection and clinical staging of BOS will be performed according the current criteria established by the International Society of Heart and Lung Transplantation. Spirometry was performed according to ATS/ERS guidelines (19). Clinical acute rejection was defined as any biopsy > grade 1 or clinically as a reversible deterioration of graft function responding to steroid pulse therapy (15 mg/ kg methylprednisolone for three days, maximal 1000 mg/d) after ruling out other conditions.

BAL will be performed according to ATS standard recommendations.

9.2 Study Monitoring and Auditing

The Sponsor (KS-MHH assigned by MHH) of this study is responsible according to ICH GCP guidelines for assuring proper study conduct as regards protocol adherence and validity of the data recorded on the CRF´s.

Data Quality Control (QC) will be performed by the appropriate departments of the MHH.

Quality Assurance (QA), in form of protocol, ICF, CRF, report, in-house and on-site audits, will be performed by the QA Unit of the MHH.

10 STUDY ORGANIZATION

10.1 Sponsor responsibilities

It is an Investigator Initiated Trial by the Department of Respiratory Medicine of the Hannover Medical School. All organizational issues will be done by the department. The Hannover Medical School will take the Sponsor role and will assign the KS-MHH for reviewing and assuring that the clinical study obeys AMG and GCP regulatories.

According to § 4 AMG the Sponsor (KS-MHH) will take responsibility for the inducement, organization and financing of the clinical trial. Sponsor and Investigator assure that the clinical trial will be conducted in accordance with the established laws and instructions according to ICH-GCP-

Guidelines (1996), declaration of Helsinki (1996) as well as the directives of the AMG and the GCP-V (2004). The Investigators accept the requirements by signing the study protocol.

10.2 Study funding

Astellas Pharma GmbH will give a financial support to the study. The Department of Respiratory Medicine of the Hannover Medical School will take responsibility for assuring the financing and conduction of the study according to § 4 AMG.

10.3 Subject insurance

On behalf of the Sponsor the mandatory patient insurance according to § 40 para. 1 Nr. 8 and para. 3 AMG for all participating patients was concluded with the following insurance company:

Allianz Versicherungs-AG, 10900 Berlin
Probanden-Jahresvertrag Nr. GHA 30/0446/5302393/490

Due to this, any damages of health during the conduct of the study are insured with a maximal amount of coverage of 500.000 € per patient. This insurance covers all damages that will occur indirectly or directly to the patient by the study medication or interventions in connections with the clinical study.
A copy of the insurance certificate will be handed out to the participating patients together with the informed consent form.

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