AN OPEN, MULTICENTRE, RANDOMISED, PARALLEL GROUP STUDY TO COMPARE IN MARGINAL OLD-FOR-OLD RENAL TRANSPLANT PATIENTS THE SAFETY AND EFFICACY OF TWO TREATMENTS: SEQUENTIAL MYCOPHENOLATE MOFETIL / DELAYED TACROLIMUS PLUS STEROIDS VS TACROLIMUS PLUS MYCOPHENOLATE MOFETIL IN PATIENTS REQUIRING INDUCTION WITH ANTI-IL2 MONOCLONAL ANTIBODY

SHORT TITLE: TIGRE DOMATA

(Trial Italiano Gestione REni DOnatori MArginali in Trapiantati Anziani)

CLINICAL TRIAL NO: FG-506-02-IT-01

EudraCT Number:

Clinical Study Report

Study Drug Name:	Tacrolimus (FK 506)
Indication:	Renal transplant
Document Number/MGC Number:	FG-506-02-IT-01
Date of Document:	May 26th ,2004
Study Initiation Date:	3rd Quarter 2004
Study Completion Date:	1st Quarter 2008
Responsible Officer or Designee:	Dr. [redacted] and Dr. [redacted]
Sponsor:	Original sponsor was Fujisawa GmbH and following a company merger this became Astellas Europe.

This study was performed in compliance with Good Clinical Practice (GCP).

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List of Abbreviations

AE	Adverse Event
ALT	(SGPT) Alanine Amino Transferase (Serum Glutamic-Pyruvic Transaminase)
AST	(SGOT) Aspartate Amino Transferase (Serum Glutamic-Oxaloacetic-Transaminase)
ATN	Acute Tubular Necrosis

ATG	Anti-human T-Lymphocyte Globulin
AZA, Aza	Azathioprine
Cl	Clearance
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitors
Cr, Creat	Creatinine
CRF	Case Report Form
Cya	Ciclosporin
CYP	Cytochrome
CV	Curriculum Vitae
CVA	Cerebral Vascular Accident
d	day
DGF	Delayed Graft Function
dL	deciliter
DKG	Double Kidney Transplant
ECD	Expanded Criteria Donor
ESP	Eurotransplant Senior Program
FK	Tacrolimus
FKBP	Tacrolimus Binding Protein
FPG	Fasting Plasma Glucose
g	gram
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
H	hour
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
i.v.	Intra Venous
ICH	International Conference of Harmonization
IL	Interleukin
INN	International Non-Propietary Name
kg	kilogram
KTx	Kidney Transplant
LBW	Lean Body Weight
L	Liter
LDL	Low Density Lipoprotein
mg	milligram
µg	Microgram
mL	Milliliter

min	minute
MMF	Mycophenolate Mofetil
mMol	Millimolar
MPA	Mycophenolic Acid
ng	nanogram
n	Number
NCR	No Carbon Required
NSAIDS	Nonsteroidal Anti-Inflammatory Drugs
OKT3	Anti-CD3 Monoclonal Antibody
OPTN	Organ Procurement and Transplantation Network
p.o.	per os
PGL	Post Glucose Load
PRA	Panel of reactives antibodies
PTD	Post Transplant Diabetes
PVC	Packed Cell Volume
SAE	Serious Adverse Event
SOP	Standard Operatives Procedures
SPC	Summary of Product Characteristics
TAC, Tac	Tacrolimus
UNOS	United Network for Organ Sharing
WBC	White Blood Cell
WHO	World Health Organisation

List of Terms

Treatment Arm 1 Sequential mycophenolate mofetil / tacrolimus / steroids

Treatment Arm 2 Tacrolimus / mycophenolate mofetil / steroid one single dose (peri-operatively)

1 ETHICS

The study protocol (dated 26 May 2004) and the subject information documents and informed consent forms were submitted to independent ethics committees in the participating centers. An ethics committee's approval was a pre-condition for study initiation in the country, details, including names and addresses of all ethics committees are in Appendix 13.3.

The study protocol was amended 3 times overall; these amendments are detailed in Section 4.1.3.

The study was conducted in accordance with the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice and with the principles of the Declaration of Helsinki.

All patients were informed of the nature and purpose of the study and their written informed consent was obtained before screening. The investigator discussed the nature of the study, its

requirements and restrictions with each potential participant and/or his/her parents or legal representative. The patient and/or parents or legal representative was granted adequate time to read and understand the patient information and to ask questions. Written informed consent had to be obtained before any study-related procedures were performed.

The protocol and amendments are provided in Appendix 13.1. The IEC consulted and the subject information documents and informed consent forms are provided in Appendix 13.4.

2 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Investigators

The study was performed in 14 centers located in Italy in which patients were recruited.

The coordinating investigator was [redacted].

List of Participating Investigators and Centres

Center code	Principal investigator	Institution	City
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Dr. [redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Dr. [redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Dr [redacted]	[redacted]	[redacted]
[redacted]	Dr [redacted]	[redacted]	[redacted]

		[redacted]	
[redacted]	Dr. [redacted]	[redacted]	[redacted]

2.1 Monitoring

The study was monitored by qualified and trained clinical monitors from CRO (Contract Research Organisation) [redacted] until 30 May 2007, [redacted] from 1 June 2007.

2.2 Study Management

Study Managers: [redacted]

Study Biostatistician: [redacted].

Data Manager: [redacted] – [redacted] (until 30 June 2008); [redacted] (from 1 July 2008).

Representative Regulatory Affairs: [redacted]

2.3 Data Management and Statistics

Data management was performed by [redacted] (until 30 November 2006) and [redacted] (from 1 December 2006). Randomization sequences were prepared by [redacted].

2.4 Clinical Trial Supplies

Study medication was managed by Fujisawa GmbH until March 2005, and, [redacted] until August 2006, and [redacted] from September 2006.

2.5 Clinical Study Report

The clinical study report was written by [redacted], and reviewed by [redacted] and [redacted], Study Manager, [redacted] - [redacted].

INTRODUCTION

2.6 Background Information

2.6.1 Tacrolimus

Tacrolimus is a lipophilic macrolide produced by Streptomyces tsukubaensis and is a potent and novel immunosuppressive agent[1]. The compound was first isolated in 1984 in the laboratories of Fujisawa Pharmaceutical Company Ltd, Japan.

In vitro experiments have shown that tacrolimus is 10 to 100 times more potent than ciclosporin in inhibiting T-cell proliferative responses including murine and human mixed lymphocyte reactivity, cytotoxic T-cell generation, the production of T-cell-derived soluble mediators such as IL-2 and γ-interferon, and the expression of IL-2 receptors in the mixed lymphocyte reaction[2].

Tacrolimus and ciclosporin have a similar primary mode of action. Both tacrolimus and ciclosporin bind with high affinity to intracellular proteins, their respective cytoplasmic receptors, called immunophilins (tacrolimus to FK binding protein (FKBP) and ciclosporin to cyclophilin). Tacrolimus is mainly metabolised by cytochrome P450 3A4, and can therefore interact with other drugs using the same metabolic pathways, including ciclosporin [3,4].

Tacrolimus has been used to prevent allograft rejection in a variety of animal transplant models including heterotopic heart transplantation in rats[5] and mice[6], renal transplantation in dogs[7], liver transplantation in rats[8], liver transplantation in dogs[9] and renal transplantation in primates[9]. These studies demonstrated the potent immunosuppressive effects of this drug in vivo and, in some cases, the establishment of tolerance[9]. Clinical studies[10, 11] have confirmed the efficacy of tacrolimus in liver transplantation, and indicate that tacrolimus has advantages over conventional ciclosporin-based immunosuppressive regimens with regard to incidence of rejection and steroid requirements.

Twelve month results from a European multicentre kidney transplant study, which included a total of 448 patients in a comparative study of tacrolimus and ciclosporin based therapy, showed a significant advantage for patients treated with tacrolimus in terms of prevention of rejection episodes but with a comparable safety profile to that of ciclosporin[12]. Superior efficacy of tacrolimus was significantly demonstrated for steroid-sensitive acute rejection, steroid-resistant acute rejection and antibody-sensitive acute rejection[12]. These results are confirmed by a US multicentre study, in which 205 patients were treated with a tacrolimus based regimen and 207 patients were treated with a ciclosporin based regimen[13].

Tacrolimus has regulatory approval in Europe, USA, and Japan for a variety of indications ranging from primary liver and kidney transplantation, to rescue use in liver, kidney and heart transplantation.

3.1.2 Tacrolimus in Combination with Mycophenolate mofetil

Mycophenolate mofetil is an inhibitor of the de novo purine synthesis with apparent selectivity for B and T lymphocytes[14] and has been developed as a replacement for azathioprine for use in conjunction with ciclosporin. Recent phase III studies demonstrate that MMF is superior to both placebo and azathioprine when used in combination with ciclosporin and steroids[15, 16, 17]. 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 ciclosporin and steroids.

Recently, the combination of tacrolimus and MMF has been evaluated in a dose ranging study comparing tacrolimus/steroids, tacrolimus/1g MMF per day/steroids and tacrolimus/2g MMF per day/steroids in 232 patients. The combination of tacrolimus with 1g and 2g 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 1g and 2g MMF groups. All three treatment arms had a comparable safety profile, although diarrhoea and leucopoenia - known to be more frequently observed with the use of MMF[15, 16, 17] - were most pronounced in the 2g MMF arm. It was concluded that the combination of tacrolimus, 1g MMF, and steroids is a safe and effective regimen for rejection prophylaxis following kidney transplantation[18]. In a US multicentre dose comparison study of MMF in combination with tacrolimus the control arm received a tacrolimus-Aza-steroid triple regimen[19]. The 2g/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 patients 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 patients received antibody induction.
In a more recent study comparing three different immunosuppressive regimens, 223 Kidney transplanted patients were randomised to receive either Tac-MMF-steroids, Tac-Aza-steroids or CyA-MMF-steroids based regimen. Study results show a similar incidence of acute rejection, patient and graft survival for the three different treatments schedules. The combination of Tac-MMF (2g/d) demonstrated, never-theless, its superiority in terms of incidence of steroid resistant rejection at one year[20]. Study results were confirmed at two years[21].
A pilot study conducted in Spain has also proved the efficacy of a Tac-MMF-steroids based regimen in the treatment of renal transplanted recipients receiving grafts from old donors. The mean age of patients was 65.8 years while donors mean age was 63.3 years. A total of 35 patients was treated with Tacrolimus 0.1 mg/kg/d, MMF 2g/d and steroids 0,5 mg/Kg/d. Patients and graft survival were 94% and 88% at one and two year respectively. No cases of graft loss other then in patient exitus were reported[24].

3.1.3 Steroid Withdrawal

The use of steroids in combination with other agents has been the mainstay in immunosuppression for three decades despite the well known deleterious side effects associated with their long-term use. These are Cushing's Syndrome (moon face and trunk obesity) and secondary disorders such as cardiovascular problems, gastrointestinal complications, and diabetes. Hyperlipidaemia poses a significant management issue following renal transplantation and has been linked to post- transplant cardiovascular disease with reported high morbidity and mortality rate[23-29]. There is also a considerable amount of evidence associating hyperlipidaemia with the progression of chronic transplant nephropathy in addition to its correlation with atherosclerosis[30-35]. Registry data show that patients in which steroids were withdrawn from a ciclosporin regimen had a significantly higher 5-year graft survival than those where steroids were maintained.
There is also evidence that the steroid withdrawal can be considered safe in patients receiving a tacrolimus-based regimen. Shapiro et al. reported that 44% of Tac patients who had functioning kidneys were taken off steroids at a median follow-up of 1.12 year[36]. All ciclosporin patients required chronic steroid therapy. In a later publication Shapiro et al. reported that 49% of the successfully transplanted adult patients receiving tacrolimus had stopped steroids. A detailed follow up confirmed that these patients had excellent long term graft survival and function[37]. 62% of paediatric patients also achieved steroid withdrawal[38].
In another prospective trial using tacrolimus for the treatment of refractory acute rejection (i.e. patients with a need for increased immunosuppression) 10 out of 11 patients who participated were withdrawn from steroids and remained free of steroids[39].
There have been a number of studies demonstrating that steroid withdrawal leads to a 15-30% reduction in serum cholesterol levels bringing many patients back to normal levels[40-48].
A comparison of ciclosporin- and tacrolimus-based regimens in renal transplant recipients revealed significantly lower serum cholesterol and LDL levels, and less use of lipid-lowering drug treatments, in the tacrolimus group at 12 months[49]. Patients switched from ciclosporin to tacrolimus showed a drop in serum cholesterol to pre-transplant levels suggesting that

ciclosporin promotes hyperlipidaemia while tacrolimus is neutral in this respect[50]. It would therefore be expected that the benefit of steroid withdrawal in terms of cardiovascular parameters should be most dramatic with a tacrolimus-based regimen.
In 27 patients treated with Tacrolimus / MMF triple regimen it was possible to withdraw steroids in some patients as early as 7 days post transplant[51]. In this study there was an overall 27% acute rejection incidence (mean follow-up 6 months) but only 17% experienced rejection after withdrawal of steroids. It was suggested in this study that the early rejection episodes were due to a delay in attaining therapeutic MPA levels. Immunosuppressive cover during this period using monoclonal antibody, may facilitate a steroid free regimen at an acceptable acute rejection incidence.
A recent trial is investigating early steroid withdrawal or steroid free regimes. This is a Canadian study in which daclizumab/MMF/ciclosporin regimen, totally without steroids, is being tested[52]. Fifty-seven patients were enrolled into the study and
although short term at present, these results are comparable to those achieved with steroid containing protocols.

2.2 Rationale for the Study

The number of patients in dialysis waiting for transplantation is currently increasing at a very fast rate, while the number of organ available is not sufficient to meet this growing request.
In an effort to overcome the disparity between supply of cadaveric donors and demand, various strategies have emerged to expand the existing donor selection criteria.
A recent analysis of American Society of Transplantation data showed that in 15-20% of transplantations organs from "expanded criteria donors" are used.
The use of marginal kidney has been shown to be associated with a significant survival benefit when compared with maintenance dialyses[53].
The growing interest on the possible use of organs from marginal donors has led the scientific community to investigate which are the best strategies for transplant performance and allocation of marginal organs[22, 53-58].
Nevertheless, available data on the use of suboptimal organs have not completely overcome the reluctance in their use mainly for the lasting perception of possible poorer outcomes.
According to US OPTN/UNOS[62] (Organ Procurement and Transplantation Network/United Network for Organ Sharing) kidney allocation policy, effective October 30, 2002, expanded criteria donor (ECD) kidneys are defined by donor characteristics that are associated with a 70% greater risk of kidney graft failure when compared to a reference group of nonhypertensive donors of age 10 through 39 years whose cause of death was not a cerebral vascular accident (CVA) and whose terminal creatinine was less than or equal to 1.5 mg/dl.
While for the definition of ECD multiple criteria are applied for donors younger than 60 years of age, all donors greater than or equal to 60 years of age meet the ECD threshold and are included in the definition of expanded criteria donors[62].
Criteria of the ESP (Eurotransplant Senior Program) on donor's age are even stricter, and age of old marginal donor is established at 65 years [64,66].
As the consensus on multiple criteria is not univocal, this study included only kidney(s) from donors older than 60 years of age, defined as "marginal donors" and "marginal kidney(s)", transplanted in recipients older than or equal to 50 years of age (old-for-old allocation).

The choice of elderly recipients is due to ethical consideration, as the benefits of marginal old-for-old kidney transplant in this age group are demonstrated[62], while the use of marginal kidneys in other patient populations may be of concern.
The therapeutic trend towards the use of marginal kidneys is aimed to minimise the risk of nephrotoxic effects due to immunosuppression.
New protocols have been presented which evaluate the possibility of a delayed introduction of calcineurin inhibitors[59,60] or a total withdrawal of steroids[35-52], with encouraging results.
A report from the University of Torino[59] described data on the efficacy and safety of an experimental sequential protocol in an old-for-old allocation. Patients (mean age 76.9) receiving a single or double kidney transplantation (donor mean age 67.7) were initially treated with an induction therapy based on antilymphocyte globulins or antibodies anti CD25, MMF or AZA and Steroids. When serum creatinine reached a value lower than 2.5 mg/dL, MMF (or AZA) was withdrawn and replaced by tacrolimus (though levels 15 ng/mL). Steroid withdrawal starts at day 46.
Data analysis on 83 patients showed an overall low incidence of acute rejection: 12% (graft loss 2.4%) and low rate of infections 7.5%. DGF reported for 51% of patients with no advantage compared with historical data for conventional tacrolimus based therapy (46%), nevertheless observed DGF mean duration was significantly lower (6.1 vs 14.5 days, - P>0.001). A significant advantage was also observed in creatininemia values for non-DGF patients compared to conventional therapy. 82% of patients were also able to undergo a complete steroids stop within the 24th month of treatment.
A tacrolimus / MMF regimen is viewed by many as the best current immunosuppressive regimen in kidney transplantation and therefore its evaluation in this population is appropriate.
Both study groups were experimental, as experimental is the population in study, hence the character of this study is exploratory, because no reference standard regimen has reached a consensus.
Differentiated profiles were foreseen in the two groups: group 1 would have benefited of the delayed introduction of calcineurin inhibitor, aimed to a positive effect on renal function; group 2 would have benefited of the lack of steroids, aimed to a positive effect on the incidence of post-transplant diabetes.

3 STUDY OBJECTIVES

4.1 Objectives

To explore the safety and efficacy of a tacrolimus based regimen with delayed tacrolimus introduction and a steroid free tacrolimus based immunosuppressive regimen.

4.2 Primary Enpoints

4.2.1 Primary Efficacy Endpoint

Renal function measured by calculated creatinine clearance (Cockcroft formula) at month 6.

4.2.2 Primary Safety Endpoint

Incidence of PTD, evaluated according to WHO criteria at month 6.

4.3 Secondary Endpoints

- Acute rejection:
    - Incidence of and time to first biopsy proven acute rejection
    - Overall frequency of biopsy proven acute rejection episodes
    - Incidence of and time to first steroid-resistant acute rejection
    - Severity of biopsy-proven acute rejections (Banff 97 criteria)
- Patient survival
- Graft survival
- Renal function evaluated as creatinine value at months 3 and 6
- 24 h Proteinuria, at months 3 and 6
- GFR (calculated) and months 3 and 6
- Incidence and duration of DGF
- Incidence of adverse events

4 INVESTIGATIONAL PLAN AND METHODS

4.1 Study Design and Plan Description

4.1.1 Overall

This was an open, multicentre, randomised, parallel group, comparative phase III study, in elderly patients undergoing cadaveric kidney allograft transplantation from marginal donors (defined as donors > 60 years).

Patients were randomised to one of the following treatment arms:

Arm 1 : Sequential mycophenolate mofetil / tacrolimus / steroids

Arm 2 : Tacrolimus / mycophenolate mofetil / steroid one single dose (peri-operatively)

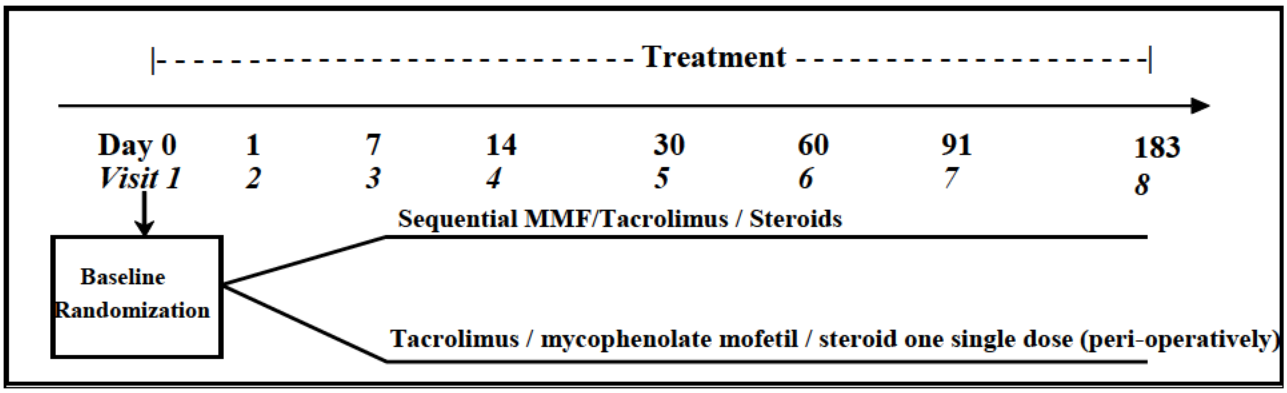
A total of 300 patients (150 patients per treatment arm) in 20 centers were to be included in the study. The duration of patient participation in the study was 6 months.

However the required number of recruited patients was not reached, due to low recruitment rate in most centres and active participation in the study of 14 centres only.

A total of 8 assessment visits were scheduled during the 6-month individual patient study period. Serious adverse events, as defined in the protocol, were recorded for each patient for an additional 28-day period after study end or study withdrawal.

The sequence and duration of the study treatment phase is depicted in the study flow diagram below:

Figure 1: Study Flow Diagram



The character of this study was exploratory, because no reference standard regimen has reached a consensus.
The statistically sufficient sized phase III study had to provide detailed efficacy and safety information to compare two investigational treatment groups, i.e. delayed administration of tacrolimus and withdrawal of steroids. Both groups were aimed to minimise immunosuppression and related risks while not increasing the risk of rejection, but different efficacy and safety profiles were foreseen, i.e. a better early renal function in group 1 and a lower incidence of PTD in group 2.

4.1.3 Amendment and Administrative Change Summary

The clinical study protocol was amended 3 times, the dates and reasons for amendment are as detailed in this section.

Amendment 1 (see appendices 13.1), a non-substantial amendment, dated August 19th 2005, affecting the following topics :

- Name of the sponsor and study medication manufacturing facilities

Amendment 2 (see appendices 13.1), a substantial amendment, dated April 4th 2006, affecting the following topics:

- Donor age
- Allowed steroid administration
- Exclusion criteria malignancies
- Initial dose 0,10 to 0,15 according to clinical judgment
- Clarification on analyses of single and double kidney blocks
- Change of patients number per center
- Prolongation of enrollment period to 3rd quarter, 2007

Amendment 3, (see appendices 13.1), a substantial amendment, dated November 1st 2007, related to change in sponsorship of clinical trial and name of study medication manufacturing facilities

4.2 Selection of Study Population

It was planned to recruit 150 patients in each treatment arm, thus 300 patients in total, with a minimum of 12 and a maximum of 40 patients per site.

However, due to low recruitment rate in most centres and active participation of 14 centres only, the foreseen number of recruited patients was not reached, even if recruitment period was prolonged.

4.2.1 Screening Assessments

Patients were assessed for compliance with inclusion and exclusion criteria and signed (or their parent, legal representative signed) informed consent prior to enrollment into the study.

4.2.2 Inclusion Criteria

1. Male or female patient ≥ 50 years of age with end stage kidney disease and suitable candidate for marginal cadaveric single or double kidney transplantation with compatible AB0 blood type
2. Patient requires induction with anti-IL-2 monoclonal antibody
3. Donor is > 60 years of age.
4. First transplant or re-transplant.
5. Patient has been fully informed and has given written informed consent according to ICH-GCP. Patient unable to write and/or read but who fully understands the oral information given by the investigator (or nominated representative) has given oral informed consent witnessed by an independent person.

4.2.3 Exclusion Criteria

1. Patients with an immunological high risk, defined as a PRA grade >50% in the previous 6 months .
2. Loss of the first transplant within 6 months from transplantation for immunological reason.
3. Patient is allergic or intolerant to steroids, macrolide antibiotics (e.g. erythromycin, clarithromycin), tacrolimus, mycophenolate mofetil (including other ingredients of steroids, MMF and Tac).
4. Patient or donor is known to be HIV positive.
5. Patient has significant liver disease, defined as having during the past 28 days

continuously elevated AST (SGOT) and/or ALT (SGPT) levels 3 times greater than the upper value of the normal range of the investigational site.

6. Patient with malignancy or history of malignancy < 5 years, except non metastatic basal or squamous cell carcinoma of the skin that has been treated successfully.
7. Patient has significant, uncontrolled concomitant infections and/or severe diarrhoea, vomiting, or active peptic ulcer.
8. Patient is participating or has participated in another clinical trial and/or is taking or has been taking an investigational drug in the past 28 days.
9. Patient has previously received or is receiving an organ transplant other than kidney.
10. Ischemia time > 24 hours.
11. Non heart beating donors.

4.2.4 Criteria for Withdrawal or Discontinuation

The patient was free to withdraw from the study for any reason and at any time without giving reason for doing so and without penalty or prejudice. The investigator was also free to terminate a patient's involvement in the study at any time if the patient's clinical condition warrants it. It was also possible that the sponsor or the competent authorities requested termination of the study if there were concerns about conduct or safety. All patients withdrawn from the study were followed up for serious adverse events for 28 days. Patients withdrawn from the study were not replaced by additional new included patients

Reasons for withdrawal were:

- the patient death
- the patient withdrew consent
- the patient lost to follow up
- the use of prohibited concomitant medications
- a protocol violation occurred, which led to withdrawal, as specified in the protocol
- graft failure (or retransplantation)
- failure in treatment of a steroid resistant rejection episode
- Investigator feeling that it was in the patient's best interest due to an adverse event

Reasons specific for patients in Arm 1 :

- Steroids dosing modification for > 15 cumulative days in days 0-45, unless associated with an adverse event (including current and previous rejection episodes and delayed graft function lasting more than 10 days)
- Delay of steroids withdrawal for > 5 days, unless associated with an adverse event (including current and previous rejection episodes and delayed graft function lasting more than 10 days)
- If MMF is withdrawn before day 14 with creatinine ≥ 3 mg/dL, unless related to an adverse event including rejection
- If MMF is not withdrawn when the conditions described in section 7.1.1 are reached
- If MMF dose is reduced to < 1.5 g/day or reduced to 1.5 g/day for more than 15 cumulative days

Reasons specific for patients in Arm 2 :

- If MMF dose is modified for > 15 cumulative days, unless associated with an adverse event (including rejection episodes)

The reasons for withdrawal had to be recorded in the CRF.
In the event of patient withdrawal, the investigator needed to complete the provided enrolment / withdrawal form stating the reason and date of withdrawal. This form had to be faxed to the sponsor within 24 hours after withdrawal**.** The fax number, as well as a contact name and telephone number in case of transmission difficulties had to be printed on the enrolment / withdrawal form.

At the end of each patient's participation in the study, the investigator had to complete all outstanding forms in the CRF. For patients prematurely withdrawn from the study the assessments of Visit 8 (Day 183) had to be performed, the study completion page completed, a follow-up to month 3 realised, and month 12 follow up information collected unless the patient specifically requests that the latter or the latter two was/were not done. Patients who prematurely discontinued were treated and followed according to established acceptable medical practice. All pertinent information concerning the outcome of such treatment were entered in the Case Report Form. For patients that discontinued prematurely due to death or a SAE a narrative description of the events leading to withdrawal had to be provided.

4.3 Treatments

4.3.1 Treatment Arm 1

4.3.1.1 Dosing and administration of mycophenolate mofetil

Initial mycophenolate mofetil dose

The initial daily dose was 2g/day p.o. given in two doses (equals 1g twice daily).
The first dose of 1g of mycophenolate mofetil had to be administered within 12 hours prior to reperfusion.
The second dose of 1g had to be administered within 72 hours of after reperfusion.
For subsequent mycophenolate mofetil dose, the daily dose of 2g had to be given orally and split into 2 equals doses. See Table "Arm 1 for Shift from MMF to tacrolimus".

TABLE 1. Arm 1 - Shift from MMF to tacrolimus

Day 0 – 14	MMF was withdrawn and tacrolimus administration started when creatinine < 3 mg/dL. MMF could be still administered 3 days after tacrolimus administration start, until tacrolimus has reached the target levels. If MMF was withdrawn and tacrolimus administration started before day 14 when creatinine ≥ 3 mg/dL, unless related to an adverse event including rejection, this was be regarded as a protocol violation and the patient was withdrawn.
Day 15 – 21	After day 14 and until day 21, MMF withdrawal and tacrolimus administration started when creatinine < 3 mg/dL and may start at Investigator's clinical judgment even if creatinine was ≥ 3 mg/dL. MMF could be still administered 3 days after tacrolimus administration start,

	until tacrolimus has reached the target levels.
Day 21 onwards	If a serum creatinine level < 3 mg/dL was not reached within day 21 under whichever treatment, this was regarded as a treatment failure and treatment was continue at investigator's discretion, but patients were not withdrawn from the study.
Day 0 - 21	If MMF was not withdrawn and/or tacrolimus administration not started when the above conditions are reached, unless related to an adverse event, except ATN, this was regarded as a protocol violation and patients were withdrawn from the study.

It was recommended that mycophenolate mofetil was administered on an empty stomach.
The occurrence of gastrointestinal disorders and leucopoenia might have required transient dose changes within the accepted ranges of the protocol or a modification of the daily dose split (e.g. into three doses).
Dose reduction to 1.5 g/day in two equal divided doses was allowed for a maximum of 15 cumulative days.
Dose interruption was not allowed. When the administration of MMF was stopped, tacrolimus administration had to be started.

4.3.1.2 Dosing and administration of steroids

Methylprednisolone or equivalent:

Day 0*: 1000 mg or less i.v. bolus * pre-, intra-, or post-operatively
Day 1: 125 mg i.v. bolus

Prednisone or equivalent:

Day 2-14: 20 mg p.o.
Day 15-23: 15 mg p.o.
Day 24-34: 10 mg p.o.
Day 35-45: 5 mg p.o.
Day 46-183: 0 mg

Oral administration had to be as a single daily dose.
Deviations from dosing:
- Day 0-45: dosing modification for > 15 cumulative days, unless associated with an adverse event (including current and previous rejection episodes and delayed graft function lasting more than 10 days) represented a protocol violation and resulted in patient withdrawal.
- Day 46-183:

a. delay of steroids withdrawal for > 5 days, unless associated with an adverse event (including current and previous rejection episodes and delayed graft function lasting more than 10 days) represented a protocol violation and resulted in patient withdrawal.
b. re-introduction of steroids for > 10 cumulative days after steroid stop, with the exception of steroid maintenance treatment, represented a treatment failure, but patients were not withdrawn from the study

4.3.1.3 Dosing and administration of tacrolimus

Initial tacrolimus dose

Tacrolimus administration had to be started when MMF was withdrawn, see Table: "Arm 1 – Shift from MMF to tacrolimus".
The initial daily dose was 0.10 to 0.15 mg/kg given orally in two doses (equals 0.050 to 0.075 mg/kg twice daily) according to clinical judgement.

Subsequent tacrolimus dose
Subsequent oral tacrolimus doses were adjusted on the basis of clinical evidence of efficacy and occurrence of adverse events, and observing the following recommended whole blood trough level ranges:

Day* start of administration-21:	10 -15 ng/mL (target 12 ng/mL)
Day* 22-41:	8 -12 ng/mL
Day* 42-183:	5 -10 ng/mL

The patient's status regarding rejection and toxicity always took precedence over whole blood trough levels when assessing the appropriate dose. As tacrolimus requires dosing defined for individual patients, the optimal whole blood trough level might have been outside the recommended ranges.

Tacrolimus dose modification:

The investigator could adjust the patient's dose and modify the tacrolimus dose regimen as deemed necessary to minimise adverse events and maintain effective immunosuppression. Due to a long half life of tacrolimus (approximately 16 hrs), it was recommended that dosing adjustments be limited to a maximum of 2 times per week as changes in trough blood levels occur slowly, usually only 48 to 72 hours after dose adjustment. Changes in tacrolimus dose were to be made in steps of 25% of the current dose. Temporary tacrolimus dose interruption could be considered if unacceptable drug-related side effects were observed. However, tacrolimus interruption for more than 7 days, unless this was related to an adverse event or the trough level being outside the assigned ranges, constituted a protocol violation and resulted in patient withdrawal.

Oral administration of tacrolimus

Tacrolimus was administered at the same time each morning and evening throughout the study (every 12 hours). Tacrolimus capsules were to be swallowed with fluid (preferably water) at least one hour before meals or two hours after meals in the morning and in the evening.

4.3.2 Treatment Arm 2

4.3.2.1 Dosing and administration of tacrolimus

Initial tacrolimus dose
The initial daily dose was 0.10 to 0.15 mg/kg given orally in two doses (equals 0.050 to 0.075 mg/kg twice daily), one pre-operatively and one post-operatively.
The pre-operative dose of 0.050 to 0.075 mg/kg of tacrolimus had to be administered after randomisation and within 12 hours prior to reperfusion and if possible within 3 hours prior to anaesthesia.
The first post-operative dose of 0.050 to 0.075 mg/kg of tacrolimus had to be administered either in the morning or evening according to normal hospital schedule. This dose was not to

be administered less than 4 hours after pre-operative dose or more than 12 hours after reperfusion.

Subsequent tacrolimus dose
Subsequent oral tacrolimus doses were adjusted on the basis of clinical evidence of efficacy and occurrence of adverse events, and observing the following recommended whole blood trough level ranges:

Day 0-21:	10 -15 ng/mL (target 12 ng/mL)
Day 22-41:	8 -12 ng/mL
Day 42-183:	5 -10 ng/mL

The patient's status regarding rejection and toxicity always took precedence over whole blood trough levels when assessing the appropriate dose. As tacrolimus requires dosing defined for individual patients, the optimal whole blood trough level might be outside the recommended ranges.

Tacrolimus dose modification:

The investigator could adjust the patient's dose and modify the tacrolimus dose regimen as deemed necessary to minimise adverse events and maintain effective immunosuppression. Due to a long half life of tacrolimus (approximately 16 hrs), it was recommended that dosing adjustments be limited to a maximum of 2 times per week as changes in trough blood levels occur slowly, usually only 48 to 72 hours after dose adjustment. Changes in tacrolimus dose were to be made in steps of 25% of the current dose. Temporary tacrolimus dose interruption could be considered if unacceptable drug-related side effects were observed. However, tacrolimus interruption for more than 7 days, unless this was related to an adverse event or the trough level being outside the assigned ranges, constituted a protocol violation and resulted in patient withdrawal..

Oral administration of tacrolimus

Tacrolimus was administered at the same time each morning and evening throughout the study (every 12 hours). It might have been necessary, depending on the time of administration of the initial dose, to adjust the time of administration of the second dose to allow subsequent dosing morning and evening in accordance with the normal hospital schedule. If this occurred an interval of at least 4 hours should have been maintained between administrations. Tacrolimus capsules were to be swallowed with fluid (preferably water) at least one hour before meals or two hours after meals in the morning and in the evening.

4.3.2.2 Dosing and administration of mycophenolate mofetil

Initial mycophenolate mofetil dose
The initial daily dose was 2g/day p.o. given in two doses (equals 1g twice daily).
The first dose of 1g of mycophenolate mofetil was administered within 12 hours prior to reperfusion.
The second dose of 1g was administered within 72 hours of transplantation, defined as time of reperfusion.

Subsequent mycophenolate mofetil dose

Day 0 – 14:	The daily dose of 2g should be given orally and split into 2 equals doses.
Day 15 – 183:	1g/day in 2 divided doses (equals 500 mg twice daily).

Patients had to stay on MMF for the duration of the trial. It was recommended that mycophenolate mofetil was administered on an empty stomach. The occurrence of gastrointestinal disorders and leucopoenia might have required transient dose changes within the accepted ranges of the protocol or a modification of the daily dose split (e.g. into three doses). Dose reduction or interruption for > 15 cumulative days, unless associated with an adverse event (including rejection episodes) constituted a protocol violation and resulted in patient withdrawal.

4.3.2.3 Dosing and administration of steroids

Methylprednisolone or equivalent:
Day 0*: 1000 mg or less i.v. bolus * pre-, intra-, or post-operatively
Day 1-183: 0 mg
Introduction of steroids for > 5 cumulative days, unless associated with an adverse event (including current and previous rejection episodes and delayed graft function lasting more than 10 days) constituted a treatment failure, but the patient was not be withdrawn from the study.

4.3.3 Treatment of rejection

Episodes of rejection were to be verified by biopsy and graded using the BANFF 97 classification
First line therapy for an acute rejection episode was steroids according to local practice. It was suggested to keep Methylprednisolone doses between 100 mg to 1000 mg per day.
If a biopsy indicated a severe vascular rejection antibodies might be given as first line therapy if it was local practice and continue subsequently on maintenance steroids.
If the rejection episode did not respond to steroids additional agents such as OKT3 or polyclonal antibodies might be used according to local practice.
A maximum period of 28 days was allowed to return to pre-rejection therapy.
Should treatment of rejection episodes necessitate the introduction of an immunosuppressant not foreseen by the protocol for the relevant arm, or necessitate the anticipated introduction of tacrolimus (Arm 1), patients were considered treatment failures but were not to be withdrawn from the study.

4.3.4 Identity of Investigational Products

A detailed description of the study treatments, including composition, packaging, labeling, and storage of study drugs, can be found in the study protocol (Appendix 13.1.1).

Tacrolimus Capsules, 0.5 mg, 1 mg, and 5 mg capsules were manufactured/packaged by [redacted]./[redacted].

Mycophenolate mofetil, 250 mg capsules were manufactured by [redacted].

4.3.5 Method of Assigning Subjects to Treatment Groups

Allocation of the patients to treatment was performed locally at each center using sealed randomization envelopes provided by the Data Operations department of Astellas Pharma GmbH.

4.3.6 Blinding

This was an open label study so there was no blinding necessary.

4.3.7 Treatment Compliance

Tacrolimus whole blood trough level measurements were used as guides to assess patient compliance during the study. Poor compliance in taking the baseline immunosuppressant was also used as an indicator for overall patient compliance.

4.3.8 Prior and Concomitant Medications and Therapies

Records of all concomitant medications taken seven days before randomization and throughout the study were entered in the CRF. Details of anaesthetics or other medications related to surgery were not required, except intraoperative steroids.

For a description of possible interactions of study medication with other drugs, please see Appendix 13.1, (Protocol appendix 4) .

Patients were instructed not to start any new medication, including any over the counter products, without first consulting the investigator.

Prohibited Concomitant Medications:

During the study all other systemic immunosuppressive medications apart from tacrolimus, mycophenolate mofetil, anti-IL-2 monoclonal antibody for induction, steroids, and mono-/polyclonal antibodies used for the management of rejection were prohibited.
During the study period all non-licensed medications and study medication administered as part of another clinical study were prohibited. They had to be discontinued, at latest, 28 days prior to transplantation.
Should the investigator consider the use of a prohibited concomitant medication as the most appropriate regimen, then the patient had to be be withdrawn.

4.4 Assessments, Endpoints, and Appropriateness of Measurements

4.4.1 Overview of Schedule of Procedures

Table 2 displays the study visit schedule.

Table 2: Study Visit Schedule

ASSESSMENTS	Visit 1 baseline (Day 0)	Visit 2 Day 1	Visit 3 Day 7	Visit 4 Day 14	Visit 5 Month 1	Visit 6 Month 2	Visit 7 Month 3	Visit 8 Month 6
Informed Consent[1]	x							
In-/Exclusion criteria	x							
Randomisation	x							
Patient data[2]	x							
Donor and donor organ data [3]	x							
Surgical details	x							
Blood pressure, pulse and body weight	x	x	x	x	x	x	x	x
Serum creatninine[4]	x	x	x	x	x	x	x	x

Dispensing/Collect study medication	x	x	x	x	x	x	x	x
Laboratory assessment [5]	x	x	x	x	x	x	x	x
Ongoing data collection: [6] – Rejection episodes – Serum creatinine – Serum glucose (fasting) – Adverse Events – Concomitant medication – Mycophenolate mofetil dose – Steroids dose – Days on dialysis – Days hospitalized								
– Tacrolimus dose & trough levels [7]	x.....x----------							x

1 Informed Consent must be obtained before any study related procedures are performed. Enrolment and randomisation into the study must occur prior to the pre-operative dose of any study medication.
2 Includes date of birth, sex, ethnic group, height, reason for the patients own kidney failure, start of dialysis, previous transplants, medical personal history and family history for diabetes, and secondary diagnoses, smoking habit, pre-study medication (7 days), viral status (HBV – superficial and core antigens and antibodies -, HCV, CMV. HIV) and date, AB0 blood type, HLA main types, PRA grade (last value in the previous 6 months) and date.
3 Includes date of birth (or age), sex, weight, viral status (HBV, HCV, HIV, CMV, EBV), AB0 blood type, HLA main types, cause of death, diuresis in the last 24 hours (if 24 hours value unknown, but if value for different period available, value and corresponding period), if non heart beating and if anatomical abnormalities or not, initial and final calculated creatinine clearance (Cockcroft formula), proteinuria, initial and final creatinine, biopsy score.
4 Serum creatinine must be measured at every protocol-defined visit. Renal function will be assessed by calculated creatinine clearance (Cockcroft formula).
5 If possible, all samples should be taken in the morning after a fasting period of at least six hours, preferably before study drug administration; list of laboratory assessments: see 4.2.2.
6 In addition to routine laboratory, clinically significant serum creatinine values obtained between study visits must also be recorded. Tacrolimus levels will need to be collected and recorded 2-3 times per week during hospitalisation. Thereafter, tacrolimus levels will be collected at every outpatient visit or when clinical/laboratory signs indicate an acute rejection and if signs of toxicity are observed. Data collection of various parameters relevant to drug treatment will be performed according to group assigned by randomisation.
7 Tacrolimus dose will start according to group assigned by randomisation. Measurement of tacrolimus trough levels will start the day after the first dose, according to group assigned by randomisation.
Visits dates and study time-points are calculated from the day of transplantation which is the day of reperfusion and defined as day 0. Visit 2 or post-operative assessment should be performed within 12 hours after reperfusion.
No deviations from scheduled visits are allowed for visit 1 and 2. Deviations from scheduled visits and specified time-points must not exceed ±1 day for visit 3, ±3 days for visits 4 and 5 and ±7 days for Visits, 6, 7 & 8.

4.4.2 Efficacy

4.4.2.1 Diagnosis and grading of rejection episodes

If clinical and/or laboratory signs indicated the occurrence of a rejection episode a renal biopsy must be performed.
The biopsy had to 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 was performed by the local histopathologist following the Banff (97 version) criteria, who should be unaware of the patient's treatment allocation at the time of this evaluation. The slides had to be stored locally, for a later central evaluation, if necessary.

4.4.2.2 Classification of acute rejection episodes

See Section 4.5.3.3.2 for definitions.

4.4.3 Safety

Safety parameters of interest were incidence of post-transplant diabetes mellitus (PTD), incidence of documented infections (confirmed by culture, biopsy or serology), overall incidence of adverse events, lipid profile.

4.4.3.1 Adverse events

Adverse Events, including clinically significant laboratory abnormalities, were assessed by investigators and recorded in the Case Report Form as described in Appendix 13.2, Protocol section 11.

An AE was defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

An AE could therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

4.4.3.1.1 Serious Adverse Event (SAE)

A serious AE was defined as any untoward medical occurrence that at any dose:

- resulted in death
- was life-threatening (the term life-threatening in the definition. "Serious" refers to an event in which the patient was 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 had been more severe)
- required inpatient hospitalization or prolongation of existing hospitalization
- resulted in persistent or significant disability/incapacity
- was a congenital anomaly/birth defect
- was an important medical event that requires intervention to prevent one of the above

Medical and scientific judgment had to be exercised in deciding whether expedited reporting was 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 patient or may require intervention to prevent one of the outcomes listed in the definition above. These had always to be considered serious.

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.

Additionally manifested signs and symptoms caused by overdose, and reports of cancer had to be handled in the same manner as serious AEs.

Hospitalization was not assessed as an SAE if hospitalization:

- was solely for the purposes of performing protocol-related procedures (e.g. blood collection for PK profiles)
- was solely for the purposes of performing routine procedures.

Any complication of a protocol-related procedure which fulfilled the previously-stated criteria for a SAE had to be reported as an SAE.

Furthermore, hospitalizations occurring under the following circumstances were not considered as SAE:

• hospitalizations which were planned before study entry or occured without being scheduled before study entry for a pre-existing, non-worsening condition

• hospitalizations not fulfilling the criterion of untoward medical occurrence (e.g. hospitalizations for elective cosmetic surgery, social and/or convenience admissions)

Inpatient hospitalization means the subject was formally admitted to a hospital for medical reasons. This may or may not be overnight.

Pregnancy was not considered an AE but to be reported on an SAE form and in the same timeframe as SAEs.

4.4.3.1.2 Relationship to Drug

The causality of an AE in relation to study medication as a whole was categorized as follows:

Highly Probable:

Apparent relationship in time between AE and drug administration or drug concentration in body and fluids or tissues. Relationship between AE and drug already known or to be expected. Disappearance or diminution of symptoms after stopping the drug, or reducing its dosage. Reappearance of symptoms after renewed administration of the drug.

Probable:

Apparent relationship in time between AE and drug administration. Relationship between AE and drug already known or to be expected. Disappearance or diminution of symptoms after stopping the drug or reducing its dosage. The adverse reaction cannot be explained by the patient's clinical status.

Possible:

Apparent relationship in time between AE and drug administration. Relationship between AE and drug already known or to be expected. Adverse reaction could also be explained by a number of other factors.

Unlikely:

Relationship in time between AE and drug administration not probable. Adverse reaction to be explained rather by other factors, a relationship to the drug however could not definitely be ruled out.

Definitely not:

Sufficient information to affirm that the AE was unrelated to the drug.

4.4.3.1.3 Severity of AE

The intensity of AEs was categorized as follows:

Mild: Patient was aware of symptoms, but is easily able to tolerate them.

Moderate: Patient experienced enough discomfort to interfere with normal activity.

Severe: Patient was unable to carry out normal activities.

4.4.3.1.4 Detection, Reporting and Responsibilities

The obligation to report AEs started with enrolment of the patient in a study. On an ongoing basis the investigator determined whether any clinical or laboratory AEs had occurred. The follow-up of all AEs was continued until the overall clinical outcome was ascertained. New SAEs occurring within 28 days after the patient completed the study or were withdrawn had to be reported to the sponsor (using the SAE report form).

Each AE had to be recorded in the CRF, describing date of onset, date stopped, seriousness, maximum intensity, relationship to study medication, therapy, outcome and, in case of an infection, site and type of infection.

In case of an AE, the investigator was responsible:

- for the correct and complete recording of all adverse events in the CRFs.
- for following up ongoing adverse events after completion of the clinical trial until the patient has recovered, or until no further change in the clinical condition could be expected, and to report their outcome.
- for reporting all SAEs to the sponsor within 24 hours by telephone or fax (using the SAE report form). This responsibility continued for 28 days after the end of the study for each patient.

The initial SAE report was to contain the following details: study number and centre code, patient number, initials, gender, date of birth, drug allocation, date of event onset, description of the event, name, address, and telephone number of the investigator.

As soon as more information were available, a follow-up SAE report was to be sent that included the clinical course of the SAE, relevant background and history, diagnostics, histology, outcome etc. For follow-up reports the same timelines applied as for initial notifications.

The investigator and sponsor were responsible for informing the Ethics Committee and competent authorities of SAEs where required.

4.4.3.2 Blood Pressure and Body Weight

Blood pressure and body weight were measured at each visit defined as per protocol, according to the hospital's routine procedure. Blood pressure had to be measured after 5 minutes of rest.

4.4.3.3 Laboratory Examinations

The laboratory values taken at Visit 1 (day 0) could not be older than 48 hours at the time of reperfusion.

Table 3 details at which visits the safety laboratory screens were to be performed. No deviations from scheduled visits were allowed for visit 1 and 2; deviations from scheduled visits and specified timepoints had not to exceed ±1 day for visit 3, ± 3 days for Visit 4 and Visit 5 and ± 7 days thereafter. All samples were to be taken in the morning after a fasting period of at least 6 hours, preferably before study drug administration. Each local laboratory had to provide a current and approved list of reference ranges including units for each parameter and a laboratory certificate.

Table 3: Laboratory Assessment Schedule

Lab parameters	Visit 1 baseline (Day 0)	Visit 2 Day 1	Visit 3 Day 7	Visit 4 Day 14	Visit 5 Month 1	Visit 6 Month 2	Visit 7 Month 3	Visit 8 Month 6
Biochemistry								
Sodium	X						x	x
Potassium	X						x	x
Calcium	X						x	x
Blood glucose	X						x	x
Glucose tolerance test (PGL)							x	x
Hb_{1AC}	X						x	x
SGOT/AST	X						x	x
SGPT/ALT	X						x	x
Total Bilirubin	X						x	x
Cholesterol	X						x	x
HDL	X						x	x
Triglycerides	X						x	x
Serum creatinine	X	x	x	x	x	x	x	x
Haemoglobin	X						x	x
Haematocrit	X							
WBC	X						x	x
Platelets	X						x	x
Urine Analysis								
Proteinuria 24 hrs							x	x
Viral Status								
CMV (early antigen)	X				x	x	x	x

HBV, HCV,EBV	X						x	x
HIV	X							

4.4.4 Additional measurements and ascertained data

4.4.4.1 Whole Blood Tacrolimus Trough Level Measurements

Monitoring of tacrolimus whole blood trough levels was performed using enzymatic immunoassay. Blood samples were drawn before the morning dose of tacrolimus was administered. Tubes and tubing made of PVC had to be avoided.
Blood samples (1.0 mL) were collected for determination of whole blood trough levels of tacrolimus 2-3 times per week during the first 4 weeks and thereafter routinely at visit.

4.4.4.2 Other Additional Data

A follow-up questionnaire asking for patient and graft survival, renal function, immunosuppression therapy, was used at month 12.

4.5 Statistical Methods

4.5.1 Planned Sample Size

In spite of the exploratory character of the study formal sample size calculations were carried out for the primary efficacy endpoint as well as for the primary safety endpoint.
The sample size calculation for the primary efficacy endpoint was based on the two-sided t-test for differences in the mean creatinine clearance (calculated from serum creatinine values by Cockcroft formula) at month 6 between the two treatment arms. A difference of 10 ml/min/1.73m2 in the mean creatinine clearance was considered as clinically relevant. For specified $\alpha = 0.05$ and assuming a standard deviation of 25 ml/min/1.73m2, with 133 patients per treatment arm the power was assumed at least 90% to detect a difference of 10 ml/min/1.73m2.

For the primary safety endpoint incidence of post transplant diabetes (PTD) the power was assumed 90% with 133 patients for incidence of PTD of 10% and 25% in the respectively treatment arms ($\alpha = 0.05$, chi-square test).
Assuming a dropout rate of about 10%, 150 patients per treatment arm were required, resulting in a total of 300 enrolled patients.
However the required number of patients was not reached, due to low recruitment rate in most centres and active participation of 14 centres only, even if recruitment period was prolonged. In total, 142 patients only were enrolled into the study in 14 centers (2 centres did not recruit any patients), 72 in the Sequential Tacrolimus/ MMF/ Steroids arm (Arm 1) and 70 in the Tacrolimus/MMF arm (Arm 2).

Hence, all results of following analyses are to be regarded as highly explorative.

Further details are provided in Section 14.1 of the study protocol (Appendix 13.1).

4.5.2 Populations for Analysis

All possible measures were taken to ensure that the primary endpoint variables were available for all patients included in the study. The difference between Full Analysis Set and Per Protocol Set was anticipated to be negligible. Therefore the intention was to show the consistency of results for the two study populations in all efficacy parameters. Should the results, however, differ between the two populations then the Full Analysis Set were to be considered primary. All safety analyses were done with the Safety Analysis Set. A patient listing was produced, containing assignment to the analysis set(s), and reasons for exclusion from each analysis set.

Full Analysis Set (FAS) include all randomized patients who received at least one dose of study medication and who were transplanted, with results attributed to the treatment group they were randomized.

Per Protocol Set (PPS) is a subset of the Full Analysis Set and include all patients who have the measurement of the primary endpoint variables available. In addition, a patient could be excluded from the Per Protocol Set in case of major protocol violations. The Per Protocol Population was defined during a blinded review before database closure.

Safety analysis set
The Safety Analysis Set included all randomized patients who received at least one dose of study medication.

4.5.3 Statistical Methodology

4.5.3.1 Definitions, Data Conversions, and Handling of Missing Data

All patients not withdrawn during *the whole 6-month period* of the study are called *completers*. The results for completers are additionally displayed where appropriate (e.g. tacrolimus dosing and trough levels, laboratory values, vital signs).

4.5.3.1.1 General Time Definitions

Day 0 was defined as the date of transplantation (recorded as 'date of reperfusion'). For patients who were not transplanted, Day 0 was the day of randomization. All other day definitions are relative to Day 0. Analysis of medication, adverse events, rejections, patient survival, graft survival, and treatment failure will be based on dates and days relative to Day 0.

Time-to-event analysis was performed on a Day basis. The analysis of laboratory data and vital signs was visit-based.

4.5.3.1.2 Definition of Days, Weeks and Months and Their Corresponding Time Windows

CRF Visit	CRF Visit Name	Analysis Day		Analysis Visit window	
Visit 1	**Baseline**	Day	0		
Visit 2	**Day 1**	Day	1		
Visit 3	**Day 3**	Day	7	±	1 day
Visit 4	**Day 14**	Day	14	±	3 days
Visit 5	**Month 1**	Day	30	±	3 days
Visit 6	**Month 2**	Day	60	±	7 days
Visit 7	**Month 3**	Day	91	±	7 days
Visit 8	**Month 6**	Day	183	±	7 days

Time Point

Time Period					
Week 1	Day	0	to	Day	7
Week 2	Day	8	to	Day	14
Week 3	Day	15	to	Day	21
Week 4	Day	22	to	Day	28
Month 2	Day	29	to	Day	61
Month 3	Day	62	to	Day	91
Month 6	Day	92	to	Day	183

Time Interval

EOS Maximum day of Visit 8 or follow-up visit, whichever was higher (this value was the same value for all patients).

Individual EOS Last visit for completers and lost to follow-up patients and follow-up visit for withdrawn patients.

Table 4: Event and censor times for the Kaplan-Meier analyses

Parameter	Event Time	Censor Time
Patient survival	Day of death	Day of last follow-up[*]/ day of last visit[#]
Graft survival	Day of graft loss	Day of last follow-up[*]/ day of last visit[#]
Rejection	Onset of first rejection	Day of withdrawal[*]/ day of last visit[#]

* for withdrawn patients
\# for completers and lost to follow-up

4.5.3.1.3 Handling of dropouts or missing values

1. The "worst-case scenario" approach was used when estimating the unknown parts of partial dates. For an incomplete start date the first day of the respective month was used if only the day was unknown. The first of January of the respective year was used if both day and month were unknown. For an incomplete stop date, the last day of the month or the 31st of December of the year was used, respectively. Estimated stop dates after the date of final visit or date of withdrawal were replaced by the date of final visit or date of withdrawal and were flagged as ongoing. Estimated start dates prior to Day 0 were replaced by the date of Day 0 if the known part of the date did not clearly indicate that the start date was prior to Day 0 (e.g. MAR2005 is clearly before 01APR2005). Otherwise estimated start dates prior to Day 0 were replaced by the last day of the respective month or the 31st of December of the respective year. That is, the estimated date was as close to Day 0 as possible without overwriting the known parts of the date.

2. Completely missing start dates were estimated by Day 0; completely missing stop dates were estimated by the date of final visit (for completers) or by the date of withdrawal (for withdrawn patients).

3. If a ***start date of medication dose change*** was missing or incomplete, it was estimated by making the time interval for the higher dose as long as possible, and the duration of the lower dose as short as possible. For example:

Medication	Start Date	Dose
tacrolimus	23/05/00	0.15 mg/kg
tacrolimus	**/05/00	0.2 mg/kg

was estimated as follows:

Medication	Start Date	Dose
tacrolimus	23/05/00	0.15 mg/kg
tacrolimus	24/05/00	0.2 mg/kg

Remark: The underlying assumption was that the sequence in the CRF reflected the sequence of dose administration.

4. If a stop date of medication, adverse event, or hospitalization was after the date of final visit (for completers), or after withdrawal (for withdrawn patients), it was flagged as 'stopped after study end' and estimated for duration calculations with the "cut day". The cut day was day of last visit for completers and day of withdrawal for withdrawn patients.

5. Birth dates with missing day, but given month and year were not estimated. The month and the year were used for calculation of age, when age was not given in the CRF, according to the SAP.

6. Days calculated from estimated dates were flagged in listings (see SAP Appendix D for details). All missing stop dates were flagged as ongoing.

7. Only start dates of tacrolimus administration were recorded in the CRF. Duration was calculated by estimating the stop date as the start date of the next dose minus 1 (if next start date > current start date). If the next start date was equal to the current start date, the stop date was estimated with the current start date. The stop date of the last administration period starting before study end/withdrawal was estimated with the date of study end/withdrawal.

8. Calculation of duration for given start and stop dates always included the start and stop day (i.e. duration = stop day – start day + 1).

9. Only days between Day 0 and the day of last visit (day of withdrawal for withdrawn patients) were used for the calculation of concomitant medication duration. For example, if an episode is recorded from Day –2 until Day 10 for a patient withdrawn at Day 9, the episode duration was 10 days.

10. Missing lab values were not estimated.

4.5.3.1.4 General calculation rules

1. Percentages were always quoted using number of 'known' values in the denominator unless otherwise stated.

2. Percentages were given to one decimal place unless otherwise stated. Percentages greater than 0, but rounded to 0.0 were presented as '< 0.1'. If any frequency was zero, the respective percentage will be written as '0.0'.

3. P-values were quoted to three decimal places only. Confidence intervals were also quoted to three decimal places. However, if SAS printed four decimal places, values were not rounded again, but printed to four decimal places.

4. Chi-square tests in contingency tables were replaced by Fisher's exact tests if any expected cell frequency was less than five.

4.5.3.2 Demographics, Other Baseline Characteristics, and Prior and Concomitant Medications

Demographic and appropriate key prognostic variables were summarized overall and by dose group using descriptive statistics (mean, standard deviation, median, minimum and maximum) for continuous measures and frequency counts and percentages for categorical measures. Comparisons among treatment groups were performed using a t-test for continuous measures and a chi-square test for categorical measures,

Distribution of viral status, medical history, primary and secondary diagnoses, and total ischemia times were tabulated by treatment group.

Treatment groups were compared using the Wilcoxon rank-sum test.

Concomitant medications of special interest were summarised separately by type of medication:

- antidiabetic medication
- serum lipid-reducing agents
- antihypertensive medication
- diuretics

4.5.3.3 Efficacy

4.5.3.3.1 Primary endpoint: definition and method for analysis

According to the exploratory character of the study the following hypotheses for primary safety and efficacy endpoints were tested for exploratory purposes on significance level $\alpha = 0.05$:

Primary efficacy endpoint:

H_{0A}: $\mu_1 = \mu_2$ versus H_{1A}: $\mu_1 \neq \mu_2$

With μ_i = mean creatinine clearance at month 6 (calculated from serum creatinine values by Cockcroft formula and corrected for body surface area) in treatment arm i (i = 1, 2).

The hypothesis were tested by ANOVA including the factors treatment, centre and treatment per centre

Primary safety endpoint:

H0B: $p_1 = p_2$ versus H_{1B}: $p_1 \neq p_2$

With pi = incidence of post transplant diabetes (PTD), according to WHO criteria at month 6 in treatment arm i (i = 1, 2).

The hypothesis were tested by Cochran-Mantel-Haenszel test controlling for centre

4.5.3.3.2 Secondary efficacy and safety endpoints

The following secondary efficacy and safety endpoints were analyzed:

- Acute rejection:
  - -Incidence of and time to first biopsy proven acute rejection

-Overall frequency of biopsy proven acute rejection episodes
-Incidence of and time to first steroid-resistant acute rejection
-Severity of biopsy-proven acute rejections (Banff criteria)
-Patient survival
-Graft survival
-Renal function measured by calculated creatinine clearance (Cockcroft formula) at month 3
-Renal function evaluated as creatinine value at months 3 and 6
-24 h Proteinuria, at months 3 and 6
-GFR (calculated) at months 3 and 6
-Incidence and duration of delayed graft function
-Incidence of PTD according to WHO at month 3
-Incidence of adverse events
-Incidence of documented infections (confirmed by culture, biopsy or serology)
-Lipid profile

All secondary endpoints were summarised per treatment group using appropriate descriptive statistics, i.e. number and percentage of patients for categorical variables, and mean, standard deviation, median, minimum, maximum for continuous variables. Where appropriate, summaries will be provided over time.
Incidence of and time to acute rejection, biopsy-proven acute rejection, corticosteroid-resistant acute rejection, biopsy-proven corticosteroid-resistant acute rejection as well as patient and graft survival will be analysed using Kaplan-Meier procedures. Wilcoxon-Gehan-tests will be used for comparison of the survival functions in the two treatment groups.

Diagnosis and grading of rejection episodes
When a clinical and/or laboratory signs indicated the occurrence of a rejection episode a renal biopsy had to be performed.
The biopsy should have been 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 was performed by the local histopathologist following the Banff (97 version) criteria, who should be unaware of the patient's treatment allocation at the time of this evaluation. The slides had to be stored locally.

Acute rejection: definition, start and stop dates

An acute rejection episode was defined on the basis of the rejection page from the CRF. For each rejection episode there was at least one corresponding page in the CRF.

The start of a rejection episode was recorded on the rejection CRF page as start date. In case no date was recorded as start date, it was estimated with either the start date of the corresponding adverse event, the date of biopsy with positive result, or the start date of rejection treatment – whichever was earlier.

The end of a rejection episode was defined as the stop date of the rejection documented in the corresponding field on the rejection page of the CRF.

Categories of acute rejections

Biopsy proven and non-biopsy proven acute rejections

All acute rejection episodes were classified first into biopsy proven rejection episodes and non-biopsy proven acute rejection episodes.

An acute rejection episode was biopsy proven if one biopsy result between the start date and the stop date was classified as 'mild acute rejection (Banff I)', 'moderate acute rejection (Banff II)' or 'severe acute rejection (Banff III)'.

All other acute rejection episodes were non-biopsy proven and defined as " suspected rejections".

Clinical classification of rejections

All acute rejection episodes were further classified into the following categories:

Spontaneously resolving acute rejection

A spontaneously resolving acute rejection was defined as a rejection episode which was not treated with new or increasing corticosteroid medication, antibodies or any other medication and resolved regardless of any tacrolimus or MMF dose changes.

Steroid sensitive acute rejection

A steroid sensitive acute rejection was defined as a rejection episode which was treated with new or increased corticosteroid medication only and resolved, regardless of any tacrolimus dose changes.

Steroid resistant acute rejection

A steroid resistant acute rejection was defined as a rejection episode which did not resolve following treatment with corticosteroids. Rejection episodes which were initially treated with antibodies only will also be included in this category. These were further classified into episodes which resolved with further treatment and those which did not respond to that treatment or were ongoing at the time of study end or patient withdrawal.

Other acute rejection

This category comprises rejection episodes which cannot be classified into any of the above categories.

Rejection follow-up was recorded for rejections which were ongoing at withdrawal or study completion. Since classification into the above categories may change between withdrawal/study end and rejection follow-up, summaries were provided for classification at both time points.

Acute rejection: methods for analysis

A listing of AR episodes was created for review prior to analysis which included a start and a stop date for each episode, as well as its classification into one of the categories described above.

Incidence of and time to rejections as well as patient and graft survival were analysed using Kaplan-Meier procedures. Wilcoxon-Gehan-tests were used for comparison of the survival functions in the two treatment groups.
All time-to-event related parameters were analysed relative to Day 0, defined as the day of reperfusion.

Patient and graft survival

Graft loss was defined as the need to return to long term dialysis, retransplantation, nephrectomy or death. The date of graft loss is the earliest date of any of these events. Graft survival is defined as the absence of graft loss. In case of long term dialysis, the date of graft loss is the first day of dialysis as reported on the dialysis form. Patient and graft survival were analyzed using Kaplan-Meier cumulative survival probability estimates. Frequency tables of patient death and graft loss are provided by treatment arm. The adverse event leading to graft loss was also listed.

4.5.3.4 Safety

4.5.3.4.1 Coding of adverse events

Adverse events were coded using version 8.0 of the Medical Dictionary for Regulatory Activities (MedDRA).

4.5.3.4.2 Analysis of adverse events

Incidences of adverse events during treatment were summarised separately for each treatment group.

Only treatment-emergent adverse events were analyzed. An adverse event was considered treatment-emergent if it started on or after the day of first study medication intake (tacrolimus, MMF or steroids). Non treatment-emergent adverse events, if any, were flagged in the listings.

The overall incidence of adverse events were compared using descriptive p-values from Fisher's exact test.

Adverse events leading to patient's premature discontinuation were summarized separately. With the exception of this table, the term "graft rejection" does not appear in the AE tables, but is included in the patient listings.

Events of special interest

- Infections
- Cardiac/ vascular events
- Neurological disorders
- Nephrological disorders
- Glucose metabolism disorders
- Gastrointestinal disorders
- Malignancies

- AE's leading to dose modification, premature discontinuation, or death

Adverse events were classified in the above categories using the MedDRA terms in the SAP, Appendix H. Frequency of occurrence of events of special interest were summarized in tables. The 2 treatment arms were compared with the chi-square (Fisher's exact) test.

4.5.3.4.3 Laboratory data

Laboratory data were analyzed on a visit basis. Laboratory data were converted into SI units if recorded in any other unit.

Table 3 displays the laboratory assessment schedule according to Visit number and Day of study participation.

4.5.3.4.4 Diabetes

Occurrence of post-transplantation diabetes mellitus (PTDM) was defined as follows, according to Criteria for the Diagnosis of Diabetes Mellitus and Impaired Glucose Homeostasis [65]

Diabetes mellitus

Positive findings from any two of the following tests on different days:

Symptoms of diabetes mellitus (i.e. polyuria, polydipsia or unexplained weight loss) plus casual (at any time of day without regard to time since last meal) plasma glucose concentration ≥200 mg per dL (11.1 mmol per L)
or FPG ≥126 mg per dL (7.0 mmol per L)
or 2hr PGL ≥200 mg per dL (11.1 mmol per L) after a 75-g glucose load

Impaired glucose homeostasis

Impaired fasting glucose: FPG from 110 to <126 (6.1 to 7.0 mmol per L)

Impaired glucose tolerance: 2hr PGL from 140 to <200 (7.75 to <11.1 mmol per L)

Pre-transplant diabetes mellitus was recorded as a secondary diagnosis in order to distinguish de novo occurrence. Number and percentages of patients with diabetes mellitus were presented by treatment group for all patients, for patients with pre-existing condition, and for de novo cases. The 2 treatment arms were compared with the Chi-square (Fisher's exact) test. Additionally, Glycosylated Hemoglobin (HbA1c) was measured at baseline and during the study (visits 7 and 8). It was summarized with the rest of lab measurements, visit-based. The incidence of pathologic occurrences (HbA1c > 6%) at study end was also compared between treatment arms using the Chi-square test (Fisher's exact test).

4.5.4 Changes from Planned Analyses

None

4.5.5 Interim Analyses

No interim analyses were performed for this study.

4.6 Data Quality Assurance and Data Issues

The study was conducted in accordance with ICH-GCP guidelines and the principles of the declaration of Helsinki. In order to ensure the collection of accurate, consistent, complete, and reliable data, the study was monitored by the sponsor 's designee by means of on-site visits and inspection of CRFs, source document verification and cross-checks of data.

Throughout the study, crf pages were collected by [redacted] visual and computer-assisted review of the data was performed on an ongoing basis during the study conduct by [redacted] and, after collection of all CRFs, by [redacted] thereby-ensuring that the data satisfied the criteria as defined in the protocol and internal data validation plan. The internal data validation plan specified which type of data problems were to be queried to the investigator and which type of data errors could be resolved internally by [redacted] data manager. Resolutions of queries were implemented in the database.

All (i.e., for 101 patients) critical data and safety data (AEs), and all data from a random selection of patients were compared with the original CRFs. Discrepancies found in the critical data have been updated. The calculated error rate on the random sample was close to 0%, as documented in the relevant report, which was well within the predefined acceptable limit of 0.5%.

Medical terminologies coded for the study included adverse events, medical history, secondary diagnoses and previous and concomitant medications. MedDRA version 12.0 was used for coding adverse events, medical history and secondary diagnoses. The world health organization drug reference list (WHO-DRL) dictionary version 2009 was used to code previous and concomitant medications.

When data were received, all data problems were resolved, and all data checks and quality control checks were performed, the database was locked on 17 December 2009.

5 RESULTS: STUDY POPULATION

The primary endpoint analyses are presented for the FAS and PPS where appropriate, as well as the results of the secondary efficacy analyses. All safety analyses are presented for the FAS.

5.1 Disposition of Subjects

Source documents for this subsection:

Source	Location
ETT	
12.1.1	Populations for analysis
12.1.2	Number of patients in each treatment group, by centre
12.1.3	Disposition of patients
12.1.4	Number of patients withdrawn from the study, by time
12.2.1	Patient Demographics

5.1.1 Number and Distribution of Patients

In total, 142 patients were enrolled into the study at 14 centers (see Table 5). Of the 142 patients randomized to receive treatment, three patients in arm 2 were randomized but never transplanted and therefore were excluded from all analysis sets; one patient in Arm 1 and two patients in Arm 2 did not receive any dose of tacrolimus. Therefore 139 patients received at least one dose of study medication (72 in arm 1 and 67 in Arm 2) and thus were eligible for the FAS.

A total 31 of FAS patients were excluded from the PPS, 17 and 14 in the 2 arms respectively.

Table 5: Populations for Analysis

	Number of Patients		
	SequentialTacrolimus/ MMF/ Steroids	**Tacrolimus/MMF**	**Total**
Patients enrolled	72	70	142
Not randomized	0	0	0
Randomized to treatment	72	70	142
Excluded from full analysis set	0	3	3
Not transplanted, no study med. Received	0	3	0
Full Analysis Set	72	67	139
Excluded from Per-protocol Analysis Set	17	14	31
Per-protocol Analysis Set	55	53	108
Safety Analisys Set	71	67	138

Source: Table 12.1.1.

The full analysis set (FAS) contains all randomized and transplanted patients with results attributed to the treatment group that they were randomized to and who received at least one dose of study medication (tacrolimus, MMF or steroids).

The per protocol set (PPS) contains all patients in the FAS excluding patients withdrawn for any reason (i.e. intake of prohibited concomitant medications , graft loss, investigator decision in the best interest of patient).

The most common reason for exclusion from the PPS was on the grounds of a major protocol deviation and investigator decision in the best interest of patient; see Section 5.2 for details of the major protocol deviations.

5.1.2 Reasons for Withdrawal

Approximately 78% of the patients included in the FAS completed the study: 55 patients in treatment Arm 1 and 53 patients in treatment Arm 2. A summary of patient disposition is provided in Table 6.

Table 6: Patient Disposition - FAS

	Number of Patients (%)		
	Sequential Tacrolimus/ MMF/ Steroids N =72	**Tacrolimus/MMF N =67**	**Total N = 139**
Completed	55 (76.4)	53 (79.1)	108 (77,7)
Total deaths	3 (4.2)	2 (3.0)	5(3.5)
During study	2 (2.8)	2 (3.0)	4 (2.8)
After withdrawal/EOS	1 (0.0)	0 (0.0)	1 (0.7)
Withdrawn †	15 (20.8)	12 (17.9)	27 (19.4)
Graft failure	3 (4.2)	3 (4.5)	6 (4.3)
Lost to follow-up	1 (1.4)	0 (0.0)	1 (0.7)
Investigator feels it is in patient's best interest toAE	3 (4.2)	3 (4.5)	6 (4.3)
Protocol violation	7 (9.7)	3 (4.5)	10 (7.1)
Prohibited medication	0 (0.0)	3 (4.5)	3 (2.1)
Other	1 (1.4)	3 (4.5)	4(2.8)

Source: Table 12.1.3.

† For reasons other than death

The most common reason for withdrawal in both treatment arms was protocol violation and /or use of prohibited medication. A total of 13 (9.2%) patients were withdrawn due to protocol violation and/or use of prohibited medication , the percentage in each treatment arm was comparable. Withdrawn due to Investigator decision in patient's best interest due to AEs occurred in 6 patients and the percentage in each treatment arm was comparable (4.2 % in Arm 1 and 4.5 % in Arm 2). AEs resulting in discontinuation are described in Sections 9 Three patients in both arms withdrew due to graft loss. Graft loss is discussed in Section 8

Two patients died in both arms during the study. In Arm 1 one patient died due to sudden cardiac death and one due to haemorrhagic shock; in Arm 2 one patient died due to arrhythmia and one due to haemorrhagic shock. Details are presented in Section 8.

5.2 Protocol Deviations

Patients with major protocol deviations were excluded from the PPS. Details of all major deviations can be found in Appendix 13.1.

13 patients were withdrawn due to major protocol deviations. The 13 major protocol deviations are described in the following paragraph.

5.2.1 Major Protocol Deviations

5 Patients (n [redacted]) had protocol deviations use of prohibited concomitant medication.

The remaining 8 patients (n. [redacted]) were withdrawn from the study due to deviations from study treatment schedule.

5.3 Demographics and Other Baseline Characteristics

Source documents for this subsection:

Source	Location
ETT	
12.2.1	Patient Demographics
12.2.2	Age and sex distribution
12.2.3	Patients Viral Status at Baseline
12.2.4	Primary Diagnosis
12.2.5	Donor demographics
12.2.6	Donor Viral Status
12.2.7	Surgical Details
12.2.8	Summary of medical history and secondary diagnoses

5.3.1 Demographics and Viral Status at Baseline

A summary or patient demographics is provided in Table 7.

Table 7: Summary of Patient Demographics - FAS

	Number of Patients (%)		
	Sequential Tacrolimus/ MMF/ Steroids N =72	**Tacrolimus/MMF N =67**	**p-value**
Male n (%)	46 (63.9)	44 (65.7)	0.826†
Female n (%)	26 (36.1)	23 (34.3)	
Age (years) n (%)			
≤19	0 (0.0)	0 (0.0)	
20-29	0 (0.0)	0 (0.0)	
30-39	0 (0.0)	0 (0.0)	
40-49	0 (0.0)	0 (0.0)	
50-59	30 (41.7)	16 (23.9)	0.026†
≥60	42 (58.3)	51 (76.1)	
Mean (SD) Age	60.51(5.19)	61.72(4.18)	0.134‡
Height (cm)			
Mean (SD)	168.4 (8.71)	166.2(7.7)	0.112‡
Median (range)	168 (145-187)	166 (150-188)	
Weight (kg)			
Mean (SD)	70.10 (12.17)	69.58 (10.77)	0.792‡
Median (range)	69.5 (48.0-96.0)	71.0 (48.0-93.0)	

Source: Tables 12.3.1 ; 12.3.2
† Chi-squared test; ‡ Student's t-test;

The 2 treatment arms were largely comparable in terms of patient demographics, except the age distribution. A summary or patient viral status at baseline is provided in Table 8.

Table 8: Summary of Patient Viral Status at Baseline - FAS

		Number of subjects (%)				
		Sequential Tacrolimus/ MMF/ Steroids N =72		Tacrolimus/MMF N =67		p-value†
Viral status at baseline						
CMV	Positive	62	(86.1)	61	(91.0)	
	Negative	8	(11.1)	6	(9.0)	0.3470
	Not recorded	2	(2.8)	0	(0.0)	
HCV	Positive	1	(1.4)	1	(1.5)	
	Negative	71	(98.6)	66	(98.5)	
	Not recorded	0	(0.0)	0	(0.0)	0.9591
EBV	Positive	51	(70.8)	49	(73.1)	
	Negative	9	(12.5)	8	(11.9)	
	Not recorded	12	(16.7)	10	(15.0)	0.9508
HIV	Positive	0	(0.0)	0	(0.0)	
	Negative	71	(100.0)	67	(100.0)	
	Not recorded	0	(0.0)	0	(0.0)	NA
HBsAb	Positive	44	(61.1)	40	(59.7)	
	Negative	17	(23.6)	19	(28.4)	
	Not recorded	11	(15.3)	8	(11.9)	0.7322
HBcAb	Positive	19	(26.4)	19	(28.4)	
	Negative	44	(61.1)	41	(61.2)	
	Not recorded	9	(12.5)	7	(10.4)	0.9156
HBsAg	Positive	2	(2.8)	0	(0.0)	
	Negative	70	(97.2)	67	(100.0)	
	Not recorded	0	(0.0)	0	(0.0)	0.1694
HBcAg	Positive	0	(0.0)	0	(0.0)	
	Negative	2	(2.8)	3	(4.5)	
	Not recorded	70	(97.2)	64	(95.5)	0.5907

Source: Tables 12..2.3
† Chi-squared test NA = Not applicable
CMV = cytomegalovirus; HCV = hepatitis C virus; EBV = Epstein-Barr virus; HIV = Human Immunodeficiency virus; HBsAb= hepatitis B virus surface antibody; HBcAb= hepatitis B virus core antibody; HBsAg= hepatitis B virus surface antigen; HBcAg= hepatitis B virus core antigen

The 2 treatment arms were comparable in terms of patient viral status at baseline.

5.3.1.1 Primary Diagnosis

Glomerulonephritis was the most common underlying disease leading to end-stage renal failure (primary diagnosis) for patients overall, with 21 and 16 patients in the 2 arms respectively

having this diagnosis. Polycystic disease was the second most common primary diagnosis with 15 patients in both arms. The two arms were comparable for primary diagnosis.

Further details of patients' primary indication for transplant are displayed in Table 9 for the FAS.

Table 9: Primary Diagnosis - FAS

	Number of subjects (%)				
	Sequential Tacrolimus/ MMF/ Steroids N =72			**Tacrolimus/MMF** N =67	
Glomerulonephritis	21	(29.2)		16	(23.9)
Uropathy (incl. Chronic pylenephritis)	3	(4.2)		2	(3.0)
Nephrosclerosis (incl. Hypertensive nephropathy)	7	(9.7)		11	(16.4)
Polycystic disease	15	(20.8)		15	(22.4)
Other hereditary nephropathy	1	(1.4)		0	(0.0)
Diabetic nephropathy	1	(1.4)		1	(1.5)
Congenital nephropathy	1	(1.4)		1	(1.5)
Systemic vasculitis	1	(1.4)		0	(0.0)
Other	6	(8.3)		3	(4.5)
Acute cortical necrosis	1	(1.4)		0	(0.0)
Interstitial nephritis	1	(1.4)		0	(0.0)
Microangiopaty thrombotic	0	(0.0)		1	(1.5)
Nephrolithyasis	1	(1.4)		0	(0.0)
Renal amyloidosis	0	(0.0)		1	(1.5)
Retroperitonal fibrosis	1	(1.4)		0	(0.0)
Suspected interstitial nephropathy	1	(1.4)		0	(0.0)
Tubulo interstitial nephropathy	1	(1.4)		1	(1.5)
Unknown	16	(22.2)		18	(26.9)

Source table 12.2.4

5.3.1.2 Secondary Diagnosis

The 2 treatment arms were comparable for secondary diagnoses. In the FAS, 75% of patients in Arm 1 and 85% in Arm 2 reported a secondary diagnosis of hypertension. Coronary heart diseases was reported in 16% of patients in Arm 1 and in 19% in Arm 2. Diabetes was reported in 4% of patients in Arm 1 and 3% of patients in Arm 2. Other secondary diagnoses

reported were: “other”, 54% and 65% respectively. Further details of patients’ primary secondary diagnoses are displayed in Table 10

Table 10 – Summary of secondary diagnoses and medical hystory

	Number of subjects (%)			
	Sequential Tacrolimus/ MMF/ Steroids N =72		**Tacrolimus/MMF** N =67	
Major thoracic or cardiovascular surgery	0	(0.0)	4	(6.0)
Major abdominal surgery	20	(27.8)	21	(31.3)
Malignancy	1	(1.4)	6	(9.0)
Congenital abnormalities	2	(2.8)	1	(1.5)
Diabetes mellitus	3	(4.2)	2	(3.0)
Hypertension	54	(75.0)	57	(85.1)
Glucose intolerance	0	(0.0)	1	(1.5)
Coronary (or other) heart disease	12	(16.7)	13	(19.4)
Psychiatric disorder including drug or substance abuse	1	(1.4)	0	(0.0)
Disability	0	(0.0)	0	(0.0)
Other	39	(54.2)	46	(65.7)

5.3.1.3 Medical History

For the FAS, 27,8% of patients in Arm 1 and 31,3% of patients in Arm 2 had a medical history that included major abdominal surgery. Major thoracic surgery had been performed on 6% of patients in Arm 2. Malignancy was reported in 1,4% and 9% of patients respectively. Congenital abnormalities were reported in 2,8% of patients in Arm 1 and 1,5% in Arm 2.

5.3.2 Donor Organ Demographics and donor viral staus

A summary of donor demographics is presented in Table 11.

Table 11: Summary of Donor Demographics – FAS

	Number of subjects (%)				
	Sequential Tacrolimus/ MMF/ Steroids N =72		**Tacrolimus/MMF** N =67		p-value
Age of Donor (years) <=19	0	(0.0)	0	(0.0)	

20 -29	0	(0.0)	0	(0.0)	
30 - 39	0	(0.0)	0	(0.0)	
40 - 49	0	(0.0)	0	(0.0)	
50 - 59	1	(1.4)	0	(0.0)	0.3224^
>= 60	71	(98.6)	70	(100.0)	
N		72		70	
Mean		69.74		71.35	
SD		4.61		5.25	
Median		69.41		70.47	
Minimum		56.96		61.07	0.0545~
Maximum		82.54		84.41	
Sex					
Male	35	(48.6)	23	(32.9)	0.0562^
Female	37	(51.4)	47	(67.1)	

~ Student's t-test
^ Chi-square test
Source: Tables 12.2.5

Donor demographics were comparable for both treatment arms. The 2 treatment arms were comparable in terms of donor viral status at baseline.

5.4 Concomitant Therapies

Source documents for this subsection:

Source	Location
ETT	
12.5.5.6	Concomitant antihyperlipidaemic medication
12.5.5.7	Concomitant antihypertensive medication
12.5.5.8	Concomitant diuretics

5.4.1 Overall Concomitant Medication Use

The most common concomitant medications administered during the study were as depicted in Table 12 .

Table 12: Concomitant Medications - FAS

Class of medication	Sequential Tacrolimus/ MMF/ Steroids N =72	Tacrolimus/MMF N =67
Antihyperlipidaemic – at any time during the study	15.3%	16.4%
Antihypertensive agents - at any time during the study	81.9%	77.9%
Diuretics- at any time during the study	84.7%	83.8%

Source Table 12.5.5.6 ; 12.5.5.7 ; 12.5.5.8

No differences on concomitant study medication administration across the treatment arms were identified.

6 RESULTS: TREATMENT COMPLIANCE AND STUDY DRUG EXPOSURE

6.1 Treatment Compliance

Tacrolimus whole blood trough level measurements were used to estimate patient compliance with study medication. Poor compliance in taking the baseline immunosuppressant was also used as an indicator of overall patient compliance.

6.2 Administration of Study Drugs, Corticosteroids, and Anti-Rejection Therapy

Source documents for this subsection:

Source	Location
ETT	
12.3.1	Tacrolimus administration – Total daily dose
12.3.2	Tacrolimus whole blood trough levels (ng/mL)
12.3.3	Corticosteroid medication – Rejection therapy
12.3.4	Corticosteroid medication – Maintenance therapy – Mean Values and Cumulative Values - All patients
12.3.5	Corticosteroid medication – Maintenance therapy – Mean Values and Cumulative Values - Completers
12.3.6	Basiliximab Medication
12.3.7	Distribution of Basiliximab Medication
12.3.8	Antibody Preparation – Rejection therapy
12.3.9	Other immunosuppressive medication
12.3.10	Immunosuppressive medication at Study End

6.2.1 Tacrolimus Administration

One patient in Arm 1 received no tacrolimus.

The mean total daily dose of tacrolimus was similar for both treatment arms for the duration of the study (Table 13).

Table 13: Tacrolimus administration : total daily dose (mg/kg) – FAS

	Sequential Tacrolimus/ MMF/ Steroids N =72				Tacrolimus/MMF N =67			
Time	N	Median	Mean	SD	N	Median	Mean	SD
Week 1 ~	27	0.10	0.10	0.05	66	0.08	0.08	0.04
Week 2 ~	45	0.08	0.09	0.05	52	0.06	0.06	0.04
Week 3 ~	43	0.09	0.10	0.05	36	0.05	0.05	0.03
Week 4 ~	31	0.08	0.09	0.04	30	0.06	0.06	0.03
Month 2 ~	51	0.07	0.08	0.05	38	0.05	0.05	0.04
Month 3 ~	36	0.05	0.06	0.03	24	0.05	0.05	0.03
Month 6 ~	16	0.05	0.06	0.02	19	0.05	0.05	0.02

~Mean during time period
Source Table 12.3.1

Whole blood trough levels of tacrolimus measured throughout the study are presented in Table14.

Table 14: Whole Blood Trough Levels of Tacrolimus (ng/mL) - FAS

	Sequential Tacrolimus/ MMF/ Steroids N =72			Tacrolimus/MMF N =67		
	n	**Mean (SD)**	**Median**	**n**	**Mean (SD)**	**Median**
Day 1	03	7.7 (8.6)	3.5	57	17.8 (10.6)	14.7
Day 7	32	9.6 (6.2)	8.7	62	13.5 (6.8)	12.9
Day 14	32	10.5 (5.6)	9.6	59	10.8 (4.4)	10.0
Month 1	58	11.5 (4.7)	10.9	57	9.8 (4.0)	9.5
Month 2	53	10.1 (4.0)	9.9	51	8.7 (3.4)	8.0
Month 3	47	9.0 (3.1)	8.8	48	9.1 (3.8)	8.5
Month 6	48	8.3 (2.7)	8.3	48	8.6 (2.9)	8.2

Source: Table 12.3.2

The mean whole blood trough levels of tacrolimus were comparable between the 2 treatment arms throughout the study.

6.2.2 MMF Administration

The administration of MMF occurred in most of patients as detailed in table 15.

Table 15: Patients receiving MMF

Subjects receiving treatment during:	Number of subjects (%)			
	Sequential Tacrolimus/ MMF/ Steroids N =72		**Tacrolimus/MMF** N =67	
Week 1	67	(93.1)	63	(94.0)
Week 2	62	(86.1)	62	(92.5)
Week 3	52	(72.2)	53	(79.1)
Week 4	41	(56.9)	50	(74.6)
Month 2	40	(55.6)	49	(73.1)
Month 3	35	(48.6)	42	(62.7)
Month 6	34	(47.2)	40	(59.7)

6.2.3 Corticosteroid Administration

In Arm 1 (results for the FAS, all patients) the mean dose of corticosteroid was 1.96 mg/kg in week 1, then it was progressively reduced to 0.14 mg/kg at Week 2 , up to 0.009 mg/kg at Month 6 (for further detail, refer to table 16). In treatment Arm 2 the mean dose of corticosteroid ranged from 7.7 mg/kg in week 1 to 1.12 mg/kg in Week 2 and 0.31 mg/kg in Month 6.

Table 16: Cumulative doses Corticosteroid Medication – Maintainance therapy- FAS

	Sequential Tacrolimus/ MMF/ Steroids N =72			**Tacrolimus/MMF** N =67		
	N	Mean	SD	N	Mean	SD

Daily dose (mg/kg) ~ during:							
Week	1	70	1.968	1.355	67	7.701	5.416
Week	2	70	0.141	0.121	64	1.122	2.185
Week	3	67	0.069	0.045	61	0.826	1.903
Week	4	64	0.067	0.032	61	0.763	1.906
Month	2	60	0.041	0.027	54	0.507	1.727
Month	3	60	0.013	0.022	54	0.468	1.726
Month	6	58	0.009	0.016	54	0.316	1.586
Daily dose (mg) ~ during:							
Week	1	70	132.2	76.78	64	521.0	349.6
Week	2	70	9.498	7.679	64	76.62	150.2
Week	3	67	4.676	2.784	61	57.57	133.4
Week	4	64	4.578	1.789	61	52.40	133.3
Month	2	60	2.808	1.606	54	36.53	126.4
Month	3	60	0.892	1.416	54	33.69	126.2
Month	6	58	0.589	1.064	54	23.84	119.0

^ Based on equivalents prednisolone, excluding treatment given for indications other than routine immunosuppression

~ Mean during time period

6.2.4 Basiliximab Administration

Data of basiliximab administration are summarized in table 17.

Table 17: Distribution of Basiliximab Medication

	Sequential Tacrolimus/ MMF/ Steroids N =72		**Tacrolimus/MMF** N =67	
	Subjects		Subjects	
	N	(%)	N	(%)
Day 0	38	(52.8)	37	(55.2)
Day 1	0	(0.0)	0	(0.0)

Day 3	0	(0.0)	2	(3.0)
Day 4	37	(51.4)	32	(47.8)
Day 5	1	(1.4)	0	(0.0)
Day 6	0	(0.0)	1	(1.5)
Day 9	0	(0.0)	1	(1.5)

7 RESULTS: EFFICACY

7.1 Efficacy/Clinical Evaluation Results

Source	Location
ETT	
12.6.1	Laboratory data:creatinine
12.4.1.1	Overall frequency of rejection
12.4.1.2	Overall frequency of rejection confirmed by byopsy
12.4.1.3	Frequency of biopsy proven and treatment requiring acute rejection by centre
12.4.2.1	Overall estimated rate of subjects free from acute rejection (Kaplan-Meier method)
12.4.2.2	Overall estimated rate of subjects free from acute rejection –confirmed by byopsy (Kaplan-Meier method)
12.4.2.3	Overall estimated rate of subjects free from biopsy proven and treatment requiring acute rejection - (Kaplan-Meier method)
12.4.2.4	Overall estimated rate of subjects free from corticosteroid-resistant acute rejection (Kaplan-Meier method)
12.3.2.5	Overall estimated rate of subjects free from corticosteroid-resistant acute rejection confirmed by biopsy (Kaplan-Meier method)
12.4.3.1	Histological grade of acute rejection
12.4.3.2	Histological grade of all biopsies
12.4.3.3	Summary of graft loss and death
12.4.3.4	Overall estimated graft survival rate (Kaplan-Meier method)
12.4.4	Overall estimated rate of subjects free from treatment failure (Kaplan-Meier method)
12.5.1	Overall estimated subject survival rate (Kaplan-Meier method)

7.1.1 Primary Enpoint: 6 months creatinine clearance difference

Descripton of distribution of serum creatinine over time and test results for comparison between the two groups are reported in table 18. No statistical difference was found at any time.

Table 18: Laboratory data: creatinine

	Sequential MMF/Tacrolimus - Steroids N=72			Tacrolimus/MMF N=67			
Serum creatinine~	N	Mean	SD	N	Mean	SD	p-value+
Visit 1 (Day 0)	71	07.63	02.23	67	07.56	02.20	0.7166
Visit 2 (Day 1)	71	07.29	02.24	67	07.25	02.16	0.9195
Visit 3 (Day 7)	70	05.36	03.89	64	05.20	03.28	0.8089
Visit 4 (Day 14)	70	03.41	02.38	64	04.35	03.44	0.0699
Visit 5 (Month 1)	64	02.29	01.38	60	02.94	02.12	0.0480

Visit 6 (Month 2)	59	02.16	01.04	54	02.31	01.47	0.5410
Visit 7 (Month 3)	59	02.07	0.872	54	01.91	0.570	0.2488
Visit 8/End of study (Month 6)	68	02.33	01.54	64	02.42	01.81	0.7796

~ SI-Unit: serum creatinine mg/dl;
+ Student's t-test

Creatinine Clearance, calculated using the Cockroft Gault formula, did not show any difference between groups at the end of study (Table 19)

Table 19: Calculated creatinine clearance: visit 8/end of study (Month 6)

	Sequential MMF/Tacrolimus - Steroids N=72			Tacrolimus/MMF N=67			
creatinine clearance~	N	Mean	SD	N	Mean	SD	p-value+
Visit 8/End of study (Month 6)	58	40.12	16.96	54	40.22	11.59	0.9657

~ml/min Normal range 70-150
+ ANOVA Analysis, stratified by center

7.1.2 Rejections

A summary of frequency of rejection is provided in table 20 (Source: Table 12.4.1.1)
Source documents for this subsection

Table 20 Overall frequency of rejection

	Sequential MMF/ Tacrolimus - Steroids N=72			Tacrolimus/ MMF N=67			
	Subjects		Episodes	Subjects		Episodes	
	N	(%)	N	N	(%)	N	p-value
Acute rejections	18	(25.0)	20	26	(38.8)	27	0.0804>
Spontaneously resolving acute rejections ~	0	(0.0)	0	0	(0.0)	0	NA
Corticosteroid sensitive acute rejections +	11	(15.3)	12	22	(32.8)	22	0.0151>
Corticosteroid resistant acute rejections #	2	(2.8)	3	3	(4.5)	4	0.6720<
Resolved with further treatment	0	(0.0)	0	1	(1.5)	1	1.0000<
Unresolved with further treatment	2	(2.8)	3	2	(3.0)	3	
Other	4	(5.6)	4	0	(0.0)	0	0.1206<
Suspected rejections *	1	(1.4)		1	(1.5)		1.0000<
Suspected chronic rejections	0	(0.0)			0	(0.0)	NA

~ A spontaneously resolving acute rejection is defined as a rejection episode which was not treated with new or increasing corticosteroid medication, antibodies or any other medication and resolved irrespective of any tacrolimus dose changes
+ A corticosteroid sensitive acute rejection is defined as a rejection episode which was treated with new or increasing corticosteroid medication only and resolved, irrespective of any tacrolimus dose changes
# A corticosteroid-resistant acute rejection is defined as a rejection episode, which did not resolve following treatment with corticosteroids In the case that a rejection episode was not treated with corticosteroids first but only with antibodies, it was nevertheless be included in this category
* A suspected acute rejection is defined as a rejection, which was not histologically confirmed
> Chi-square test comparing the numbers of subjects
< Fisher's exact test comparing the numbers of subjects

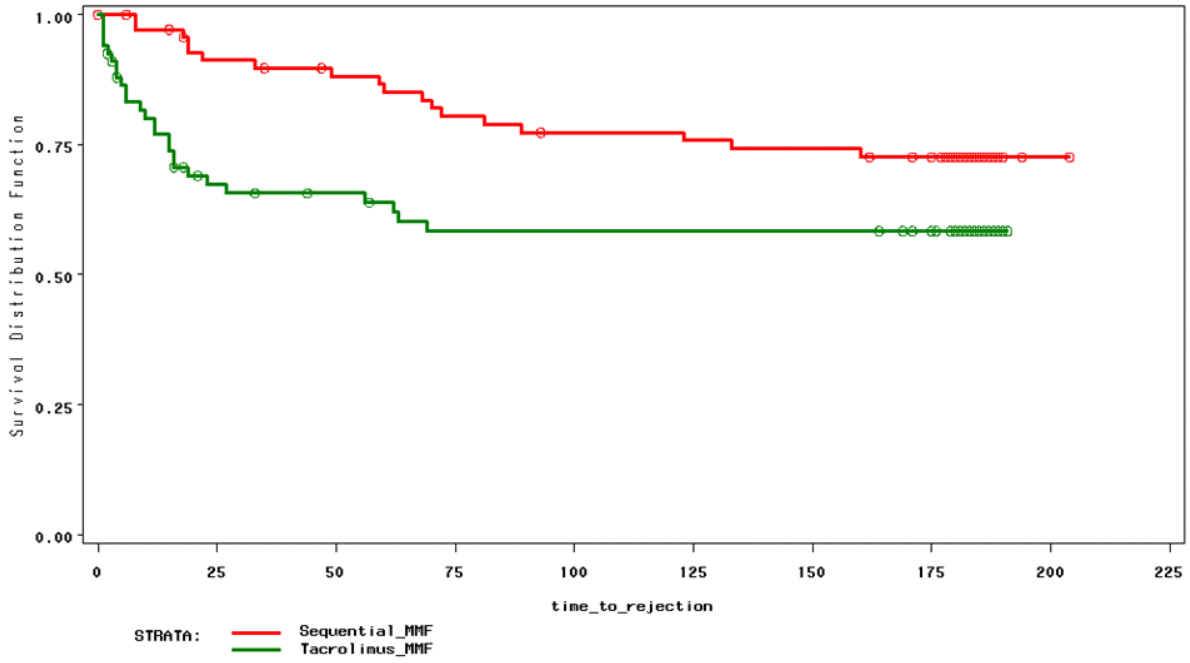
Overall, 24 pts experienced acute rejection (Arm 1: 18 (25%); Arm 2: 26 (38.8%) most corticosteroids sensitive (Arm 1: 11 (15.3%); Arm 2: 22 (32.8%)), showing a statistical

significant association with treatment (p=0.0151, χ^2 test). When considering rejections with confirmed biopsies only, the distribution was as follows: Arm 1: 12 (16.7%); Arm 2: 7 (10.5%); corticosteroids sensitive: Arm 1: 8 (11.1%); Arm 2: 5 (7.5%), (Source: ETT 12.4.1.2). For further details see also ETT 12.4.1.3).

Estimate of probability of being free of rejections is reported in figure 2.

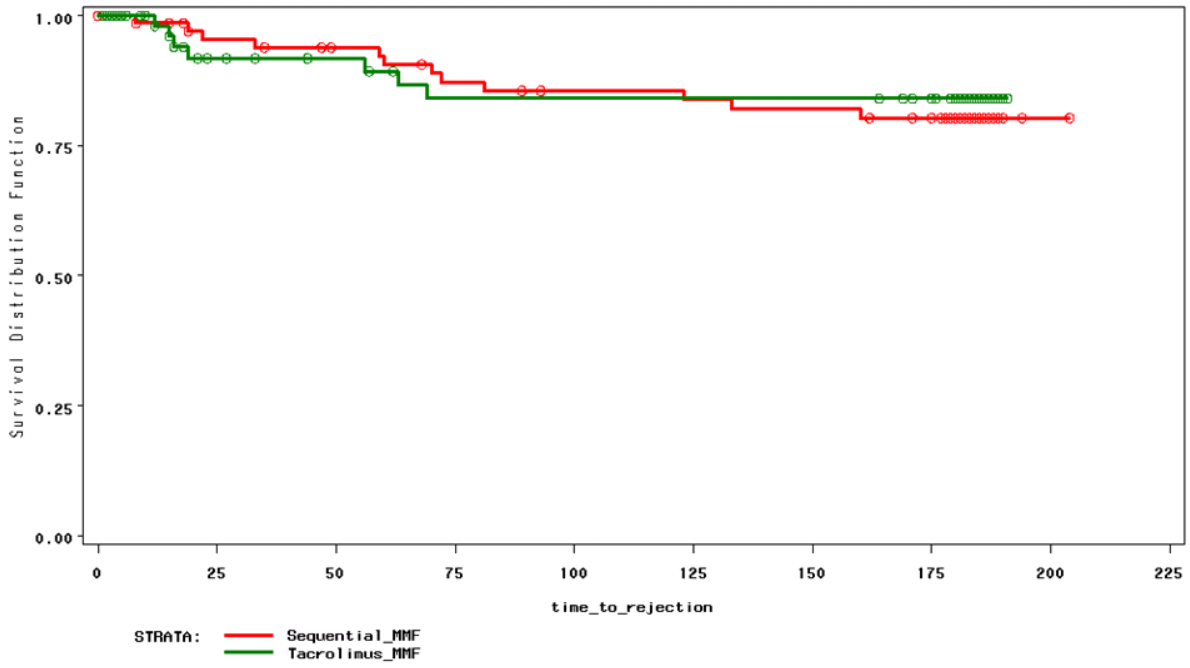
The figure shows a statistically significant difference between treatments (Wilcoxon Gehan test for a difference between treatments over 6 months: Chi-squared (1df) = 7.7677 p-value = 0.0053; 95% confidence limits for 6 month survival: Arm 1 (0.67 – 0.87); Arm 2 (0.46 – 0.71) (source: ETT 12.4.2.1)

Fig. 2 Overall estimate of probability of being free from rejections



However, when considering the probability of being free from biopsy proven rejection, data re comparable in the two arms (figure 3) (Wilcoxon Gehan test for a difference between treatments over 3 months: Chi-squared (1df) = 0.0028 p-value = 0.9557; 95% confidence limits for 3 month survival: Arm 1 (0.70 – 0.90); Arm 2 (0.73 – 0.90) (source: ETT 12.4.2.2)

Fig. 3 Overall estimate of probability of being free from biopsy proven rejections



Similar patterns emerged when comparing probability of being free from biopsy proven, treatment requiring rejection (Wilcoxon Gehan test for a difference between treatments over 3 months: chi-squared (1df) = 0.0054 p-value = 0.8733; 95% confidence limits for 3 month survival: Arm 1 (0.72 – 0.91); Arm 2 (0.73 – 0.95) (source: ETT 12.4.2.3). Similar estimates were also observed in the comparison of probability of being free from corticosteroid-resistant acute rejection (Wilcoxon Gehan test for a difference between treatments over 3 months: chi-squared (1df) = 1.3063 p-value = 0.2531; 95% confidence limits for 3 month survival: Arm 1 (0.91 – 1.00); Arm 2 (0.87 – 1.00) (source: ETT 12.4.2.4) and in the comparison of being free from corticosteroid-resistant biopsy proven acute rejection (Wilcoxon Gehan test for a difference between treatments over 3 months: chi-squared (1df) = 0.4111 p-value = 0.5214; 95% confidence limits for 3 month survival: Arm 1 (0.91 – 1.00); Arm 2 (0.90 – 1.00) (source: ETT 12.4.2.5). ETT tables 12.4.3.1 and 12.4.3.2 reports the histological grade of biopsies for acute rejections and overall, respectively, with very similar pattern of distribution in the two arms.

7.1.3 Graft loss and death

A summary of frequency of graft loss and deaths is provided in table 21 (Source: Table 12.4.3.3)

Overall, 8 graft loss occurred (4 in each arm), 6 (3 in each arm) during study. Five deaths were observed (Arm 1: 3; Arm 2: 2), all for cardiovascular problems.

Table 21: Frequency of graft loss and deaths

	Number of subjects (%)			
	Sequential MMF/ Tacrolimus - Steroids N=72		Tacrolimus/ MMF N=67	
Graft loss	4	(5.6)	4	(6.0)
During study	3	(4.2)	3	(4.5)
After withdrawal	1	(1.4)	1	(1.5)
Cause of graft loss				
During study				
• Kidney explanted	1	(1.4)	0	(0.0)
• Return of long term dialysis	1	(1.4)	0	(0.0)
• Transplantectomy/nephrectomy	1	(1.4)	2	(3.0)
• Kidney transplant esplant due to severe ischemia of the kidney	0	(0.0)	1	(1.5)
After withdrawal	1	(1.5)	1	(1.5)
• Transplantectomy	1	(1.5)	1	(1.5)
Deaths	3	(4.2)	2	(3.0)
During study	2	(2.8)	2	(3.0)
After withdrawal	1	(1.4)	0	(0.0)
Cause of death for subjects				
Who died during the study				
• Haemorrhage, shock	1	(1.4)	0	(0.0)
• Sudden cardiac death	1	(1.4)	0	(0.0)
• Arrhythmia	0	(0.0)	1	(1.5)
• Shock haemorrhagic	0	(0.0)	1	(1.5)
After withdrawal				
• Cardiac arrest	1	(1.4)	0	(0.0)

Estimate of graft survival is presented in figure 4, showing no difference between arms. (Wilcoxon Gehan test for a difference between treatments over 6 months: chi-squared (1df) = 0.0459 p-value = 0.8303; 95% confidence limits for 6 month survival: Arm 1 (0.90 – 1.00); Arm 2 (0.90 – 1.00) (source: ETT 12.4.3.4). No differences were detected also for time to treatment failure (Wilcoxon Gehan test for a difference between treatments over 6 months: chi-squared (1df) = 0.0984 p-value = 0.7573; 95% confidence limits for 6 month survival: Arm 1 (0.80 – 0.96); Arm 2 (0.80 – 0.96) (source: ETT 12.4.4) nor for overall survival comparison (Wilcoxon Gehan test for a difference between treatments over 6 months: chi-squared (1df) = 0.0288 p-value = 0.8651; 95% confidence limits for 6 month survival: Arm 1 (0.93 – 1.00); Arm 2 (0.93 – 1.00), figure 5 (source: ETT 12.5.1) .

Figure 4 Estimated graft survival rate

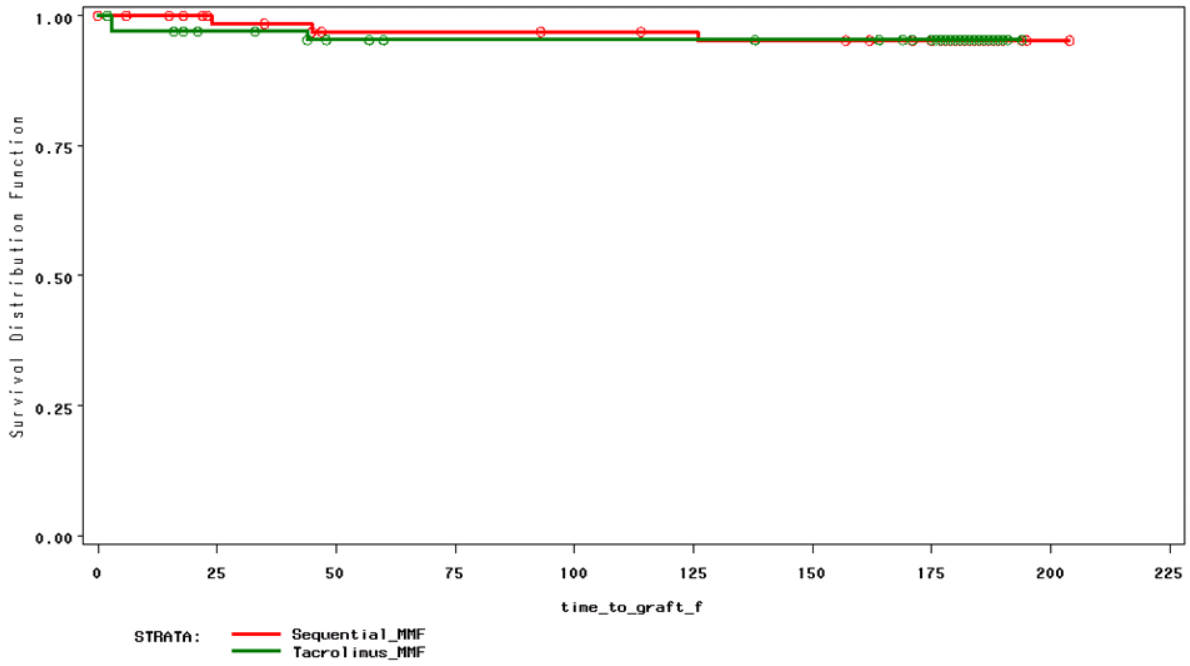
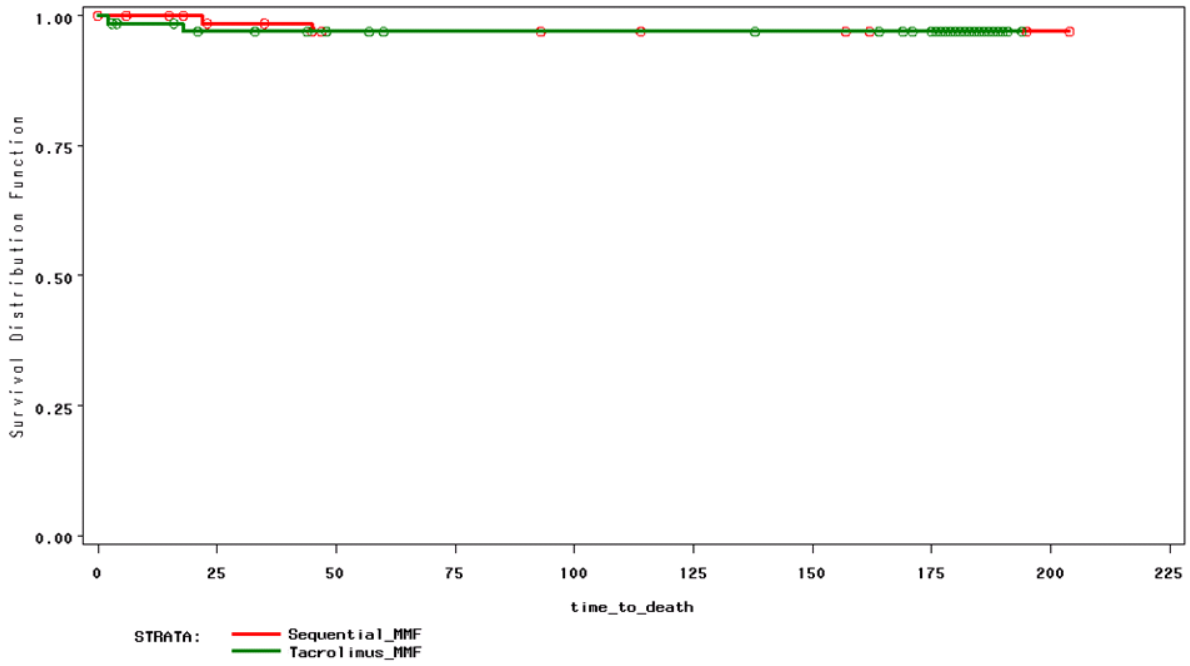


Figure 5 Estimated overall survival rate



7.2 Efficacy/Clinical Evaluation Conclusions

Based on the results of this study, Arm 1 did not show any difference regarding creatinine clearance. Arm 1 gets a prolonged time free of rejection and a reduced rate of corticosteroid sensitive acute rejections compared to Arm 2. No evidences of differences between the two arms were observed for other efficacy outcomes.

8 RESULTS: SAFETY

Source documents for this subsection:

Source	Location
ETT	
12.5.2.1	Glucose metabolism disorders - subjects without pre-existing glucose metabolism disorders (post tranplant diabetes)
12.5.2.2	Glucose metabolism disorders - subjects worsening of pre-existing glucose metabolism disorders
12.5.2.3	Concomitant antidiabetic medication - subjects without pre-existing glucose metabolism disorders
12.5.3	Overall adverse event summary
12.5.3.1	Overall incidence of the most frequently^ reported adverse events regardless of relationship to study medication
12.5.3.2	Overall occurrence of the most frequently^ reported adverse events regardless of relationship to study medication, by time
12.5.3.3	Overall incidence of the most frequently^ reported adverse events assessed by the investigator as being causally related+ to study medication
12.5.4.1	Overall incidence of the most frequently^ reported serious adverse events regardless of relationship to study medication
12.5.4.2	Overall incidence of the most frequently^ reported serious adverse events regardless of relationship to study medication, by time
12.5.5.1	Infections
12.5.5.2	Overall occurrence of infections
12.5.5.3	Nephrological disorders
12.5.5.4	Neurological disorders
12.5.5.5	Cardiac events
12.5.5.6	Concomitant antihyperlipidaemic medication
12.5.5.7	Concomitant antihypertensive medication
12.5.5.8	Concomitant diuretics
12.5.5.9	Gastrointestinal disorders
12.5.5.10	Malignancies
12.5.5.11	Adverse events resulting in patient premature discontinuation
12.6.1	Laboratory data: haematology
12.6.2	Laboratory data: clinical chemistry
12.7	Vital signs
12.8.1	Hospitalization
12.8.2	Intensive care unit

8.1 Adverse Events

8.1.1 Summary of adverse events

Table 22 reports the summary of adverse events. Percentage of adverse events was 98.5% in both arms. Of note, subjects with MMF causally related adverse events, as assessed by investigators, were 29.2% in Arm 1 compared to 71.6% in Arm 2 while Tacrolimus causally related adverse events, were 29.2% in Arm 1 compared to 71.6% in Arm 2. MMF causally related serious adverse events, were 8.3% in Arm 1 compared to 17.9% in Arm 2 while

Tacrolimus causally related serious adverse events, were 20.8% in Arm 1 compared to 16.4% in Arm 2 (Source: Table 12.5.3).

Table 22 Summary of overall adverse

	Sequential MMF/Tacrolimus - Steroids N=72			Tacrolimus/ MMF N=67		
	Subjects		Events	Subjects		Events
	N	(%)	N	N	(%)	N
Adverse events	69	(95.8)	315	66	(98.5)	370
Serious adverse events	36	(50.0)	53	38	(56.7)	57
MMF Causally-related adverse events~	21	(29.2)	25	48	(71.6)	93
TACROLIMUS Causally-related adverse events~	34	(47.2)	65	41	(61.2)	76
MMF Serious causally-related adverse events~	06	(8.3)	07	12	(17.9)	15
TACROLIMUS Serious causally-related adverse events~	15	(20.8)	17	11	(16.4)	17

~ Causally-related is defined as a highly probable, probable and possible as assessed by the investigator

8.1.2 Summary of adverse events: Deaths, other serious adverse events, and adverse events resulting in discontinuaton

8.1.2.1 Deaths

Five deaths were observed (3 in Arm 1, 2 during study - 1 hemorrhage, 1 sudden cardiac death - 1 after withdrawal, cardiac arrest; 2 in Arm 2 during study - 1 arrytmia, 1 hemorrhagic shock). Refer to table 21 for details.

8.1.2.2 Serious adverse events other than deaths

Table 23 reports the overall rate of reported serious adverse events, assessed by investigator as causally related to treatment (Source: Table 12.5.4.3). Overall, proportion of serious adverse events ranged 1.4% to 11.1% (kidney transplant rejection, in Arm 1). No evidence of difference in distribution between the two arm was detectable. For further details see ETT: Table 12.5.3.1-12.5.3.3, 12.5.4.1-12.5.4.3.

Table 23: Overall incidence of the most frequently^ reported serious adverse events assessed by the investigator as being causally related+ to study medication

	Sequential MMF/Tacrolimus - Steroids N=72			Tacrolimus/ MMF N=67			
	Subjects		Events	Subjects		Events	
Adverse event~	N	(%)	N	N	(%)	N	p-value*
Kidney transplant rejection	8	(11.1)	9	5	(7.5)	6	0.4604
Nephropathy toxic	1	(1.4)	2	5	(7.5)	5	0.1059
Delayed kidney graft function	1	(1.4)	1	1	(1.5)	1	1.0000

~Coded using modified MedDRA
^ All causally related serious adverse events are reported in this table
+Causally-related is defined as a highly probable, probable and possible as assessed by the investigator
*Fisher's exact test comparing the number of subjects

8.1.2.3 Adverse events resulting in discontinuation

Table 24 reports the adverse events resulting in discontinuation. Overall 16 patients discontinued treatment due to an adverse event (8 in each arms). Most serious adverse events were related to renal complication or cardiovascular events (source: Table 12.5.5.11). No evidence of significant difference in distribution between the two arms was detected.

Table 24: Adverse events resulting in patient discontinuation

	Sequential MMF/Tacrolimus - Steroids N=17			Tacrolimus/ MMF N=14			
	Subjects		Events	Subjects		Events	
Adverse event~	N	(%)	N	N	(%)	N	p-value+
Arrhythmia	0	(0.0)	0	1	(7.1)	1	0.4516
Cerebrovascular accident	0	(0.0)	0	1	(7.1)	1	0.4516
Coma	1	(5.9)	1	0	(0.0)	0	1.0000
Complications of transplanted kidney	1	(5.9)	1	0	(0.0)	0	1.0000
Delayed kidney graft function	1	(5.9)	1	2	(14.3)	2	0.5764
Haemorrhage	1	(5.9)	1	0	(0.0)	0	1.0000
Kidney transplant rejection	2	(11.8)	2	1	(7.1)	1	1.0000
Nephropathy toxic	1	(5.9)	1	1	(7.1)	1	1.0000
Renal tubular necrosis	1	(5.9)	1	0	(0.0)	0	1.0000
Renal vein thrombosis	0	(0.0)	0	1	(7.1)	1	0.4516
Shock	1	(5.9)	1	0	(0.0)	0	1.0000
Shock haemorrhagic	0	(0.0)	0	1	(7.1)	1	0.4516
Sudden cardiac death	1	(5.9)	1	0	(0.0)	0	1.0000
Total	8	(47.1)	10	8	(57.1)	8	0.5761

~Coded using modified MedDRA
+Fisher's exact test comparing the number of subjects

8.1.3 Adverse events of special interest

8.1.3.1 Glucose metabolism disorders

Table 25 reports the occurrence of glucose metabolism disorders (Diabetes mellitus, Glucose tolerance decreased, Hyperglycemia). Overall 15 (21.7%) patients in Arm 1 and 19 (29.7%) pts in Arm 2 experienced glucose metabolism disorders. Previous type 1 diabetes worsened in three subjects (1 in Arm 1 and 2 in Arm 2). Among 25 pts without pre-existing diabetic disorders, 4 subjects, 3 of 14 in Arm 1 and 1 of 11 in Arm 2 used concomitant antidiabetic medications (source tables 12.5.2.1 -12.5.2.3).

Table 25 Glucose metabolism disorders

	Sequential MMF/Tacrolimus – Steroids N=69			Tacrolimus/ MMF N=64			
	Subjects		Events	Subjects		Events	
Adverse event~	N	(%)	N	N	(%)	N	p-value^
SUBJECTS WITHOUT PRE-EXISTING GLUCOSE METABOLISM DISORDERS							
Diabetes mellitus type 1	12	(17.4)	12	9	(14.5)	9	
Diabetes mellitus type 2	2	(2.9)	2	6	(8.7)	6	
Glucose tolerance decreased	2	(2.9)	2	6	(8.7)	6	
Impaired fasting glucose	17	(1.4)	1	1	(1.5)	1	
Hypoglycaemia	1	(1.4)	1	0	(0.0)	0	
Hypoglycaemia coma	0	(0.0)	0	1	(1.5)	1	
Total	*15	(21.7)	18	*19	(29.7)	24	0.2937

~ Coded using modified MedDRA

^ Fisher's exact test comparing the number of subjects

* Some patients had more than one Glucose metabolism disorder

8.1.3.2 Infections

Table 26 reports the overall proportion of infections, by type (Source: Table 12.5.5.1). 50% of patients in Arm 1 and 42% in Arm 2 experienced infection. The most frequent type of infection were due to bacterial (20%) and viral (33%) agents. All types of infection were similar in the 2 groups, with the exception of CMV infection, which affected 12.5% of patients in Arm 1 vs. 7.5% in Arm 2 (Source: Table 12.5.5.2). No evidence of significant difference in distribution between the two arms was detected.

Table 26 Infections

	Sequential MMF/Tacrolimus - Steroids N=72			Tacrolimus/ MMF N=67			
	Subjects		Events	Subjects		Events	
Type of infection~	N	(%)	N	N	(%)	N	p-value*
Unknown	9	(12.5)	9	2	(3.0)	3	0.0569
Bacterial	13	(18.1)	19	15	(22.4)	20	0.5245
Viral	11	(15.3)	13	7	(10.5)	8	0.3967
Fungal	3	(4.2)	4	4	(6.0)	4	0.7111
Protozoal	0	(0.0)	0	0	(0.0)	0	NA
Other	0	(0.0)	0	0	(0.0)	0	NA

*Fisher's exact test comparing the number of subjects

8.1.3.3 Nephrological disorders

Table 27 reports the occurrence of nephrological disorders. Overall 59 pts (42.4%) were affected by Nephrological disorders, 34 in Arm 1 and 25 in Arm 2. (Source Table: 12.5.5.3). Glomerulonephritis was the most frequent AE (15.8%). No evidence of significant difference in distribution between the two arms was detected.

Table 27 Nephrological disorders

	Sequential MMF/Tacrolimus - Steroids N=72			Tacrolimus/ MMF N=67			
	Subjects		Events	Subjects		Events	
Adverse event~	N	(%)	N	N	(%)	N	p-value^
Blood creatinine increased	9	(12.5)	10	5	(7.5)	5	0.3241
Dysuria	3	(4.2)	3	2	(3.0)	2	1.0000
Fluid retention	12	(16.7)	14	10	(14.9)	10	0.7787
Glomerulonephritis	1	(1.4)	1	0	(0.0)	0	1.0000
Haematuria	3	(4.2)	3	2	(3.0)	2	1.0000
Nephritis	0	(0.0)	0	1	(1.5)	1	0.4820
Nephropathy	2	(2.8)	3	0	(0.0)	0	0.4970
Nephropathy toxic	4	(5.6)	6	6	(9.0)	7	0.5215
Obstructive uropathy	2	(2.8)	3	1	(1.5)	2	1.0000
Proteinuria	4	(5.6)	4	0	(0.0)	0	0.1206
Renal impairment	4	(5.6)	4	5	(7.5)	5	0.7383
Renal tubular necrosis	9	(12.5)	9	4	(6.0)	4	0.2477
Ureteric stenosis	0	(0.0)	0	1	(1.5)	1	0.4820
Urinary fistula	1	(1.4)	1	0	(0.0)	0	1.0000
Total	34	(47.2)	61	25	(37.3)	39	0.2376

~Coded using modified MedDRA
^Fisher's exact test comparing the number of subjects

8.1.3.4 Neurological disorders

Table 28 reports the occurrence of neurological disorders. Overall 25 pts (18.0%) were affected by neurological disorders, 12 in Arm 1 and 13 in Arm 2. (Source Table: 12.5.5.4). Anxiety was the most frequent neurological disorders (4.3%). No evidence of significant difference in distribution between the two arms was detected.

Table 28 Neurological disorders

	Sequential MMF/Tacrolimus - Steroids N=72			Tacrolimus/ MMF N=67			
	Subjects		Events	Subjects		Events	
Adverse event~	N	(%)	N	N	(%)	N	p-value^
Acute psychosis	0	(0.0)	0	1	(1.5)	1	0.4820
Anxiety	4	(5.6)	4	2	(3.0)	2	0.6818
Cerebrovascular accident	0	(0.0)	0	1	(1.5)	1	0.4820
Convulsion	1	(1.4)	1	0	(0.0)	0	1.0000
Headache	1	(1.4)	1	1	(1.5)	1	1.0000
Insomnia	1	(1.4)	1	1	(1.5)	1	1.0000
Neurotoxicity	0	(0.0)	0	2	(3.0)	2	0.2305
Paraesthesia	1	(1.4)	1	0	(0.0)	0	1.0000
Psychomotor hyperactivity	0	(0.0)	0	1	(1.5)	1	0.4820
Psychotic disorder	1	(1.4)	1	0	(0.0)	0	1.0000
Syncope	1	(1.4)	1	0	(0.0)	0	1.0000
Tremor	3	(4.2)	3	4	(6.0)	4	0.7111
Total	12	(16.7)	13	13	(19.4)	13	0.6747

~ Coded using modified MedDRA

^Fisher's exact test comparing the number of subjects

8.1.3.5 Cardiovascular disorders

Table 29 reports the occurrence of cardiovascular disorders. Overall 61 pts (43.9%) were affected by cardiovascular disorders, 29 in Arm 1 and 32 in Arm 2 (source Table: 12.5.5.5). Hypertension was the most frequent cardiovascular disorders (25, 18.0%), accounting for 17 (23.6%) pts in Arm 1 and 8 (11.9%)in Arm 2. No evidence of significant difference in distribution in the two arms was detected (source Table: 12.5.5.6-12.5.5.8).

Table 29 Cardiovascular disorders

	Sequential MMF/Tacrolimus - Steroids N=72			Tacrolimus/ MMF N=67			
	Subjects		Events	Subjects		Events	
Adverse event~	N	(%)	N	N	(%)	N	p-value^
Arrhytmia	0	(0.0)	0	1	(1.5)	1	0.4820
Arteriovenous fistula thrombosis	1	(1.4)	1	0	(0.0)	0	1.0000
Atrial fibrillation	1	(1.4)	1	3	(4.5)	3	0.3522
Deep vein thrombosis	2	(2.8)	2	1	(1.5)	1	1.0000
Extrasystoles	0	(0.0)	0	1	(1.5)	1	0.4820
Fluid overload	2	(2.8)	2	0	(0.0)	0	0.4970
Graft complication	0	(0.0)	0	1	(1.5)	1	0.4820
Haemorrhage	2	(2.8)	2	0	(0.0)	0	0.4970
Haemorrhoidal haemorrage	1	(1.4)	1	0	(0.0)	0	1.0000
Haemorrhoids	1	(1.4)	1	1	(1.5)	1	1.0000
Haemorrhoids aggravated	0	(0.0)	0	1	(1.5)	1	0.4820
Hypertension	17	(23.6)	17	8	(11.9)	8	0.0734
Hypertensive crisis	1	(1.5)	1	0	(0.0)	0	1.0000
Hypotension	4	(5.6)	4	1	(10.5)	7	0.3542
Hypovolaemic shock	1	(1.4)	1	0	(0.0)	0	1.0000
Intra-abdominal haemorrage	0	(0.0)	0	1	(1.5)	1	0.4820
Myocardial infarction	0	(0.0)	0	1	(1.5)	1	0.4820
Necrosis	0	(0.0)	0	1	(1.5)	1	0.4820
Peripheral ischaemia	1	(1.4)	1	0	(0.0)	0	1.0000
Phlebitis	0	(0.0)	0	1	(1.5)	1	0.4820
Pulmunary embolism	0	(0.0)	0	1	(1.5)	1	0.4820
Renal artery stenosis	1	(1.4)	1	0	(0.0)	0	1.0000
Renal embolism	1	(1.4)	1	0	(0.0)	0	1.0000
Renal haemorrhage	0	(0.0)	0	2	(3.0)	2	0.2305
Renal vein thrombosis	0	(0.0)	0	1	(1.5)	1	0.4820
Retroperoitoneal haemorrhage	1	(1.4)	1	1	(1.5)	1	1.0000
Shock	1	(1.4)	1	0	(0.0)	0	1.0000
Shock haemorrhagic	0	(0.0)	0	1	(1.5)	1	0.4820
Sudden cardiac death	1	(1.4)	1	0	(0.0)	0	1.0000
Tachycardia	2	(2.8)	2	2	(3.0)	2	1.0000
Thrombophlebitis	0	(0.0)	0	5	(7.5)	5	0.0240
Vena cava injury	1	(1.4)	1	0	(0.0)	0	1.0000
Venous injury	1	(1.4)	1	0	(0.0)	0	1.0000
Total	29	(40.3)	43	32	(52.5)	41	0.3743

~Coded using modified MedDRA
^Fisher's exact test comparing the number of subjects

8.1.3.6 Gastrointestinal disorders

Table 30 reports the occurrence of gastrointestinal disorders. Overall 43 pts (30.9%) suffered for gastrointestinal disorders, 16 (22.2%) in Arm 1 and 27 (40.3%) in Arm 2. Evidence of significant difference in distribution of all gastrointestinal disorders between the two arms was detected (p=0.0212). Diarrhoea was the most common gastrointestinal disorder, affecting 22 (15.8%) pts, 8 (11.1%) in Arm 1 and 14 (20.9%) in Arm 2 (source Table: 12.5.5.9).

Table 30 Gastrointestinal disorders

	Sequential MMF/Tacrolimus - Steroids N=72			Tacrolimus/ MMF N=67			
	Subjects		Events	Subjects		Events	
Adverse event~	N	(%)	N	N	(%)	N	p-value^
Abdominal pain	3	(4.2)	3	4	(6.0)	4	0.7111
Acute abdomen	1	(1.4)	1	0	(0.0)	0	1.0000
Constipation	0	(0.0)	0	1	(1.5)	1	0.4820
Diarrhoea	8	(11.1)	8	14	(20.9)	16	0.1143
Dry cough	0	(0.0)	0	1	(1.5)	1	0.4820
Dyspesia	1	(1.4)	1	0	(0.0)	0	1.0000
Enteritis	0	(0.0)	0	1	(1.5)	1	0.4820
Gastritis	2	(2.9)	2	2	(3.0)	2	1.0000
Gastrointestinal disorder	1	(1.4)	1	2	(3.0)	3	0.6090
Intestinal obstruction	0	(0.0)	0	1	(1.5)	1	0.4820
Large intestinal ulcer	0	(0.0)	0	1	(1.5)	1	0.4829
Nausea	1	(1.4)	1	2	(3.0)	2	0.6090
Pancreatitis	0	(0.0)	0	3	(4.5)	3	0.1094
Stomatitis	0	(0.0)	0	1	(1.5)	1	0.4820
Vomiting	0	(0.0)	0	2	(3.0)	2	0.2305
Total	16	(22.2)	17	27	(40.3)	38	0.0212

~Coded using modified MedDRA
^Fisher's exact test comparing the number of subjects

8.1.3.7 Malignancies

One case of cancer (seminoma) occurred in Arm 1 (source table 12.5.5.10).

8.1.4 Relation of adverse events to study drug exposure

No evidence of drug exposure dependent AEs was detected, both for adverse and serious adverse events, regardless their relationship with treatment. (Source: Table 12.5.3.2-12.5.4.2)

8.2 Clinical Laboratory evaluation

Haematologic data and clinical chemistry data remained constant over time and were very similar in the two arms (source Tables 12.6.2 and 12.6.3). Regarding renal function, mean serum creatinine decreased from values of 7.6 mg/dL at baseline to 2.4 mg/dL at 6 month, very similarly in both groups (source: table 12.6.1)

8.3 Vital signs

Calculation of weight, systolic and diastolic blood pressure over time showed very similar distribution in both group. Mean weight tended to remain constant, as well as blood pressure, over time, similarly in the two groups (source: table 12.7).

8.4 Other safety related observations

Table 31 reports hospitalizations and ICU occurrence. Overall no difference between the 2 groups was detected. (Source Table 12.8.1, 12.8.2)

Table 31: Hospitalization and Intensive Care Units

	Sequential MMF/Tacrolimus – Steroids N=72		Tacrolimus/ MMF N=67		Student' t test P value
	Mean	SD	Mean	SD	
HOSPITALIZATIONS					
Number of episodes of hospitalisation:	2.05	1.19	1.99	1.03	
Number of days of hospitalisation	9.66	8.78	10.01	9.03	0.6258
ICU					
Number of episodes of ICU:	1.08	0.28	1.11	0.42	
Number of days of ICU	9.46	9.82	9.60	10.06	0.9443

8.5 Safety conclusion

Overall in this study good safety level were achieved in both treatment groups. Non statistically significant differences in occurrence of CMV infection were noted in favor of Arm 2.

9 DISCUSSION AND OVERALL CONCLUSIONS

Based on the results of this study, Arm 1 did not show any difference regarding creatinine clearance. Arm 1 gets a more prolonged rejection free time and a reduced rate of corticosteroids sensitive rejections compared to Arm 2. Regarding the other efficacy outcomes, no evidences of differences between the two arms were observed. Either, no differences in safety profile were noted. However the lack of statistical power due to due to smaller than planned accrual does not allow to draw firm conclusions from these data, and these indications prompt confirmation from more powered studies.

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11 END-OF-TEXT TABLES AND FIGURES