CLINICAL STUDY REPORT

Prospective, randomized, open, 2-arm national multi-center study to evaluate the value of Rituximab in humoral chronic rejection after renal transplantation

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Protocol Code: VAL-518-HEE-0200-S

EudraCT-number: 2006-006137-41

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SYNOPSIS

Protocol No.	VAL-518-HEE-0200-S
Protocol Version/Date	8k from 8.7.2008
Title	Value of rituximab in humoral chronic rejection after renal transplantation
EudraCT No.	2006-006137-41
Sponsor	Prof. Dr. Dr. Uwe Heemann, München
Project phase	II
Indication	Renal transplantation
Objectives Primary objective Secondary objectives	To evaluate the benefit of rituximab in patients with CAN with histologically proven C4d deposits and/or plasma cell and/or B-Lymphocyte (CD20+ cells) infiltration of their grafts. Renal function at 1 year Tolerability/ safety Graft survival at 1 and 2 years
Trial design	Prospective, randomized, open, 2-arm, national multi-center study
Target population	Renal transplant patients beyond 1 year post Tx, suffering from CAN and histologically proven C4d deposits and/or plasma cell and/or B-Lymphocyte (CD20+ cells) infiltration of their grafts
Planned sample size	200 patients (100 patients each group)

Inclusion/Exclusion criteria	Inclusion criteria: GFR > 25 ml/min x 1,73m^2 (Cockroft & Gault) Time after transplantation < 3 months Single organ recipient Age >18 years Informed consent Biopsy proven CAN with C4d+ and/or plasma-cell and/or B-Lymphocyte (CD20+ cells) within the last 4 weeks before inclusion ACE/AT1 blocker started more than 1 week before inclusion Exclusion criteria: History of Hepatitis B, HIV, Hepatitis C (active/chronic) Signs of acute cellular rejection Proteinuria >4g/24h History of malignancy within the last 5 years, (except non-metastatic basal or squamous cell carcinoma of the skin) Systemic infection Leukopenia Pregnancy Participation in another investigational drug study
Total number of centers	8-12
Sample size per center	>= 15
Length of study	1 year recruitment, 1 year protocol treatment period and 1 year additional follow up per patient
Investigational medicinal product(s) (Dose/Route/Regimen)	Rituximab (MabThera®): 375mg/m² as IV infusion over >= 6h each at time point 0 and 2 weeks.

	Initial infusion rate of 50 mg/h, stepwise rise is possible after 30 min (see PI)
Comparator drug(s) (Dose/Route/Regimen)	None
Background medication (Dose/Route/Regimen)	Immunosuppression: Tacrolimus: Trough level 4-8 ng/ml Mycophenolatmofetil (MMF): 1.5-2.0 g/d, Steroids: Optional (as given before study entry); a single dose of 100 mg Methylprednisolon i.v. was to be given at baseline (day 0) in both groups. ACE-inhibitor or AT1-receptor-antagonist
Main parameter(s) of Efficacy Safety	**Primary endpoint** Serum creatinine level 12 month **Secondary endpoint** GFR (calculated) Proteinuria Graft survival after 1 and 2 years Tolerability of rituximab Incidence and severity of acute rejections Patient survival after 1 year Incidence and severity of adverse events (serious and non-serious)
Study procedure	Patients with histologically proven C4d deposits and/or plasma cell and/or B-Lymphocyte infiltration (CD20+ cells) of their grafts within the last 3 months before inclusion (centrally confirmed), fulfilling the inclusion/exclusion criteria, were to be randomized 1:1 into one of the 2 groups:

	A) Treatment with rituximab B) Treatment without rituximab All patients were to be treated with baseline medication of Tacrolimus, MMF, steroids and ACE-inhibitor or AT1-receptor-antagonist. Each patient will be followed for 1 year, with study visits at day 0, 1, 3, 7 and 14, month 3, 6 and 12
Randomization procedure	1:1 Procedere: All biopsies were analyzed by histopathological centers and the comment will be faxed to the central managing unit (Munich). Upon receipt, it was decided whether the patient is eligible for the study and if yes, the patient was randomized
Statistical considerations Sample size calculation	Sample size calculation was based on the primary endpoint of the study. From previous investigational data a standard deviation (SD) of 0,8 mg/dl for serum creatinine was expected. Thus a sample size of 64 per group (n=128) is necessary to detect a difference of 0,4 mg/dl in serum creatinine level (effect size: 0,5) with 80% power and alpha-error 5% using the two sample t-test. In consideration of an expected drop-out rate of approximately 30%, a group sample size of 100 (n=200) was chosen. Pre-analysis of the data was planned 8 month after recruitment to prove assumptions of sample size calculation with adjustment of sample size in case of aberrance.

Statistical Analysis	Usual descriptive statistics were to be displayed for the efficacy and safety endpoints. Safety and tolerability parameters were tabulated according to frequency, and analyzed descriptively. Comparison of the main parameter serum creatinine between the therapy groups was to be performed by the two sample t-test. Secondary endpoints were to be analyzed explorative: For analysis for categorical data Chi-square and Fisher Exact-Tests respectively were to be used. The mean of numeric parameters between the therapy groups were to be compared using two sample t-test or Mann-Whitney-U-test where appropriate. For the analysis of graft survival Kaplan-Meier curves were to be estimated and compared with the log-rank-test between the therapy groups. All analysis was to be performed on a significance level.

1 ETHICS

1.1 INDEPENDENT ETHICS COMMITTEE (IEC) OR INSTITUTIONAL REVIEW BOARD (IRB)

The study and any amendments were reviewed by the Ethics Committee of the medical faculty at TU München. The committee Chair is Prof. Dr. A. Schömig.

1.2 ETHICAL CONDUCT OF THE STUDY

The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

1.3 PATIENT INFORMATION AND CONSENT

Informed patient consent was obtained before randomisation and enrolment. A sample patient information and consent form is attached in the appendix section.

2 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Function	**Name**	**Institution**	**Adress**
Sponsor (Dekan)		TUM	
LKP	Prof. Dr. med. Dr. h.c. Uwe Heemann	Abteilung Nephrologie an der TU München Klinikum rechts der Isar	Ismaninger Str. 22 81675 München
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Data bank	Dr. Rainer Blaser	Institut für Medizinische Statistik und Epidemiologie	Ismaninger Str. 22 81675 München
Data management	Dorothea Küster	Münchner Studienzentrum	Ismaninger Str. 22 81675 München
AMS		Roche Pharma AG	Emil-Barell-Straße 1 79639 Grenzach-Wyhlen
Drug Manufacturer		Roche Pharma AG	Emil-Barell-Straße 1 79639 Grenzach-Wyhlen
Statistics	Nephrology	TUM	Ismaninger Str. 22 81675 München

3 PARTICIPATING CENTRES

Centres in grey did not enrol any patients.

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4 INTRODUCTION

Kidney transplantation has become an established treatment of end-stage renal disease. Chronic allograft nephropathy (CAN) belongs to the important causes of long-term kidney graft loss. The rate of long-term graft failure due to CAN still remains at 50% to 80%. So far no proven treatment of CAN is available, but special emphasis has been put on the reduction of acute rejection episodes, as their frequency is one of the main factors in the development of CAN. Numerous studies indicate an unfavourable influence of antibody-mediated alloimmunity on kidney allograft outcome. Antibodies were present in 96% of 826 patients who rejected a kidney graft, and were associated with chronic rejection in 33 studies of kidney, heart, lung and liver grafts and appeared in 3 studies in the circulation. The impact of humoral alloreactivity, indicated by C4d deposits in peritubular capillaries of a renal allograft, on the development and progression of CAN, is therefore a significant problem in transplantation. Capillary C4d-deposits, as presumed marker of antibody-mediated rejection AMR, have been associated with inferior renal function and graft survival. The high rate of steroid- and antibody-resistant rejection episodes in patients with evidence of AMR may suggest limited anti-humoral efficacy of drugs primarily directed at cellular immune mechanisms and further stresses the need for specific antihumoral therapy. Varies therapeutic strategies have been proposed to treat AMR. These include apheresis i.e plasmapheresis (PP), immunoadsorption (IA) and intravenous immunoglobulin (IVIG).

Rituximab, a high affinity CD20 antibody that depletes B-cells, could be a therapeutic strategy to control humoral rejection and thus delay the progression of CAN. Rituximab is well established in the therapy of various types of lymphoid malignancies. Furthermore there is increasing evidence for the efficacy of Rituximab for the treatment of various autoimmune disorders. The aim of this study was to evaluate the efficacy of Rituximab in the treatment of humoral chronic renal allograft rejection.

5 STUDY OBJECTIVES

To evaluate the benefit of rituximab in patients with CAN having deposits of C4d+ and/or plasma-cells and/or B-lymphocytes in the allograft biopsy.

5.1 PRIMARY OBJECTIVE AND ENDPOINT

Renal function after treatment with rituximab measured by creatinine level 12 month after therapy.

5.2 SECONDARY OBJECTIVE AND ENDPOINT

Glomerular filtration rate (Cockroft & Gault formula), graft survival and proteinuria as further parameters to evaluate efficacy at 1 and 2 years.
Tolerability and safety of Rituximab (MabThera) treatment (incidence and adverse events and serious adverse events).

5.3 SAFETY

Tolerability of rituximab
Incidence and severity of acute rejections
Patient survival after 1 year
Incidence and severity of adverse events

6 INVESTIGATIONAL PLAN

6.1 OVERALL STUDY PLAN

This study was a prospective, randomized, open, 2-arm, national multi-center study to evaluate the value of rituximab in humoral chronic rejection after renal transplantation in approximately 150-200 patients (75-100 patients each group). Due to slow enrolment, the study was terminated prematurely after 13 patients had been enrolled.
All biopsies were analysed by Prof. Groene (Heidelberg) or an alternative pathological institute and the results immediately communicated to the central managing unit (Munich). Upon receipt, patients with biopsy proven CAN with C4d+ and/or plasma-cells and/or B-lymphocytes within the last 4 weeks before inclusion (centrally confirmed), fulfilling the inclusion/exclusion criteria, were randomized 1:1 into one of the 2 groups:
Arm A: Treatment with rituximab
Arm B: Treatment without rituximab

All patients were treated with baseline medication of Tacrolimus, MMF, steroids (optional, with same dose as given before study entry) and ACE-inhibitor or AT1-receptor-antagonist. A single dose of 100 mg Methylprednisolon i.v. was given at baseline (day 0) in both groups (in the rituximab group 30 min before start of the rituximab infusion). Study visits were at 1,3,7 and 14 days (rituximab group), 3, 6 and 12 months. The follow-up period of 1 year with a study visit at 24 months was not completed for any patient because of the early termination of the study.

6.2 SELECTION OF STUDY POPULATION

6.2.1 Total number of patients

Initially the enrolment of 200 patients was planned. The total number of patients enrolled was 13 before the trial was prematurely terminated due to slow enrolment. Seven patients were randomised to the Rituximab treatment arm, and 6 patients were randomised into the control arm.

6.2.2 Gender Distribution

In this clinical trial the gender distribution happened arbitrarily.

6.2.3 Inclusion Criteria

On inclusion in the study, the basic immunosuppression of the transplant patients was not changed as the patients continued on their previous immunosuppression consisting of tacrolimus, MMF and (optional) steroids, if the patients were on steroids before inclusion. Patients who fulfilled the following inclusion criteria were eligible for inclusion in the study:

- Renal allograft recipients at least 1 year after transplantation with GFR > 25 ml/min x 1,73m^2 (MDRD)
- Single organ recipients of renal allograft
- Patients who have provided informed consent
- Patients who are >= 18 years of age
- Patients who have biopsy proven CAN with C4d+ deposits and/or plasma-cells and/or B-lymphocytes within the last 4 weeks before inclusion
- Patients who are treated with ACE/AT1 Blocker more than 1 week before inclusion

6.2.4 Exclusion Criteria

Patients meeting any of the following exclusion criteria were not eligible for inclusion in the study:

- Patients who suffer from HIV infection
- Patients with a history of Hepatitis B
- Patients with Hepatitis C (active/chronic)
- Patients who have a contraindication for the use of rituximab, such as leukopenia or experienced infusion-related adverse events to former antibody treatment
- Patients who showed signs of acute cellular rejection in the biopsy
- Patient has a malignancy or history of malignancy within the last 5 years, except non-metastatic basal or squamous cell carcinoma of the skin that has been treated successfully.
- Patient has a systemic infection requiring treatment.
- Female patients who are pregnant or lactating
- Patients who have any form of substance abuse, psychological illness or any other condition, which, in the opinion of the investigator, may interfere with the patient's ability to understand the requirements of the study.
- Patients who have a proteinuria >4g/24h
- Patient is unlikely to comply with the visits scheduled in the protocol.
- Patient is simultaneously participating in another investigational drug study or has participated in such study within 28 days before entry in this study.

6.2.5 Patient randomisation and allocation to treatment

The indication for performing a kidney biopsy, done by each study center, had to be due to clinical reasons such as declining graft function of the transplanted kidney with the goal to investigate possible causes, i.e. rejection. Consequently, the biopsy performed in each centre was not a study related procedure. The biopsy result led to randomisation. Before randomisation took place, written informed consent was obtained.

Allocation of the patients to treatment was performed in an open manner according to a randomisation schedule provided by the central managing unit Munich. The randomisation was 1:1, stratified by centre.

The following paragraph gives an overview of the sequence of events.

- The patient was informed of nature, purpose and possible hazards of the clinical trial and asked to sign the Informed Consent form.

- It was confirmed that the patient met all inclusion/exclusion criteria. The biopsy result was communicated to Munich. If eligible, the patient was then be enrolled in the study and randomised.

6.2.6 Discontinuation criteria for individual patients

The patient was free to withdraw from the study at any time without penalty or prejudice. The investigator was also free to terminate a patient´s participation in the study at any time if the patient´s clinical condition warrants it. All patients withdrawing from the study were followed up for Serious Adverse Events for 28 days. Patients were discontinued from receiving the study drug for the following reasons:

1. If the investigator thinks a discontinuation of therapy would be in the best interest of the patient.
2. If the patient requested discontinuation.
3. If a patient was unable to comply with the protocol.
4. If a patient became pregnant or failed to use adequate birth control (for those patients who are able to conceive).
5. If prohibited concomitant immunosuppressive medication was required as described in section 9.3.4
6. If the patient was lost to follow up

6.3 TREATMENT

6.3.1 Identity of Investigational Product

6.3.1.1 Physicochemical Properties of study drug

Generic Name: rituximab

Code Number: IDEC-C2B8, IDEC-102, Ro 45-2294

Trade Name: MabThera® (European Union)

EU Approval Number: EU-1-98-067-001

Synonyms: Chimeric Pan-B, C2B8, mouse-human chimeric antibody to CD20 antigen

Labeling of study medication: no study specific labelling will be performed. The drug will be used with its marketed presentation.

6.3.1.2 Clinical Formulation

Rituximab is formulated for intravenous administration as a sterile product in 9.0 mg/mL sodium chloride, 0.7 mg/mL polysorbate 80, 7.35 mg/mL sodium citrate dihydrate, and sterile water for injections (pH 6.5). The antibody is supplied for market use in 10 mL and 50 mL vials containing 100 mg of antibody/10 mL of solution or 500 mg of antibody/50 mL of solution at a concentration of 10.0 mg/mL. No preservative is used since the vial is designed for single use. The product packaging consists of USP Type I borosilicate glass vials (10 mL and 50 mL) with 20 mm plug stoppers and 20 mm plastic flip-off caps. For this study only the 100mg vials were used.

6.3.1.3 Stability, Storage and Handling Instructions

Prepared infusion solutions of rituximab are biologically and chemically stable at 2°C to 8°C (36°F to 46°F) for 24 hours and at room temperature for an additional 12 hours. The product was not used beyond the expiration date stamped on carton. Rituximab vials were protected from direct sunlight. No incompatibilities between rituximab and polyvinylchloride or polyethylene bags have been observed.

6.3.1.4 Preparation and Dosing of rituximab (MabThera)

Mabthera® was administered as an intravenous infusion. The calculated total dose of rituximab was dissolved in 1000 – 2000 ml NaCl 0.9 %. A final concentration of at least 1 mg/ml (maximum 4mg/ml) was aimed for.

During the rituximab infusion the patients were either connected to a monitor or pulse and blood pressure were measured every 30 minutes during the first infusion, later every 60 minutes. For the first administration an initial infusion rate of 50 mg/h was recommended. If this was well tolerated it could be increased by 50 mg/h every 30 minutes up to a maximum infusion rate of 400 mg/h. If the first administration of rituximab was well tolerated all subsequent administrations could be started at a rate of 100 mg/h which was then increased every 30 minutes up to 400 mg/h.

In general, a slow infusion over a period of 8 hours was used in this protocol to minimise first adverse reactions. If any of the following side-effects occured the rituximab infusion was interrupted and only continued after clinical improvement:

• Fever > 38.5°C
• Shaking chills
• Bronchospasm
• Hypotension by more than 30 mmHg or clinical judgement of the treating physician

6.3.1.5 Treatment

Patients with CAN and C4d+ deposits and/or plasma-cells and/or B-lymphocytes in the biopsy were enrolled into the study. Patients randomised to the rituximab arm, received rituximab with 375mg/m² as an intravenous infusion over up to 8h on days 0 and 14. During the rituximab infusion the patients were either be connected to a monitor or pulse and blood pressure was measured every 30 minutes during the first infusion, later every 60 minutes. If any side effect occured, see above, the infusion was immediately discontinued.

6.3.1.6 Basic Medication Rituximab

The patients received rituximab (375 mg/m^2) as i.v. infusion over ≤ 6 hours each at time points 0 and two weeks.

6.3.1.7 Dosing and administration of additional immunosuppressive medication

Patients were required to be on an immunosuppressive regimen with tacrolimus and mycophenolatmofetil. 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 4-8 ng/ml.
The daily dose of mycophenolatmofetil (MMF) was in the target range of 1.5-2.0 g/d. If gastrointestinal disorders or leucopenia occured, dose changes were accepted.
A single dose of 100 mg Methylprednisolon i.v. was given at baseline (day 0) in both groups (in the rituximab group 30 min before start of the rituximab infusion). Administration of oral steroids was optional.

6.3.2 Prior and Concomitant Therapy

6.3.2.1 Concomitant Medication Tacrolimus

Tacrolimus was administered as concomitant immunosuppression with a trough level between 4 and 8 ng/ml.

6.3.2.2 Concomitant Medication Mycophenolatmofetil (MMF)

MMF was administered as a concomitant immunosuppression at a dose of 1.5 to 2.0 g/day.

6.3.2.3 Concomitant Medication Methylprednisolone

Methylprednisolone was administered as a concomitant immunosuppression at a dose of 100 mg i.v. at day 0 (baseline).

6.3.2.4 Concomitant Medication Steroids (Prednisolone)

Prednisolone was administered as a concomitant immunosuppression according to the prestudy dose.

6.3.2.5 Other concomitant medication

Each patient was administered an ACE-inhibitor or AT1-receptor-antagonist on the basis of clinical evidence of efficacy and occurrence of adverse events. Any other concomitant therapy was administered according to the disease status.

6.3.2.6 Premedication

For a prevention of an anaphylactic reaction and a cytokine release syndrome patients were pretreated with antihistamines (e.g. Tavegil® 2 mg i.v., Tagamet® 200 mg) 30 minutes prior to rituximab application and thereafter if clinically indicated (e.g. in case of infusion-related symptoms). Other medications could also be administered as clinically indicated at the investigator's discretion except for contraindicated medications. In the event of severe infusion-related side-effects the rituximab infusion was stopped immediately and the symptoms treated aggressively with administration of NaCl 0.9 %, methylprednisolone 100 mg i.v., pethidine (Dolantin®) i.v. bronchodilators/antihistamines etc. as necessary. The rituximab infusion was not continued until all symptoms have subsided.

6.3.2.7 Administration of prophylactic antiviral treatment

No prophylactic antiviral treatment was administered. Blood samples of patients were regularly screened for viral activity whenever clinically indicated.

6.3.2.8 Prohibited concomitant therapy

Drugs that were prohibited during this study:

• All non-licensed medication and other investigational drugs

• Antibody therapy other than rituximab

• Chemotherapeutics

6.4 EFFICACY AND SAFETY VARIABLES

6.4.1 Efficacy and Safety Measurements Assessed and Flow Chart

Table 1: Schedule of Assessments for Cohort A and Cohort B

	At time of steroid/rituximab infusion	Study Month = Number of months after steroid/rituximab infusion®							
	Baseline (Day 0)	Day 1	Day 3	Day 7	Day 14	Mo. 3	Mo. 6	Mo. 12	Mo. 24
Informed consent	X								
Eligibility[1]	X								
Demographics[2]	X								
Medical history of renal disease[3]	X								
Renal transplantation[4]	X								
Clinical assessments[5]	X	X[9]	X[9]	X[9]	X[9]	X	X	X	X
Laboratory (serum chemistry) I	X	X[9]	X[9]	X[9]	X[9]	X	X	X	X
Laboratory (serum chemistry) II	X				X[9]	X	X	X	X
Laboratory (hematology)	X	X[9]	X[9]	X[9]	X[9]	X	X	X	X
rituximab Infusion in Arm A	X				X				
Comorbidity[6]	X					X	X	X	X
Immunosuppressive therapy[7]	X	X[9]	X[9]	X[9]	X[9]	X	X	X	X
Concomitant therapy[8]	X	X[9]	X[9]	X[9]	X[9]	X	X	X	X
De novo or recurrence of renal disease	X	X[9]	X[9]	X[9]	X[9]	X	X	X	X
Acute rejection episodes, graft loss and patient death	X					X	X	X	X
Adverse event reporting		X[9]	X[9]	X[9]	X[9]	X	X	X	X
B-cell counts[10]	X	X			X	X	X	X	X

Informed consent and eligibility will be performed prior to enrollment in the study

[1] Inclusion and exclusion criteria, Chest X-ray, PSA, abdominal ultrasound, pregnancy test (see section 8)

[2] Gender, date of birth and race

[3] Etiology and History of renal disease (see appendix 3)

[4] Donor and recipient information, clinical data on transplantation, CMV and EBV serology and serum creatinine, proteinuria, biopsy results of all acute and chronic rejections *(gelöscht. And malignancies since transplantation)*

[5] Blood pressure, weight, height (gelöscht: for pediatric patients), for calculated creatinine clearance see appendix 5

[6] See appendix 6

[7] Concomitant immunosuppressive therapy

[8] Anti-hypertensives, anti-hyperlipids and anti-diabetics and drugs known to affect renal function (see appendix 7)

[9] only in patients treated with rituximab

[10] only in patients treated with rituximab if routine in center

[Lab. I] Sodium, potassium, phosphorus, calcium, AST, ALT, creatinine, urea, urine analysis

[Lab. II] fasting lipid profile (total cholesterol, Triglycerides), serum protein, LDH, total bilirubin and serum glucose

6.4.1.1 Laboratory analysis

Laboratory analysis in so far as necessary to comply with the protocol was performed by the local laboratory at each investigational site.

6.4.1.2 Baseline and Follow up Assessments

After obtaining a signed informed consent form, a screening evaluation to determine patient eligibility was conducted at the day of randomisation. Safety and concomitant medication use was assessed throughout treatment. Adverse events were documented.

In all patients, at baseline, at 3, 6 and 12 months, additionally in patients treated with rituximab on days 1, 3, 7 and 14 the following assessments were performed (also see Flow chart).

1) Only at baseline: Medical history including reason for renal transplantation and grading of biopsy proven rejection. All rejection episodes and immunosuppressive therapy prior to enrolment into this study were documented, as well as current concomitant medication. Furthermore, chest X-ray, PSA, abdominal ultrasound, pregnancy test was performed.

2) At all study visits physical examination included:

- Vital signs (blood pressure, pulse, temperature)
- Height, weight, and calculation of body mass index (BMI)

3) At all study visits laboratory, diagnostic assessments included:

- Hematology (WBC including Diff. count, Hb, RBC and Platelets)

3.1) At baseline, day 1, 3, 7, 14 (if applicable), month 3, 6, and 12:

-Serum chemistry (sodium, potassium, calcium, phosphorus, creatinine, urea, urine analysis, AST, ALT)

- Glomerular filtration rate was calculated by Cockroft & Gault formula and reported in the CRF

3.2) At baseline, day 14 (if applicable), month 3, 6, and 12.

-Serum chemistry (total bilirubin, serum glucose, serum protein, LDH, fasting lipid profile (cholesterol, triglyceride))

4) At baseline, month 3, 6, 12 and 24 urine analysis (proteins)

5) Pregnancy test:

A pregnancy test was performed in all female patients of child bearing potential upon hospitalisation or within 7 days prior to enrolment.

6) At all study visits microbiological testing:

If infection was suspected, appropriate samples were taken for microbiological analysis and any positive findings recorded as adverse events.

7) Comorbidity

8) At all study visits concomitant immunosuppressive therapy

9) At all study visits selected concomitant therapy: Anti-hypertensives, anti-hyperlipids and anti-diabetics (generic names) and drugs known to affect renal function

10) De novo or recurrence of renal disease

11) Presence of acute rejection episodes (presumed or biopsy proven)

12) Graft loss or patient death

6.5 CHANGES IN CONDUCT OF THE STUDY

Due to slow patient enrolment, the study was terminated prematurely. At this point 13 patients had been enrolled. Follow-up for 24 months was not completed for any of the patients due to premature termination of the trial.

7 STUDY PATIENTS

7.1 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Table 2: Patient Demographic and other baseline characteristics

Patient-ID	Arm	Date of Birth	Age	Sex	Renal disease	Donor	Donor Age
02001	A	24.06.1981	27	female	tubular intersitial nephropathy	cadaveric	47
04002	A	01.01.1983	26	male	hemolytic uremic syndrome	cadaveric	53
07008	A	02.05.1963	46	male	chronic glomerulonephritis	cadaveric	16
01001	A	19.06.1954	54	male	IgA nephropathy	cadaveric	55
01004	A	25.01.1984	24	male	Himon-Syndrome	cadaveric	42
01005	A	06.07.1967	41	male	chronic glomerulonephritis	cadaveric	61
01008	A	14.12.1960	48	male		living	15
Mean			**38**				**41**
Standard deviation			**12**				**19**
03001	B	03.11.1971	37	male	chronic glomerulonephritis	living	58
04001	B	04.01.1988	20	male	tubular intersitial nephropathy	living	44
01002	B	12.05.1944	64	male	IgA nephropathy	cadaveric	50
01003	B	02.12.1945	63	male	Polycystic kidney disease	living	58
01006	B	21.01.1944	64	male	Polycystic kidney disease	cadaveric	68
01007	B	13.03.1967	41	female	secondary amyloidosis, Crohn's disease	cadaveric	56
Mean			**48**				**56**
Standard deviation			**18**				**8**

8 EFFICACY AND SAFETY EVALUATION

8.1 DATA SETS ANALYSED

8.1.1 Primary endpoint

Serum creatinine level 12 month

8.1.2 Secondary endpoint

GFR (calculated)

Proteinuria

Graft survival after 1 year

8.2 ADVERSE EVENTS (AES)

8.2.1 Definition of an adverse event

An adverse event (AE) is any untoward medical occurrence after starting study drug treatment, regardless of whether the event is considered related or unrelated to the study drug. The investigator will evaluate changes in physical signs, laboratory values, or other diagnostic procedures in determining AEs. Subjective AEs should be elicited by first questioning the patient in a non-directive manner, then, if any unfavorable symptoms are reported, questioning in a more detailed manner to obtain the information necessary for reporting the event. The NCI Common Terminology Criteria for Adverse Events (NCI-CTC), Version 3, is used for assessing the severity of AEs.

8.3 STATISTICAL ISSUES

Due to the reduced number of patients, statistical analysis of the primary endpoint and secondary endpoint data was not performed. Patient by patient descriptive analysis is included here.

Since only 5 patients participated long enough to reach the primary endpoint after 1 year, comparative analysis will be performed using data gained after 6 months. Out of the 4 patients which received Rituximab, 3 had slightly worse levels of creatinine (on average 19 % change to baseline value) and 1 had reduced serum creatinine after 6 months. The 4 patients which did not receive rituximab, had increased creatinine after 6 months (on average 23% change to baseline creatinine). A detailed overview of the course of the serum creatinine values is shown in table 3 below.

Table 3: Serum creatinince values

Arm	PatientNumber	Baseline	1 day	3 days	7 days	14 days	3 months	6 months	12 months
A	02001	2,86							
A	04002	1,89	3,02	2,3	2,14	2,41	2,15		
A	07008	3,28	2,99	2,65	2,82	2,82	3,09	3	
A	01001	1,4		1,4	1,4	1,4	1,5	1,6	2,9
A	01004	2,3	2,4	2,4	2,4	2,4	2,9	3	3
A	01008	1,7		2,2	2,1	1,8	2,6	2,4	
B	03001	3,69					4,02	4,33	
B	04001	3,96					4,66	5,28	4,65
B	01002	1,6					1,4	1,7	1,8
B	01003	1,1					1,4	1,5	1,4
B	01005	2,4							
B	01006	3,3							
B	01007	2,4					3,3		

8.4 BY PATIENT ANALYSIS

Patient 01001:

54-year old male patient with IgA nephropathy as underlying renal disease. The patient was randomised to the rituximab treated arm. This patient was treated for 13 months according to the study protocol, until discontinuation due to adverse event (severe leucopenia). The patient was transplanted using a cadaveric donor kidney. The biopsy result confirmed plasma cell infiltration, an antibody-mediated reaction and a chronic active antibody-mediated rejection. Baseline creatinine was 1,4 mg/dl, calculated baseline GFR was 56. At 6 months, creatinine was 1,6 mg/ml, calculated GFR was 45. At 12 months, creatinine was 2,9 mg/ml, calculated GFR was 24, and proteinuria was 338 mg/g crea. No further acute rejection episode occurred. The following AEs occurred: month 3 (25.02.08) chicken pox, severe, unrelated to study drug, resolved; month 6 (5.7.08) deterioration of renal function, mild, unrelated to study drug, resolved; month 12 (5.7.08) urinary tract infection, moderate CTCAE 2, possible relation to study drug, resolved; (1.1.08) herpes labialis, moderate, possible relation to study drug, resolved; (22.1.08) rise in creatinine, moderate, possible relation to study drug, unresolved; rise in CRP, mild, possible relation to study drug, unresolved; (23.1.08) leucopenia, severe, possible relation to study drug, unresolved.

Patient 01002:

64-year old male patient with IgA nephropathy as underlying renal disease. The patient was randomised to the non rituximab treated arm. This patient was treated for 18 months according to the study protocol, until premature study termination. The patient was transplanted using a cadaveric donor kidney. The biopsy result confirmed C4d deposits, an antibody-mediated reaction and a chronic active antibody-mediated rejection. Baseline creatinine was 1,6 mg/dl, calculated baseline GFR was 47, baseline proteinuria was 116 mg/g crea. At 6 months, creatinine was 1,7 mg/ml, calculated GFR was 43, and proteinuria was 107 mg/g crea. At 12 months, creatinine was 1,8 mg/ml, calculated GFR was 41, and proteinuria was 96 mg/g crea. No further acute rejection episode occurred. The following AEs occurred: month 6 (16.09.08) cataract, mild, unrelated to study drug, resolved; obstipation (2.10.08), mild, unrelated to study drug, resolved; diarrhea (25.06.08), mild, possible relation to study drug, resolved; cold (15.06.08), mild, unrelated to study drug, resolved; month 12 (5.1.09), loss of diuresis, mild, unrelated to study drug, resolved.

Patient 01003:

63-year old male patient with polycystic kidney disease as underlying renal disease. The patient was randomised to the non rituximab treated arm. This patient was treated for 17 months according to the study protocol, until premature study termination. The patient was transplanted using a living donor kidney. The biopsy result confirmed plasma cell infiltration, an antibody-mediated reaction, T-cell-mediated rejection, and acute T-cell-mediated rejection. Baseline creatinine was 1,1 mg/dl, calculated baseline GFR was 60, baseline proteinuria was 318 mg/g crea. At 6 months, creatinine was 1,5 mg/ml, calculated GFR was 50. At 12 months, creatinine was 1,4 mg/dl, calculated baseline GFR was 54, baseline proteinuria was 5270 mg/g crea. No further acute rejection episode occurred. The following AEs occurred: month 6 (23.07.08) edema, mild, unrelated to study drug, unresolved; diarrhea, mild, unrelated to study drug, resolved; (14.6.08) pulmonary infection with cough and secretion, mild, unrelated to study drug, resolved; month 12 (26.3.09) diarrhoe, mild, probable relation to study drug, resolved; edema, mild, unrelated to study drug, resolved.

Patient 01004:

24-year old male patient with Himon syndrome as underlying renal disease. The patient was randomised to the rituximab treated arm. This patient was treated for 17 months according to the study protocol, until premature study termination. The patient was transplanted using a cadaveric donor kidney. The biopsy result confirmed C4d deposits, an antibody-mediated reaction and a chronic active antibody-mediated rejection. Baseline creatinine was 2,3 mg/dl, calculated baseline GFR was 37, baseline proteinuria was 834 mg/g crea. At 6 months, creatinine was 3,0 mg/ml, calculated GFR was 27, and proteinuria was 362 mg/g crea. At 12 months, creatinine was 3,0 mg/ml, calculated GFR was 27, and proteinuria was 448 mg/g crea. No further acute rejection episode occurred. The following AEs occurred: day 3 (25.04.08) cough and secretion, mild CTCAE Grade 1, unrelated to study drug, unresolved; same day, nausea, mild, unrelated to study drug, resolved; day 7 cough (29.04.08), mild CTCAE Grade 1, unrelated to study drug, resolved; secretion, mild, unrelated to study drug, resolved; hyperparathyroidism, mild, unrelated to study drug, unresolved; day 14 (6.5.08), rituximab infusion adjustment, mild, probable relation to study drug, resolved; month 3 (3.7.08) scrotal abscess, mild, unrelated to study drug, resolved; (18.6.08) epididimitis, mild, unrelated to study drug, resolved; month 6 (21.7.08) urinary tract infection, moderate, possible relation to study drug, resolved.

Patient 01005:

41-year old male patient with chronic glomerulonephritis as underlying renal disease. The patient was randomised to the rituximab treated arm. This patient was treated for 3 days according to the study protocol, until premature withdrawal of consent. The patient was transplanted using a cadaveric donor kidney. The biopsy result confirmed CD20-positive cells and C4d deposits, an antibody-mediated reaction and a chronic active antibody-mediated rejection. No AEs were reported.

Patient 01006:

64-year old male patient with polycystic kidney disease as underlying renal disease. The patient was randomised to the non-rituximab treated arm. This patient was treated for 1 months according to the study protocol, until Cellcept had to be stopped due to BK-virus infection. The patient was transplanted using a cadaveric donor kidney. The biopsy result

confirmed plasma cell infiltration and C4d deposits, an antibody-mediated reaction and a chronic active antibody-mediated rejection. No other AEs were reported.

Patient 01007:

41-year old female patient with secondary amyloidosis due to Crohn's disease as underlying renal disease. The patient was randomised to the non rituximab treated arm. This patient was treated for 3 months according to the study protocol, until occurance of adverse event. The patient was transplanted using a cadaveric donor kidney. The biopsy result confirmed CD-20-positive cells, an antibody-mediated reaction and a chronic active antibody-mediated rejection. No further acute rejection episode occurred. The following adverse events occurred: month 3 (29.8.08) CMV infection, moderate, possible relation to study drug, resolved; (5.11.08) BK-Virus infection, moderate, possible relation to study drug, resolved.

Patient 01008:

48-year old male patient. The patient was randomised to the rituximab treated arm. This patient was treated for 11 months according to the study protocol, until Cellcept had to be terminated. The patient was transplanted using a living donor kidney. The biopsy result confirmed CD-20 positive cells and C4d deposits, an T-cell-mediated reaction and a chronic active T-cell-mediated rejection. Baseline creatinine was 1,7 mg/dl, calculated baseline GFR was 46, baseline proteinuria was 103 mg/g crea. At 6 months, creatinine was 2,4 mg/ml, calculated GFR was 31, and proteinuria was 92 mg/g crea. No further acute rejection episode occurred. The following AEs occurred: day 14 (16.09.08) leucopenia, mild CTCAE 1, possible relation to study drug, resolved; month 3 (7.11.08) cough, mild, unrelated to study drug, resolved; month 6 (15.12.08) diarrhoe, mild, possible relation to study drug, resolved; month 12 (28.4.09) diarrhoe, mild, unrelated to study drug, resolved; (16.3.09) gastrointestinal infection, moderate, probable relation to study drug, resolved.

Patient 02001:

27-year old female patient with tubular interstitial fibrosis as underlying renal disease. The patient was randomised to the rituximab treated arm. This patient dropped out of the study after baseline measurements, due to early withdrawal of consent. No AEs were reported.

Patient 03001:

37-year old male patient with chronic glomerulonephritis as underlying renal disease. The patient was randomised to the non rituximab treated arm. This patient was treated for 9 months according to the study protocol, until dialysis was necessary. The patient was transplanted using a living donor kidney. The biopsy result confirmed CD20-positive cells and C4d deposits, a T-cell-mediated reaction and a chronic active T-cell-mediated rejection. Baseline creatinine was 3,69 mg/dl, calculated baseline GFR was 19, baseline proteinuria was 2000 mg/g crea. At 6 months, creatinine was 4,33 mg/ml, calculated GFR was 15, and proteinuria was 3647 mg/g crea. No further acute rejection episode occurred. Graft-loss due to terminal failure of graft function occured. The following AEs were reported: month 3 (05.01.09) anaemia, mild, unrelated to study drug, resolved; worsening hypertension, mild, unrelated to study drug, unresolved.

Patient 04001:

20-year old male patient with tubular interstitial nephropathy as underlying renal disease. The patient was randomised to the non rituximab treated arm. This patient was treated for 14 months according to the study protocol, until adverse event #5 occured. The patient was transplanted using a living donor kidney. The biopsy result confirmed CD20-positive cells and C4d deposits, an antibody-mediated reaction and a chronic active antibody-mediated rejection. Baseline creatinine was 3,96 mg/dl, calculated baseline GFR was 21, baseline proteinuria was 5190 mg/g crea. At 6 months, creatinine was 5,28 mg/ml, calculated GFR was 15. At 12 months, creatinine was 4,65 mg/ml. Further episode of acute rejection occurred in month 12 on 15.7.09. No biopsy was performed; treatment was dialysis, which was ongoing. The following AEs were reported: month 3 (15.01.08) campylobacter infection, mild, unrelated to study drug, resolved; (15.7.08) infection respiratory tract, mild, unrelated to study drug, resolved; (15.8.08) conjunctivitis, mild, unrelated to study drug, resolved; month 6 (15.10.08) common cold, moderate, unrelated to study drug, resolved; month 12 (28.6.09) pneumonia, life-threatening (CTCAE 4), unrelated to study drug, resolved; (15.7.09) diarrhoe, mild, unrelated to study drug, resolved.

Patient 04002:

26-year old male patient with haemolytic uremic syndrome as underlying renal disease. The patient was randomised to the rituximab treated arm. This patient was treated for 3 months according to the study protocol, until premature study termination. The patient was transplanted using a cadaveric donor kidney. The biopsy result confirmed C4d deposits, an antibody-mediated reaction and a chronic active antibody-mediated rejection. Baseline creatinine was 1,89 mg/dl, calculated baseline GFR was 46, baseline proteinuria was 945 mg/g crea. At 3 months, creatinine was 2,15 mg/ml, calculated GFR was 40, and proteinuria was 1045 mg/g crea. No further acute rejection episode occurred. No AEs were reported.

Patient 07008:

46-year old male patient with chronic glomerulonephritis as underlying renal disease. The patient was randomised to the rituximab treated arm. This patient was treated for 8 months according to the study protocol, until premature study termination. The patient was transplanted using a cadaveric donor kidney. The biopsy result confirmed C4d deposits, an antibody-mediated reaction, a chronic active antibody-mediated rejection, a T-cell-mediated reaction and a chronic active T-cell-mediated rejection. Baseline creatinine was 3,28 mg/dl. At 6 months, creatinine was 3 mg/ml, calculated GFR was 45, and proteinuria was 98 mg/g crea. No further acute rejection episode occurred. One AE was reported: day 1 (13.01.09) mild CTCAE Grade 1, non-related to study drug, resolved.

8.5 EFFICACY CONCLUSIONS

No conclusions may be drawn about the efficacy of the treatment due to low numbers of patients.

8.6 DEATHS, OTHER SERIOUS ADVERSE EVENTS, AND OTHER SIGNIFICANT ADVERSE EVENTS

8.6.1 Definition of serious adverse event

A serious adverse event (SAE) is any AE occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution. An SAE is any AE that results in any of the following outcomes:

- Death
- A life-threatening AE (in the view of the investigator, the event placed the patient at immediate risk of death from the AE as it occurred. It does not include an AE that, had it occurred in a more severe form, might have caused death),
- Inpatient hospitalization, or prolongation of existing hospitalization,
- Persistent or significant disability or incapacity,
- Congenital anomaly,
- An important medical event that, while not fatal, life threatening, or requiring hospitalization, and based on appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of these outcomes.

8.6.2 Listing of Deaths, other Serious Adverse Events and Other Significant Adverse Events

Table 4: Serious Adverse Events

PatNr	SAE	Relation to study drug	Outcome
001/01	Urinary tract infection	Probable	Resolved
001/01	Varizella zoster infection	Probable	Resolved
001/04	Epididymitis left-sided	Unlikely	Resolved
001/04	Scrotal abscess right-sided	Unlikely	Resolved
001/06	Renal polyoma virus infection	Unlikely	Drop out of study
001/07	Skin ulcerations	Unlikely	Resolved with consequence
001/07	Urinary tract infection	Unlikely	resolved
001/08	Diarrhoea	Unlikely	resolved
002/04	Diarrhoea	Unlikely	Resolved
001/04	Pneumonia	Unlikely	Resolved with consequence

8.6.3 Narratives of Deaths, Other Serious Adverse Events and Certain Other Significant Adverse Events

Patient 00101: Varizella zoster infection/chicken pox

The patient presented with disseminated skin lesions on head, shoulder, and thorax on 25.2.08 The patient's mother had been diagnosed with varizella zoster infection at that time. There was no history of chicken pox in the patient's childhood. After dermatological diagnosis of chickenpox, the patient was submitted and treated with aciclovir i.v. and later valaciclovir until symptoms disappeared.

Patient 00106: Polyomavirus Infection of the renal allograft

The patient presented with an elevation of serum creatinine on 15.5.08, after renal transplantion in 01/2008. A graft biopsy was performed, which showed a polyomavirus infection of the graft. Ciprofloxacin treatment was started, and in the meantime Cellcept was terminated. Since this violated the study protocol, the patient terminated the trial at this point.

Patient 00107: skin ulcerations

On 27.04.09, this patient presented with worsening skin ulcerations on the lower leg, which had been present since the beginning of April 2009. The skin biopsy showed a leukocytoclastic vsculitis, constistent with either Pyoderma gangraenosum or Calciphylaxia. The patient had a pre-existing hyperparathyroidism and dialysis-therapy, which are risk factors for calciphylaxia. Since there was no clinical response to cortisone treatment, thiosulfate treatment was started, which halted the progression of the ulcerations. Surgical treatment using a skin graft was used to close the skin defect.

Patient 00107: urinary infection

The patient presented with fever and myalgia. Following the diagnosis of a urinary tract infection, the patient was submitted and treated with amoxicillin/clavulanic acid. Mycophenolat-Mofetil was stopped for 2 days. The patient recoved quickly under this therapy.

Patient 00108: Diarrhoea

On 28.04.2009 the patient presented with diarhoea. The patient was submitted, and a colonoscopy was performed since hemorrhagic colitis had occurred since March 2009. The colonoscopy showed no specific pathology.

Patient 00402: Diarrhoea

On 15.06.2009, this patient presented with diarrhoea, vomitting, and weight loss (3 kg) in 1 day. The patient was submitted for treatment. Cellcept was changed to Myfortic. This led to an improvement of the symptoms.

Patient 00401: Pneumonia

The patient presented on 28.06.09 with progressive dyspnia, and following repiratory insufficiency and intubation. The cause for pulmonary infection was probably pulmonary edema due to renal graft failure. The patient received antibiotic therapy, tacrolimus was reduced, and Myfortic was stopped temporarily. Furthermore, dialysis treatment was necessary. The patient recovered quickly, however due to terminal graft failure, dialysis treatment had to be continued.

8.7 SUSPECTED UNEXPECTED ADVERSE EVENTS

No previously unknown drug side-effects were observed in this study.

8.8 ADVERSE DRUG REACTION

No allergic reaction to Rituximab was observed in this trial. The SAE's, which are due to infections are likely to be due to the overall immunesuppression of kidney-transplant patients; for example, patient 001/06 in the placebo group, had a Polyomavirus infection without having received Rituximab at all. In the case of patient 001/04, the situation is similar. In patient 001/07 the skin ulcerations were most likely due to a case of calciphylaxia, which may have developed due to long-standing hyperparathyroidism, which is further confirmed by the good clinical response to thiosulfate, the treatment for calciphylaxia. The urinary infection is likely to be due to immunesuppression, but is probably not specifically due to Rituximab. In patient 001/08, the ischemic colitis is most probably due to intermittent atrial fibrillation and embolus formation together with pre-existing atherosclerosis. In patient 004/02, the diarrhea could have been related to cellcept medication, since a change to Myfortic led to an end of the diarrhea. Patient 004/01 suffered from pneumonia, which is probably related to pulmonary edema in the context of renal graft failure.

8.9 SAFETY CONCLUSION

In summary, the registered SAE's are due to the known side-effects of the study drug, concomitant medication, or their combination.

9 DISCUSSION AND OVERALL CONCLUSIONS

Due to the reduced number of patients enrolled in the study, who reached the primary endpoint no conclusions can be drawn about the efficacy of Rituximab treatment in humoral chronic allograft nephropathy after renal transplantation. As was discussed above, from previous investigational data a standard deviation (SD) of 0,8 mg/dl for serum creatinine was expected. Thus a sample size of 64 per group (n=128) is necessary to detect a difference of 0,4 mg/dl in serum creatinine level (effect size: 0,5) with 80% power and alpha-error 5% using the two sample t-test. Due to the early termination of the study and the reduced number of patients participating in the trial, adequate data analysis was not possible. Only 5 patients reached the primary endpoint after 1 year. Out of the 4 patients which received Rituximab, 3 had slightly higher levels of creatinine (on average 19 % change to the baseline creatinine value) and 1 had reduced serum creatinine after 6 months. Four patients which did not receive Rituximab, had increased creatinine after 6 months (on average 23% change to baseline creatinine). Therefore, a difference in the result of these small groups cannot be seen.

In terms of the tolerability and safety related to Rituximab in this group of patients, the adverse and serious adverse events, which were related to the study drug were among the known side-effects of Rituximab. These were primarily infections, although immunosuppression was effective in the control patients as well due to standard post-transplantation immunosuppressive therapy. No allergic reactions to the study drug occurred in the treated patients in this trial.

10 APPENDICES

10.1 Patient information and consent form

10.2 Protocol 8.07.2008