Clinical Study Protocol

A Randomized Open-label Study

Efficacy and safety of basiliximab combined with tacrolimus, mycophenolate and low-dose corticosteroids vs thymoglobulin, tacrolimus, mycophenolate and high-dose corticosteroids in Pancreatic Allograft Recipients

Study number: OUS-PTx-01

EudraCT number: 2012-005335-83

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Version 1.2, 2013-03-09

We have read the protocol of this study and confirm that all information necessary to conduct the study is provided by these documents. We are prepared to perform the study according to this protocol

Investigator

_____	_____	___/___/___
Print Name	Signature	Date

Co-Investigators

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1 INTRODUCTION AND BACKGROUND

The first pancreas transplantation (PTx) was performed in Minnesota in 1966 by Kelly and colleagues (1). In recent years the number of procedures has grown considerably worldwide, and is now a well established treatment option for patients with diabetes mellitus with and wihtout concomitant diabetic End-Stage Renal Disease (ESRD) (2-4). The indication for PTx is advanced and/or badly controlled diabetes mellitus (”brittle” diabetes, severe hypoglycemic episodes, ”unawareness”, etc). Solitary pancreas transplantation (SPT; without concomitant kidney transplantation) is usually classified as PTx alone (PTA), PTx after kidney transplantation (PAK) or PTx after islet transplantation (PAI). Kidney transplantation of the diabetic uremic population increases survival compared to long-term dialysis (5, 6). Transplant options for patients with diabetic end-stage nephropathy include simultaneous pancreas-kidney (SPK), live donor kidney (LDK) and deceased donor kidney (DDK) transplantation. SPK transplantation relieves not only the patient’s uremia, but also alleviates the hyperglycaemic state of diabetes. Large international patient registries show that patient survival rates after SPK have reached more than 95% at 1 year and 87% at 5 years post-transplant, respectively (2). Nevertheless, PTx as treatment for type 1 diabetes has not gained the same popularity as transplantation of other organs, partly because PTx have been associated with a high rate of surgical complications; particularly bleeding, thrombosis and exocrine leakage. Furthermore, there has been a lack of reliable, non-invasive rejection monitoring instruments, and the invasive, percutaneous pancreas biopsies have been associated with a high rate of complications.

The difficulties encountered with PTx have to some extent been compensated by a very selective attitude towards the donors, but thereby making pancreas grafts a scarce resource. In contrast to other abdominal transplantations such as liver transplantation (LTx) and kidney transplantation (KTx), where repeated biopsies have been used for immunosurveillance, percutaneous biopsies of the pancreas-graft have traditionally been avoided due to a high rate of biopsy-related complications (exocrine leaks/fistulas and bleeding episodes). Thus, fear of acute rejections and lack of adequate rejection markers, have led to a rather intensive immunosuppressive load in PTx recipients.
Solitary pancreas transplantation (SPT) has traditionally been subjected to even higher complication and rejection rates, with inferior graft and patient survival - thus favoring the combined SPK procedure. This has been attributed to an even worse rejection monitoring capability, without a ”reporter” allograft kidney. No biochemical markers have proven to be effective in rejection surveillance.

Pancreas graft thrombosis is a feared complication in the postoperative course, partly due to the oversized vessels used (coeliac trunk/superior mesenteric artery/portal vein) in conjuction with the low blood flow through an isolated pancreas graft. In the native setting, these vessels also serve the intestines and spleen. Therefore, PTx poses a delicate balance between thrombosis and bleeding complications.

End stage type 1 diabetes is a devastating chronic disease. PTx offer long term insulin-independency. Efforts should be made to define robust patient selection criteria and offer eligible patients insulin-independency before severe diabetic complications appear.

The Norwegian experience
PTx is performed at one single national centre in Oslo, and from 1983 to date 300 procedures have been performed (7-11). In recent years, the activity has increased; 22 were performed in

2011 and we will probably reach 30 in 2012. Approximately 9 out 10 PTx's have been SPK's, hence only about 10% have been SPTs. In the first period from 1983 through 1987, a duct-occluded segmental pancreas was used for transplantation. From 1988, the whole pancreas graft was used, and the exocrine secretion was drained by anastomosing the duodenal segment to the urinary bladder. This technical solution was chosen partly because it offered some sort of rejection monitoring, by urine amylase counts and cystoscopic pancreas biopsies. However, many patients suffered from chemical cystitis and metabolic acidosis, due to loss of bicarbonate. In 1998 the urinary bladder anastomosis was abandoned, in favor of the more physiological enteric anastomosis, the duodenal segment being connected to the proximal jejunum. However, this solution offered even less options to monitor upcoming rejections, as percutaneous biopsies was mostly avoided due to the previously mentioned hazards.

We have recently examined (12) all PTx's performed at our hospital during 2006-2010 (n=61; 59 SPK, 2 PTA). Our overall surgical complication rate has decreased from earlier years, but we still suffer a substantial rate of reoperations (about 30% of patients), mainly caused by exocrine leakage, bleeding and vein thrombosis. When comparing the populations with or without reoperation, higher donor age had a significant negative impact. No significant effect of donor age on graft survival was observed. There was a tendency towards better results in female recipients, both regarding surgical complications and graft survival. The rejection rate (altogether about 30%) was significantly higher in the graft loss group, and reoperations were insignificantly associated with graft loss.

From late 2011, several measures have been implemented to improve outcome and reduce the rate of surgical complications. In line with most Tx centres in Scandinavia, we have switched the prophylactic anticoagulation treatment from our traditional Macrodex® regime to a Fragmin® regime. Several surgical/technichal changes have also been implemented during recent years; tentatively more atraumatic graft procurement, preserving the entire coeliac arterial axis including the gastroduodenal artery, obtaining a long portal vein without the need for elongation, as well as extended in situ dissection by means of of Ligasure™. Due to the conventional lack of rejection monitoring parameters, we launched an investigatory surveillance program, with protocol biopsies of the duodenal segment via double balloon enteroscopy (13). The impact and value of this program has yet to be investigated. Previous reports have described separate rejection of the pancreas or kidney in the SPK setting, and the gold standard for proving rejection of the pancreas is undoubtedly a biopsy of the pancreas itself. This encouraged us to further develop techniques for better surveillance, such as endoscopic transduodenal ultrasound-guided biopsies of pancreas (EUSBP). Inferior outcome of PTA and lack of valid tools for immunosurveillance in the abscense of a simultaneous kidney graft, have led some centers to evolve the duodeno-duodenostomy (DD) for drainage of the exocrine pancreas, making the EUSBP possible. There are many theoretical advantages with the DD, especially regarding rejection surveillance, and we have recently adopted this technique. The endoscopic access afforded by the DD also makes it possible to stent the pancreatic duct in case of exocrine leakage.

Immunosuppressive therapy

Over time, the induction therapy and maintenance immunosuppressive protocols have changed. From 1983 to 2000, all recipients received triple immunosuppressive regimens with cyclosporine, azathioprine and prednisolone (CS). During the last part of the 1990's azathioprine was substituted by Mycophenolate mofetil (MMF), and cyclosporine was substituted by tacrolimus. After 2000, the immunosuppression has been intensified by induction therapy both for PTx (Antithymocyte globulin (ATG)) and for kidney transplants

alone (basiliximab). Thus in recent years, PTx recipients have received a quadruple immunnosuppressive regimen, that includes tacrolimus, MMF, CS and ATG. The dosage of ATG has been directed by T-cell counts.

2 OBJECTIVES OF THE STUDY

Several studies have shown acceptable results after PTx by substituting ATG with basiliximab (14-18), which is considered to convey a considerably lower number of adverse events. However, the issue of ATG vs basiliximab in PTx has not yet been solved. The potential advantages of reducing the overall cortiocosteroid (CS) load is obvious, as CS is a well-known pro-diabetic agent and causes severe long term adverse effects. *On this background, we intend to investigate a PTx low-grade immunosuppressive protocol, with Basiliximab and low-dose CS versus the conventional protocol with ATG and high-dose CS.*

The rationale for the study is that; *i*) a high immunosuppressive load may be partly responsible for the high rate of PTx associated complications/reoperations; *ii*) a high immunosuppressive load is related to infectious complications; *iii*) improved PTx rejection surveillance by DD and EUSBP allows a low-graded immunosuppressive protocol ; *iv*) the study will contribute to the unresolved issue regarding the use of ATG vs Basiliximab in PTx.

2.1 Primary objectives

- Compare the incidence of *acute rejection episodes* at 6, 12, 36 and 60 months after pancreas transplantation, between two quadruple immunosuppressive regimens; basiliximab combined with tacrolimus, mycophenolate and low-dose corticosteroids vs thymoglobulin, tacrolimus, mycophenolate and high-dose corticosteroids. The incidence of rejection is defined as the fraction of patients in which rejections episodes (one or more) have been proven by biopsies. For SPK rejection in either organ, pancreas or kidney, counts.
- Compare the incidence of *surgical complications*, involving reoperations and reinterventions, in the two study groups.

2.2 Secondary objectives

- Compare the number and severity of rejection episodes in the pancreas allograft to the ones occurring in the kidney allograft (SPK), and the ones diagnosed by the duodenal segment biopsies.
- Compare pancreas graft survival at 12, 36 and 60 months after transplantation between the study groups.
- Monitor kidney (and pancreas) graft survival (SPK) 12, 36 and 60 months post-Tx.
- Compare patient survival at 12, 36 and 60 months post-Tx.
- Compare the incidence of non-surgical complications (infections, cardial complications, pulmonary complications and neurological complications).

2.3 Immunological studies

- Scheduled biopsies will be taken according to our present routine protocol; simultaneosly from these four transplant/organ sources at predestined points of time:
    - Pancreas transplant (*P*)
    - Kidney transplant (*K*)
    - Duodenal segment of pancreas transplant (*tD*)
    - Duodenum of recipient = native Duodenum (*nD*) – Serves as ‘control’

- Baseline (Day 0; at Tx): *K +tD + nD* - These will be taken during surgery
- 3 weeks post-Tx: *P +tD + nD* - Endoscopically
- 6 weeks post-Tx: *P + K + tD + nD* - Endoscopically (P/tD/nD) + Percutaneous (K)
- 12 months post-Tx: *P + K + tD + nD* - Endoscopically (P/tD/nD) + Percutaneous (K)

In addition, indication biopsies will be taken whenever there is suspicion of rejection in either organ. Preferably, simultaneous P + tD + nD endoscopic biopsies and K percutaneous biopsies should be obtained.

All biopsies will be examined at our local pahology unit; the pancreas and duodenal biopsies by prof. Tor Jacob Eide and dr. Krzysztof Grzyb; the kidney biopsies by prof. Helge Scott and dr. Erik Heyerdahl Strøm. The pancreas and kidney biopsies will be evaluated by well-known BANFF criteria, while duodenal biopsies will be rated according to Wu et al. (19).

- Histological evaluation of the scheduled and indication biopsies will involve comparative studies between the two groups and comparisons between biopsies from the various transplants/organs of the same patient.
    - Rejection histology scores.
    - Immunoshistochemistry on immunologic markers (CD25, FOXP3, CD4, CD3, CD8, CD45RO, perforin, granzyme A/B, etc).

- Blood samples will be obtained at the time of transplantation and the later scheduled appointments indicated above.
    - Study of immune cell activation (CD25, FOXP3, CD4, CD3, CD8, CD45RO, perforin, granzyme A/B, etc) and cytokines (IL-2, TNF-a, IFN-g, IL-10, IL-12 etc).
    - Compare biomarkers in serum, indicative of acute rejection, by at least weekly blood sampling post-Tx (RNA microarray on a series of genes related to rejection, quantitative PCR on selected genes) (20-21).

2.4 Endoscopic mucosal imaging and ultrasound

- During upper endoscopy for scheduled biopsies (see 2.3 above), pictures of the transplant duodenal mucosa will be taken.
    - The mucosal images will be rated regarding rubor, edemea villous atrophy etc. and compared to biopsy rejection scores.
- Endoscopic ultrasound (EUS) will be used during biopsying of the pancreas. EUS images will be sampled and stored, for comparative analysis.
    - The EUS analysis will mainly involve circulatory parameters.

2.5 Donor and recipient baseline characteristics

- We will investigate relationships between the below mentioned donor/recipient charactheristics and graft survival/surgical complications/non-surgical complications.
    - Donor age
    - Donor gender
    - Donor BMI
    - Recipient age
    - Recipient gender
    - Recipient BMI

- Recipient PRA (Panel Reactive Antibody) status
- Recipient comorbidity status; particularly cardiovascular status

2.6 Non-immunological rejection markers

- The following analyses will be performed and correlated to rejection, functional parameters (glucose levels/need for insulin (P) and creatinine (K)) and graft survival.
  – By daily blood samples during the first 10 days, thereafter 3 times a week until week 10.
  - Amylase (pancreas specific amylase)
  - Lipase
  - CRP
  - Amylase/Lipase/CRP combined parameter
  - C-peptide
  - Pancreas Auto-Antibodies
- In addition, amylase in drainage fluid will be measured daily, untill the drains are removed (usually at day 4-8 post-Tx).

3 STUDY DESIGN

This is an open-label, randomized, comparative study that will be conducted at our single, national centre for organ transplantation in Oslo. All pancreas recipients > 18 years of age, who fulfill the inclusion criteria, will be randomized before transplantation to group I or II.

4 DURATION OF STUDY

All consecutive PTx recipients during five years are planned to be enrolled, with a minimum of 60 patients. The study will continue untill all patients have completed 60 months of follow-up or have discontinued participation in the study.

5 NUMBER OF PATIENTS

At least 60 patients will be enrolled in the study and randomised to group I and II at a 1:1 ratio. Solitary pancreas (PAK/PTA/PAI) recipients and combined pancreas/kidney (SPK) recipients will be randomised separatively, to assure a similar ratio of Solitary PTx : SPK in each group.

6 SELECTION OF PATIENTS

6.1 Inclusion Criteria

Patients will be eligible for study entry if **ALL** of the following criteria are met:

6.1.1 Age ≥18 years

6.1.2 Patients who receive a primary or secondary pancreas transplant, with or without a simultaneous kidney transplant (SPK).

6.1.3 Women who are of childbearing potential must have a negative serum pregnancy test at baseline.

6.1.4 Operability has to be ascertained by preop. examination, performed by nephrologist, transplant surgeon and anaesthesiologist.
6.1.5 Signed and dated informed consent form.

6.2 Exclusion Criteria

Patients will not be eligible if **ANY** of the following criteria are met:

6.2.1 Evidence of systemic infection
6.2.2 Presence of unstable cardiovascular disease.
6.2.3 Malignancy < 5 years prior to entry into the trial (with the exception of adequately treated basal cell or squamous cell carcinomas of the skin).
6.2.4 Panel-reactive antibodies (PRA) > 20% or the presence of donor-specific antigens (DSA).
6.2.5 Use of investigational agents <1 month prior to entry into the trial.
6.2.6 Any positive test for HBV, HBC or HIV.

7 DOSAGE AND ADMINISTRATION

All study drugs will be labelled as such:

- "PTx-studien"
- Dosing according to the below paragraphs 7.1 og 7.2
- "Hovedutprøver: Ole Øyen"
- "Henvendelser via telefon: 23070500 evt. 92264777"

7.1 Group I (Trial arm)

Patients randomised to group I will receive an immunosuppressive regimen based on basiliximab, tacrolimus, mycophenolate mofetil and low-dose corticosteroids as follows:

7.1.1 *Basiliximab:*
One dose of 20 mg, administered intravenously over 15 minutes, at day 0 (perop., prior to revascularization) and at day 4 post-Tx; 2 doses in total.

7.1.2 *Tacrolimus:*
Initiated at day 0 (the first dose preop.) at a dose of 0,6 mg/kg x 2 p.o., later adjusted to achieve steady state whole-blood trough levels as follows:
Month 1-3 8-12 ng/ml
Month 3-6 4-8 ng/ml

7.1.2.1 *Tacrolimus concentration determination*
Whole blood trough concentrations for tacrolimus will be obtained daily from day 1-5, therafter at least 3 times a week. Concentrations will also be determined at the time of any serious adverse event.

7.1.3 *Mycophenolate mofetil (MMF):*
MMF will be given 1000 mg twice daily. It can be reduced to 750mg twice daily in case of adverse events and further down to 500mg in case of persisting adverse events.

7.1.4 *Low-dose Corticosteroids:*
Day 0 (perop.): Methylprednisolone 250 mg i.v.
Day 1-14: Prednisolone 20 mg x 1 p.o.
Day 15-28: Prednisolone 15 mg x 1 p.o.

Day 29-60: Prednisolone 10 mg x 1 p.o.
Day 61- 180: Prednisolone 7,5 mg x 1 p.o.
Day 181 - : Prednisolone 5 mg x 1 p.o.

7.2 Group II (Control arm)

Patients randomised to group II will receive an immunosuppressive regimen based on thymoglobulin, tacrolimus, mycophenolate mofetil and high-dose corticosteroids as follows:

7.2.1 *ATG (Thymoglobulin):*

Initiated at day 0 (the first dose preop.) at a dose of 2,5 mg/kg i.v. Later dosing is directed by T-cell counts once daily. The T-cells are kept suppressed for 10 days post-Tx, and new dose of 2,5 mg/kg i.v. is given when the T-cell count rises above 0,050. Altogether, 2-4 doses of ATG is usually needed.

7.2.1.1 *T-cell counts*

Whole blood T-cell counts will be obtained daily from day 1-10.

7.2.2 *Tacrolimus:*

Initiated at day 0 (the first dose preop.) at a dose of 0,6 mg/kg x 2 p.o., later adjusted to achieve steady state whole-blood trough levels as follows:
Month 1-3 8-12 ng/ml
Month 3-6 4-8 ng/ml

7.2.2.1 *Tacrolimus concentration determination*

Whole blood trough concentrations for tacrolimus will be obtained daily from day 1-5, therafter at least 3 times a week. Concentrations will also be determined at the time of any serious adverse event.

7.2.3 *Mycophenolate mofetil (MMF):*

MMF will be given 1000 mg twice daily. It can be reduced to 750mg twice daily in case of adverse events and further down to 500mg in case of persisting adverse events.

7.2.4 *High-dose Corticosteroids:*

Day 0 (perop.): Methylprednisolone 500 mg i.v.
Day 1: Methylprednisolone 40 mg x 2 i.v.
Day 2-8: Prednisolone tapered from 40 mg x 2 p.o. to 10 mg x 2 p.o. (10 mg reduction/day)
Day 9-28: Prednisolone 20 mg x 1 p.o.
Day 29-60: Prednisolone 15 mg x 1 p.o.
Day 61-180: Prednisolone 10 mg x 1 p.o.
Day 181- : Prednisolone 5 mg x 1 p.o.

7.3 Concomitant Treatments

7.3.1 Required treatment

i) Prophylaxis against the development of *Pneumocystis carinii*, with trimetoprim-sulfa is required for all patients during the first 6 months of treatment.

ii) Prophylaxis against Cytomegalovirus (CMV) with valganciclovir for 3 months, if the donor is CMV + and the recipient is CMV ÷.
By all other CMV constellations, *preemptive* valganciclovir treatment is given, based on weekly CMV-PCR analyses (cut off: CMV-PCR count > 0).

iii) Antibiotic prophylaxis with meropenem (2 doses) and vancomycin (1 dose) at day 0.
iv) Proton pump inhibitor (Somac) is given for at least 2 months post-Tx.

7.3.2 Prohibited treatment
i) Other investigational drugs
ii) NSAID's should be avoided
iii) Terfenadine, cisapride, astemizole, pimozide, cimetidine and ketoconazole are not allowed.

8 RANDOMISATION

Patients will be allocated to Group I or Group II at random according to computer generated randomisation envelopes, made by the Oslo University Hospital Science Support Department. The block size will be 20 (10 + 10; totally 3 blocks x 20 = 60). The inclusion and randomisation of each patient at admission will be performed by the transplant surgeon on duty, in collaboration/communication with the investigators. The assigned randomisation number and therapy group will be recorded on the patient's case report form. Once the patient number and randomisation number has been assigned, they cannot be reassigned. If a patient withdraws from the study before or after study participation the patient, the patient number and randomisation number cannot be reissued.

9 TREATMENT OF ACUTE REJECTION EPISODES

P-, tD and K-biopsies must be examined in all suspected cases of rejection. This investigation should be performed before anti-rejection therapy is commenced, or at least within 24 hours of start of treatment. Wherever possible, anti-rejection therapy should be postponed until a histological diagnosis of rejection is confirmed. Acute rejection should first-line be treated with boluses of Methylprednisolone according to our local practice for both pancreas- and Kidney-Tx. For *steroid resistant rejections* (defined as: no pancreas/kidney functional improvement after at least 4 boluses of Methylprednisolone or rejection in repeat D-, P- or K-biopsies), ATG therapy should be initiated and administered for 7-14 days.
All biopsies will be examined at our local pahology unit; the pancreas and duodenal biopsies by prof. Tor Jacob Eide and dr. Krzysztof Grzyb; the kidney biopsies by prof. Helge Scott and dr. Erik Heyerdahl Strøm.

10 ADVERSE EVENTS

All adverse events will be recorded in the appropriate section of the case record form, regardless of whether or not they are assumed to be related to the study drugs. The nature of adverse event, details or severity, together with the date of onset, duration and outcome will be recorded. The investigator's opinion on the relationship of the adverse event to the treatment will also be recorded.
All adverse events will be reported annually to the Norwegian Medicines Agency. Regarding reports of Serious Adverse Events and Serious Unexpected Adverse Drug

Reactions see 10.2/10.3.

10.1 Definitions

An adverse event is any adverse change from the patients baseline (pre-Tx) condition, including intercurrent disease which occurs during the course of the study after the treatment has started, whether considered related to treatment or not. Treatment includes all investigational agents administered during the course of the study.

Clinical adverse events must be graded on a three-point scale (mild, moderate and severe) and be reported in the appropriate sections of the case record form.

Mild: Awareness of symptoms but easily tolerated
Moderate: Discomfort enough to interfere with normal activities
Severe: Completely prevents normal activities

The relationship between the adverse event and the treatment must also be assessed as follows:

Definite: The experience meets the following criteria:
- followed a reasonable temporal sequence from drug administration
- compatible with known drug profile
- abated upon discontinuation of the drug (dechallenge)
- with or without documentation that the experience was confirmed by reappearance of the reaction on repeat exposure (rechallenge)

Probable: The experience meets one or more of the following criteria:
- follows a reasonable temporal sequence from drug administration
- compatible with known drug profile and can not be reasonably explained by the known characteristics of the patient's clinical state
- with or without documentation that the experience abates upon discontinuation of the drug (dechallenge)

Possible: The experience meets the following criteria:
- follows a reasonable temporal sequence from drug administration
- could have been produced by the patient's clinical state or by the drug in question
- compatible with known drug profile

Remote:
- It is not likely to be any reasonable association between the drug and the observed experience

Definitely Not:
- The experience is definitely produced by the patient's clinical state, or by other modes of therapy administered to the patient and not due to the administration of the study drug

Unknown:
- Information provided is insufficient for a confident drug relationship to be classified

Pre-existing Condition:
- In this trial, a pre-existing condition (ie, a disorder present before the adverse event reporting period started and noted on the pre-treatment medical history/physical examination form) should not be reported as an adverse event unless the condition worsens or episodes increase in frequency during the adverse event reporting period.

10.2 Serious Adverse Event

Any clinical adverse experience or abnormal laboratory test value that is SERIOUS (including life-threatening surgical complications, grave rejection episodes, death), occurring during the course of the study, irrespective of the treatment received, have to be recorded and highlighted in our study database

A serious adverse event is any untoward medical occurrence that, at any dose:

- Results in death
- Is life-threatening (immediate risk of death as the event occurred)
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity (a substantial disruption in a person´s ability to conduct normal life functions)
- Is a congenital anomaly/birth defect
- Is an overdose (whether by accident or deliberate)
- Is a significant hazard to the patient or requires intervention to prevent a serious outcome.
- Pregnancy will be recorded in the same time frame as serious AEs.

All Serious Adverse Events which occur during the study period will be communicated to the Norwegian Medicines Agency within 15 days (by means of the CIOMS formula)..

10.3 Serious Unexpected Adverse Drug Reaction (ADR)

A Serious Unexpected adverse drug reaction is defined as reaction which in the opinion of the Investigator is thought to be definitely, probably or possibly drug related and has not previously been known to occur for that drug either from the literature, adverse event listings or Investigator experience - but is not a common clinically insignificant illness.
All Serious Unexpected Adverse Drug Reactions which occur during the study period will be communicated to the Norwegian Medicines Agency within 15 days (by means of the CIOMS formula).

10.4 Follow Up of Adverse Events

Any abnormal laboratory values, abnormal clinical findings and adverse events which are of clinical significance, in the opinion of the investigator, must be followed with appropriate medical management until resolved.
Individual patients will be excluded from the study if serious adverse effects, related to the trial medication are observed; practically this will mainly regard anaphylactic reactions to Basiliximab.

11. ANALYSIS OF RESULTS

The primary end-points ‘Incidence of rejection’ and ‘Rate of surgical complications’ are both cathegorical parameters and can be treated similarly in power calculations.

11.1 Strategy regarding 0-hypothesis

Regarding the '*Incidence of rejection*', a non-inferior approach seems reasonable. The most important aspect is to avoid the following (Type 1) error: Not detecting an elevated rejection rate in Group I, when there in fact is a difference in favor of the Group II (control) immunosuppressive regimen.
One could argue to use a one-sided test/"rejection region", because it is highly unlikely that the low-immunosuppressive regimen will yield a lower incidence of rejection. However, according to statistical tradition, a two-sided test will be demonstrated.
Thus, the *0-hypothesis* will have to be:

H0: *Group I will have a significantly different rejection rate compared to Group II* (*Gr. I ≠ Gr. II*)

- Between groups comparison of total biopsy-proven rejection rate; based on scheduled and 'ad hoc' biopsies from the duodenal segment, pancreas and kidney.
  -- Biopsy-proven rejection in either organ (for SPK) contribute to the rejection incidence.

Regarding the '*Rate of surgical complications*', the most important aspect is to avoid the following (Type 1) error: Demonstrating an elevated complication rate in Group II, when there in fact is no difference.
Also here, one could argue to use a one-sided test/"rejection region", because it is no reason to believe that the low-immunosuppressive regimen will yield a higher rate of complications. However, according to custom, a two-sided test will be demonstrated.
Thus, a natural *0-hypothesis* will be:

H0: *Group I and Group II will have similar rates of surgical complications* (*Gr. I = Gr. II*)

- Between groups comparison of surgical complication rate; defined as the fraction of patients experiencing one or more surgical complications, involving reoperation or reintervention.

11.2 Sample size and Power calculation

These statistical deductions are based on the ***binomial*** distribution/response and a two-sided test (22, 23):

$$\text{N patients required per arm:} \quad \frac{Pn\,(1-Pn) + Ps\,(1-Ps)}{(Pn - Ps)^2} \times C$$

Pn: Reference probability; Ps: Probability to be detected; c: Test constant

The assumed reference rates (Pn) for the primary end-points (both actually about 30%) are based on recent data from Norway (12).
By convential presumptions; Power 1-β = 80% (➔ c = 7,9), and 33% relative change in rejection/complication rates to be detected, these will be the figures:

End-point	**Rejections**	**Surgical complications**
Null hypothesis to be tested	H0: Gr. I ≠ Gr. II H1: Gr. I = Gr. II	H0: Gr. I = Gr. II H1: Gr I ≠ Gr. II
Statistical model	Binomial distribution; two-sided test	
Assumed reference rate (Pn)	0,30	0,30
Effect to be detected (Ps)	≥ 0,40 (≥ 33% increase)	≤ 0,20 (≤ 33% decrease)

Type I error (α)	5%	
Power (1-β) ➔ c	80% ➔ 7,9	
Number of pats required	355 per arm	292 per arm

If we significantly release on the stastistical presuppostions/demands - by only claiming 60% Power (➔ c = 5,4) and 100% relative change in detected rejection/complication rates - these will be the figures:

End-point	**Rejections**	**Surgical complications**
Null hypothesis to be tested	H0: Gr. I ≠ Gr. II H1: Gr. I = Gr. II	H0: Gr. I = Gr. II H1: Gr I ≠ Gr. II
Statistical model	Binomial distribution; two-sided test	
Assumed reference rate (Pn)	0,30	0,30
Effect to be detected (Ps)	≥ 0,60 (≥ 100% increase)	≤ 0,15 (≤ 100% decrease)
Type I error (α)	5%	
Power (1-β) ➔ c	60% ➔ 5,3	
Number of pats required	27 per arm	80 per arm

Practicability with regard to statistics:

(i) It is totally unrealistic – for any Tx-center in the world – to include 600-700 PTx patients, during any reasonable time frame. In Oslo, we are by far the highest volume center in Scandinavia. Our 28 PTx's performed in 2012 represent 5,6 p.m.p. (per million population), which actually is far higher than any other country in the world, according to figures presented by the Council of Europe in cooperation with the Spanish Tx organization (24). Even if we cooperated/coincluded with all the other PTx centers in Scandinavia (Uppsala/Göteborg/Helsinki), the potential would not exceed 50 patients per year.

(ii) The maximally realistic number of PTx patients to be included in Oslo during a reasonable time frame (2-3 years) will be about 60 (30 + 30).

(iii) Thus, our intentions with regard to statistical Power have to be more modest. The above figures (lower table) do however show, that a doubled rejection rate in the trial arm can be detected at 60% Power with 27 x 2 = 54 patients.

(iv) The prospects/visions of this study consists of a lot more than detecting significant changes in rejection/complication rates. Please, cfr. paragraphs 2.2 – 2.6 of this protocol. The simultaneous biopsy strategy (D- + P- + K-biopsies) is unique. And the 'molecular biology' analyses of these simultaneous biopsies and blood samples have the potential to provide new insights. Furthermore, "new" potential rejection markers (C-peptide; CRP/Amylase/Lipase combined parameter) will be explored.

(v) Regarding safety, provisional analyses on rejections and surgical complications will be performed after 20 pts. with 3 mts follow-up; please cfr. 11.4 below.

11.3 Statistical methods in data analysis

- The primary evaluation criteria 'Incidence of rejection' and 'Rate of surgical complications' will be evaluated for several populations:
  - All patients who receive at least one dose of study medication, defined as the 'Intention-to-Treat' population.
  - All patients who complete 12 months of intended study medication, defined as the 'Intention completed' population.

The analysis of these categorical parameters will consist of:

1. Comparisons of groups using the Fisher exact test.
2. Confidence intervals of 95% of the percentage of incidence of these events.

- The loss of grafts and deaths will be analysed by the Kaplan-Meier method for estimating the time to events.
- Continous (non-cathegorical) variables will be analysed by student t-tests and chi-square tests.
- Any other methods that are not planned can be considered as alternative methods.

11.4 Provisional analysis

Summaries of provisional data will be carried out (descriptive statistics, graphs) during the course of the study when it is considered necessary, particularly with regard to the rejection rate in Group I. In any case, an intermediary/provisional analysis will be performed when the first 20 patients have completed 3 months follow-up. These summaries will be used only for control purposes and will not necessarily include formal statistical analysis.

Discontinuation of the study will be considered, at least at the 20 pts/3 mts follow-up point of time, according to these criteria:

- If the biopsy-proven combined rejection rate (pancreas + duodenal-segment + kidney) in the trial arm is ≥ doubled compared with the control arm.
- If the rate of surgical complications/reoperations in the trial arm is ≥ doubled compared with the control arm.

12. DATA HANDLING AND RECORD KEEPING

12.1 Case Report Forms

a) All data from each included patient should be recorded on case report forms (CRF's), separate from the hospital files. Ballpoint pens will be used.

b) Case report forms and other pertinent records are to be submitted to the Norwegian Medicines Agency, if requested, upon completion of the study.

d) The investigator must also submit all incomplete case report forms that reflect patient experience with the drug, including retrievable data on patients who withdrew before completion of the study.

12.2 Record Retention

The investigator must arrange for the retention of the subject identification codes for at least 15 years after the completion or discontinuation of the trial. Subject files and other source data must be kept for the maximum period of time permitted by the hospital.

13 ETHICAL CONSIDERATIONS

13.1 Institutional Review Board (IRB)/Ethics Committee (EC)

It is the responsibility of the investigator to obtain approval of the trial protocol/ amendments from the IRB/EC before commencement of the study. All correspondence with the IRB/EC should be filed by the investigator.

13.2 Informed Consent

It is the responsibility of the investigator to give each subject (or the subject´s acceptable representative) prior to inclusion in the trial, full and adequate verbal and written information regarding the objective and procedures of the trial and the possible risks involved. The subjects must be informed about their right to withdraw from the trial at any time. Written subject information must be given to each subject before enrolment. Furthermore, it is the responsibility of the investigator to obtain signed informed consent from all subjects prior to inclusion in the trial.

13.3 Declaration of Helsinki

This study will be conducted in accordance with the Declaration of Helsinki.

13.4 Good Clinical Research Practice (GCP) and Forskrift om klinisk utprøving av legemidler til menneske

The study will be performed in accordance with the European 'Guidelines on Good Clinical Research Practice' (Consolidated guideline, CPMP/ICH/135/95) and the Norwegian 'Forskrift om klinisk utprøving av legemidler til menneske' (FOR 2009-30-10).

13.5 Unanticipated Problems

Any changes in the study or unanticipated problems involving risks to subjects must be reported promptly to the Ethics Committee and the Norwegian Medicines Agency.

14 PUBLICATIONS

Upon completion of the study, the investigator will seek to publish the results in recognised scientific journals, within the field of transplantation.

15 REFERENCES

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16 STUDY FLOW CHART

	BASELINE DAY 0	**DAY 4**	**DAY 10**	**WEEK 3**	**WEEK 6**	**WEEK 10**	**MONTH 3**	**MONTH 6**	**MONTH 12+36+60**
Basiliximab – **Group I**	**X**	**X**							
Low-dose Corticosteroids – **Group I**	----------Continuously, tapered----------								
MMF – Both groups	----------Continuously----------								
Tacrolimus – Both groups	----------Continuously----------								
Corticosteroids – Both Groups	----------Continuously, tapered----------								
Thymoglobulin **Group II**	**X**								
High-dose Corticosteroids – **Group I**	----------Continuously, tapered----------								
PCP-prophylaxis	----------Continuously----------								
Medical History	**X**								
Previous Treatment	**X**								
Physical examination incl. vital signs	**X**			**X**			**X**	**X**	**X**
PA Chest X-ray	**X**	To be obtained when clinically indicated							
Complete blood count	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Fasting Blood Chemistries incl. amylase, lipase	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Blood T-cell counts **Group II**	**Daily**								
Blood C-peptide, HbA1c, Auto-Ab	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Blood fasting total, LDL and HDL-cholesterol and triglycerides	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Scheduled biopsies	**K + tD + nD**			**P + tD + nD**	**P + K + tD+nD**				**P + K + tD+nD**
Blood immunology: RNA arrays. Quantitative RT-PCR	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
CMV-PCR	**X**			**X**			**X**	**X**	**X**
Pregnancy Test	**X**	Whenever clinically indicated							
Concomitant Medication	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Tacrolimus Trough Levels		**Week 1**	**Week2+ 3**	**X**	**X**	**X**	**X**	**X**	**X**
MMF Through Levels	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Adverse Event Monitoring		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**