Transplant International

Oral Sessions

Monday, 5 September 2011

Kidney (double kidney transplants and surgical aspects)

O-001 MAXIMIZING THE SUCCESS OF TRANSPLANTATION WITH KIDNEYS FROM OLDER DONORS: LONG-TERM RESULTS OF A MULTICENTER, PROSPECTIVE STUDY

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Background: Kidney biopsy (KB) has been proposed for the assessment of all donors ≥60 yrs old (ED). However, the reliability of an histological evaluation is still controversial.
Objective: to investigate the efficacy of a protocol aimed at performing KB only in the donors clinically judged at higher risk of kidney damage.
Patients and Methods: This is a prospective multicentre study involving 14 transplant centres. By clinical criteria, ED were classified at low-risk (=LRD: age between 60-69 years and without risk factors) or at high risk (=HRD: age: ≥70 yrs or between 60-69 but with a risk factor: creatinine clearance <60mL/min, proteinuria ≥3g/24h, severe hypertension, diabetes, cardiovascular disease). Kidneys from LRD were used for two single transplants (SKT), without KB. Kidneys from HRD were always biopsied and, according to Remuzzi score, were allocated either to SKT, or dual kidney transplant (DKT) or discarded.
Results: From January-2003 to December-2004, 287 of 796 donors harvested were ≥ 60 years old (36%). 97 (33%) were excluded mainly for macroscopic vascular lesions or histology. 190 donors entered the study, 126 (66%) LRD, 64 (34%) HRD (41 and 23 were then used for dual and single transplant).

Main results

	HRD-DKT	HRD-SKT	LRD-SKT
Biopsy	YES	YES	NO
No. Donors	41	23	126
Mean Donor Age	72.5	70.4	64.3
S. creatinine clearance (mL/min)	77±35	78±25	93±27
No. Transplants	41	40	234
DGF	17%	45%	34%
5 yrs - Mean(SD) S-Cr (mg/dL)	1.8 (0.8)	2.1 (1.4)	1.8 (0.8)
5 yrs - Graft Survival	95%	89%	87%

Conclusions: Clinical evaluation is a reliable tool to distinguish LRD from HRD and it narrows down the use of KB only to HRD. At 5-years, the results from both HRD and LRD have been remarkably good

O-002 LONG TERM RESULTS OF A LARGE EXPERIENCE OF DOUBLE KIDNEY TRANSPLANTATION (DKT) PERFORMED AT A SINGLE CENTER

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Body: DKT technique has allowed to widen the donor acceptance criteria for kidney transplantation. This study reports the long term results of a large series of DKT performed at our Center.
Methods: From October 1999 to December 2010, 161 patients (mean age 61.8±6.1yr) underwent DKT at our Center with a mean follow-up of 53±33 months. The mean donor age was 72±8 yrs and the mean creatinine clearance was of 68±27 mL/min. DKT were selected based on clinical and histological assessment. In 43 cases (26.7%) kidneys were placed bilaterally and in 118 (73.2%) monolaterally. 138 (85,7%) recipients received induction therapy with Thymoglobulin, 16 (9.9%) with basiliximab and 7 (4.3%) received no induction. Maintenance immunosuppression was based on CNI, MMF, steroids in 43 patients (26.7%); sirolimus, MMF, steroids in 78 (48.4%); low dose-CNI, everolimus, steroids in 40 (24,8%).
Results: Average cold ischemia time was 16.3±2.5hours. Incidence of DGF was 35.5% (mean duration 6 days). Acute rejection occurred in 26 cases (16%). 20 patients experienced surgical complications: 2 renal vein thrombosis, 10 wound dehiscences, 4 lymphocele, 2 ureteral anastomosis stenosis, 2 urinary leaks. Renal function was satisfactory at 1, 3 and 5 yrs with a mean S-creatinine of 121±50, 127±48 and 137±43 μmol/L, respectively. The actuarial 5-year patient and graft survivals were respectively 94.7% and 93%. The cause of death was cardiovascular in 7 patients, infective in 3, bowel complications in 2. 10 grafts were lost due to: PNF (1), renal vein thrombosis (1), vascular rejectjon (1), chronic rejection (5), immunosuppression withdrawal for Kaposi sarcoma (2).
Conclusions: Very satisfactory long term results of DKT in terms of graft survival and renal function may encourage the use of very elderly donors beyond the old for old allocation.

O-003 USE OF INFERIOR EPIGASTRIC ARTERY AS A CONDUIT FOR ACCESSORY VESSELS IN RENAL TRANSPLANTATION

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Background: Donor kidney shortages have caused increased use of organs with multiple vessels. This may cause pitfalls including thrombosis in small accessory arteries, with particular significance in lower polar arteries due to their importance for ureteric supply. Novel techniques are therefore available to revascularise lower polar renal arteries. We aimed to assess the results of lower polar artery anastomosis to the inferior epigastric artery (IEA).
Methods: A retrospective analysis was performed of adult renal transplants over 10 years (2001 to 2010; 1269 patients) assessing cases of inferior polar arterial anastomosis to IEA. Main renal arteries were anastomosed to common, external or internal iliac artery, and accessory inferior polar artery to IEA. Primary endpoints were urinary complications. Secondary endpoints included graft and patient survival, Creatinine at 1 year and post-transplant arterial complications (hypertension from renal artery stenosis).
Results: 19 patients underwent IEA anastomosis (10 live donors kidneys, 9 cadaveric; median age 49 (range 23-66)) 2 (11%) were Diabetics whilst none had peripheral vascular disease. Median anastomotic time was 38 minutes (range 30-70.) There were no urinary complications and 1 episode of post-transplant hypertension due to polar arterial stenosis. 2 patients (2/15 time censored 1 year, 4 within last year and grafts functioning) suffered graft loss with no mortalities. 1 year Creatinine was 135.5 umol/l (median, time censored 15 patients; range 91-354) with a median Glomerular Filtration Rate of 52.5ml/min (range 15-80).
Conclusion: Strategies allowing revascularisation of polar arteries allow utilisation of a larger donor pool. IEA's use has been proven in the largest case series to date, to have excellent graft and patient outcomes. There were no associated ureteric complications with a low incidence of post-operative arterial stenosis. This novel surgical approach therefore offers a viable option to maintain vascularity.

O-004 EFFICACY AND SAFETY OF ROUTINE INTRAOPERATIVE INSERTION OF DOUBLE-J STENTS IN KIDNEY TRANSPLANTATION: A SYSTEMATIC REVIEW

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Objective: To systematically evaluate the efficacy and safety of routine insertion of double-J stents to prevent urine leak and urinary obstruction in kidney transplant recipients.
Method: Medline, Embase, Cochrane Library, and Chinese Biomedicine database were searched to locate relevant randomized controlled trials (RCTs). Data extraction and assessment of methodologic quality were performed independently by two reviewers. Meta-analysis was performed by Revman 5.0 software.
Result: Ten RCTs (including 1616 patients) were identified. By comparing the routine stent group with the no stent group, the meta-analysis showed: (1) incidence of urine leak, urinary obstruction and urinary tract infecion (UTI) was 4 times lower, 6 times lower, increased by 52%, respectively ($P<0.05$); (2) Patient and graft survival, rate of acute rejection, delayed graft function and hematuria were of no significant difference ($P>0.05$).
Conclusion: Routine stenting reduces the incidence of urine leak and urinary obstruction. Although the double-J stent increases the risk of UTI, it seems that UTI doesn't affect the outcome of transplantation.

O-005 HAND-ASSISTED SCOPIC LIVING DONOR NEPHRECTOMY: A VERY SAFE PROCEDURE

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Background: When subjecting perfectly healthy persons to living donor nephrectomy (LDN). *safety* is of utmost importance. Major complications experienced with the strictly laparoscopic technique (including two intestinal perforations) during our randomised study (2001-2004; laparoscopic versus open) urged us to explore alternative approaches.
Methods/Materials: Since 2005, 333 hand-assisted procedures have been followed in a prospective manner and compared to data from our randomised study. A Pfannenstiel incision (7-9 cm) has been employed for hand-assistance and kidney retrieval. The *laparoscopic* approach has by time and experience been found superior to the retroperitoneoscopic (RP) technique. In 2007 dissection/hemostasis with Ultracision was replaced by latest generation LigaSure.
Results: There has not been any major surgical complications (inside fascia), particularly no bleedings or visceral lesions. In one patient persistent abdominal pain was suspected to be due to subileus, and laparoscopic exploration was performed 3 mts post-donation. However, no distinct pathology was found.

Table 1. Results

[mean (range)]	2001–2004 Strictly laparoscopic [Randomised study] (N=64)	2005–2011 Hand-assisted Scopic (N=333)
Laparoscopic:RP-scopic	64:0	295:38
Right kidney:Left kidney	64:0	84:249
Op. time (min)	180 (110–295)	158 (95–275)
Perop. incidents	Vascular lesions: 2 (3%) Conversions: 2 (3%)	Vascular lesions: 4 (1,2%) Conversions: 1 [0,3%; GIA-failure]
Major Complications/Reop. – Inside fascia	Total: 6 reop. (10%) Serious: 2 (3%; intest. perf.)	No major/No reop. within d. 30 Percutaneous drainage of lymphocele: 1
Minor Complications – Subcutaneous region	2 (3,1%; infection/ persist. secretion)	6 (1,8%; wound sinus/infection/ persist. secretion)
Multiple Linear Regression – Significant factors	– Hand-assistance on Major complications + Op. time – Sex on Op. time (longer in males)	

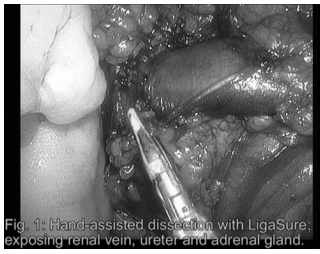
Fig. 1: Hand-assisted dissection with LigaSure; exposing renal vein, ureter and adrenal gland.

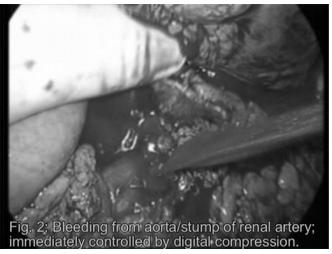
Fig. 2: Bleeding from aorta/stump of renal artery; immediately controlled by digital compression.

Conclusion: The hand-assisted scopic procedure has at our center turned out to be very safe, demonstrated by 333 consecutive cases without major surgical complications/reoperations. We consider the hand-assisted technique to afford increased security, both towards vascular incidents and gastrointestinal complications - and fast/efficient dissection.
However, the learning curve/experience with time has obviously contributed to these results.
Furthermore, the technical improvements during recent years have been important: Hemostatic devices (LigaSure; taking care of all venous branches without clips), "High Definition imaging", blunt/non-cutting trochars.
In the case of the very rare major bleeding incidents, hand-assistance may be critically beneficial. And these incidents are too rare to be accounted for statistically by most LDN series.
We have abandoned the RP-approach and now consider the laparascopic access to be more plain, straightforeward and faster. [VIDEO-presentation]

O-006 OUTCOME OF RENAL TRANSPLANT INTO PATIENTS WITH LOWER URINARY TRACT ABNORMALITY

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Introduction: Patient with lower urinary tract abnormality (LUTA) are often considered as high risk for transplantation. The commonest risk includes urinary tract infection (UTI) and serious morbidity related to urosepsis.
Method: Retrospective analysis of patients undergoing transplantation with LUTA from January 2000 to December 2010 were analyzed. Prevalence of UTI, urosepsis and the microbial pattern together with graft outcome were analyzed comparing different groups of LUTA.
Results: There were 83 patients comprising 38 primary vesicouretic reflux (VUR), 12 posterior urethral valves (PUV), 5 neuropathic bladders (NEU), 9 enterocystoplasties (ENC), 5 Ileal conduits (ILC) and 8 miscellaneous (3 neobladders, 2 urethral strictures, 3 prune belly syndrome etc). Reconstructive surgery when required was undertaken prior to transplantation. Six patients were lost to follow-up. Median follow-up was 59 months (range 8-127). UTI occurred in 60/77 (80%). Overall 547 episodes of UTI were treated with an average of UTIs/patient/year were VUR=1.45, PUV= 0.58, NEU=3.39, ENC=1.56, ILC=4.09. Urosepsis was similar in all groups. Graft loss occurred in 12 of which 7 were UTI related and spread across all groups. One patient died of an unrelated cause with a functioning graft. E.coli was the commonest organism identified (VUR=34%, PUV=38%, ENC=26%, NEU=65%) except the ILC which enterococci was the commonest (26%).
Discussion: Though the prevalence of UTI is high in patients with LUTA with neuropathic bladders and Ileal conduits are most prone. Incidence of urosepsis and the pattern of uropathogens of Enterocytoplasty and Ileal conduit patients were similar possibly reflecting importance of bowel mucosa as a causative factor. Enterococci were most commonly seen with Ileal conduits possibly due to bowel or stoma bag colonization which requires needs further evaluation.

Liver transplantation (hepatocellular carcinoma)

O-007 PRE-LIVER TRANSPLANT BIOPSY IN MULTIFOCAL HCC: a potential EXCLUSION CRITERION FOR TRANSPLANTATION?

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Background: In cirrhotic patients with multifocal HCC pre-liver transplant (LT) biopsy of the largest tumour is increasingly routinely undertaken in some centres. Poor differentiation is used as an exclusion criterion for LT.
Methods: Analysis of a prospective database of 65 consecutive patients undergoing orthotopic LT for radiologically diagnosed HCC at St James's University Hospital between 2006-2011.
Results: The median age at LT was 57 years with a predominance of male recipients (84.5%). The majority of patients had hepatitis C virus infection (47.9%), alcoholic liver disease (19.7%) or a combination of both. In 5 patients no HCC was found in the explant; a 7.7% misdiagnosis rate.
Based on pre-transplant MRI the mean number of tumours was 2.1 (1-6) with a mean size of 18.2mm (4-60), compared to 2.1 (0-9) and 15.9mm (3-55) on histological examination of the explant. A discrepancy between the definition of the largest lesion on pre-transplant radiology and the largest explant tumour occurred in 5 (7.7%) of cases. Based on radiological staging 56 (83.6%) patients were transplanted within the Milan criteria (MC) and 11 outside (16.4%), compared to 40 (67.8%) and 19 (32.2%) on histological examination.
Tumours were classified as well, moderately or poorly differentiated in 39 (31.7%), 60 (48.8%) and 24 (19.5%) cases. In patients with multifocal HCC, 8 (26.7%) had tumours of differing grades. In two (6.7%) patients the largest tumour was well differentiated whilst smaller tumours in the explant were poorly differentiated. In one patient the largest lesion was benign with other smaller invasive tumours confirmed histologically.
Conclusion: There is a need to optimise selection strategies for LT for HCC. In our UK series the largest tumour was not always representative of tumour burden or biological aggression and its potential use to exclude patients from curative treatment is questionable.

O-008 THE IMPACT OF WAITING LIST ALPHA-FETOPROTEIN CHANGES ON THE OUTCOME OF LIVER TRANSPLANT FOR HEPATOCELLULAR CARCINOMA

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Background & Aims: Liver transplantation is a recognized treatment for selected patients with hepatocellular carcinoma (HCC), but transplant criteria still need to be refined, especially in the case of more advanced or downstaged tumors.
Methods: The present study investigated alpha-fetoprotein (AFP) as a predictor of outcome in 9303 patients listed with a diagnosis of HCC in the Scientific Registry of Transplant Recipients.

Results: Local pre-transplant HCC treatment was used in 37% of patients on the waiting list. Patients with AFP levels >400 ng/ml at the time of listing who were downstaged to AFP ≤400 ng/ml had better intent-to-treat survival than patients failing to reduce AFP to ≤400 (77% vs. 39% at 3 years, p≤0.001) and similar survival to patients with stable AFP ≤400 ng/ml (69%, p=0.14). Patients with AFP levels decreased ≤400 ng/ml and patients with levels persistently ≤400 ng/ml also had similar drop-out rates from the list (10% in both groups) and post-transplant survival rates (86% vs. 75% at 3 years, p=0.11). Such an AFP downstaging was associated with good survivals whatever the level of the original AFP (even if originally >1000 ng/ml). Only the last pre-transplant AFP independently predicted survival (p≤0.005), unlike AFP at listing or AFP changes.
Conclusions: Overall, downstaging HCC patients with high AFP is feasible and leads to similar intent-to-treat and post-transplant survivals to those of patients with AFP persistently low. Only last AFP appears relevant for patient selection before transplantation and should be used in combination with morphological variables.

O-009 HEPATOCELLULAR CARCINOMA ON CIRRHOSIS: LIVER RESECTION, LIVER TRANSPLANTATION OR BOTH SURGICAL TREATMENTS?

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Background: The management of patients with cirrhosis and early hepatocellular carcinoma (HCC) is controversial, especially due to low availability of cadaveric liver donors.
Methods: From January 1996 to December 2010, 503 patients with transplantable HCC according to Milan criteria were treated by liver resection (LR) (n=180) or liver transplantation (LT) (n=323 of 512 listed for LT at our institution).
Results: Among 180 patients elegible for transplantation who underwent LR, 72 (40%) developped HCC recurrence and 53/72 (74%) of these, presented HCC recurrence into Milan criteria. Only 30 of the 72 patients underwent LT as a salvage procedure, with a transplantation rate of 41% of patients with HCC recurrence. According to intention to treat analysis of transplantable HCC patients who underwent LR (n=180), compared to all those listed for LT (n=512), 5-year overall survival was 71% in the LR group versus 62% in patients listed for LT, respectively (p=NS); 5-year disease-free survival was 41% in the LR group versus 54% in patients listed for LT (p=NS). The median time from resection to transplantation was 2.1 years (0.8-5.5) in the subgroup of 30 patients with HCC recurrence after LR. Five-year overall (83% vs. 71%, p=NS) and disease-free (62% vs. 68%, p=NS) survival rates and the mean time on the waiting list was similar (5.5±6.4 vs. 8.2±6.5 months) for salvage LT and primary LT for HCC, respectively. Among 36 salvage LT, 5/36 (13%) developped HCC recurrence versus 33/323 (10%) primary LT (p=NS).
Conclusion: For patients with early HCC and well compensated cirrhosis selectively to perform resection as first-line therapy followed by salvage transplantation, is the best strategy that spares the use of liver grafts, avoids potential problems with prolonged waiting times, and provides the patient with rapid access to an effective therapy.

O-010 INCREASED ALPHA-FETOPROTEIN AFTER BRIDGE THERAPIES AS PREDICTOR OF RECURRENCE IN PATIENTS WITH HEPATOCELLULAR CARCINOMA TREATED WITH LIVER TRANSPLANTATION

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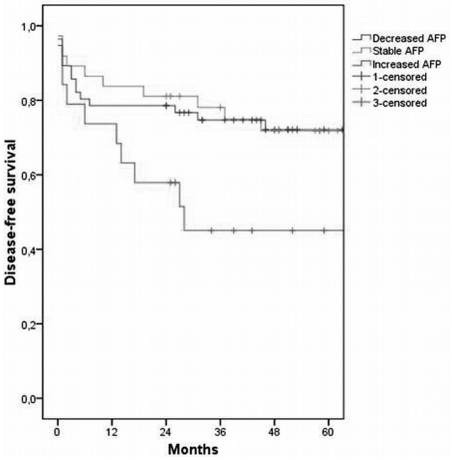
Background and aims: Locoregional therapies are routinely adopted for the treatment of hepatocellular carcinoma (HCC) as bridge for liver transplantation (LT). However, their role has not been completely investigated. Moreover, pre-LT recurrence predictors usable in clinical practice may be extrapolated by these therapeutic approaches. The aim of the study is to analyze the group of HCC patients treated with bridge therapies before LT and to find predictive parameters for the risk of post-LT recurrence.
Methods: A cohort of 112 HCC patients treated with bridge therapies and then transplanted in 3 roman LT centers from 1999 to 2009 has been retrospectively analyzed.
Results: At linear regression, increased AFP after bridge therapies resulted the sole risk factor for the risk of HCC recurrence after LT (*p*-value: 0.002; Odds Ratio: 3.7).
Patients with increased AFP values showed the worst 5-year disease-free survival rates (45.0 *vs* 72.1% in patients with decreased values, *p*-value = 0.047).



Conclusions: Good response to bridge therapies consents to obtain better post-LT results with regard to the risk of recurrence. Increased AFP values seem to be a strong predictor of recurrence. Biological selection performed by bridge therapies may consent to exclude too aggressive tumors from LT: integration of new parameters such as AFP with previous selection criteria (morphology, grading) could increase the results in terms of survival.

O-011 BARCELONA CLINIC LIVER CANCER STAGING PREDICTS THE SURVIVAL BENEFIT OF LIVER TRANSPLANTATION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Background: The Barcelona Clinic Liver Cancer (BCLC) staging is now considered the best prognostic system for patients with hepatocellular carcinoma (HCC).
Aim: To create a prediction model linking the BCLC stage of HCC patients to their 5-year liver transplant (LT) benefit.
Methods: Data from the ITALICA database (n=2951) were used to identify a large cohort of consecutive HCC patients (n=1328) judged potentially eligible for LT according the following criteria: absence of macroscopic vascular invasion and/or metastases, age ≤70 years and absence of relevant extrahepatic co-morbidities. We performed a Cox's multivariate analysis to evaluate the prognostic power of BCLC staging adjusted for the following covariates: age, sex, aetiology of cirrhosis, MELD score, alphafetoprotein, therapy. Liver transplant survival benefit for a given candidate was calculated using two-dimensional simulation analysis as that candidate 5-year life expectancy (LE) with a transplant (http://89.96.76.14/metroticket/calculator/) minus his/her 5-year life expectancy without a transplant according to BCLC stage.
Results: In the Cox non-LT survival model, hazard ratios (95% confidence interval) associated with increasing BCLC stages were 1.50 (1.10-2.10) for BCLC A vs. 0, 1.55 (1.32–1.82) for BCLC B-C vs. A, and 1.40 (1.10-1.79) for BCLC D vs. B-C respectively.
One-hundred-twenty-six patients (9%) with a predicted 5-year survival after LT < 50% were excluded from LT-benefit analysis. Median 5-year transplant benefit in months (25%–75% quantiles) significantly increased according to

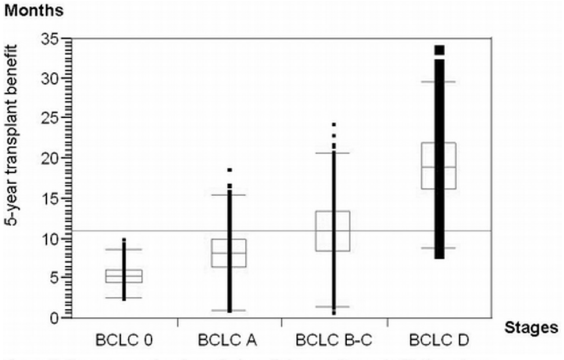

Figure 1. 5-year transplant benefit (months) according to BCLC staging

BCLC stage: BCLC 0 = 5.29 (4.54-6.16), A = 8.28 (6.46-10.10), B-C=11.26 (9.00-14.00), D=19.39 (16.18-22.41).
Conclusion. We calculated the net (adjusted for age, sex, aetiology of cirrhosis, MELD score, therapy) 5-year transplant benefit according to BCLC stage in HCC patients without absolute contraindications to LT.

O-012 TEN-YEAR FOLLOW-UP OF A RCT: NEOADJUVANT CHEMOTHERAPY IN LIVER TRANSPLANTATION FOR HCC

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Background: Liver transplantation (LT) for hepatocellular carcinoma (HCC) yields similar survival rates as LT for non-HCC patients by strict selection of patients according to Milan criteria or similar staging procedures. Neoadjuvant chemotherapy with Doxorubicin failed to improve results on the short term1. This study aims at follow-up data of a RCT after ten years.
Methods/Materials: 75 patients where initially included in a RCT to study treatment effects on patient survival within five years. Follow-up data were collected at scheduled outpatient visits and endpoint data were taken from hospital or autopsy reports.
Groups were analysed according to original randomization, subgroups to UICC classification I-III vs. IV by Kaplan-Meier estimation and log rank testing. Distributions of reasons for death were tested by Chi-Square.
Results: Out of 75 recruited patients, 64 entered randomization and were enrolled to either chemotherapy (CT) (n=34) or control group (CG) (n=28). Ten-year patient survival for CT and CG was 24% and 29% respectively (p=0.42). The most dramatic difference in survival was seen in subgroup analysis UICC I-III vs. UICC IV, with 10-year survival of 45% vs. 14% (p= 0.02). Female vs. male survival at 10-years was 56% and 21% respectively (p=0.014). Recurrence of HCC was the predominant reason for death 43% in CT and 62% in CG (p=0.18), followed by sepsis and de-novo tumor of 15% vs. 5% (p=0.2) for either endpoint.
Conclusion: Chemotherapy with Doxorubicin has no impact on overall survival neither in five years nor in the ten-year analysis. The real difference in survival is seen in our subgroups UICC I-III (within Milan) and UICC IV (beyond). Among reasons for death there is a trend of lower HCC recurrence but higher sepsis and de-novo tumor in CT.
Reference:
1. Pokorny H. et al. AJT 2005;5:788-94.

Clinical pancreas transplantation

O-013 RETROPERITONEAL PANCREAS TRANSPLANTATION WITH ENTERIC DRAINAGE INTO THE RECIPIENT DUODENUM

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Introduction: Simultaneous pancreas-kidney transplantation has been widely considered as the most effective way to obtain normoglycemia in patients with type 1 diabetes mellitus and end-stage renal disease. The surgical techniques for duct management changed from bladder drainage to enteric drainage. The main disadvantage of enteric drainage is the difficulty for rejection monitoring.
Methods: We evaluated a case-series of twelve patients with retroperitoneal pancreas transplantation performing enteric drainage into the recipient duodenum. Therefore after midline laparotomy, the right hemicolon and caecum were mobilized. The pancreas was placed with the tail inferiorly and vascular anastomosis were performed to recipient inferior caval vein and aorta or iliac artery. Exocrine drainage was established with a side-to-side duodeno-duodenostomy. Induction therapy was performed with T-cell-depleting polyclonal antibodies (anti-thymocyte globulin) and a steroid taper. The maintenance therapy consisted of Tacrolimus and Mycophenolate Mofetil. Upper gastrointestinal endoscopy was performed for biopsy and rejection monitoring.
Results: Initial function of both organs was excellent in 96%. Early technical problems requiring relaparotomy did not appear in this case-series. In one case bleeding of the recipient duodenum in the area of the anastomosis was diagnosed by gastroscopy. Graft thrombosis and acute rejection were not seen. The most frequent reason for pancreas graft failure was death with a functioning graft (myocardial infarction 1, cardiovascular arrest 1). One patient developed graft pancreatitis two years after transplantation, which could be managed without complications.
Biopsies were performed via gastroscopy without any complications and allowed reliable rejection monitoring.
Conclusion: Side-to-side duodeno-duodenal anastomosis is technically feasible. It provides the ability to visualize the donor pancreas with gastroscopy. Biopsies for rejection monitoring can be performed easily.

O-014 IMPACT OF PANCREAS DONOR SCORING SYSTEM IN GRAFT COMPLICATIONS AND OUTCOMES: SINGLE CENTER EXPERIENCE

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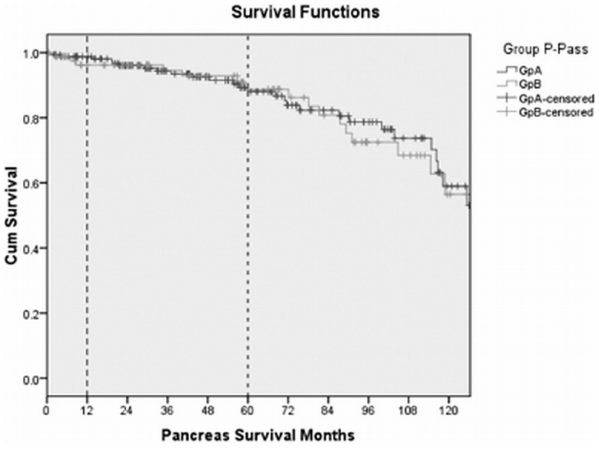
Introduction: Due to scarcity of organs and the strict criteria of acceptance for pancreas donors, the number of transplants are decreasing. Recently the P-PASS score predicted rate of acceptance of graft based on donor characteristics, making it less likely in scores over 17. Extending the criteria of acceptance might not affect graft outcome but increase the complications.
Aim: Evaluate the complication rate in pancreas recipients comparing the donor P-PASS scores and the graft outcome.
Methods: P-PASS score was retrospectively calculated for all donors for pancreas transplanted between 2000 and 2010. Graft thrombosis, collections, anastomotic leaks and other major complications are evaluated.
Results: Among 249 grafts into 232 patients, donor score was <17 (gpA) in 165 (66.2%) and ≥ 17 (gpB) in 32.5%. Number of retransplants was 26 in gpA and 11 in gpB. Overall complication rate of any kind was 29% in gpA and 33.3% in gpB. Thrombosis occurred in 49 patients (31 in gpA vs 18 in gpB) (p=0.05). Pancreatic collections rate was 8% in gpA and 5% in gpB (p=NS). Patients required surgical intervention for other reasons than thrombosis or collections occurred in 14.5% in gpA and 25.9% in gpB (p=0.05). Recipient age, cold ischemia time, intensive care unit and hospital stay is presented in the table.

Demographics

	GpA (±SD)	GpB (±SD)	p value
Recip Age	39.3±7.25	39.2±7.5	NS
ICU (day)	3.7±5	4.1±4.3	NS
HD (day)	19.1±14	25.2±26.3	0.03
CIT	685.4±224	659.2±220	NS

Pancreas survival at 1 and 5 years was 98.8% and 94.4% for gpA and 96.2% and 72.5% for gpB (p=NS).



Postoperative mortality occurred in 3 patients.
Conclusions: Rate of complications might increase with higher score donors but the long term survival does not get affected. Acceptance criteria for pancreas donor can be widened with acceptable complication risk.

O-015 ACCEPT OR REJECT? MAJOR INCONSISTENCIES IN ASSESSING DONOR CHARACTERISTICS FOR ORGAN ACCEPTANCE IN PANCREAS TRANSPLANTATION

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Background: The majority of pancreata, offered in allocation, is discarded.

This pancreas under-utilization is not well understood yet. We aimed to analyse whether consistent turndown criteria can be identified.
Methods: We analysed the allocation protocols of all Eurotransplant-registered German whole pancreas donors in 2005-2009 (n=1769). Several reasons were categorized according to their relevance for rejecting the organ for transplantation. Donor-related characteristics, e.g. BMI, age and P-PASS, were compared between transplanted and rejected pancreata.
Results: 63% of the offered pancreata were not transplanted. The most frequent turndown reasons were "long ICU stay", "resuscitation", "age" and "lab results". Those donors where "long ICU stay" was named stayed longer on ICU than those whose pancreas was transplanted (10.8±5.1 vs. 3.5±3.4 days). However, there was a considerable overlap: Of 442 donors who stayed 4-7 days, the pancreata of 7% were rejected because of "ICU stay" while 34% were transplanted (59% cases: rejected for other reasons). The pancreata of those who stayed on ICU 8-11 days were rejected due to "ICU stay" in 29%, transplanted in 24%. Even a stay of 0-3 days was given as a discard reason in 12 cases, whereas 15 pancreata were transplanted whose donors stayed on ICU ≥12 days. Similar results were found for age and BMI. The P-PASS was lower in the used than in the rejected organs (mean/median 16 vs. 18), but the range was similar (9-24 vs. 9-25).
Discussion: There is no consistency in the decision-making process using the single donor characteristics (e.g. BMI, ICU stay, age) or the P-PASS. A better understanding of the organ rejection process is needed in order to facilitate the acceptance of pancreata used for transplantation. Prospective qualitative interviews are planned to investigate the relevance of non-medical reasons.

O-016 SINGLE CENTRE EXPERIENCE WITH DCD PANCREAS TRANSPLANTATION

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This article summarizes a single centre experience with transplanting pancreases from controlled DCD.
From March 2004 to February 2011, 407 pancreas transplants were performed: 54 from DCD and 353 from DBD. DCD pancreases were accepted if donor age< 60 years, BMI <32, and time to cardiac arrest from withdrawal <1 hour. Campath induction, Tacrolimus & MMF maintenance and TEG directed anticoagulation were employed. Kaplan-Meier estimates were used to compare 1-year graft & patient survival. There were 17 SPK, 22 PTA and 15 PAK from DCD; 282 SPK, 38 PAK and 33 PTA from DBD, resulting in more isolated pancreases (PAK & PTA) from DCD (p=0.0001). DCD had 14 months median follow-up (range 0-45); DBD had 30 months' follow up (range 0-83). DCD and DBD recipients were of similar age (43±9 vs. 42±8) and BMI (25±2.9 vs. 25±5). DCD donors were younger (33±12 vs. 37±13, P=0.03) but had similar BMI as DBD (23±3vs. 24±4). DCD initial warm ischemia was 13 minutes (0-21) and had longer cold ischemia (732±149 vs. 663±167 minutes, p=0.01). DCD donors had less vascular cause of death (33% vs. 52%, p=0.02). SPK pancreas survival & overall patient survival were similar (94% DCD vs. 89% DBD, p=0.5), (95% DBD vs. 96% DCD). Higher isolated DBD pancreas graft survival (89% DBD vs. 73% DCD, p=0.03) contributed to better overall pancreas survival (89 vs. 80%, p=0.04) in DBD. DCD pancreas graft loss 2° to thrombosis was more frequent (11% vs. 0.8%, p=0.0008) despite anticoagulation. DCD pancreases and kidneys had more frequent DGF (13% vs. 2%, p<0.0001) & (29% vs. 11%, p=0.02). DCD grafts had similar PNF of the kidney (0% vs. 1%, P=NS) and of the pancreas (3.7% vs. 1.4%, P=NS). These single-centre results suggest that DCD pancreases are better utilized if implanted simultaneously with a kidney.

O-017 FUNCTIONAL CAPACITY OF PANCREAS AND KIDNEY GRAFTS FROM "EXTREME" PEDIATRIC DONORS

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Introduction: We describe 3 cases of pancreas transplantation with pediatric donors between 6 weeks and 25 months of age.
Patients: Patient 1 (age: 46 years): Pancreas and kidney transplantation (PNTx) with a 26 months old donor and a body weight of 15 kg. Patient 2 (age: 54 years): Single pancreas transplantation (pancreas after kidney) with a 13 months old donor and a body weight of 10 kg. Patient 3 (age: 35 years): En bloc dual PNTx with a 6 weeks old donor and a body weight of 4.1 kg
Immunosuppression included in all patients ATG, tacrolimus, MMF and steroids.
Results: In patients 1 and 2, surgical procedures were uneventful. In patient-3, the postoperative course was complicated due to bleeding and leak from enteric anastomosis requiring reoperations. No vascular complications were observed. In all patients, normoglycemia was established hours after reperfusion. In all 3 patients donor specific antibodies were detected within the first 2 weeks. All patients were treated with IVIG administrations at 2g/kg. Patient-3 received additionally bortezumib. Kidney and pancreas function in patient 1 at 1.5-year and pancreas function in patient 2 at 1-year were excellent. In patient-3, both kidneys nearly doubled their size within 4 weeks and increased their functional capacity, reflected by continuous decrease of serum creatinine. At 4 months, patient-3 is normoglycemic (B-HbA1c 36 mmol/L, c-peptide > 1.2 nmol/L) without requiring exogenous insulin or dialysis treatment (current creatinine 84 μmol/l).
Conclusion: Pancreas and kidney transplants from "extreme" pediatric donors have excellent functional capacity and short-term outcomes

O-018 ABO-INCOMPATIBLE DECEASED-DONOR PANCREAS AND KIDNEY TRANSPLANTATION USING A NOVEL PROTOCOL INCLUDING ECULIZUMAB AND MONITORING OF COMPLEMENT FUNCTION

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Backgrounds: We describe 2 consecutive and successful ABO-incompatible kidney and pancreas transplantations (PNTx) using a novel protocol for prompt inhibition of complement activation in case of ABO activity.
Patients/Methods: Patient 1: 43-years, ABO-phenotype: A1 to 0, baseline anti-A1 IgG and IgM titers were 1:4.
Patient 2: 46-years, ABO-phenotype: A1 to 0, baseline anti-A1 IgG and IgM titers were 1:16.
Both patients suffering from poorly controlled Type I diabetes underwent a deceased-donor ABO incompatible PNTx. Patients were given single-plasmapheresis and rituximab preoperatively. The immunosuppression included basiliximab, tacrolimus, MMF and steroids. Postoperatively, regular antigen-specific immunoabsorptions were performed. In patient 2 immunoadsorption was discontinued in favour of plasmapheresis due to lack of efficacy. Eculizumab was used as rescue treatment for humoral rejection.
Results: In both cases, PNTx was uneventful with excellent primary kidney and pancreas function. Patient 1 experienced a rebound of isoagglutinins and a distinct biopsy-verified vascular rejection on day 9. Eculizumab was given at 600 mg on days 10 and 14. Complement analysis documented complement activation and confirmed that eculizumab completely blocked complement function. At 3 months, patient I had normal kidney and pancreas function. Double-balloon endoscopy with biopsy of the duodenal segment of the pancreas on day 60 showed normal macroscopical and histological architecture of the mucosa.
In patient 2, isoagglutinins consistently increased on day 9. Unlike patient 1, however, no humoral rejection or complement activation was observed. Currently, at 1 month, the patient has normal kidney and pancreas function.
In both patients, repeated Luminex examinations revealed no donor-specific HLA antibodies.
Conclusions: With the current protocol, including eculizumab, ABO-incompatible deceased-donor transplantations might routinely be feasible. Furthermore, pancreas and its attached duodenal segment seem to have the same capacity as the kidney to develop accommodation.

DGF / IRI

O-019 THE EFFECTS OF INFLIXIMAB ON ISCHAEMIC-REPERFUION (I/R) INJURY IN A MODEL OF RENAL TRANSPLANTATION

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Background: Infliximab is a Tumour Necrosis Factor (TNF) antagonist used to treat rheumatoid arthritis and inflammatory diseases. It is monoclonal antibody that can block TNFα, an inflammatory mediator that is involved in renal ischaemic reperfusion (I/R) injury. The aim of the study was to investigate the effects of Infliximab on I/R injury in an *ex-vivo* model of renal transplantation.
Methods: Porcine kidneys were retrieved after 10 minutes of warm ischaemia and then statically stored for 24 hours at 4°C. After preservation kidneys were reperfused on an isolated organ perfusion system with oxygenated autologous blood at 38°C for 3 hours. Infliximab (5mg/kg) was added to the system just before reperfusion (n=5) and compared to a control group (n=6). Haemodynamic and functional parameters were then assessed during 3hr reperfusion.

Results: Levels of TNF-α were significantly reduced in kidneys treated with Infliximab (309±179 vs control 1529±823 pg/ml; P=0.029). During reperfusion infliximab treated kidneys had significantly enhanced renal blood flow (RBF) and lower intra-renal resistance (IRR) compared to the control group [Area under the curve (AUC) RBF 402±55 vs control 261±29.5 ml/min/100g·h; P=0.003, IRR 2.1±0.4 vs 3.9±1.1 mmHg/min/100g·h; P=0.014)]. Oxygen consumption was also significantly improved after 3 hours of reperfusion (37.8±9.8 vs 20.8±6 ml/min/g; P=0.014). However, no improvement was observed in renal function (AUC serum creatinine, 2547±403 vs control 2415±532 μmol/L·h; P=0.673).
Conclusion: Infliximab significantly improved kidney perfusion, oxygen delivery and reduced TNF-α levels in this porcine kidney model. Although, immediate renal function was not recovered, further investigation to assess Infliximab's potential to ameliorate I/R injury in renal transplantation is warranted.

O-020 BORTEZOMIB PROTECTED AGAINST ISCHEMIA-REPERFUSION INJURY ASSOCIATED WITH ORTHOTOPIC LIVER TRANSPLANTATION IN THE RAT: AN IGL-1 SOLUTION APPROACH

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Antecedents: Hepatic ischemia reperfusion injury (IRI) is the main cause of both initial poor function and primary non-function of liver allograft. Ubiquitin proteasome system (UPS) is the main no-lysosomal, multicatalytic proteinase complex involved in the degradation of most intracellular short lived proteins and stress response, including the endoplasmatic reticulum (ER) stress. UPS inhibitors used at a non toxic low dose has potential protective effects against oxidative stress but its role on liver IRI associated with orthotopic liver transplantation (ROLT) is unclear. We evaluated Bortezomib (BZ) effects at non toxic low and single dose) to donors/recipients subjected to ROLT.
Experimental: Sprague Dawley (200 g b.w.; n=6 each group) rats were classified as follows Group 1: Sham; Group 2 (ROLT)= By removing the lateral and the two caudates lobes, the grafts preserved in IGL-1.Solution (1 h; 4C) were transplanted. Recipients rats were sacrificed at 6 hours for sample collection and Group 3 (BZ+ROLT) = Same as group 2 but with BZ i.v. administration (10mg/kg) to donor/recipients rats. AST/ALT and mitochondrial (GLDH activity) injury, as well as oxidative stress (MDA), expression of cytoprotective factors (HO-1 and HSP70) and ER stress (GRP78, CHOP and ATF4) were determined. BZ effects on apoptosis (TUNEL) and earlier regeneration (PCNA) were also evaluated.
Results: A significant reduction in AST/ALT was observed in UPS -treated rats when compared to ROLT alone. This was consistent with a significant diminution of GLDH and MDA and concomitant with HO-1 and HSP70 increase. Also GRP78, CHOP and ATF4 changes were observed.
Conclusions: BZ used as a single and therapeutic single dose contributes to prevent efficiently IRI associated with ROLT. Up regulation of cytoprotective HO-1 and HSP 70 would contribute to liver graft protection.

O-021 PROSPECTIVE TRIAL ON ERYTHROPOIETIN IN CLINICAL TRANSPLANTATION

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Purpose: to study the effect of high dose erythropoietin on the incidence of delayed graft function/primary non function (DGF/PNF) and renal function in non-heart beating donor (NHBD) kidney transplant recipients.
Methods: All consecutive NHBD transplant recipients were included in this prospective, randomized, double blind, placebo-controlled study. Erythropoietin-β was administered to the recipient as an intravenous bolus of 3.3×10^4 IU, 3-4 hours before the transplantation as well as 24 and 48 hours after reperfusion.
Primary end point was the composite of DGF/PNF. Secondary end points included DGF duration, acute rejection, measured creatinine clearance (mGFR) and survival at 1 year after transplantation. For safety purposes any arterial or venous thrombosis was recorded.
Results: A total of 92 patients were included in the study. DGF occurred in 86.7% in the EPO group as compared to 87.2% in the placebo group (NS).
The incidence of PNF was 6.7% in the EPO group and 2.1% in the placebo group.
If DGF developed, median duration was 10 days in the EPO group and 9 days in the placebo group (NS).
Acute rejections occurred in 20.5% in the EPO group and in 26.1% in the placebo group (P=0.62).
The measured creatinine clearance showed no difference at 6 weeks (44±19 ml/min and 46±18 ml/min in EPO and placebo group respectively), but was significantly better in the EPO group 1 year after transplantation (68 vs 57 ml/min) ($P<0.05$).
One-year patient and graft survival were respectively 96/93% and 96/96% in the EPO and placebo group.
Thromboembolic events occurred in 14 patients in the total group, the majority consisted of thrombosis of the vascular access.
Conclusion: High-dose Erythropoietin did not reduce the incidence or duration of DGF. However, treatment with EPO resulted in a significantly better recovery of mGFR, but also more thrombotic events.

O-022 MOLECULAR PREDICTORS OF DELAYED GRAFT FUNCTION OF KIDNEY ALLOGRAFT

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Background: Delayed graft function (DGF) as a consequence of ischemia/reperfusion injury (IRI) negatively influences the outcome of kidney transplantation. Little is known about molecular changes during cold organ storage and after reperfusion. The aim of this study was to identify new molecular markers that may be associated with DGF.
Methods: To investigate changes of the intrarenal transcription profile during IRI, three graft biopsies were performed in each allograft prior or during the transplantation (baseline biopsy at the time of organ retrieval, pre-implantation and post-implantation biopsy). The intragraft expression of 90 target genes known to be implicated in the pathogenesis of ischemia-reperfusion injury was measured using quantitative real-time RT-PCR ($2^{-\Delta\Delta Ct}$) method in DGF (n=9) and primary function patients (n=26). Generalized linear mixed models (GLMM) were used to analyze correlated, repeated measures data.
Results: While the cold storage led to induction of gene transcripts related to apoptosis (BCL2, BCL2L11, BID, CASP8, CYCS, MAPK8) and decreased expression of proinflammatory markers (C3, CCL21, CCL5, CD68, CD8A, ENG, IL2RA, TNFRSF1), reperfusion caused the up-regulation of inflammation (CD69, CDKN1A, ICAM1, IFN, IL10, IL12A, IL2, IL6, IL8, PTGS2, SELE, TNF). The tubular atrophy score in baseline biopsy but not donor age was predictive of DGF. Candidate markers were further tested for prediction of DGF development. Logistic regression analysis revealed, that combination of mRNA gene expression of caspase 9 (CASP9), intercellular adhesion molecule 1 (ICAM-1), spermidine/spermine N1-acetyltransferase (SAT1) with histological score of tubular atrophy (ct) discriminates between grafts with DGF and primary function with sensitivity 0.64 and specificity 0.77 (AUC=0.77).
Conclusion: The intrarenal transcriptome analysis during transplantation may identify grafts at risk for DGF and thus help to adapt the immunosuppressive regimen.
This project was supported by Internal Grant Agency (NS10516-3/2009).

O-023 TETRAHYDROBIOPTERIN PREVENTS ISCHEMIA REPERFUSION INJURY INDUCED LETHAL GRAFT PANCREATITIS TARGETING THE NEURONAL NITRIC OXIDE SYNTHASE

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Donor pretreatment with the essential nitric oxide synthase (NOS) cofactor tetrahydrobiopterin was shown to attenuate ischemia-reperfusion-injury (IRI) induced graft pancreatitis in a murine model. Since the mechanism of tetrahydrobiopterin-mediated protection is still unclear, we investigated, whether the two constitutively active NOS - isoforms represent its major targets using endothelial (eNOS-/-) and neuronal NOS (nNOS-/-) knockout mice. In a heterotopic pancreas transplantation model syngeneic C57Bl6 mice were used as donor-recipient pairs, with donors beeing either wild types, eNOS-/- or nNOS-/-. Donors were pretreated with 50mg/kg bw tetrahydrobiopterin or were untreated. Non-transplanted animals served as controls. Pancreatic grafts were exposed to 16h cold ischemia time. Following 2h and 4h reperfusion,

microcirculation was analyzed by intravital fluorescence microscopy. Parenchymal damage was evaluated histopathologically and immunohistochemically, intragraft tetrahydrobiopterin-levels were determined by HPLC. All groups were tested for recipient survival.
Compared to non-transplanted controls, prolonged cold ischemia time resulted in significantly impaired microcirculation in untreated wild type as well as eNOS-/- grafts ($p<0.05$). Only in untreated nNOS-/- grafts microcirculatory deficits were absent. While tetrahydrobiopterin pretreatment preserved blood flow in wild type and eNOS-/- grafts, it did not further increase microcircualtion in nNOS-/- grafts. Tetrahydrobiopterin substantially reduced parenchymal damage and nitrotyrosine formation in wild type ($p<0.05$) and, in a lesser degree, in eNOS-/- grafts. nNOS-/- grafts were less prone to develop IRI-associated parenchymal damage ($p=0.07$) and showed no differences between treated and untreated grafts. Significantly prolonged recipient survival was only observed in animals receiving nNOS-/- pancreatic grafts and in grafts treated with tetraydrobiopterin, independent of their genotype (all $p<0.01$).
nNOS knockout of the donor prevented IRI-induced lethal graft pancreatitis in this model. These data suggest a crucial role of the neuronal NOS isoform in the pathogenesis of IRI following organ transplantation.

O-024 REMOTE ISCHAEMIC PRECONDITIONING PROTECTS THE HEPATIC MICROCIRCULATION FROM LIVER ISCHAEMIA REPERFUSION INJURY THROUGH THE NITRIC OXIDE/cyclicGMP PATHWAY

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Background: Ischaemia reperfusion (IR) injury is a major cause of morbidity and mortality in liver transplantations. Remote ischaemic preconditioning (RIPC) protects the liver from IR injury. Here we investigated: 1.the effects of RIPC on liver microcirculatory blood flow (MBF); 2.the role of the nitric oxide (NO)-soluble guanylyl cyclase (sGC)-cyclicGMP (cGMP) pathway in mediating the effects of RIPC.
Methods: C57BL6 mice were used in 6 groups: 1.Sham operation; 2.RIPC: 6-cycles of 4x4 minutes of hindlimb ischaemia/reperfusion using microvascular clamping of the femoral vessels; 3.IR: 40-minutes of partial (70%) liver ischaemia followed by 2-hours of reperfusion; 4.RIPC+IR; 5.C-PTIO+RIPC+IR: Carboxy-PTIO (NO scavenger) was administered prior to RIPC+IR; 6.ODQ+RIPC+IR: ODQ (sGC inhibitor) was administered prior to RIPC+IR.
Hepatic MBF was measured using Laser Doppler Flowmetery. Sinusoidal damage was evaluated with transmission electron microscopy (TEM). Liver damage was assessed using plasma liver enzymes levels. Hepatic cGMP was measured using an enzyme immunoassay.
Results: The RIPC+IR group showed significantly increased hepatic MBF compared to the IR group ($P<0.05$) throughout liver reperfusion. There were no significant differences between the RIPC+IR and either the sham or RIPC groups ($P>0.05$). Both the C-PTIO+RIPC+IR and ODQ+RIPC+IR groups had significantly reduced MBF compared to RIPC+IR throughout reperfusion ($P<0.05$).

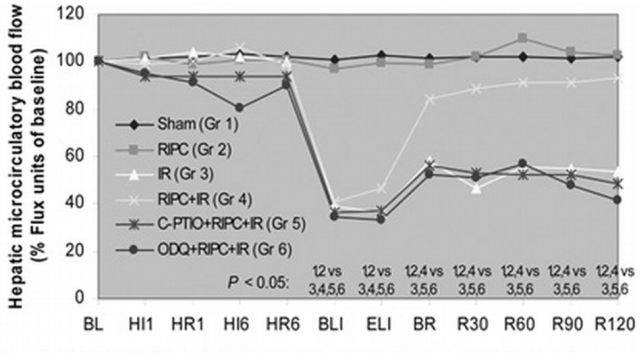

Figure 1. Hepatic MBF illustrated as mean percentage compared to pre-ischaemic baseline. Significant ($P < 0.05$) inter-group differences are shown vertically below each time point on the x-axis. The time points of MBF measurements were: *BL*, baseline; *HI1*, first hindlimb ischaemia; *HR1*, first hindlimb reperfusion; *HI6*, sixth hindlimb ischaemia; *HR6*, sixth hindlimb reperfusion; *BLI*, beginning of liver ischaemia; *ELI*, end of liver ischaemia; *BR*, beginning of liver reperfusion; *R30 to R120*, liver reperfusion at 30, 60, 90, and 120 min post ischaemia.

Liver TEM showed sinusoidal damage in the IR, C-PTIO+RIPC+IR, and ODQ+RIPC+IR groups; with relatively intact sinusoidal structure in the remaining groups. Plasma ALT and AST levels were significantly increased in the IR, C-PTIO+RIPC+IR, and ODQ+RIPC+IR groups compared to sham, RIPC, and RIPC+IR groups ($P<0.05$). Hepatic cGMP was significantly elevated in the RIPC compared to sham ($P<0.05$).
Conclusion: RIPC protects against liver IR injury by preservation of hepatic MBF. This is achieved through the NO-sGC-cGMP pathway during hindlimb RIPC.

Applied biology I

O-025 UP-REGULATION OF EXTRACELLULAR SIGNAL-REGULATED KINASE 1/2 SIGNALLING IN A MHC MISMATCHED MODEL OF CHRONIC RENAL ALLOGRAFT INJURY

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Background: A primary goal in the development of novel therapeutic strategies is improvement in long term renal allograft outcome. Extracellular signal-related kinases 1 and 2 (ERK 1/2) are serine/threonine kinases of the MAPKinase cascade. T cell proliferation and Th1 differentiation, as well as transduction of fibrogenic stimuli (e.g. TGF-β1) is ERK dependent. ERK 1/2 signalling may play a role in the pathogenesis of chronic immunological graft injury. To investigate this further, activated (phosphorylated) ERK 1/2 expression was examined in vivo in MHC mismatched rat kidney allografts. Dark Agouti (DA) to Wistar Furth (WF) strain transplantation provides an immunologically accelerated model of chronic injury with histological changes of chronic antibody mediated rejection.
Methods: Orthotopic kidney transplantation following left nephrectomy was performed in male rats weighing 200-250 g. Recipient native right kidney was removed 2 weeks post-transplant. Recipients received 1.5 mg/kg ciclosporin daily subcutaneously.

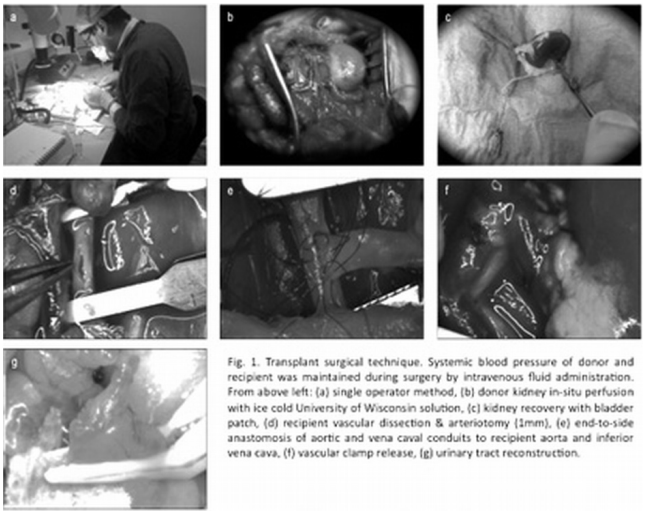


Fig. 1. Transplant surgical technique. Systemic blood pressure of donor and recipient was maintained during surgery by intravenous fluid administration. From above left: (a) single operator method, (b) donor kidney in-situ perfusion with ice cold University of Wisconsin solution, (c) kidney recovery with bladder patch, (d) recipient vascular dissection & arteriotomy (1mm), (e) end-to-side anastomosis of aortic and vena caval conduits to recipient aorta and inferior vena cava, (f) vascular clamp release, (g) urinary tract reconstruction.

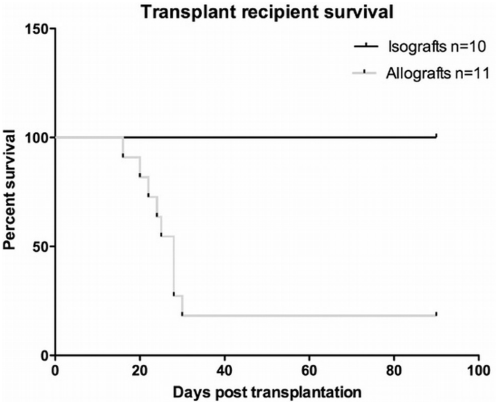
Results: Control DA-DA isografts survived for 12 weeks post transplant with normal tissue histology at termination and no change in functional parameters between measurements at 4 and 12 weeks. In marked contrast, 9 DA-WF allografts were lost to acute rejection following removal of the right native kidney. Allografts surviving to 12 weeks developed inflammatory cell infiltration, tubulo-interstitial fibrosis, vascular neo-intimal hyperplasia and transplant glomerulopathy in addition to functional impairment.

Chronic allograft injury model

	Serum [creatinine] μmol/L	Systolic BP mmHg	Urinary protein mg/24hrs	Glomerulo-sclerosis %	Interstitial fibrosis %
Allografts	160	148	77	19	13
Isografts	54	135	46	3	3
Significance level	<0.01	<0.05	NS	<0.001	<0.001

12 week outcomes.

Phosphorylated ERK 1/2 expression as measured by western blotting was sig-



nificantly higher in allografts than isografts and almost non-existent in the naive DA kidney.
Conclusion: Allografts which survived to 12 weeks showed features chronic rejection. This was associated with increased phosphorylated ERK 1/2 expression as compared to controls which showed stable renal function and normal histology. Inhibition of ERK 1/2 signalling may attenuate the immunological component chronic allograft injury.

O-026 mTORC1 PATHWAY ACTIVATION IS INSTRUMENTAL IN VASCULAR LESION INDUCED BY ANTIPHOSPHOLIPID ANTIBODIES

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Background: Antiphospholipid antibodies (APA) induce severe vascular lesions in both native and transplanted kidneys which lead to progressive renal failure (APA-nephropathy). The key regulator of cell growth, mTOR complex 1 (mTORC1), has been implicated in vascular narrowing after mechanical injury. Hence, we wondered whether APA might activate mTORC1 in endothelial cells to promote vascular lesions.
Methods: We evaluated the phosphorylation of two downstream targets of mTORC1 (S6RP and 4EBP1) and vascular cell proliferation in either native or transplanted kidney of patients with (n=7 and 37, respectively) or without (n=7 and 59, respectively) APA-nephropathy.
Results: APA resulted in mTORC1 pathway activation in both native and transplanted kidney. Remarkably, the activation concerned selectively the endothelial cells. Moreover, this activation was correlated with endothelial and smooth muscle cell proliferation and, more importantly, with the increase of vascular lesion scores. Among kidney transplant recipients with APA, 10 were treated by the mTORC1 inhibitor sirolimus, as an immunosuppressive regimen. Remarkably, these patients showed reduced vascular lesions and enhanced allograft survival rate. This beneficial effect was associated with a complete inhibition of mTORC1 pathway in endothelial cells and a significant decrease of endothelial and smooth muscle cell proliferation.
Conclusion: Our results suggest that endothelial mTORC1 activation is instrumental in APA-nephropathy. Further prospective trials are mandatory to confirm the promising beneficial effect of sirolimus.

O-027 FUNCTIONAL TOLERANCE BY DONOR-SPECIFIC BLOOD TRANSFUSION (DSBT) IN RATS IS ACCOMPANIED BY MICROVASCULAR C4D DEPOSITION.

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We previously reported: 1) DSBT induces functional tolerance in a rat model of cardiac Tx, although with histology of chronic rejection ;2) renal allografts can be functionally tolerized by DSBT. We now examine whether 1) "tolerized" renal allografts show signs of chronic rejection; 2) C4d deposition intervenes in this process.
Inbred male RA/PVG rats were used as fully mismatched donor/recipient pairs: DSBT-group (DSBT 12 days pre-allogeneic Tx) (n=25), rejection-group (no DSBT pre-allogeneic Tx) (n=8), control-group (no DSBT pre-syngeneic Tx) (n=14). Animals were sacrificied at 3 days/2 weeks/6 weeks/12 weeks/$\geq$20 weeks. Glomerular basement membrane splitting (cg) and intimal fibrosis (cv) were considered features of chronic rejection. C4d was scored in the peritubular capillaries (PTC), vasa recta (VR) and renal arteries (ART).
1) In the DSBT-group, cg and/or cv appeared at 2 weeks and were strongly pronounced at $\geq$6 weeks postTx. 15 (65%) grafts harvested $\geq$2 weeks postTx showed cv and/or cg. In the rejection-group no signs of chronic rejection were noted, while in the control-group no cv was detected, but mild cg was noted in 2 cases $\geq$20 weeks postTx. 2) In the DSBT-group, no C4d deposition was present at 3 days, but C4d appeared in the microvasculature in all allografts at 2 weeks, to gradually diminish over time (86% at 6 weeks/75% at 12 weeks/12.5% at $\geq$20 weeks). In the rejection-group, 0% 3 day-grafts showed C4d, while 100% $>$3 days were C4d-positive. All syngeneic grafts were C4d negative. 3) C4d in PTC and VR/ART correlated with acute cellular rejection ($p<0.0001$), while only C4d in VR/ART correlated with chronic rejection (p=0.0284).
1) Despite inducing functional tolerance, DSBT did not prevent chronic rejection; 2) C4d deposition correlated with acute and chronic rejection; 3) C4d in PTC is a feature of acute rejection, while C4d in VR/ART correlates with chronic rejection; 4) cg is not a specific lesion for chronic rejection.

O-028 MONOCLONAL ANTIBODY INHIBITION OF TLR2 PROMOTES GRAFT FUNCTION IN A MURINE MODEL OF RENAL TRANSPLANTATION

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Toll-Like Receptors (TLRs) are critical molecules involved in the activation of innate and subsequent development of adaptive immunity. TLRs sense danger signals elicited by exogenous pathogens and by endogenous ligands released in inflammatory diseases. Their activation leads to activation of NF-κB and release of proinflammatory cytokines. Increased expression of TLRs, and the cytokines produced following activation, have been associated with ischaemic kidney damage, acute kidney injury, end-stage renal failure, acute renal transplant rejection and delayed allograft function.
OPN301 is a mouse anti-human TLR2 antibody that cross reacts with mouse TLR2. We have shown that the half life is approximately 8-9 days and there is functional bioavailability up to at least 14 days post administration. Here we show that inhibition of TLR2 promotes graft function in a kidney isograft model of ischaemia reperfusion injury. Recipient mice (n=10 per group), were treated i.v. with OPN301, isotype-control or saline prior to surgery. The kidney then underwent 30 minutes of cold ischaemia prior to transplantation. The native kidney was removed at day 5 and renal function assessed 24 hours later by measurement of blood urea nitrogen (BUN). BUN concentrations in saline and isotype-treated mice were similar, but were significantly decreased in OPN301-treated mice. The histopathological appearance corresponded well with renal functional results. Tubular structure was well preserved in the OPN301-treated groups, with only sparse necrosis, which was also reflected in the severity score.
This is the first demonstration that inhibition of TLR2 with a therapeutic agent (OPN-301) provides significant protection from ischaemia/reperfusion injury in a model of kidney transplantation.

O-029 THE ROLE OF RORγT IN T17-MEDIATED ALLOIMMUNITY

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IL-17-producing T cells (T17) may play a major role in allograft rejection, as T17 cells in Th1-deficient mice mediate a pro-inflammatory alloimmune response resulting in accelerated allograft rejection. However, the mechanisms underlying T17-mediated alloimmune responses in transplantation are poorly understood, particularly with respect to regulation by the hallmark T17 transcription factor RORγt.
To precisely explore the role of RORγt in T17 alloimmunity in the absence of Th1, we created RORγt reporter and knockout mice on a T-bet-deficient (T-bet$^{-/-}$) background by crossbreeding T-bet$^{-/-}$ with Rorγt$^{gfp/wt}$ reporter and Rorγt$^{gfp/gfp}$ knockout mice, respectively.
First, we explored the fate-mapping capacity of our reporter model by polarizing T cells from Rorγt$^{gfp/wt}$T-bet$^{-/-}$ mice towards T17 and measuring GFP expression in vitro. We found that GFP was clearly up-regulated in T17-polarized T cells from Rorγt$^{gfp/wt}$T-bet$^{-/-}$ mice and that the expression of GFP paralleled the expression of RORγt (RT-PCR and FACS). Importantly, the T17-polarized T cells from the reporter mice were as capable of producing IL-17 as T cells from controls, as shown by ELISA and FACS, thus demonstrating the efficacy of our reporter model. Next, we tested the hypothesis that RORγt sig-

nificantly contributes to the differentiation of alloreactive T17 cells by studying T cells from Rorγt$^{gfp/gfp}$T-bet$^{-/-}$ double-knockout mice under T17-polarizing conditions, in terms of expression of T17 effector cytokines in vitro. As expected, we found that IL-17 expression was substantially lower in the double-knockout mice when compared to Rorγt$^{gfp/wt}$T-bet$^{-/-}$ and T-bet$^{-/-}$ controls, as indicated by RT-PCR, ELISA, and FACS. Clearly, RORγt plays a major, but not exclusive, role in driving IL-17-producing T cells in the absence of T-bet.
In conclusion, our novel model is an unprecedented tool in studying RORγt^{+} T17 cells in alloimmunity. In transplantation, our studies point to an important and as yet understudied role of RORγt.

O-227 GENETIC VARIANTS OF FOXP3 INFLUENCE GRAFT SURVIVAL IN KIDNEY TRANSPLANT PATIENTS

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Background: Regulatory T cells (Treg) play a role in controlling alloreactivity. A functional $(GT)_n$ dinucleotide repeat polymorphism has been described within the promoter region of the transcription factor FOXP3, the master gene for Treg development and function. Increased promoter activity is associated with 15 or less (GT) repeats. The present study aimed to investigate the influence of the $(GT)_n$ FOXP3 gene polymorphism on renal allograft survival.
Methods/Materials: Genotypes of 632 first-time transplant patients (mean follow-up time 6.7 years) were determined and grouped according to the length of the (GT) repeats; alleles with $\leq (GT)_{15}$ were categorized as short (S) and alleles with $\geq (GT)_{16}$ as long (L). Hemizygous males were included in the respective female homozygous SS or LL groups leading to the three genotype groups S/SS, SL and L/LL.
Results: S-allele carriers with graft survival of at least 3 months and acute rejection episodes showed superior graft survival when compared to patients who only expressed the L-allele (Kaplan-Meier, p = 0.009). No impact of the FOXP3 genotypes on renal graft survival was found when patients did not experience acute rejection (p = 0.311). Carriers of the S-allele who experienced acute rejection showed similar graft survival rates as non-rejecting S-allele carriers. No association between the causes of graft failure and the FOXP3 $(GT)_n$ polymorphism was observed (p = 0.56). Cox proportional hazard regression analysis defined the $(GT)_n$ FOXP3 gene polymorphism as an independent factor for renal allograft survival (S/SS: HR = 0.67, 95% CI 0.46 -0.98, p = 0.037; SL: HR = 0.54, 95% CI 0.32 - 0.9, p = 0.018; L/LL: regarded as reference).
Conclusion: This study is the first gene association analysis in kidney transplant patients which identified the beneficial effect of FOXP3 gene variants on graft survival.

Tissue injury / preservation

O-030 HEPATIC PRECONDITIONING BY CILOSTAZOL IMPROVE BILE SECRETION AND REDUCE VACUOLIZATION AND APOPTOSIS IN AN ISOLATED RAT LIVER REPERFUSION MODEL

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Introduction: Although hypothermia is pivotal to organ preservation, it has negative side effects like microcirculatory failure, mediated by sinusoidal endothelial cell injury as an imbalance of vasoconstrictors and vasodilators. Liver allograft failure may occur. In the following setting, we analyzed the preconditioning effects of cilostazol, a PDE3-inhibitor, in an isolated cold liver reperfusion model in rats.
Materials and Methods: Sprague-Dawley-rats were divided into 5 groups (n=6 each), including a sham group (Sham; $<$ 2h cold storage), a vehicle-treated (NaCl 0,9%) control group (Con; 24h cold storage) and 3 cilostazol groups (Cilo 0.1, 1.0 and 10mg; applied 180min before 24h cold storage). All livers were stored at 4°C in HTK solution before ex situ reperfusion with 37°C Krebs Henseleit buffer for 60min in a non-recirculating system. Afterwards outflow was collected for enzyme analysis and tissue samples were taken for histology and Western blot analysis.
Results: During organ retrieval a significantly increased bile flow in cilostazol pretreated livers was observed (Cilo 10: 1.35$\pm$0.08; Cilo 1.0: 1.46$\pm$0.13; Cilo 0.1: 1.39$\pm$0.11 versus Con: 1.01$\pm$0.12 μl/gLG*min; $p<0.05$).
After 24h cold storage and 60min reperfusion a significantly reduced vacuoles positive cells and apoptotic cell death in cilostazol preconditioned rats were measured. Westernblot analaysis of cleaved caspase-3 showed a dose depending anti-apoptotic effect of cilostazol (Cilo 0.1: 1.39$\pm$0.25; Cilo 1.0: 2.44$\pm$0.68; Cilo 10: 0 5.08$\pm$0.90; $p<0.05$).
Conclusion: Pre-treatment with PDE3 inhibition can reduce hepatic tissue injury and apoptotic cell death after 24h cold storage and reperfusion without compromising hepatic excretory function. The anti-apoptotic effect of cilostazol is dose-depended. PDE-III inhibitors may represent effective drugs to improve hepatic function and reduce I/R-injury after liver transplantation.

O-031 FROM PERI-OPERATIVE COMPLEMENT ACTIVATION TO ALLOGRAFT PROTECTION AND REGENERATION – A PRELIMINARY REPORT

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Background: Recently we reported that complement activation during acute organ injury may lead to mobilization of stem cells (SC) and therefore to stimulation of damaged organ's regeneration (*Ratajczak MZ et al. Leukemia 2010;24:1667-75*). In this paper we wanted to examine peri-operative complement activation during ischemia-reperfusion injury (I/R) following kidney transplantation, and establish whether activation of the complement creates a pro-regenerative environment that may result in SC mobilization, as well as, if it is associated with post-transplant allograft function.
Methods/Materials: Renal transplant recipients (n=69) were divided into early, slow and delayed graft function group (EGF, SGF, DGF). Blood samples were collected intra-operatively directly before, and in the 1st and 5th minute of allograft reperfusion from the renal vein. C3a, C5a, C5b-9 levels were measured using ELISA.
Results: During I/R injury 2-times higher C3a levels were observed in SGF and DGF patients, comparing to EGF individuals ($p<0.01$). These were associated with duration of pre-transplantation dialysis treatment, and correlated with peri-operative thromboxane concentrations ($R=0.69; P<0.005$). C5a levels were significantly lower in patients with graft activation problems (SGF, DGF) than in EGF individuals ($P<0.05$). Our preliminary results suggest that peri-operative complement activation correlates with circulating sphingosine-1-phosphate levels. No correlation between peri-operative activity of pro-oxidative enzymes (xanthine oxidase) and C3a or C5a levels was observed. Enhanced peri-operative activation of complement was associated with worst early and long-term (1 year) allograft function.
Conclusion: During human renal transplantation complement activation occurs, is more evidently pronounced in SGF/DGF patients, and in these creates a biochemical environment that seems to be less pro-regenerative than is observed in EGF individuals. Our results provide indirect evidence that it is the platelets' metabolism (rather than oxidative stress) that seems to be associated with complement activation during human renal I/R injury.

O-032 ASSESMENT OF CONTINIOUS VERSUS SHORT TERM OXYGENATED PULSATILE MACHINE PERFUSION IN A PIG AUTOTRANSPLANT MODEL

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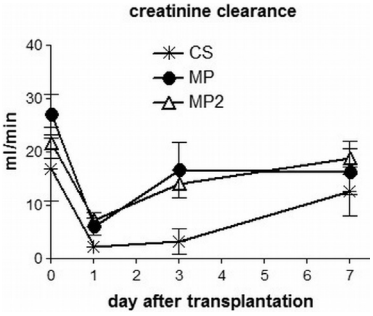
Background: Clinical data suggest that pulsatile machine perfusion (MP) from procurement until transplant might be the preferred method for kidney preservation. However, it is unclear whether short term MP following cold storage (CS) is equally effective.
Methods: Kidney function after cold preservation (4°C, 21 hours) and transplantation was studied in an autotransplant model using Landrace pigs (25-30 kg; n=5 per group) with one week follow up. Preservation was performed by conventional CS or pulsatile MP with a Lifeport Kidney Transporter using oxygenated KPS either for the entire preservation period (MP) or only for 2 hours subsequent to 19 hours CS (MP2).
Results: Vascular resistance at the end of machine perfusion was lower after MP than after MP2, but perfusate activities of LDH were significantly lower (factor 2) after MP2 than after MP. MP and MP2 tendentially improved cortical microcirculation upon early reperfusion (erythrocyte flux) and significantly reduced maximal serum creatinine levels by 50% compared to CS. No differences were seen whether MP was performed continuously or only 2 hours prior to reperfusion. However, compared to CS, blood urea peak was lowered

to 50% in MP and to 25% after MP2. Fractional excretion of Na^+ was unaltered after MP and MP2 but significantly increased till POD 5 in CS. Recovery of creatinine-clearance to normal values was obtained after 2-3 days in MP2 and MP but not before day 7 after transplantation in CS.



Conclusion: While MP consistently improves preservation of porcine kidneys, short term oxygenated MP after CS proves to be as effective as continuous oxygenated MP during the whole storage time. Due to its logistical convenience, the concept of an a posteriori MP treatment recommends itself for future clinical investigation.

O-033 NMR ANALYSIS OF SOLUTION DURING MACHINE PERFUSION: PREDICTING GRAFT OUTCOME?

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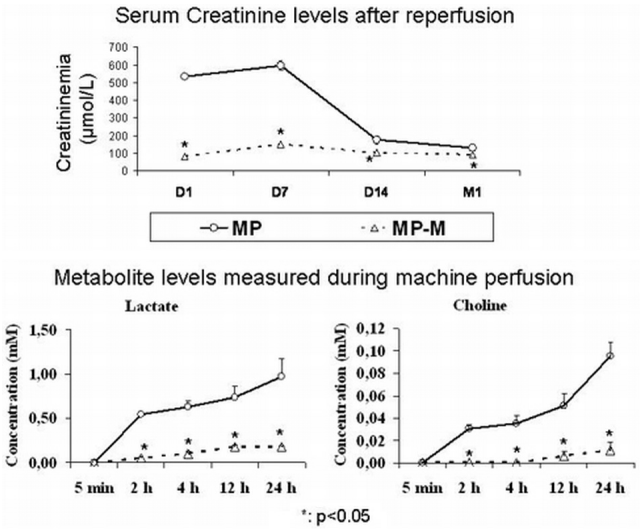
Background: Supplementation of preservation solution with specific molecules such as melagatran®, a thrombin inhibitor, showed important benefits in static storage. As machine perfusion also offers protection to the organ, we measured the benefits of combining technological and pharmacological optimization using classical biochemical tests and 1H NMR metabonomic approach.
Methods: In a kidney auto-transplantion pig model, organs were submitted to 60 min warm ischemia prior to machine preservation on the ORS Lifeport. We compared perfusats of MP alone (MP group) to perfusats of MP with melagatran (MP-M group). NMR data were acquired at 500.13 MHz using an Avance 500SB spectrometer with 5mm broadband inverse probe.
Results: Kidney perfused with Melagatran showed superior function recovery compared to MP alone. During perfusion, lactate levels were higher in MP than in MP-M and increased with time. Concentration of choline also increased during MP while it remained at a lower value in the MP-M group (figure, $p<0.05$). We observed similar tendencies with amino-acids such as valine, glycine or glutamate. Moreover, we showed a diminution of total glutathione (Gth) during this period. Statistical analysis revealed a strong association between elevated levels of these metabolites and poor function recovery.



Conclusion: Those observations confirm that there are fewer lesions at the cell level in presence of melagatran. These data highlight the potential of a metabonomic approach by NMR. This technique can be transposed to the clinic to be included in the diagnostic algorithm in order to better tailor the therapeutic interventions on a given organ.

O-034 MULTI-DRUG DONOR PRECONDITIONING OFFERS AN EFFECTIVE PROTECTION AGAINST ISCHEMIA/REPERFUSION INJURY FOR STEATOTIC LIVERS

Maximilian von Heesen[1], Pascal Jeanmonod[1], Stefan Dold[1], Otto Kollmar[1], Michael D. Menger[2], Martin K. Schilling[1], Mohammed R. Moussavian[1]. *[1]General-, Viszeral-, Vascular- and Paediatric Surgery, Saarland University Hospital, Homburg/Saar, Saarland, Germany; [2]Clinical Experimental Surgery, University of Saarland, Homburg/Saar, Saarland, Germany*

Introduction: Drug preconditioning in heart beating brain-dead donors induce high bioavailability of pharmacological substances in liver cells at 37°C. In an isolated liver perfusion model in the rat we evaluated the effects of a short term multidrug pretreatment model in steatotic liver organs compared to non-steatotic livers.
Methods: A total of 24 Sprague Dawley rats were divided into three groups; a preconditioning group (SL-MDDP; n=8), a Sham- and a steatotic livers–group (SL), which received 2.0ml vehicle (0.9% NaCl) instead of MDDP. All livers were stored for 24h in 4°C HTK solution. MDDP was initiated 30min before starting the cold perfusion with HTK solution for organ harvesting by applying simvastatin, N-acetylcysteine, erythropoietin, pentoxyphylline, melatonin, glycine and DFO. After cold storage, livers were reperfused for 60min through the portal vein in a non-recirculating system with Krebs Henseleit bicar-bonate (KHB) buffer saturated with carbogen at a flow rate of 2mL/min*g liver tissue.
Results: The cellular and mitochondrial integrity in the SL were significantly lower during the whole reperfusion compared to Sham-controls ($p<0.05$). MDDP significantly reduced the cellular and mitochondrial damage compared to controls ($p<0.05$). Liver enzymes were significantly increased in SL after a period of 60min reperfusion periode compared to the Sham group ($p<0.05$). The preconditioning of SL livers according to the MDDP protocol significantly reduced the hepatocellular damage ($p<0.05$). The release of proinflammatory cytokines (TNF alfa, IL 6, IL 1) in SL were also significantly higher compared to the Sham, while MDDP significantly reduced the proinflammatory reaction ($p<0.05$).
Discussion: 24-hours cold preserved marginal steatotic rat livers presented higher hepatocellular damage and an elevated release of cytokines, which were significantly reduced by the MDDP protocol. Taken together, short-term pharmacological donor preconditioning can be an effective, clinically applicable adjunct to improve organ preservation.

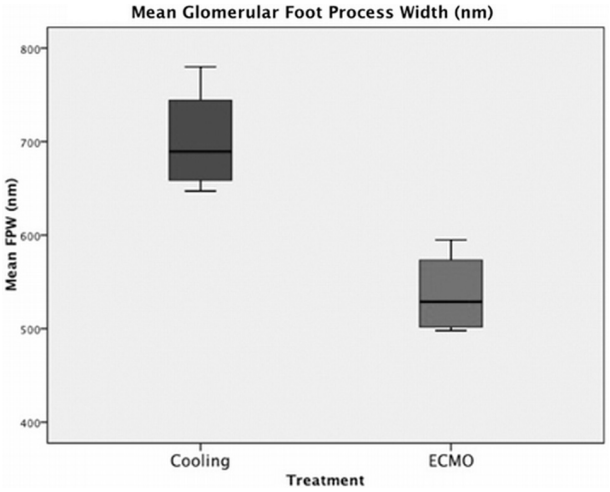
O-035 PORCINE MODEL OF EXTRA-CORPOREAL MEMBRANE OXYGENATION (ECMO) IN THE UNCONTROLLED DONATION AFTER CARDIAC DEATH (DCD) DONOR; THE EFFECT ON RENAL VIABILITY

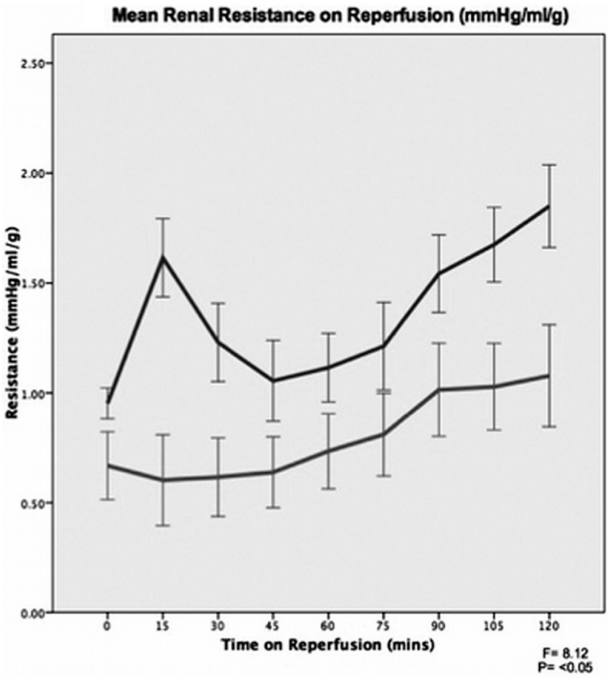
Christopher Ray[1,2], Aditya Kanwar[1,2], Mohammed Saleem Noormohamed[2,3], Stephen Ray[3], Susan Stamp[3], Soroush Sohrabi[1,2], Alex P. Navarro[1,2], Katrina Wood[3], Kathryn White[3], Robert Peaston[2], Steven White[2,3], Jon Smith[2], Anne Cunningham[3], Noel Carter[3], David Talbot[1,2]. *[1]Department of Pharmacy, Health and Wellbeing, University of Sunderland, Sunderland, United Kingdom; [2]Department of Liver and Renal Transplant, Freeman Hospital, Newcastle upon Tyne, United Kingdom; [3]Surgical and Reproductive Sciences, University of Newcastle-upon-Tyne, Newcastle upon Tyne, United Kingdom*

Aims: We sought to compare the effect of ECMO on renal viability in a Maastricht Category II donor model, with our current standard; intravascular flush and intra-peritoneal cooling.
Methods:

- Using cross-Yorkshire-landrace pigs (n=11) under general anaesthetic, an initial laparotomy, probe placement and cannulation was performed.
- All animals were euthanased, and subjected to 30mins of warm ischaemia.
- In the Cooling group (n=5), intravascular flush was administered, with peritoneal cooling, over a 2-hour period.
- In the ECMO group (n=6), a primed extra-corporeal oxygenation circuit was commenced at this stage. The abdominal organs were perfused with oxygenated normothermic blood for 2hours.
- After this 2-hour period, the abdomen was re-opened, iced and organs retrieved.
- After 18 hours cold machine perfusion they were each re-perfused on an ex-vivo oxygenation circuit to simulate transplantation and re-animation.

Results: In all parameters of viability testing the ECMO organs appeared superior to Cooling organs
Analysis of the trends with a repeated measure ANOVA revealed a significant difference between the groups for level of Glutathione-S-Transferase ($p<0.01$), renal resistance ($p<0.05$) but no significant difference for mean Lactate/Pyruvate Ratio.





Kidneys in the combined intravascular and intra-peritoneal "cooling group" demonstrated more severe histological ischaemic damage than the ECMO group. Mean score for the ECMO group was 3.3 (sd $\pm$1.5) versus "Cooling group" mean 5.4 ($\pm$1.8) $p<0.01$.
Electron microscopic examination revealed more severe damage in the cooling group. Mean glomerular foot process width (FPW) was 538μm ($\pm$45) in the ECMO group versus 702μm ($\pm$58) in the cooling group, $p<0.05$.
Conclusion: Initial results from this animal model suggest that extra-corporeal membrane oxygenation, applied in a Maastricht Category II donor model, is superior to combined arterial and peritoneal cooling in preservation of renal viability.

Liver transplantation (technical and live donor)

O-037 DONOR DOPAMINE DOES NOT AFFECT GRAFT SURVIVAL AFTER LIVER TRANSPLANTATION: DATA FROM A RANDOMIZED CONTROLLED TRIAL

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Background: Treatment of the deceased heart-beating donor with low-dose dopamine results in less dialysis requirement after kidney transplantation and appears to improve the outcome after heart transplantation. This study investigates the clinical course of liver allografts from multi-organ donors that were enrolled in the randomized dopamine trial (clinicaltrials.gov Identifier: NCT00115115).
Methods: Between March 2004 and August 2007, 264 brain-dead donors were randomly assigned to receive or to not receive low-dose dopamine. Eligibility criteria included circulatory stability under low-dose norepinephrine. The present study is nested in the randomized controlled trial of donor pretreatment with dopamine. We assessed the outcomes of 197 liver transplants performed at 32 European centers.
Results: Dopamine was infused at 4μg/kg/min for a median duration of 362 minutes (IQR 182 minutes). Donors and recipients were very similar in baseline characteristics. Thirty-four recipients (16.8%) were transplanted with high urgency and 23 (11.7%) received a repeat transplant. Pretransplant MELD score was not different in recipients of a dopamine treated vs. untreated graft (18$\pm$8 vs. 19$\pm$9, p=0.28). Mean cold ischemic time was 632$\pm$172 vs. 600$\pm$170 minutes (p=0.20). Following transplantation, no differences occurred in biopsy-proven rejection episodes (15.3% vs. 17.2%, p=0.85), requirement of hemofiltration (27.6% vs. 27.3%, p=0.99), and in-hospital mortality (13.3% vs. 12.1%, p=0.85). Graft survival was 72.5% vs. 74.8% and 61.2% vs. 63.6% at one and three years.
Conclusion: Contrasting heart and kidney transplantation, donor pretreatment with dopamine does not improve the outcome after liver transplantation. Since liver cells specifically express s-COMT with high activity, dopamine is rapidly degraded in-vivo, which most likely abrogates its potential to protect the liver graft from oxidative stress under cold storage conditions.

O-038 LIVER GRAFT ISCHEMIC PRECONDITIONING: CLINICAL OUTCOMES 3 YEARS AFTER TRANSPLANTATION

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Background: Ischemic-Preconditioning (IP) is a technique against ischemia/reperfusion injury (IRI). The clinical relevance of IP in liver transplantation (LT) is controversial.
Methods/Materials: From 9/2007 to 11/2008, 108 deceased liver donors were randomized to receive IP (n=50, IP+) or not (n=58, IP-). HCV was present in 51 of 108 recipients (47%). Grafts were classified as marginal (n=61, marg+) or not (n=47, marg-) according to donor age $\geq$65 yrs and/or macrosteatosis $\geq$15%. Depending on treatment received, 4 subgroups were created (IP+marg+/IP+marg-/IP-marg+/IP-marg- n=25/25/36/22). Donor (gender, age, ICU-stay, AST/ALT, ischemia times, macrosteatosis) and recipient pre-LT (gender, age, LT indication, MELD) variables were considered. AST/ALT, bilirubin, INR and bile production were measured in first post-LT week. With median follow-up of 34 months (0.3-42), clinical outcomes of recipients were analyzed.
Results: No significant differences for donor and recipient pre-LT variables were detected in the IP+/IP- groups. In the first post-LT week, median AST and INR were higher and bile production was lower in marg+ livers independently from IP (p=0,022, p=0,004 and p=0.03, respectively). Overall 3yrs graft survival was 84%. In IP+ grafts a significantly increased incidence of acute rejection (36% vs 16%, p=0,02) and a trend to more frequent biliary complications (24% vs 14%, p=0.2) were recorded, but no impact on survival was observed (84% IP+ vs 84.5% IP-). Among the 51 HCV recipients, IP+ grafts had a trend to better survival (85% IP+ vs 68% IP-, p=0.1); this was particularly true for the 24 HCV patients who received marg+ grafts (90% IP+ vs 57% IP-, p=0.08).
Conclusions: No solid conclusion on clinical relevance of IP in LT can be drawn. However, a beneficial effect of IP on the outcome of HCV recipients seems to emerge and deserves further investigation in the future.

O-039 ERYTHROPOIETIN ATTENUATES ISCHEMIA-REPERFUSION INJURY FOLLOWING THE PRINGLE MANEUVER: A PROSPECTIVE RANDOMIZED STUDY

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Background: Ischemia-reperfusion injury (IR/I) is a crucial obstacle for sucessful oragn transplantation. We examined the protective effect of erythropoietin (Epo) against IR/I following the Pringle maneuver (PM) during hepatic resection, in a prospective randomized trial.
Methods: Patients were randomized by age, sex, diagnosis, and surgical method, and assigned to three groups: 1. A conventional group (n=10) who received 100 mg of steroid before PM, and on postoperative days 1, 2 and 3, followed by tapering until postoperative day 7. 2. An EPO1 group (n=10) who received 30000 U of Epo before the PM and at the end of surgery. 3. An EPO2 group (n=8) who received 60000 U of Epo before the PM. Hb, Ht, AST, ALT, and TNF-α were measured before (Pre) and after (Day 0) surgery, and on postoperative days 1, 3, 7, and 14.

Results: There were no increases in Hb and Ht in the EPO1 and EPO2 groups. Median values of AST in the conventional, EPO1, and EPO2 groups were 409 IU/L, 142 IU/L, and 205 IU/L, respectively (P=0.041), on Day 0, and 275 IU/L, 157 IU/L, and 342 IU/L, respectively (P=0.018), on Day 1. The corresponding median values of ALT were 351 IU/L, 76 IU/L, and 141 IU/L, respectively (P=0.020), on Day 0, and 300 IU/L, 112 IU/L, and 289 IU/L, respectively (P=0.004), on Day 1. Median levels of TNF-α were 13.5 pg/mL, 9.7 pg/mL, and 8.0 pg/mL, respectively (P=0.0006), on Day 0, and 17.7 pg/mL, 4.6 pg/mL, and 3.5 pg/mL, respectively ($P<0.0001$) on Day 1.
Conclusion: Epo has greater potential than steroid to ameliorate IR/I after the PM. This protective effect of Epo may be applicable not only in patients undergoing hepatic resection, but also for much severer IR/I, such as that occurring in liver transplantation.

O-040 STRATEGIC BREAKTHROUGH AND PARADIGM SHIFT IN ADULT ABO-INCOMPATIBLE LIVER TRANSPLANTATION

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(Background)ABO blood-type incompatibility has long been a major obstacle to expand a exiguous donor pool in adult liver transplantation. Herein we present our current results of adult ABO-incompatible (ABO-i) living donor liver transplantation (LDLT) using a simple protocol.(Methods)Among 313 adult LDLTs, 14 cases (4.5%) were ABO-i LDLTs. The first 3 cases were managed by portal infusion therapy as previously reported. The last 11 cases were managed consecutively by the same protocol including preoperative administration of a single dose of Rituximab (375mg/m2) followed by 3 to 5 sessions of plasma exchange (PE) before LDLT without portal infusion therapy, and this is the subject of the study. Triple immunosuppression consisted of calcineurin inhibitor (CNI), mycofenolate mofetil (MMF) and steroids was exactly the same as blood-type compatible LDLTs except for MMF started 7 days before LDLT. Splenectomy was performed for all cases. A target trough level of CNI after LDLT was also the same as blood-type compatible LDLT.(Results)All of the 11 patients were alive (100% survival) with the mean follow-up of 1.3 years (1-37 months). Neither antibody-mediated nor hyperacute rejection was encountered. No serious infectious complication and biliary complication occurred. There was only one episode of mild acute cellular rejection (71POD) for which steroid argumentation was effective. Relaparotomy was performed three times in a single case for late portal vein thrombosis. The median preformed isoagglutinin antibody titers before PE was x512 while the median peak antibody titers was x32, among which only a case with fulminant hepatic failure was required PE after LDLT. (Conclusion)The management of adult ABO-i LDLT has been evolved and established. ABO-i adult LDLT is no longer a contraindication but rather a viable option.

O-041 CONTEMPORANEOUS PORTAL AND ARTERIAL REPERFUSION VERSUS PORTAL REPERFUSION FIRST DURING LIVER TRANSPLANTATION: A PROSPECTIVE RANDOMIZED STUDY

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Although sequential portal and arterial revascularization (SPAr) is the most common method of graft reperfusion at liver transplantation (LT), contemporaneous portal and hepatic artery revascularization (CPAr) has been used to reduce arterial ischemia to the bile ducts. The aim of this study is to prospectively compare SPAr (group 1 #33) versus CPAr (group 2 #34) in 67 consecutive LT from heart beating donors. There were no differences in the demographics characteristics, MELD score, and indication to LT and donors parameters between groups. LT was carried out by piggyback technique. The biliary anastomosis was performed in all cases by duct to duct with a T-tube respectively in 36% vs 34% of cases (p=0.72). In the CPAr group the liver was reperfused simultaneously by portal vein and hepatic artery. CPAr had longer warm ischemia 62$\pm$9 vs 38$\pm$12 min ($p<0.0001$), while SPAr had an arterial ischemia time longer than the warm ischemia of the CPAr 97$\pm$38 vs 62$\pm$9 min ($p<0.0001$). Recovery of graft function was similar with no PNF and DGF was 9% vs 6% (p=0.61). 15% vs 17% had reperfusion syndrome (p=0.87). Liver function tests were similar between the two groups up to 90 days of follow-up. There were no differences in ICU and total hospital stay. One-year patient's and graft's survival were respectively 80% and 92% vs 89% and 100% (p=0.29 and p=0.14) in group 1 and 2. At median follow up of 16$\pm$11 months biliary complications were anastomotic stenosis in 21% vs 23% (p=0.82) and intrahepatic non-anastomotic biliary strictures in 18% vs 0% (p=0.009) respectively in SPAr and CPAr. CPAr is safe and feasible and reduces the incidence of intrahepatic biliary strictures by decreasing the duration of arterial ischemia to the intrahepatic bile ducts.

O-042 POLYMORPHISMS OF FRACTALKINE RECEPTOR AND RISK FOR ANASTOMOSIS STENOSIS AFTER LIVER TRANSPLANTATION

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Background: Strictures at the site of the bile duct anastomosis (AS) are major complications following liver transplantation (LT) and are thought to result mainly from surgical technique and/or prolonged ischemia times. For late AS, others than surgical factors should be incriminated. Genetic polymorphisms in chemokine receptors which mediate leucocytes trafficking to inflammatory sites, may be associated with AS occurrence.
Aim: To investigate the role of immunologic and genetic risk factors for development of AS after LT.
Material and Methods: We prospectively genotyped 3 chemokine receptors (CCR2-V64I, CCR5-D32, CX3CR1-V249I and T280M) in 137 LT recipients (44 with AS, 93 controls) by PCR or PCR-restriction fragment length polymorphism assay. Serum concentration of chemokines CCL3 and CCL5, as ligands of CCR5, and CX3CL1 as ligand of CX3CR1 were measured by enzyme linked immunosorbent assays.
Results: Median time to AS development was 6.3 months (range 0.5-191 months). The following risk factors with immunologic involvement were identified for AS occurrence: CX3CR1-249I/I (p=0.02), acute liver failure as indication for LT (p=0.03), ABO compatible, non-identical LT (p=0.005). Independent variables associated with AS were identified by multivariate logistic regression analysis: CX3CR1-249I homozygous allele (p=0.03), acute liver failure as indication for LT (p=0.01) and ABO compatible, non-identical LT (p=0.03). Serum concentrations of CCL3, CCL5 and CX3CL1 were similar between patients with AS and controls.
Conclusions: Proinflammatory and immunologic factors predispose to AS development. CX3CR1-249I homozygous allele, as a promoter of liver fibrosis, was identified as an independent risk factor for AS after LT. CCR2 and CCR5 polymorphisms had no influence in occurrence of this type of biliary complication.

O-043 HEPATIC ARTERY KINKING DURING LIVER TRANSPLANTATION: SURVEY AND INTRAOPERATIVE FLOW MEASUREMENT

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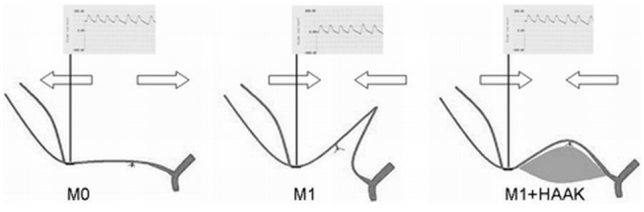
Background: Hepatic artery thrombosis (HAT) is the most common vascular complication after liver transplantation (LT). Thus may be associated with the arterial kinking (AK) in case of long arteries. Herein, we report the results of a survey about the procedures used to avoid AK and the measures of the HA flow (HAF) during the abdominal wall closure.
Materials/Methods: We sent by mail to 55 surgeons a survey about the procedures utilized to avoid AK. Furthermore, we prospectively measured the HAF at the end of the biliary anastomosis (M0) and during the abdominal wall closure (M1) in 21 consecutive patients with a long HA. We compared the results of these measures (M1) with or without arterial interposition of surgicel or omentum.
Results: We received 44 surgeons replies: 42 (96%) surgeons cut the artery shorter, of these 28 (65%) combined a surgicel interposition, 10 (22%) omentum and 4 (9.1%) used other systems. Two (4.5%) surgeons leave a long artery. Fourteen (32%) surgeons cut the artery shorter but do not use an arterial interposition.



In our expereince, we measured a HAF (mL/min) of 152 (89-205) at M0, of 114 (66-168) at M1 without hepatic artery anti kinking procedure (HAAK) and 158 (91-219) at M1 with a HAAK procedure.
Conclusions: The HAK is a major vascular complication especially in patient with a long HA, it may be a *primum movens* of HAT. As our survey shows

any consensus is available about the system to avoid AK. The HAK appeared when the liver is in its final position as demonstrated by our HAF measures (HAF drop-down in all patients). The surgicel or the omentum interposition guarantee a maintenance of a good HAF also when the abdominal wall closure is performed.

O-044 SINGLE CENTER EXPERIENCE OF CONSECUTIVE 500 CASES OF HEPATIC ARTERY ANASTOMOSIS IN LIVING DONOR LIVER TRANSPLANTATION

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Background: Hepatic arterial complication after liver transplantation (LTx) is a major source of morbidity and mortality. Only early diagnosis and treatment will save the patient's life. We analyzed consecutive more than 500 living donor liver transplantation (LDLT) patients for risk factor and result.
Method: From August 2004 to May 2010, total 522 patients including pediatric and adult LDLT were done by our center and retrospectively reviewed. Hepatic arterial anastomosis was done under Microscope and by interrupted suture with Prolene 8-0. We routinely checked intraoperative Doppler at POD#1, #3, #5 or #7. Hepatic arterial complication included thrombosis and stricture.
Results: The overall complication rate related to arterial reconstruction in LDLT was 4.6% (24 cases). 17 patients had an arterial stricture and 7 patients had an arterial thrombosis. 7 patients of 24 patients were explored and revised hepatic artery. 2 of them underwent retransplantation because of recurrent thrombosis and hepatic failure. Another 6 patients were treated with interventions. The rest 10 patients were only observed closely under antiplatelet agents because of mild disease severity. Among 24 patients, 7 patients were died. Only preoperative alcohol ingestion (p=0.014) and POD#7 Doppler RI index below 0.6 (p=0.039) were associated hepatic arterial complication. Against noted risk factors of hepatic arterial complication, our analysis reveals that arterial complication is not associated with arterial number, anatomy and pediatric LTx. Most of the hepatic arterial complications (19 of 24 cases) occurred within 1 month after LTx.
Conclusions: The risk factors of hepatic arterial complication after LDLT were preoperative alcohol ingestion and POD#7 Doppler finding and most complications occurred within 1 month. Regular Doppler check after LDLT should be considered as early diagnosis method for arterial complication and treatment of hepatic arterial complication should be designed by individually.

O-045 IMPACT OF PREVALENT STEATOSIS ON OUR LIVING DONOR LIVER TRANSPLANT PROGRAM: ANALYSIS OF 975 LIVING RELATED LIVER DONORS

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Organ shortage has been the ongoing obstacle to expand liver transplantation world-wide.Living donor liver transplantation (LDLT) was hoped to improve this shortage. We aimed to analyse the results of the evaluation of potential living donors at our center and determine the prevalence of macrosteatosis.
Methods: From 2001 to 2010, 192 deceased donor liver transplants and 90 living donors liver transplants (LDLT) were performed. 975 potential living donors were worked up according to a step-wise evaluation protocol. Their age ranged from 18 to 60 years, with 75% in the third and forth decades. They were all first and second degree relatives of the patients.
Results: Only 90 (9.2%) were accepted for donation and 885 (90.8%) were rejected. 793 (around 80%) were excluded at the earlier stages of evaluation: either at initial screening due to high body mass index or due to incompatible blood group, positive hepatitis serology, elevated liver enzymes, miscellaneous systemic diseases, socioeconomic reasons, abnormal anatomy or insufficient volume as determined by CT volumetry. 182 reached the step of liver biopsy. Of these, 44 (24%) were rejected due to abnormal fat content. As regards the remaining 138, 48 were excluded either due to abnormal histopathology (other than steatosis) or the operation was aborted due to the recipients' condition. Finally 90 underwent donation.
Conclusion: There is no doubt that LDLT has helped in alleviating the severe shortage of deceased organs in Saudi Arabia. However suitable living donors are not easy to find especially right lobe donors. Our initial evaluation is effective in eliminating a large number of unsuitable donors.However,steatosis remains a problem encountered at a later stage of the evaluation. The donor evaluation process indeed remains to be a large burden on the resources of our program

O-046 MULTICENTER BELGIAN SURVEY ON DONOR MORBIDITY AND MORTALITY IN ADULT-TO-ADULT LIVING DONOR LIVER TRANSPLANTATION

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Background: The development of Adult Living Donor Liver Transplantation (ALDLTx) programs in many western LTx centers has decreased due to reported donor morbidity/mortality.
Methods: The Belgian Royal Academy of Medicine proposed a national survey in order to assess donor morbidity/mortality after ALDLTx in Belgium. Between 09/1999 & 09/2010, 143 ALDLTx were performed in 4 Belgian University Hospitals: UZ-Ghent, UCL-Brussels, KUL-Leuven and ULg-Liege. Median donor age was 35 years (range: 19-59). Majority of donors were children (57%) and spouses (15%).
Results: Aborted procedures due to inaccurate preoperative volume assessment were encountered in 7 (5%) donors. 136 donors actually underwent liver donation. Complications occurred in 48/136 (35%), mostly due to UTI (14%), biliary fistula (7%), nerve palsy (5.4%), pulmonary complications (5.4%), early reoperation for bleeding and biliary complications (5.4%); and late reoperation (cicatricial hernia's) (3%). Portal vein thrombosis occurred in 3%. Mean hospital stay was 12±3 days. Hospital readmission was necessary in 3/135 (2.2%) patients. One death occurred in a right lobe donor due to impaired remnant liver regeneration and who expired after a sequential cascade of complications and during urgent liver transplantation at postLTx day 49.
Conclusions: Incidence and type of donor complications after ALDLTx in Belgium are comparable to those reported in the ELTR. Donor death is a rare event (0.7%) related to impaired liver remnant regeneration. Inaccurate preoperative assessment also led to procedure abortion in 3%. This emphasizes the need to more reliably predict preoperatively the actual liver volumes and the regeneration capacity of the remnant liver.

Basic science and immunobiology

O-047 INTERLEUKIN-33 PROLONGS ALLOGRAFT SURVIVAL THROUGH REDUCTION OF ANTIBODY MEDIATED CARDIAC REJECTION

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IL-33 stimulates the generation of cells, cytokine and immunoglobulin production characteristic of a type 2 immune response. In this study, we demonstrate the effect of IL-33 on allograft function during chronic cardiac rejection in mice.
B6: C-H2bm12/KhEg hearts were transplanted into wild type MHC class II-mismatched C57BL/6J mice. IL-33 was administered i.p. daily. Cardiac allografts were harvested, graft infiltrating CD4+ T-cells were isolated and cytokine production was determined by ELISA. Isolated leukocyte populations were examined by flow cytometry and alloantibody levels were determined. Further, immunohistochemical staining of cardiac allografts was performed.
Allogeneic transplanted controls showed progressive allograft rejection within 21.5 days after transplantation, whereas allograft survival in IL-33-treated animals was extended to more than 50 days. Prolonged allograft survival was accompanied by significant changes in cytokine production by graft infiltrating CD4+ T-cells. We observed a significant decrease in the production of proinflammatory IL-17A and significantly increased levels of Th2-cytokines IL-5, IL-13 and antiinflammatory IL-10. In addition, IL-33 treatment resulted in homeostatic changes of the lymphoid and myeloid compartment in both the cardiac allografts and periphery. Flow cytometric analyses demonstrated a reduction of graft-infiltrating CD19+b220+ B-cells following IL-33 therapy. Accordingly, IL-33 treated mice showed reduced alloantibody levels in the serum and less immunoglobulins as determined by immunohistochemical analysis of the grafts. In addition, a significant decrease in graft infiltrating CD11bhigh Gr1high granulocytes coinciding with a significant increase in suppressive CD11bhigh Gr1intermediate myeloid cells was observed after IL-33 therapy.
IL-33 treatment prolongs allograft survival after cardiac transplantation in mice.

IL-33 induces changes in cytokine production of graft infiltrating cells, reduces the antibody mediated rejection and alters the composition of the lymphoid and myeloid compartment. Thus, IL-33 and its downstream effects need further evaluation as a possible therapeutic option for chronic allograft rejection.

O-048 B CELLS RECIEVE EFFECTIVE HELP FOR GENERATING ALLOANTIBODY THROUGH AQUISITION AND PRESENTATION OF ADDITIONAL GRAFT ANTIGEN

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Here we examine whether allo-MHC class I-specific B cells can receive help for generating anti-MHC class I alloantibody through acquisition and presentation of additional mismatched graft alloantigen.
As expected, $TCR^{-/-}$ mice, when reconstituted with K^d-peptide-specific TCR Tg CD4 T cells, mounted strong anti-K^d alloantibody responses to a BALB/c heart graft. Surprisingly, anti-K^d antibody responses also developed when $TCR^{-/-}$ mice were reconstituted with B6 Mar CD4 T cells (specific for H-Y peptide) and challenged with male BALB/c hearts. No alloantibody was generated when Mar-reconstituted mice received female BALB/c hearts, even when Mar CD4 T cells were activated by simultaneous challenge with male B6 APC, suggesting that help for anti-class I alloantibody responses provided through T cell recognition of an additional alloantigen requires co-expression of both antigens on the same graft cell.
To investigate the hypothesis that alloantigen-specific B cells capture neighbouring donor proteins when internalising target allantigen and process this for presentation to helper T cells, bone marrow chimeric Mar mice were created that lacked MHC II expression only on B cells. These mice did not develop anti-K^d alloantibody responses to male BALB/c hearts; in contrast strong responses developed in MHC II^{+ve} control mice, confirming that provision of help by T cells that recognise additional alloantigen still requires cognate interaction with B cell MHC II. Next, to address if additional antigen acquisition was the result of B cells that expressed dual receptor specificity, we crossed anti-HEL B cell transgenic mice onto a $Rag2^{-/-}$ background. Interestingly, when challenged with a graft expressing both HEL and K^d-antigen, anti-HEL alloantibody responses developed following reconstitution with K^d-peptide-specific TCR Tg CD4 T cells, indicating that B cells with a single BCR can nevertheless pickup additional antigen for presentation.

O-049 NON-HLA ANTIBODIES DIRECTED AGAINST AT1 AND ETA RECEPTORS INDUCE VASCULAR PROLIFERATION AND COAGULATION VIA ETS-1 AND TF ACTIVATION

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Background: Non-HLA-antibodies directed against Angiotensin II type 1 (AT1R-Abs) and Endothelin-1 type A receptors (ETAR-Abs) are associated with an earlier onset of microvasculopathy in cardiac transplant recipients. Aim of this study was to investigate complement-independent mechanisms of such non-HLA antibodies contributing to the progressive vascular obliteration and to elucidate the involved molecular signalling pathways.
Methods: AT1R-Ab and ETAR-Ab positive IgG fraction was isolated from patients with obliterative vasculopathy and used for coagulation and proliferation assays in human microvascular endothelial cells (HMEC). Activation of transcription factor Ets-1 was assessed by Western blotting and immunocytochemistry using phospho-specific antibodies. To prove Ets-1-dependent tissue factor activation, different promoter deletion constructs were applied in electromobility shift assays and chromatin immunoprecipitation studies.
Results: Both autoantibodies induced stress-kinase ERK1/2 phosphorylation which could be blocked by respective receptor inhibitors. AT1R- and ETAR-autoantibodies specifically triggered activation of transcription factor Ets-1 down-stream from ERK1/2. This activation was followed by an increase in cell proliferation (AT1R-Ab, 1.58 ± 0.24, $p<0.0001$; ETAR-Ab, 1.58 ± 0.24, $p<0.0001$). Pharmacologic inhibition of upstream kinases of ERK1/2 established a direct link between ERK1/2, Ets-1 and endothelial proliferation (reduction of 0.98 ± 0.12, $p<0.0001$ and 0.49 ± 0.27, $p<0.0001$, for AT1R-Ab and ETAR-Ab, respectively). Ets-1 induced tissue factor expression by direct binding to the promoter of TF resulting functionally in increased tissue factor procoagulatory activity of the endothelial cells induced by the AT1R- and ETAR-Abs.
Conclusions: Anti-AT1R and ETAR autoantibodies may directly contribute to key biologic mechanisms involved in the pathogenesis of obliterative vasculopathy and represent a link between the increased vascular responsiveness, intravascular coagulation and proliferation responses. Our findings support addition of respective receptor antagonists to current immunosuppressive regimens in patients harbouring non-HLA antibodies directed against vascular receptors.

O-050 MODULATING INTRA-GRAFT LYMPHOID TISSUE INFLUENCES EFFECTOR HUMORAL RESPONSES

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Tertiary lymphoid organs (TLOs) and intra-graft lymphatic vessel (LV) proliferation have been described in allografts and may contribute to rejection. The lymphotoxin-β receptor (LTβR) signalling pathway is essential for lymph node development and vascular endothelial growth factor receptor (VEGFR-3) signalling is required for lymphangiogenesis. Here we study the effect of blocking these pathways on TLO and LV formation in a model of allograft vasculopathy (AV).
The presence of TLOs in day 50 bm12 hearts in B6 recipients was confirmed by: discrete aggregates of B and T cells associated with high endothelial venules. LV density was assessed by staining with anti-LYVE-1 mAb. LTβR signalling was blocked by weekly i.p. injection of 100μg LTβR-Ig fusion protein (n=5), and VEGFR-3 signalling by injection of 25μg/g mF4-31C1 mAb three times per week for 3 weeks. Control recipients received rat IgG. Donor T cells within bm12 heart allografts provoke, in B6 recipients, anti-nuclear autoantibody (Win TS 2009); this was quantified by binding test sera to nuclear antigen expressing HEp-2 cells and by anti-ds DNA ELISA.
All bm12 heart allografts from control treated B6 recipients contained TLOs, composed predominantly of B cells. Although LV density was reduced in recipients treated with mF4-31C1, TLO formation was unaltered. Treatment with LTβR-Ig resulted in fewer TLOs, less dense LVs, and a non-significant reduction in AV severity. However, autoantibody responses were significantly diminished.
Blocking VEGFR-3 signalling prevented LV, but not TLO, formation within allografts. In contrast, LTβR-Ig treatment blocked TLO development and was associated with a reduction in autoantibody. Although LTβR-Ig treatment influences responses within conventional lymphoid tissue, our results suggest that this reduction was due predominantly to the prevention of TLO formation and confirm that the lymphoid microenvironment of the allograft plays an important role in chronic rejection.

O-051 cAMP/CREB DEPENDENT TRANSCRIPTIONAL CONTROL OF TOLERANCE ASSOCIATED GENE-1 IN BONE MARROW DERIVED DENDRITIC CELLS INHIBITS MATURATION AND RENDERS THEM TOLEROGENIC

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Cell therapy by e: g. tolerogenic donor or recipient derived dendritic cells (DCs) has become an attractive therapeutic option in transplantation. However, generation of stable tolerogenic DCs is quite challenging. We could recently show that expression of TOAG-1 is associated with stable tolerance after transplantation. Furthermore, our preliminary data also revealed that bone marrow derived dendritic cells (BMDCs) down-regulate TOAG-1 mRNA expression upon LPS-mediated maturation. Here, we investigated how transcription of TOAG-1 is controlled and can be manipulated in BMDCs to generate stable tolerogenic DCs. We have cloned the human TOAG-1 promoter and identified putative transcription factor (TFs) binding sites by site directed mutagenesis and co-transfection of TFs in luciferase reporter assays. Furthermore, BALB/c BMDCs were pre-incubated with cAMP elevating drugs (forskolin, prostaglandin E2, phosphodiesterase inhibitors) and NFkB activation inhibitors (IKK and proteasome inhibition) prior to LPS-mediated maturation. Phenotype and tolerogenic potential was tested by qPCR (TOAG-1 mRNA), flow cytometry (MHCII, CD86), CBA (TNF-α, IL-6, IL-12p70, IL-10, IL-1α) and co-culture of $CD4^+$ T cells from C57BL/6 mice. Site directed mutagenesis and reporter assays revealed a positive regulation of TOAG-1 promoter activity by NFATc1, CREB and C/EBPβ, whereas the NFkB isoform RelB represses TOAG-1 transcription. Furthermore, pre-incubation of BMDCs with cAMP elevating drugs and NFkB activation inhibitors induced high TOAG-1 expression and a stable tolerogenic phenotype. These results indicate antagonistic role of cAMP/CREB and RelB in the transcriptional control of TOAG-1 and their importance for DC maturation.

O-052 TOLL-LIKE RECEPTOR 4 (TLR4) PARTICIPATES IN THE IMMUNE RESPONSE TO PANCREATIC ISLETS: IMPLICATIONS FOR HUMAN ISLET TRANSPLANTATION

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The aim of the present study was to assess the role of TLR4 in mediating an immune response to allogeneic islets. Purified human or murine (DBA1) islets were co-cultured respectively with allogenic PBMC or lymph node cells, in the presence or absence of anti-human or anti-mouse TLR4 mAb, or relevant isotype controls. Proliferating (Ki67 staining) or IFNγ-secreting cells (ELISPOT assay) were assessed. DBA1 islets, cultured 24h in vitro with the anti-mouse mAbs, were transplanted under the kidney capsule of C57BL/6 diabetic mice, injected twice a week intraperitoneally with the anti-mouse mAbs from day 0 to 28 after transplantation. Blood sugar was monitored twice a week. In vitro results showed a decrease in proliferation of 79$\pm$2% ($p<0.001$) and 67$\pm$16% ($p=0.05$) in the human and murine mixed islet-lymphocyte cultures, respectively, as compared to controls. Similarly, a decrease of 62$\pm$9% (N=3, $p<0.05$) and 64$\pm$10% (N=3, $p<0.05$) in the numbers of IFNγ-secreting cells was observed. In vivo, treatment with the anti-mouse TLR4 mAb prolonged islet graft survival to $>$ 60 days in 80% of animals (N=5), contrasting with a graft survival of 0% at 17 days in the isotype control- (N=6) and buffer-treated mice (N=3). In conclusion, our results demonstrate that TLR4 blockade can efficiently modulate the immunogenicity of human or murine islets in vitro and is able to achieve indefinite islet graft survival in vivo. Targeting TLR-4 therefore appears to be a promising strategy in controlling allogenic rejection of islet grafts.

O-053 CXCR2 INHIBITION IMPROVES ISLET TRANSPLANTATION OUTCOME

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Objective: The aim of our work is to determine whether the CXCR2 inhibition improves islet transplantation outcome.
Methods and Results: Liver inflammatory status was studied before and after intrahepatic transplantation (Tx) of 500 syngeneic islets in diabetic C57BL/6 mice. Cytokine and chemokine transcripts 4h-24h-48h after Tx were determined using RNAse protection assays. Intrahepatic leucocyte (IHL) infiltration 1, 3, 5, 7, 10, 14 days after Tx was determined by FACS. The intrahepatic mRNA for CXCL1/KC was strongly induced immediately after islet infusion (100-fold increase after 4h). Polimorfonuclear cells (Gr1+/CD11b+/Ly6C-; PMN, 100% CXCR2+) was the first leucocytes subpopulation infiltrating the liver. To evaluate whether the block of CXCL1-CXCR2 axis improve islet engraftment, 400 syngeneic islets were alternatively transplanted in diabetic CXCR2-/- or CXCR2+/+ Balb/C mice. The absence of CXCR2 led to a significant improvement of transplant function. On this basis we tested whether Reparixin, a CXCR2 allosteric inhibitor, is able to improve islet transplantation outcome. In syngeneic marginal mass model of 250 islet Tx in C57BL/6 mice the probability and median time to reach euglycaemia were 100% and 2 days for Reparixin treated mice (n=29) as compared to 58% and 50 days for vehicle treated mice (n=34) ($p<0.001$). In an allogeneic full mismatched model of islet Tx (400 Balb/c islets in C57BL/6 mice) Reparixin significantly prolonged the time to rejection: median survival time 12$\pm$0.6 days (n=13) and 8$\pm$1.3 days (n=7) respectively for Reparixin and vehicle treated mice ($p<0.007$). Islet survival time was further improved using Reparixin in combination with Rapamycin+FK-506 or MMF+FK-506. In both models Reparixin treatment was associated to a decrease PMN and NKT cells (NK1.1+/CD3+) liver infiltration.
Conclusion: Inhibition of CXCR2 is crucial for improving islet engraftment and survival. On this basis a clinical trial (NCT01220856) is ongoing testing Reparixin in association with the conventional immunosuppressive therapy.

O-054 FUNCTIONAL IMPACT OF MICA A5.1 POLYMORPHISM ON GRAFT'S ENDOTHELIAL CELL PHENOTYPE, IMMUNOREGULATORY FUNCTIONS AND ALLOANTIBODY RESPONSES

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The MHC class I chain-related A (MICA) is a highly polymorphic surface glycoprotein expressed on graft's endothelial cells (EC) and constitutes a ligand for the activating NKG2D receptor expressed on NK, CD8+T cells. Although, specific anti-MICA antibodies have been associated with kidney allograft rejection, *MICA* genotyping is not routinely achieved and the molecular bases of anti-MICA allosensitization are unknown.
Firstly, the frequency of the various *MICA* alleles among a cohort of transplant donors was examined by genomic DNA analysis from primary cultures of EC (n=97) established from transplant donors. *MICA*008* allele was found predominant (33.3%) and associated with the *MICA A5.1* mutation, leading to a truncated protein in EC with no cytoplasmic tail as determined by western blotting. Flow cytometry analyses of EC cultures from *MICA A5.1* homozygous donors showed that *A5.1* associates with a significantly higher MICA expression on EC (5.1$\pm$0.9-fold increase; $p<0.05$). No change was found for MICB and ULBPs expression. Increase in MICA level results from a parallel increase in MICA mRNA quantified by qRT-PCR (4.0$\pm$0.9-fold increase vs controls; $p<0.01$). Mechanistically, no quantitative change in microRNAs targeting MICA (miR-20a, -636, -106b) was found. Functionally, high MICA expression on EC from *A5.1* homozygous donors significantly enhances NKG2D internalization in allogeneic NK cells (50.8$\pm$5.1% and 32.4$\pm$3.2% decrease in NKG2D expression for *A5.1* vs controls; $p<0.05$). This study provides the first evidence that circulating polyreactive anti-MICA antibodies present in sera from renal graft recipients and detected by Luminex™ preferentially bind, both qualitatively and quantitatively, to *MICA*008/A5.1* EC.
To conclude, our findings indicate that the predominant *MICA A5.1* genetic variant has a strong impact on MICA expression by graft's EC that consequently promote effector cell activation and anti-MICA alloimmunization leading ultimately to graft's EC injury.

O-055 INDUCTION OF CIRCULATING ENDOTHELIAL CELLS (CECs) AND CIRCULATING PROGENITOR CELLS (CPCs) AFTER POLYCLONAL ANTITHYMOCYTE GLOBULIN (ATG) THERAPY IN LIVER TRANSPLANTATION

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Rabbit antithymocyte globulin (rATG) is widely used as induction agent playing a pivotal role in modulating the immune system. As blood circulating endothelial cells (CECs) and circulating hematopoietic progenitor cells (CPCs) represent two minute fractions of blood mononuclear cells that are thought to play important roles in tissue vascularisation, the study of both cell types is currently suggested as surrogate markers for numerous pathologies. Especially the noninvasive endothelial evaluation as an early index of vascular injury following kidney transplantation has been already demonstrated. In order to understand the influence of rATG on both cell types, we used four surface markers to identify viable CECs as $CD31^{bright}$, $CD34^{dim}$, $CD45^{-}$, $CD133^{-}$ and viable CPCs as $CD34^{bright}$, $CD133^{+}$, $CD45^{dim}$, $CD31^{+}$cells in the peripheral blood of liver transplanted recipients (n=28) until day 20 post transplantation. An induction of CECs was exclusively observed for rATG-treated patients (n=17) increasing from 0.56% $\pm$0.98% pre transplantation to 1.83% $\pm$1.85% at day 1-2 post transplantation compared with control patients receiving standard immunosuppression (n=11) ($p<0.04$). In addition, the induction of CPCs was even more pronounced illustrating an increase in rATG treated patients from 0.20% $\pm$0.26% pre transplantation to 1.55% $\pm$1.75% at day 1-2 post transplantation ($p<0.001$). An elevation of blood CPCs is still detectable at day 5 ($p=0.0379$ compared with controls) and starts to decline at day 10 post transplantation. We illustrated that both CECs and CPCs were detectable in numbers that allow kinetic monitoring post transplantation and that rATG treatment results in a transient induction. As clinical correlations between the concentration of these two populations and the effect of immunosuppressive regimens has been already proven, validation of these cell populations as biomarkers remains to be determined.

O-056 mTORC2 SIGNALLING PATHWAY ACTIVATION IS REQUIRED FOR β-ESTRADIOL MEDIATED CARDIOPROTECTION

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Background/Aims: Use of mTOR inhibitors favours faster regression of established myocardial hypertrophy in renal recipients and prevention of maladaptive myocardial hypertrophy in cardiac transplant recipients. Hormonal status is frequently altered after transplantation and has marked influences on mTOR signalling pathways in parallel to clear inferior outcomes in women. More specific mTOR-inhibitors targeting mTORC1 or mTORC2 complexes are in development. We therefore investigated in how far is distinctive mTOR complex inhibition dependent on hormonal stimulation or deprivation and its influence on signalling pathways involved in adaptive and maladaptive cardiomyocyte hypertrophy.
Methods: Female HL-1 cardiomyocytes were treated with "physiologic" (IGF-

1) and "pathologic" (ET-1) stimuli in presence or absence of β-estradiol (E2) or mTOR inhibitor rapamycin. Raptor- and rictor silencing was induced to target specifically mTORC1 and mTORC2. Signal transduction was assessed by westernblotting using polyclonal antibodies against raptor and rictor, phosphospecific antibodies against Erk, Akt (Ser473) to monitor mTORC2-activity, p70S6K (Thr389) to monitor mTORC1-activity. Cell size was determined by FACS-analysis. Effect of rictor silencing on Akt subcellular localization was assessed by immunocytochemistry.
Results: Physiologic cell stimulation in presence of E2 resulted in increased mTORC1 and mTORC2 function, whereas pathologic stimulation decreased mTORC1 activity and had minor effect on mTORC2 activity. Rapamycin treatment and raptor silencing effectively blocked mTORC1 function and led to increased mTORC2 function probably by release of negative feedback inhibition. This resulted in decreased cell size irrespective of kind of stimulus or hormonal status. In contrast, rictor silencing decreased mTORC2 function and blocked stimulus-dependent Akt nuclear translocation resulting in abrogation of E2 antihypertrophic effect.
Conclusions: mTOR inhibition with rapamycin effectively inhibits female cardiomyocyte hypertrophy independent from E2. However, specific targeting of mTORC2 in pathological myocardial hypertrophy could be harmful especially in females by antagonizing E2 mediated cardioprotective effects.

Kidney (long-term outcomes I)

O-057 CLOSURE OF THE ASYMPTOMATIC ARTERIOVENOUS FISTULA CORRECTS LEFT VENTRICULAR HYPERTROPHY IN RENAL TRANSPLANT RECIPIENTS

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Background: Left ventricular hypertrophy (LVH) is highly prevalent in patients with end-stage renal failure, and is an independent prognostic factor. Despite the expected beneficial cardiovascular effects of kidney transplantation, the prevalence of LVH remains high in renal transplant recipients. Persistence of the patent arteriovevous fistula (AVF) may contribute to this LVH after kidney transplantation. Although some studies have shown that closure of AVF induces regression of LVH in renal transplant recipients with symptomatic patent AVFs, the effect of closure of asymptomatic fistula on LVH in renal transplant recipients is still unclear.
Methods/Materials: 39 renal transplant patients with a well graft function with an asymptomatic patent AVF were included. Before and 1 month after surgical closure of the fistula, standard echocardiogram was performed.
Results: After closure of the fistula, left ventricular end-diastolic diameter (LVEDD) (44.2±5.2 to 42.3±5.4 mm, $p<0.001$) and left ventricular mass index (LVMI) (98.9±22.9 to 87.7±22.0 g/m2, $p<0.001$) decreased. Consequently, LVH prevalence (LVMI>125 g/m2) decreased from 12.8% to 5.1% and LV concentric remodeling (relative wall thickness>0.45) slightly increased 41.0% to 46.2%. Assessing left atrial dimension (LAD) before closure of the fistula showed that 10 patients had suspicion of left atrial overload with increased LAD and other 29 patients had normal LAD. 15 of 29 patients with normal LAD (51.7%) had borderline of E/e' (greater than 8 and less than 15) before operation and closure of the fistula normalized E/e' in 8 of these 15 patients. In contrast, E/e' was not corrected in patients with increased LAD.
Conclusion: In renal transplant recipients with asymptomatic patent AVFs, closure of the fistula reduces LVMI and LVEDD, which lead to regression of LVH, and may correct LV diastolic function as well.

O-058 CIRCULATING ANGIOPOIETIN-2 LEVELS PREDICT MORTALITY IN KIDNEY TRANSPLANT RECIPIENTS: A PROSPECTIVE CASE-COHORT STUDY

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Background: Angiopoietin 2 (Angpt2) impairs endothelial function by preventing Angiopoietin 1 from binding to their common endothelial-specific receptor Tie2. Here we examined whether circulating Angpt2 predicts outcome in kidney transplant recipients.
Methods: For this case-cohort study we selected 130 kidney transplant recipients who had died or returned to dialysis within the first two years of follow-up of our cohort study, as well as 130 age and gender-matched kidney transplant recipients without an event (controls) from a total of 993 kidney transplant recipients. Serum Angpt2 at baseline was measured using an in-house immunoluminometric assay. Association of baseline Angpt2 levels with all-cause mortality was examined in Cox proportional regression analyses.
Results: Median Angpt2 concentrations were significantly higher in patients who died (median [interquartile range – IQR] 3.8 [2.8-5.9] ng/mL) as compared to patients who did not die during the study period (2.9 [2.1-4.4] ng/mL; $p<0.001$). Ln (natural log) Angpt2 levels correlated positively with C-reactive protein levels ($r=0.315$, $p<0.001$), and the Charlson Comorbidity Index ($r=0.188$, $p=0.002$), and were inversely associated with eGFR ($r=-0.301$, $p<0.001$) hemoglobin ($r=-0.269$, $p<0.001$) and serum albumin concentrations ($r=-0.382$, $p<0.001$). In multivariate analyses baseline Angpt2 levels independently predicted all-cause mortality (multivariable adjusted hazard ratio associated with one natural log unit higher Angpt2 level: 1.85 (95% confidence interval:1.15-2.99) $p=0.012$).
Conclusions: Circulating Angpt2 is elevated in kidney transplant recipients, particularly in those with higher co-morbidities and micro-inflammation, possibly reflecting pronounced endothelial activation and dysfunction. In our analysis circulating Angpt2 was an independent predictor of all-cause mortality in stable, prevalent kidney transplant recipients.

O-059 PROTEINURIA STRATIFIES RISK TO THE INDIVIDUAL FOLLOWING KIDNEY TRANSPLANTATION: DEVELOPMENT OF A PROGNOSTIC RISK SCORE

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Background: Although proteinuria is a population-level risk factor for inferior outcomes following kidney transplantation, no prognostic scores incorporating proteinuria exist to stratify individual risk. The purpose of this study was to derive such a prognostic tool for estimating graft failure risk at 5 years post transplantation.
Methods/Materials: 545 consecutive patients transplanted between 1999 and 2006, alive with graft function at 12 months, were studied. Demographic, clinical and biochemical data were analysed, with proteinuria assessed by urine albumin:creatinine ration (ACR) on an early morning "spot" urine.
Results: Median urine ACR values at 12 months were 3mg/mmol. Median follow-up for the cohort was 8 years (minimum 5 years). Independent association between ACR and death-censored and overall graft failure was seen (HR 1.89; 95%CI: 1.36, 2.63; $p<0.001$ and HR 1.95; 95%CI: 1.50, 2.54; $p<0.001$ respectively).
A backwards selection process was undertaken to identify metrics included within the risk score: 12-month ACR and eGFR, rejection during the first year, recipient race, age and sex for death-censored graft failure, and the same variables plus serum albumin at 12 months for overall graft failure. These models displayed good discrimination (c-statistics 0.82 and 0.75 respectively) and were well calibrated with good agreement between expected and observed risk. The models displayed significantly improved risk reclassification when compared to eGFR or urine ACR in isolation, and also when compared that from a recently reported risk tool derived from USRDS data (Kasiske et al. AJKD 2010), where 27.5% of patients were appropriately reclassified.
Conclusion: Proteinuria, as assessed by urine ACR is not only a risk factor for inferior renal allograft outcomes, but can also provide prognostic information to aid classifying risk to individual patients. We are currently validating this risk score in independent cohorts.

O-060 DAYTIME SLEEPINESS IS ASSOCIATED WTH TAKING & TIMING NON-ADHERENCE IN RENAL TRANSPLANT RECIPIENTS

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Background: Non-adherence (NA) to immunosuppressive drugs is a major risk factor for poor outcome in renal transplant (RTx) recipients. Sleepiness has been found to be associated with non-adherence in heart failure patients, yet this relationship has not yet been explored in transplantation.

Methods: Using a cross-sectional design, we studied a convenience sample of 427 RTx recipients (37.2% females; median age of 60 y (49-67); median time post-transplant 8 years (3.5-13.5)) at a single transplant center in Switzerland. In this study sleepiness was assessed with the Epworth Sleepiness Scale. NA with immunosuppressants was measured using the Basel Assessment of Adherence to Immunosuppressive Medication Scale. NA was reported dichotomously, with any deviation being defined as NA for both the taking and timing dimension respectively. Depressive symptomatology was assessed with the Depression, Anxiety and Stress scale. Using binary logistic regression analysis we explored if sleepiness was associated with taking & timing adherence respectively, controlling for possible confounders as age, time since transplantation and depressive symptomatology.
Results: Taking and timing non-adherence were 18.2% and 43.2%, respectively; 22.3% reported daytime sleepiness and 20.3% reported depressive symptomatology. The binary logistic regression analysis revealed that taking non-adherence was determined by longer time post-Tx (OR: 1.061 CI: 1.026-1.098), sleepiness (OR:1.101 CI: 1.037-1.170), and age (OR:0.983 CI: 0.962-1.004). Timing non-adherence was determined by longer time post-Tx (OR: 1.049 CI: 1.019-1.081), sleepiness (OR:1.060 CI: 1.009-1.113), depressive symptomatology (OR: 1.041 CI: 1.014-1.070) and age (OR: 0.977 CI: 0.960-0.994).
Conclusion: Younger recipients, being longer transplanted and experiencing more daytime sleepiness were independent predictors for timing and taking non adherence. Depressive symptomatology was independent predictors for the timing dimension. This is the first time in transplantation that daytime sleepiness has been associated with non-adherence in transplant patients.

O-063 RISK FACTORS FOR NEW ONSET DIABETES POST KIDNEY TRANSPLANT AND ASSOCIATION WITH ALLOGRAFT FAILURE: DATA FROM THE INTERNATIONAL PORT STUDY

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Background: Renal transplant patients are at risk for new onset diabetes after transplantation (NODAT) due to pre-transplant co-morbidity and immunosuppression. NODAT has also been associated with increased incidence of cardiovascular disease.
Methods: We used data from the Patient Outcomes in Renal Transplantation (PORT) international data collaboration to determine the risk factors associated with NODAT in the first 5 years post-transplant, in an international cohort of 4,140 patients transplanted at 4 North American and 2 European centers, 1999-2006. NODAT was defined as initiation of hypoglycemic medications in a recipient not previously identified as diabetic at the time of transplantation. Risk factors for NODAT were identified using a Cox proportional hazards model predicting time to NODAT. NODAT was considered as a time-varying covariate in an adjusted Cox proportional hazards model predicting all-cause graft failure. All cause graft failure by 60 months post-transplant was defined as initiation of maintenance dialysis therapy, preemptive retransplantation, or death with functioning allograft.
Results: NODAT occurred in 839 patients (20%) during the first 5 years post-transplant. Risk factors independently associated with NODAT are shown in Table 1. NODAT was associated with an increased risk of graft failure (HR=1.64 [1.13-2.39], p=0.0093).
Conclusion: In this study, NODAT was independently associated with graft failure. Potential modifiable risk factors for NODAT include tacrolimus use, BMI≥30 kg/m^2, and non-use of mycophenolate mofetil at the time of transplant.

Risk Factors Associated with NODAT, after adjusting by Transplant Center

Risk Factors	N	HR	95% C.I.	P-Value
Calcineurin Inhibitors at Baseline:				
Cyclosporine	2,270	1.00		Ref
Tacrolimus	1,060	1.60	(1.33–1.92)	<0.0001
None	810	0.83	(0.68–1.02)	0.081
Age per 10 Years	N/A	1.28	(1.21–1.36)	<0.0001
Race:				
White	3,461	1.00		Ref
Black	374	1.69	(1.34–2.12)	<0.0001
Other/Unknown	305	1.70	(1.32–2.18)	<0.0001
BMI ≥ 30 kg/m2	827	1.93	(1.66–2.25)	<0.0001
Cause of ESRD:				
Glomerulonephritis	1,478	1.00		Ref
Hypertension	563	1.34	(1.09–1.65)	0.005
Other/Unknown	2,099	1.03	(0.88–1.20)	0.710
PRA at transplant:				
<10%	3,402	1.00		Ref
≥10%	504	1.06	(0.85–1.32)	0.63
Unknown	234	1.50	(1.12–2.01)	0.007
History of MI: Yes (vs No)	151	1.42	(1.03–1.96)	0.035
History of CVA/ TIA: Yes (vs No)	168	1.34	(0.99–1.80)	0.056
Cold Ischemia Time:				
0–<12 hours	403	0.78	(0.59–1.04)	0.093
12–<24 h	3,485	1.00		Ref
24+ h	252	1.27	(0.97–1.66)	0.089
MMF use at baseline: Yes (vs No)	2,529	0.71	(0.60–0.83)	<0.0001

O-064 RECIPIENT M235T POLYMORPHISM OF THE ANGIOTENSINOGEN GENE PREDICTS LONG-TERM GRAFT SURVIVAL IN KIDNEY TRANSPLANTATION

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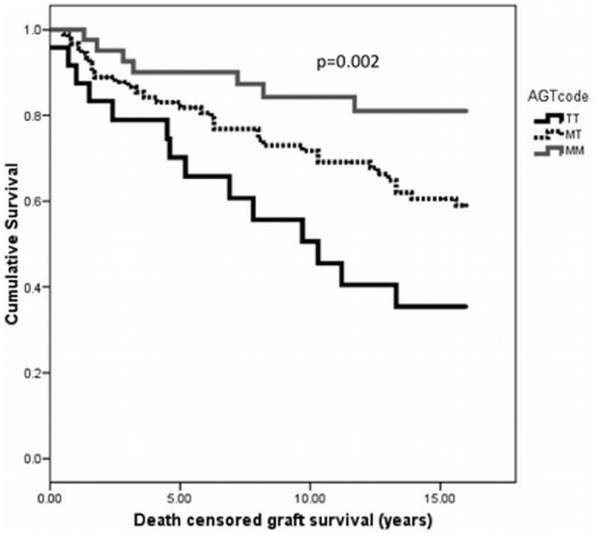
Background: Polymorphisms of genes encoding the renin angiotensin system and αadducin 1 (AAD1) have been associated with progression of renal disease and increased cardiovascular risk. These encode for factors regulating blood pressure. The relationship between these polymorphisms and outcome in renal transplant recipients is less clear.
Methods: We undertook a longitudinal follow up study of 422 first renal transplant patients (median age 42.6 years; 56.6% male; 10% live donor transplants). Patients were surveyed in 1994 and followed up after 16 years. Genotyping was performed for the M235T polymorphism of the angiotensinogen (AGT) gene, D polymorphism of the gene encoding angiotensin converting enzyme and AAD1.
Results: There were 179 (42.2%) deaths and 155 (36.7%) death-censored graft failures. Kaplan-Meier analysis demonstrated decreased patient survival in patients with TT genotype of AAD1 (p<0.001) and a trend towards poorer survival in TT genotype of AGT (p=0.07). Multivariate analysis showed reduced estimated glomerular filtration rate (eGFR) at 1 year (hazard ratio (HR) 0.97, p=0.004), recipient age (HR 1.07, p<0.001), diabetes (HR 7.058, p=0.003) and AAD1 genotype TT (HR 3.33, p=0.01) to be independent predictors of mortality. Graft survival was poorer in patients with the TT genotype of the AGT gene (see figure) but the other genetic polymorphisms studied did not influence graft survival. Multivariate analysis demonstrated reduced eGFR at 1 year (HR 0.97, p=0.006), deceased donor transplant (HR 14.87, p=0.013) and TT genotype of the AGT gene (HR 4.43, p=0.003) to be independent predictors of long-term graft failure.



Conclusions: Polymorphisms of the AGT gene may identify renal transplant patients at increased risk of long-term graft loss whilst AAD1 polymorphisms identify patients at increased risk of mortality. In this cohort, these relationships were independent of blood pressure.

O-065 IS THERE AN ASSOCIATION OF GLUTATHIONE PEROXIDASE (*GPX1)* GENE POLYMORPHISM WITH POST-TRANSPLANT GLUCOSE METABOLISM DISORDER?

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Background: Transplant recipients are at a particularly high risk of developing metabolic disorders as a consequence of factors additional to those that affect the general population including the immunosuppresive agents used in transplant management protocols. Post-transplant diabetes mellitus (PTDM) is a symptom of the metabolic disorder, which is induced by immunosupression. Several studies have suggested that oxidative stress may be associated with diabetes and its complications. The oxidative stress results from increased free radical formation which can be influenced by ischemia-reperfusion injury. The glutathione peroxidase (GPX)/glutathione system is a major defense in oxidative stress.
The aim of our study was to examine the association of the C599T *GPX1* gene polymorphism with post-transplant glucose metabolism disorder.
Methods/materials: The study included 159 patients Caucasian origin receiving kidney transplants. Patients with hemoglobin A1c continuously over 6.5 mg/100ml, fasting blood glucose $\geq$ 7.0 mmol/l, or requiring treatment with oral hypoglycemic agents or insulin continued for more than 3 months after transplantation were diagnosed as having PTDM. Standard immunosuppression consisted of tacrolimus, mycophenolate mofetil, and steroids.
Results: Analyzing the C599T polymorphism in the *GPX1* gene, the odds of PTDM were significantly higher in the carriers of T allele (with CT or TT genotype) when compared to CC homozygotes: OR=3.28, 95%CI=1.11-9.74, p=0.037 and the difference between TT and CC homozygotes was even more significant (p=0.02). Allele T was significantly more frequent among patients with PTDM compared to patients without PTDM (OR=2.14, 95%CI=1.11-4.12, p=0.024).
Conclusions: The present results suggest that C599T polymorphism of the *GPX1* gene may be associated with the risk of PTDM development in renal graft recipients. Patients with the presence of 200Leu (599T allele in TT and CT genotypes) may be more exposed to this complication.

O-066 SURVIVAL PROGNOSIS OF PATIENTS STARTING RENAL REPLACEMENT THERAPY IN THE NETHERLANDS

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Background: More than 40 years after the introduction of renal replacement therapy (RRT) in the Netherlands, it is still difficult to predict the prognosis for an individual patient. The purpose of this study is to find out whether it is possible to give a prognosis for every patient group from the start of RRT, at which time it is not known yet whether patients will or will not receive a kidney transplant.
Methods: We analysed survival for a cohort of end stage renal disease patients that started chronic RRT between 1995-2005 (N=13870) in the Netherlands; follow-up was until 1/1/2010. We excluded patients from 0-15 years old at start of RRT and the patients with missing primary disease, transplant type and intention to treat data. We stratified for age, primary disease, waitlisting (defined as registration at Eurotransplant), transplantation and type of RRT at 90 days after the start of RRT.
Results: Patients that were not waitlisted (N=7555) had the worst 10-year survival (6%); patients that were waitlisted, but not transplanted (N=1746) had a survival of 16%. Best results were found in the group of transplanted patients (N=4569) with a 10-year survival of 85%. These are overall figures; the best results are seen for the younger transplantable patients (age group 16-44 years), with glomerulonephritis or cystic kidney disease that started with peritoneal dialysis (N=384, 10 year survival: 92%); the worst results are seen in older, not transplantable, patients with diabetes that started with hemodialysis (N=278, 10 year survival: 2%).
Conclusion: Based on the analysed data a survival prognosis can be given for every patient group stratified for age and primary renal disease.

Ethics, legal and psychosocial aspects of transplantation

O-067 ALTRUISTIC NON-DIRECTED LIVING KIDNEY DONATION IN THE UK

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Introduction: A new legal framework for living kidney donation enabled altruistic non-directed donation to start in the UK in 2006. Kidneys are allocated to suitable recipients through the national scheme for deceased donor organs, ensuring equity of access for potential recipients.
Methods: The process in the UK and demographic data of altruistic donors and corresponding recipients are summarised. Univariate transplant survival rates (time to transplant failure or death) are investigated.
Results: Potential donors are evaluated in accordance with national clinical guidelines, including mandatory mental health assessment. Once fully assessed, suitable donors are registered with NHS Blood and Transplant and a suitable recipient is identified. The relationship between donor and recipient remains anonymous before transplantation and is only broken thereafter if both parties agree.
In comparison with global experience, activity has exceeded expectations: by January 2011, 60 altruistic donors had donated a kidney. Mean donor age was 53 years (range 24-82 years) and 60% were male. The mean interval between identification of a recipient and donation was 53 days (range 2-224 days) and kidneys were allocated to recipients waiting approximately three years. Kidneys are transported between donating and transplanting hospitals with median cold ischaemia time of 6 hours.
One year transplant survival (90%, 95% CI 75-96%) is comparable with that for other living donor transplants: paired donation 94%, (95% CI 83-98%), specified direct donation to compatible, unsensitised recipients 96%, (95% CI 95-97%).
Conclusions: A national programme for altruistic living kidney donation has been introduced successfully in the UK, enabling an additional 60 recipients to benefit from a living donor transplant and accounting for 3% of living donor kidney transplants in 2010. Domino (chain) paired donation will commence in 2011 and it is expected that most altruistic non-directed donors will donate to the new scheme, furthering the contribution of this valuable resource.

O-068 ALL IN THE FAMILY: HOW EARLY GROUP-EDUCATION OF FAMILIES AND FRIENDS OF CKD PATIENTS LEADS TO PRE-EMPTIVE KIDNEY TRANSPLANTATION

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Introduction: Despite evidence that pre-emptive renal transplantation offers the best treatment for patients with ESRD, many patients start with dialysis. They find it difficult to talk with relatives and friends about their illness and treatment options. Living-kidney-donation is frequently overlooked and carried out after a period of dialysis.
We hypothesized that timely education of family and friends of patients improves understanding, prevent misconceptions about future health status and stimulates discussion about LKD.
Methods: In 2008 the hospital social workers started to offer CKD patients a timely education of family and friends. They inform the patient about the possibilities of this education. When the patient agrees they organize a gathering of all relatives and friends of the patient, preferably at his home. The informative gathering involves in an intimate discussion about current and future health status of the patient and treatment modalities. Data of patient survival on dialysis, after LKD and deceased donor transplantation are given. Risks and benefits of LKD for recipient and donor are presented.
Results: Participating patients, relatives and families welcomed the approach of family counseling. All felt improved mutual understanding and bonding within the family. All patients were relieved after the hospital social worker initiated discussion about LKD. Until January 2011 group education was given to 25 families of CKD patients. Potential kidney donors showed up in 24 cases.
Conclusion: Early group-education of families and friends of patients with CKD leads to a better informed and understanding family, and to an improved family bonding. Relatives consider living kidney donation and makes pre-emptive transplantation possible.
We recommend this education to all patients with CKD stage 3-4.
Recently three hospitals in Rotterdam joined this project and started a research.

O-069 EULID PROJECT: EUROPEAN LIVING DONATION AND PUBLIC HEALTH

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Background: The EULID project, co-founded by the European Commission, has established European common standards to guarantee Living Donors (LD) health and safety through common practices and regulations about legal, ethical and LD protection practices.
Methods/Materials: 12 partners worked to reach a consensus and recommendations (April/07-Dec/09).
The project has been developed in two steps: 1) to have an overview of the European situation and design the tools, and 2) implementation of the project
Results: *Final recommendations:* Legislation: Prohibition and penalization of organ trafficking, tourism, commercialism and incentives. Prohibition of minors and persons unable to give consent. Authorization of transplant centers and LD registry controlled by authorities. Regulation of independent commission.
Ethical: Altruism should be object of the most elevated consideration. Promotion of LD should not impede cadaveric donation. Organ trafficking, commercialism or incentives are ethically unacceptable. Autonomy of the donor doesn't surpass appropriate medical decision-making.
Protection: There should be no cost to the donor. Sick-leave with 100% payment. Financial coverage in case of unforeseen events. LD should be protected of any form of disadvantage. Medical follow-up obligated and psychosocial support if needed.
Registration: Registration of all LD is obligated. Regulatory audit is mandatory. Data on identification, countries of residency, nationality, type of donation, institutions and outcome are obligatory. A central database is obligatory and supported on national authority.
Tools developed: LD Satisfaction survey: 54 questions with multiple choice answers according to Likert scale. Validation using Delphi methodology. 245 performed surveys.
Registry model data: On-line login module created and used by 9EU countries. 724 LD were registered.
Informative leaflet: Translated in 12 languages, information for each organ.
Conclusions: EULID project contributes to a European consensus that could lead to best practices and recommendations that will help to establish a protection framework on LD's health and safety.

O-070 EULOD: THE EU-FUNDED PROJECT ON LIVING ORGAN DONATION IN EUROPE

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Introduction: The project "Living Organ Donation in Europe" (EULOD) is a Coordinating Action, funded by the Seventh Framework Programme of the European Commission. It aims to establish an inventory of living donation practices in Europe, explore and promote living donation as a way to increase organ availability, and develop tools that improve the quality and safety of living organ donations in Europe. 11 institutions from 10 European countries are involved. The project runs from April 2010 – March 2012.
Methods: EULOD consists of two scientific research packages. The first package focuses on living unrelated donation practices in Europe. The second package focuses on legal restrictions and safeguards for living donations in Europe. The remaining three work packages ensure the coordination of the work, dissemination of project results and the organization of meetings. EULOD draws upon the support, knowledge and network of the European platform on Ethical, Legal and Psychosocial Aspects of Organ Transplantation (ELPAT) and the European Society for Organ Transplantation (ESOT).
Results: Current activities include a questionnaire to obtain data on living donation practices in Europe. A search strategy has been drafted to analyze the normative aspects of living organ donation. Information on European laws on living donation and against organ trade is also collected. Analysis of these laws and some organ trafficking cases is being conducted. All partners are currently collaborating to disseminate the project's activities and results to stakeholders.
Conclusion: Thirteen deliverables will result from this project, including scientific reports and recommendations. With this output, the Consortium intends to contribute to the European policy needs, including the increase of organ availability in Europe, making transplantation systems more efficient and accessible and improving the quality and safety of organ donation and transplantation at European level.

O-071 PROGRESSIVE PREDICTIVE FACTORS OF ADHERENCE TO MEDICATION AFTER RENAL TRANSPLANTATION: A FRENCH OBSERVATIONAL STUDY

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Background: Non adherent (NAd) patients (pts) present a higher risk of acute rejection and graft loss than adherent pts. However, evolution of NAd during the first year post transplantation has never been analyzed in depth.
Methods: A French multicenter prospective observational study was set up in order to describe the treatment adherence of KTR during the first year post transplant. Adherence was measured through the MAQ questionnaire.
Results: The characteristics of the 313 enrolled KTR were: mean age 49.5±13 years, men 68% and first transplantation 81.5%. The median number of daily pills intake decreased from day 0 to M12 [from 19 to 13] as well as the frequency of daily doses (QID: 43% at day 0, BID: 40% at M12). 70% of pts reported adverse events. During the first 10 days post transplant, 94% of pts consulted at least one of the 29 chapters of a transplantation information tool, the OTIS software (Organ Transplant Information System) and 24% consulted all of the OTIS software. 18%, 24% and 31% of pts were respectively non-compliant at M3, M6 and M12. Multivariate analyses showed that the daily frequency of doses was associated with good adherence at M3 (OR=1.28, p=0.006). At M6, predictive factors of good adherence were the pts' age (OR=1.02, p=0.02) and the consultation of the "infection and rejection" OTIS software module (OR=2.12, p=0.03). At M12, predictive factors were the pt's age (OR=1.02, p=0.02), the daily pills intake (OR=1.06, p=0.04), and the absence of adverse events (OR=2.0, p=0.03).
Conclusion: NAd increases over time reaching 30% of KTR at the end of the first year post transplant. NAd's risk factors change over time. So, their identification at each time of the post transplant follow-up is necessary to improve patient's adherence to treatment.

O-072 INFORMED CONSENT IN LIVING LIVER DONORS: COMPREHENSION, INFORMATION NEEDS, AND RISK PERCEPTIONS

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Background: Adult-to-adult living donation for liver transplantation poses serious health risks and no direct health benefits for living liver donors (LDs). Thus, ensuring LDs' autonomy through informed consent is critical. We investigated LDs' decision-making and perceptions of the informed consent process.
Methods: Semi-structured, voluntary interviews were conducted with LDs after they completed the final phase of evaluation and informed consent for donation according to standardized institutional and A2ALL protocols, two days before the donor operation, at our transplant center, between January 2009 and February 2011. Likert-scales measured perceptions of informed consent (1 = "not at all" to 5 = "a great deal"). Open-ended responses underwent thematic analysis.
Results: Thirty LDs participated in an interview (100% participation rate). The average age was 37 years, 63% were female, 80% were white, 63% were

married/partnered, and all had completed high school. Although 90% of LDs reported being *informed* about donation "a great deal", only 66% reported *understanding* information about donation "a great deal". LDs (40%) reported difficulty understanding medical terminology. Information LDs had desired to feel comfortable with donating included: donor complications (73%), donation procedure (47%), donor preparation (30%), recuperation (30%), and transplant center statistics (27%). Most (83%) LDs rated risks to themselves as "not at all" to "somewhat" rather than "a lot" to "a great deal" risky. LDs' comments indicate that they minimized risks to themselves: "The chance of any of these... major things [complications] happening are so minimal that they're not important."
Conclusions: The results suggest that informed consent is suboptimal for LDs. LDs insufficiently understood the donation process and downplayed the import of risks in decision-making, raising questions about adequate comprehension. Greater efforts are needed to disclose more extensive information to LDs. Further research should assess the adequacy of risk communication and LDs comprehension.

O-073 A SCHOOL-BASED ORGAN DONATION EDUCATION PROGRAM TO ENCOURAGE ORGAN DONATION REGISTRATION AND TO CHANGE PUBLIC ATTITUDES RELATED TO ORGAN DONATION

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Background: In order to increase the number of potential donors a new organ donation registration scheme was implemented in 1998 in the Netherlands. Notwithstanding a large promotion campaign registration rates failed to meet the expectations. However, in order to change public attitudes and to promote organ donation registration interventions should be targeted at separate groups. Each year, on average 190,000 Dutch 18 year-old adolescents are approached to register their organ donation preference. This group is considered to be the most important target group for encouraging donor registration.
Methods: A school-based education program (video fragments with group discussion, an interactive computer-tailored program, and a registration training) was developed to enable adolescents to make a well-considered decision about organ donation. The program is based on the results of three large-scale determinant studies, three controlled studies in which the separate draft intervention components were tested, and a randomised trial (N=2868 students). Outcome measures were intention to register, willingness to become an organ donor, negative outcome expectations, knowledge, and self-efficacy related to the registration procedure.
Results: The education program resulted in an increase in registration intention and intention to become a potential donor. Adolescents evaluated the education program positively and the majority thought that the information was interesting, credible, understandable, and useful.
Conclusion: In the past years, the education program is digitally transformed, updated and translated in English (www.organdonationeducation.azm.nl).
It can be concluded that the program is effective in enabling adolescents to make a well-considered decision about organ donation and in changing psychosocial determinants associated with donor registration.

O-074 IMPACT OF RECIPIENT'S SOCIO-ECONOMIC STATUS ON PATIENT AND GRAFT SURVIVAL AFTER LIVER TRANSPLANTATION: THE IsMeTT EXPERIENCE

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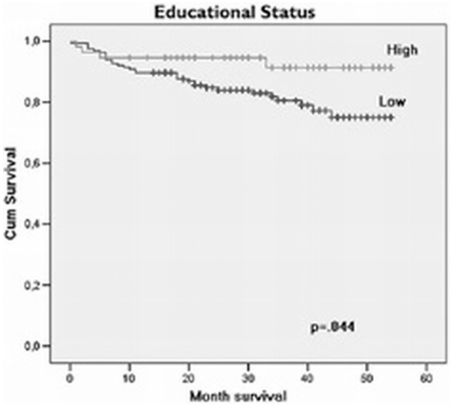
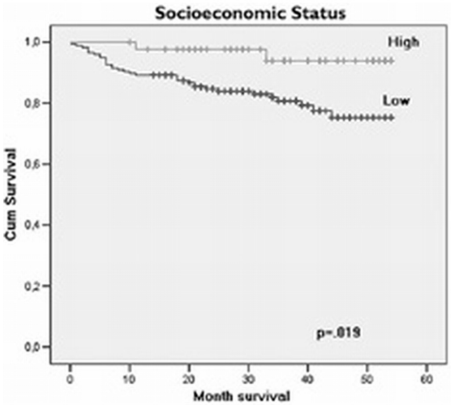
Aim: We aimed to understand if cultural and socioeconomic status (SES) level could be considered potential predictors of non-adherence in a cohort of liver transplant recipient in the South of Italy.
Methods: The retrospective study included 221 liver transplant recipients made at IsMeTT between January 2006 and September 2009. Donor gender and age; cold ischemic time, extended criteria donors, recipient age, gender, body max index, primary etiology, MELD score, co-morbidities, and patient health score assessed on the basis of clinical follow-up; highest level of education achieved and SES were collected and analyzed.
Results: Differences in survival by education and SES assessed using the Kaplan-Meier method showed a better survival rates in patients with higher cultural (p=0.04) and SES (p=0.01).
After adjusting for all co-variables, results of the multivariate Cox regression analyses showed that only SES remained an independent significant predictor of overall survival (HR=0.16,p=0.03).
Conclusion: The survival of patients after received a liver transplantation was influenced by low income, low educational level, and lack to access to quality health care. Prospective clinical investigations are necessary to fully identify the impact of SES on long-term health outcomes and to propose an evidence based guide to clinical intervention.





Cox proportional hazard of patient death according to cultural and socioeconomic status

	Patient death	
	Non-adjusted results HR (95% CI)	Adjusted results HR (95% CI)
High educational status	0.36 (0.12-0.96)*	0.68 (0.21-2.15)
High socioeconomic status	0.21 (0.05-0.89)*	0.16 (0.3-0.53)*
Recipient age	–	1.05 (1.01-1.1)*
Hepatocellular carcinoma	–	2.65 (1.113-6.32)*
Complications		
Surgical	–	4.53 (1.16-17.66)*
Metabolic	–	8.69 (1.43-52.81)**
Infective	–	13.7 (5.63-33.32)**
Neoplastic	–	3.65 (1.31-10.16)*

*P-value <0.05; **P-value <0.001.

O-075 SURVEY ABOUT CURRENT PSYCHOSOCIAL ASSESSMENT/FOLLOW-UP PRACTICES (ELIPSY PROJECT)

Chloë Ballesté[1], Ana Menjívar[2], Levent Yucetin[3], Leonídio Dias[4], Christian Hiesse[5], Christina Papachristou[6], Ingela Fehrman-Ekholm[7], Niclas Kvarnström[8], George Kyriakides[9], Martí Manyalich[10]. *[1]Surgery Department, Universitat de Barcelona, Barcelona, Spain; [2]Fundació Clínic per la Recerca Biomèdica, Fundació Clínic per la Recerca Biomèdica, Barcelona, Spain; [3]Medical Park Antalya Hospital Complex, Medical Park Antalya Hospital Complex, Antalya, Turkey; [4]Nephrology and Transplant Departments, Centro Hospitalar do Porto, Porto, Portugal; [5]Service de Transplantation Rénale, Hôpital Foch, Paris, France; [6]Medical Clinic for Internal Medicine and Psychosomatics, Charité University Hospital, Berlin, Germany; [7]Nephrology Department, Sahlgrenska University Hospital, Göteborg, Sweden; [8]Surgery Department, Sahlgrenska University Hospital, Göteborg, Sweden; [9]Paraskevaidion Surgical and Transplant Center, Paraskevaidion Surgical and Transplant Center, Nicosia, Cyprus; [10]Medical Direction, Hospital Clinic of Barcelona, Barcelona, Spain*

Background: This survey is part of the ELIPSY Project, co-funded by EAHC, which aim is to develop a common methodology to assess Living donor (LD) in the psychosocial sphere.
There is no data about the current psychosocial follow-up practices among different European centres to evaluate the key points of the methodology and find out the differences between Kidney and Liver programs.

Survey design:
– The survey contains three parts, one focused in kidney programs, the other in liver programs and the last to write comments.
– The partners reviewed the survey and some topics were rewritten or modified.
Conduction of the survey:
– Among centres from partner countries.
– Among centres from EULID partner countries: United Kingdom, Poland and Romania.
Results: The survey has been developed in 65 centres with LD programs from 10 countries. The percentage per organ of these programs was kidney 72% and liver 28%; only 42% of kidney living donor programs and 28% from liver do some psychosocial assessment.
About the tools used to perform the psychosocial follow-up, the majority of the centres don't describe the used tools and those who describe it, use the same for kidney than for liver. We don't find a standard; the centres and the professionals use different test and in some cases their own test.
To the question: Who performs the psychosocial follow-up (More that 1 option), for kidney the most frequent were nephrologists (16/26), and from liver were psychologists (6/9).
Only 58% from kidney LD centres and 67% from liver, do the analysis of the psychosocial data.
Conclusion: Results show that there is no consensus for LD psychosocial assessment practices; neither the methodology nor the professional that perform it nor psychological tests used nor the moment to carry it out.

O-076 ETHICAL AND LEGAL ISSUES IN UNCONTROLLED DONATION AFTER CARDIAC DEATH: DID WE EVERYTHING WE COULD FOR OUR PATIENTS?

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Background: Some multicentric studies have shown significant survival rates with a good quality of life (Cerebral Performance Category 1-2) in selected patients whose profile and aetiology suggested an indication for the use of non-conventional autopulse cardiopulmonary resuscitation (Bonnemeier H et al. *Resuscitation* 2011;82:155–159). Without the applications of certain manoeuvres of advanced life support–percutaneous transluminal coronary angioplasty, thrombolysis during resuscitation and extra corporeal membrane oxygenation– those patients would have been candidates for uncontrolled donation after cardiac death (DCD) (Maastricht I and II). The increasing volume of evidence of the efficacy of the latest advances in life support (Nolan JP et al. *Resuscitation* 2010; 81:1219–1276) would suggest that a significant proportion of victims of cardiac arrest who are currently transported to hospitals as potential DCD donors, can survive and have an acceptable quality of life if a series of non-conventional but available interventions are employed. The ethical and legal issues that this situation entails for professionals and society need to be discussed. To what extent do the potential benefits of these non-conventional interventions outweigh the associated clinical and economic costs?
Methods: A critical review of the clinical and bioethical literature on controlled DCD has been carried out.
Conclusions: Emergency resuscitation practices should be based on the best available evidence and professional guidelines, regardless of considerations for organ donation. Transparency about the purpose of resuscitative manoeuvres towards the family is a desirable and feasible objective in uncontrolled DCD. A positive cost-benefit ratio can be achieved with these protocols if emergency professionals are trained to select those patients who, based on the aetiology of cardiac arrest, their clinical situation and past history, could benefit from non-conventional resuscitation techniques.

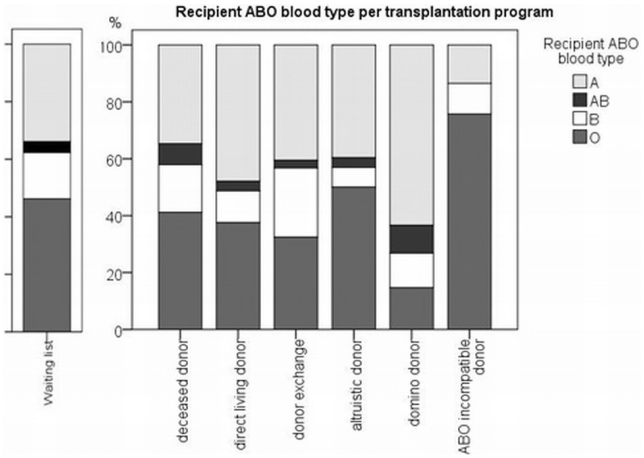
Kidney (live donation, assessment and allocation)

O-077 WHO BENEFITS FROM ALTERNATIVE LIVING KIDNEY DONATION PROGRAMS?

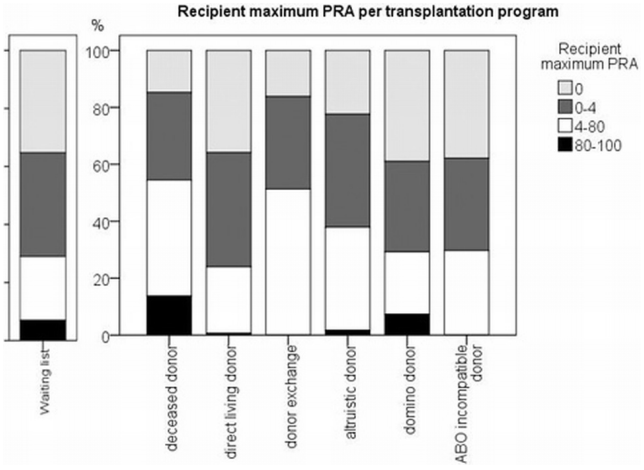
Joke Roodnat[1], Jacqueline Wetering[1], Judith Kal-van Gestel[1], Marry Klerk[1], Willij Zuidema[1], Jan IJzermans[2], Madelon Agteren[1], Willem Weimar[1]. *[1]Internal Medicine, Erasmus MC, Rotterdam, Netherlands; [2]Genaral Surgery, Erasmus MC, Rotterdam, Netherlands*

Background: 1 in 6 couples that present for living donor renal transplantation is ABO incompatible or has a positive cross-match. We compared recipients of a direct vs alternative program transplantation concerning donor relation, ABO blood type and panel reactive activity (PRA).

Methods: Between 2000 and 2010 1292 renal transplantations were performed, 501 deceased donor, 618 direct living, and 173 alternative living donor transplantations. Alternative programs in our centre are: kidney-exchange, domino-paired in combination with altruistic donation and ABO-incompatible donation.
The Rotterdam waiting list was used for comparison.
Results: There were significantly more partner donors in the alternative compared to the direct donation program (55 versus 26%, $p<0.001$). In recipients of a direct donor, blood type O was underrepresented compared to the waiting list population (37.5% vs 46%, $p<0.001$).



This was also true for recipients via the kidney-exchange (32%, ns) and domino-paired program (15%, $p<0.001$). But the prevalence of blood type O was higher in altruistic (50%, ns) and ABO-incompatible programs (76%, $p=0.012$). 7% of waiting list patients was highly sensitized (PRA>80%); significantly more than in the population of recipients of a direct donor (0.6%, $p<0.001$) and in recipients via ABO-incompatible program (0%, ns).



In the altruistic and domino-paired programs the proportion of highly sensitized patients was 1% ($p=0.026$) resp. 7.3% (ns). In the population transplanted via the donor exchange program there were no highly sensitised patients but the percentage of intermediately sensitised patients was higher ($p<0.001$).
For comparison: in the population of recipients of a deceased donor kidney 41.1% had blood type O ($p=0.01$) and 13.8% was highly sensitised ($p<0.001$).
Conclusion: In direct living donation significantly less blood type O recipients and less highly sensitised patients are transplanted than expected based on the waiting list. Various alternative donation programs can compensate this.

O-078 A KIDNEY DONOR RISK INDEX FOR THE UK

Rachel J. Johnson, Rhiannon Birch, J.A. Bradley, Chris J.E. Watson. *on behalf of the NHS Blood and Transplant Kidney Advisory Group, NHS Blood and Transplant, Bristol, United Kingdom*

Background: The simple division of deceased donor kidneys into standard and expanded criteria misrepresents the spectrum of donated kidneys. We sought to define a simple donor risk score which would predict graft outcome more closely and thus facilitate decisions regarding usability and allocation.

Methods: Data from the UK Transplant Registry on 7620 adult recipients of adult deceased donor kidney transplants 2000-2007 were analysed. Donor factors influencing transplant survival were investigated using Cox regression, adjusting for significant recipient and transplant factors. A UK donor risk index was derived from the model, which was based on 60% of the full dataset. The risk index was then validated in the remainder of the cohort and was compared to the US derived kidney donor risk index (KDRI).
Results: Increasing donor age was the most significant factor predicting poor outcome (HR 1.02 for each additional year, $p<0.0001$). Other significant donor factors were histories of hypertension (HR 1.23, p=0.009) and cardiothoracic disease (HR 1.27, p=0.05). Increased donor weight, a longer in-hospital stay and use of adrenaline were also significantly associated with poorer outcomes up to 3 years post-transplant. The resultant donor risk index was highly prognostic of outcomes in the validation cohort ($p<0.0001$), and was as effective as the US KDRI.
Conclusions: Based on six factors rather than 15, the UK risk score represents a simple index that provides an easy tool for predicting transplant outcomes and which could be incorporated into national kidney allocation policy.

O-079 COMPARISON OF PREDICTIVE RISK SCORES FOR KIDNEY ALLOGRAFT FUNCTION

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Several risk scores have been used to predict graft function after kidney transplantation. Identification of kidney allografts at increased risk of later dysfunction has important clinical consequences. The aim of the present study is to compare the predictive power of several risk scores to predict delayed graft function (DGF), serum creatinin in the 3rd, 6th and 12th month and graft function after one year.
A total of 566 consecutive patients after deceased donor renal transplantation in our centre were included. Deceased Donor Score (DDS), Donor Risk Score (DRS), Delayed Graft Function Nomogram (DGF) and Expanded Criteria Donors (ECD) were calculated in each individual. The predictive power of scores was compared using ROC analysis.
Each of the predictive scores (DDS AUC=0.62, $p<0.001$; DRS AUC=0.62, $p<0.001$; DGF AUC= 0.64, $p<0.001$; ECD AUC=0.58, $p<0.01$) was able to predict delayed graft function. The predictive power of ECD was slightly lower compared to the other scores, while none of the scores outperformed the others. No score was able to predict graft failure after one year (DDS AUC=0.56, p=0.18; DRS AUC=0.54, $p<0.37$; DGF AUC= 0.55, p=0.25; ECD AUC=0.55, p=0.25). In the 3rd, 6th and 12th month, with increasing risk score grade of DDS and DRS we observed linear increase of serum creatinin (p for all liner trends <0.001). The difference in serum creatinin between ECD positive and negative groups was significant in the 3rd, 6th and 12th month ($p<0.01$, $p<0.01$, $p<0.05$, consecutively).
This study shows, that several predictive scores may be used to predict delayed graft function and graft function during 1st year after transplantation, what may have implications in refining early postoperative management and allocation policy. No single risk score had better predictive power than the others.

O-080 LIVING DONOR EXCHANGE FOR INCOMPATIBLE COUPLES AND ITS EFFECT ON ALTERNATIVE PROGRAMS

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Living donor kidney exchange has become an efficient solution for recipients with incompatible donors. Here we describe the fate of all patients in our program. Data were collected. In seven years 422 pairs were registered. Matching couples were found for 127/185 (69%) X^+ pairs and 91/237 (38%) ABO incompatible pairs. Of 141/218 matched pairs the recipients received a kidney in an exchange procedure. However, for 26/77 match procedures that were discontinued for medical/psychological reasons, an alternative was found. So in total 167 (141 + 26) recipients received a transplant. Of the remaining 51 discontinuations 26 definitely left, for 22 an alternative transplantation was found outside the program and 3 are still waiting. For the 204 unmatched couples, 46 are still in the program while 34 others definitely left, while for 124 recipients an alternative living kidney donor was found outside the exchange program. In total after 7 years 39% received a kidney within an exchange procedure, 35% were transplanted outside the program, 14% were delisted and 12% is still waiting. The 146 patients who received a kidney outside the program were transplanted with a deceased kidney in 47 cases, 21 found another direct donor, 37 were transplanted across the blood type barrier and 41 received a transplantation in a domino-paired procedure triggered by an unspecified donor.
Conclusion: In the 7 years of our exchange program 313/422 (74%) of the participating recipients became transplanted. Approximately half of them (167/313, 53%) received a kidney within the exchange program, while 47 (15%) received a deceased donor kidney and 99 (32%) were transplanted within other living donation programs. The exchange program proved to be highly successful not only in its direct results but also indirectly by triggering alternative solutions.

O-081 ISOLATED NON-VISIBLE HAEMATURIA IN LIVING KIDNEY DONORS: CAUSES AND OUTCOMES POST-DONATION

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Background: Isolated non-visible haematuria (NVH) is common in the general population and is considered insignificant in the absence of features like reduced kidney function or proteinuria. However, for the purposes of living kidney donor assessment rigorous evaluation including kidney biopsy is recommended to exclude glomerular pathology and stratify the risk of progressive kidney dysfunction post-donation.
Methods &Materials: We retrospectively analysed all potential kidney donors who were evaluated at our centre between 1999 and 2010 and who were found to have persistent NVH. All patients were assessed according to BTS consensus guidelines including renal tract imaging, GFR measurement, screening for proteinuria and cystoscopy. All patients underwent kidney biopsy if no other cause for haematuria could be found. Provided the sample was adequate, all biopsy tissue was submitted for H&E staining, immunoperoxidase and EM.
Results: Baseline donor characteristics are given in table 1.

Table 1. Patient characteristics

Characteristic	Total = 145, Number (%)
Total of patients	145
Male	43 (29.7%)
Female	102 (70.3%)
Self reported ethnicity	
Caucasians	87 (60%)
Black	11 (7.6%)
Others	17 (11.7%)
Not available	30 (20.7%)
Donor relationship	
Genetically related	98 (67.6%)
Genetically unrelated	42 (29%)
Not available	5 (3.4%)
Smoking history	
Never smoked	79 (54.5%)
Current smokers	50 (34.5%)
Ex-smokers	14 (9.7%)
Not known	2 (1.3%)
Pre-existing hypertension	12 (8.3%)

Among 145 renal biopsy specimens 4 were inadequate hence only H&E and immunoperoxidase examination was performed. Otherwise all biopsies are included in this analysis. The final biopsy reports are given in table 2.

Table 2. Renal histo-pathological diagnoses

Diagnoses	Total 150, Number (%)
Normal	14 (9.7%)
Thin basement membrane lesion alone (TBML)	35 (24.1%)
TBML with additional pathology	35 (24.1%)
Increased mesangial cellularity	20 (13.8%)
IgA nephropathy	5 (3.4%)
IgM deposition	7 (4.8%)
Interstitial fibrosis/Arteriosclerosis	2 (1.4%)
FSGS	1 (0.7%)
Borderline TBML with other features	10 (6.9%)
Mesangial hypercellularity without immune deposits	23 (15.9%)
Arteriosclerosis/Interstitial fibrosis	8 (5.5%)
IgA nephropathy	7 (4.9%)
IgM nephropathy	8 (5.5%)
Other diagnoses	5 (3.4%)

Among the 145 donors, 63 (43.3%) were excluded from donation on the basis of the biopsy findings, 10 (6.9%) await donation and 72 (49.7%) have completed donation. 29 of 72 donors have eGFR measurement at 12 and 36 months post-donation and they were 58.9 ± 11ml/mt and 62.48 ± 13.26ml/mt (median$\pm$SD) respectively. There was no significant drop in eGFR at 36 months. Though haematuria persisted in these donors none of them developed dipstick positive proteinuria during this period.
Conclusion: In this mainly female and Caucasian population with persistent NVH, TBML was the predominant finding on kidney biopsy, but other glomeru-

lar pathologies excluded kidney donation in almost 40% of patients. Donors with TBML have a favourable short-term outcome following donation.

O-082 BODY COMPOSITION AND NUTRITIONAL STATUS IN 98 PATIENTS AWAITING KIDNEY TRANSPLANTATION: PRELIMINARY RESULTS OF THE CORPOS STUDY

Karine Moreau[1], Aurélie Desseix[1], Thomas Bachelet[1], Delphine Morel[1], Pierre Merville[1], Lionel Couzi[1], Christine Germain[2], Rodolphe Thiebaut[2], Philippe Chauveau[3]. *[1]Renal Transplant Unit, Pellegrin Hospital, Bordeaux, France; [2]USMR, Pellegrin Hospital, Bordeaux, France; [3]AURAD Aquitaine, AURAD Aquitaine, Gradignan, France*

Introduction: Chronic kidney disease is associated with impaired nutritional status and body composition (BC). However, patients on the waiting list are quite selected and BC changes before and after kidney transplantation have been little studied. We report the preliminary results of a nutritional longitudinal prospective study in awaiting kidney graft patients.
Methods: Patients were included when listed for a first kidney grafting. Fat Free Mass (FFM) and Fat Mass (FM) were estimated by Dual-energy X- ray absorptiometry. FFM and FM index (FFMI – FMI) were calculated as the ratio of FFM and FM to height squared and compared to healthy controls matched for age and sex [Coin A and al. clinical nutrition 2008, 27: 87-94]. Biochemical nutritional parameters were also recorded. Results are expressed as median (range).
Results: 28 women (W) and 70 men (M) aged 54.5 [25.3 – 65.9] were included. BC parameters are listed in this table:

Table 1

	Women (n=28)	Men (n=70)
Body Mass Index (kg/m^2)	25.1 [16.8–39.4]	25.6 [18.4–38.5]
FFMi (kg/m^2)	14.3 [11.8–21.4]	17.9 [13.9–24.2]
FMi (kg/m^2)	10.6 [3.7–18.6]	10.6 [3.7–18.6]

BC analysis shows an increase in FMi and decrease in FFMi compared to controls ($p < 0.01$), reflecting an abnormal distribution of body compartments. According to European recommendations [*Fouque D and al. Nephrol Dial Transplant 2007, 22 suppl 2: ii45-ii87*], biological parameters are quite corrects: albumin is 46 [36–53] g/l, pre albumin is 0.41 [0.24–0.74] g/l and C.R.P is 3 [1–57] mg/l, despite energy (22.9 [10–59] kcal/kg/d) and protein intakes (1.1 [0.5–2.5] g/kg/d) inadequate.
Conclusion: This study demonstrates that in patients awaiting kidney transplantation, despite a good biochemical nutritional status, there are BC abnormalities (especially an increase in FM) undiagnosed by routine investigations. The generalization of estimation of BC before renal transplantation seams essential to detect subjects at risk of developing metabolic complications after transplantation.

O-083 EXCESSIVE PREMATURE AGEING OF T LYMPHOCYTES IN END-STAGE RENAL DISEASE: AN IMPORTANT FACTOR TO CONSIDER IN PRE-TRANSPLANT IMMUNOLOGIC RISK ASSESSMENT

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Background: End-stage renal disease (ESRD) causes an impaired T cell-mediated immune function, which is associated with marked changes in T cell numbers and differentiation. We tested whether these changes are compatible with the concept of premature ageing. Such a finding would be relevant to patients undergoing kidney transplantation, as the degree of premature immunological ageing may influence the risk of allograft rejection and/or infection.
Methods: Three different approaches were chosen to assess the immunological age of T cells in 75 stable ESRD patients and age-matched healthy controls (HC) with an age range from 20-80 years. First, total numbers of circulating T cells and their differentiation pattern were analyzed by flowcytometry. In addition, the T cell receptor excision circle content (TREC) by real-time qPCR and relative telomere length (RTL) of CD4 and CD8 T lymphocytes was assessed. TRECs are generated during T cell receptor rearrangement in the thymus and are diluted with every cell division in the circulation.
Results: An age-dependent decrease in absolute T cell numbers and increased terminal differentiation was observed in both HC and ESRD patients. The T cell compartment of ESRD patients was more affected than age-matched HC, yielding an average difference in immunological age of 15-20 years. TREC content decreased with increasing age ($Rs=0.70$, $P<0.01$). A significantly greater decrease in TREC content was observed in ESRD patients compared to HC ($P<0.01$), yielding an average difference in immunological age of 15-20 years. The RTL of CD4 and CD8 T lymphocytes gave similar findings as the TREC assay but resulted in a greater immunological age difference of almost 30 years.
Conclusion: ESRD increases the immunological age of T lymphocytes by approximately 20-30 years compared to age-matched HC.

O-084 LONG TERM CARDIOVASCULAR SURVIVAL IN ESRD PATIENTS UNDERGOING SCREENING FOR RENAL TRANSPLANTATION

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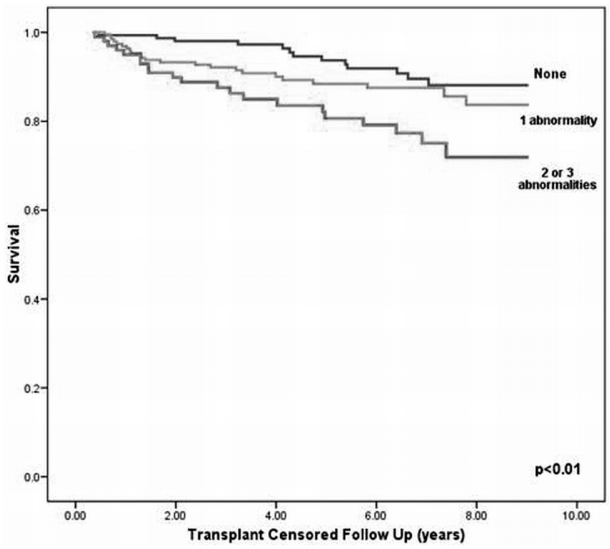
Background: Premature cardiovascular (CV) death is the commonest cause of death in ESRD patients and is associated with uraemic cardiomyopathy (left ventricular hypertrophy (LVH), LV systolic dysfunction (LVSD) and LV dilation). Abnormalities commonly occur in combination. Cardiovascular MRI (CMR) provides volume-independent assessment of myocardial structure.The effect of CMR-measured cardiac abnormalities on long term CV survival was assessed in patients who underwent CV screening for renal transplantation
Methods: 446 patients with ESRD underwent pre-transplant assessment including ventricular assessment by CMR. Data on patient outcome, including cause of death, were obtained over a 9.0 year follow up.
Results: 336 patients were accepted onto the waiting list; 128 patients were transplanted. 156 patients (35.0%) had normal cardiac structure, 276 (61.9%) LVH, 85 (19.0%) LVSD and 62 (13.9%) LV dilation. We examined the accumulation of these abnormalities: 94 (43.5%) had one abnormality and 97 (21.7%) had 2 or 3 abnormalities. LVH only was the commonest single abnormality present (41%) with 2.5% patients with LVSD alone.
114 patients died (23 post-transplantation) and 68 deaths were due to CV causes (11 post-transplantation). LVH, LVSD and LV Dilation were significantly associated with poorer transplant censored CV survival. Presence of 2 or more abnormalities also significantly reduced CV survival. Multivariate Cox regression analyses demonstrated older age at screening, past history of IHD, and presence of LVH as independent predictors of CV death. Older age and presence of 2 or 3 abnormalities independently predicted death when number of abnormalities was entered into the model.



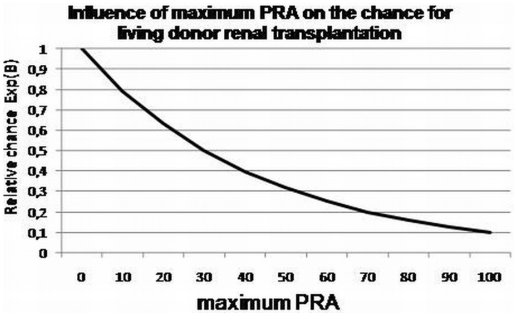
Conclusion: In ESRD patients being assessed for renal transplantation, LVH, LVSD and LV dilation are associated with poorer CV survival. Accumulation of cardiac abnormalities is also associated with a significantly poorer CV prognosis.

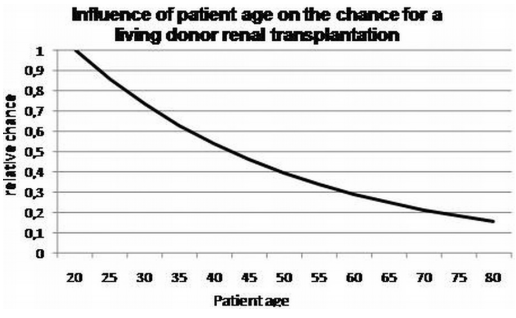
O-085 INFLUENCE OF LIVING DONOR TRANSPLANTATION ON THE COMPOSITION OF THE POPULATION THAT REMAINS DEPENDENT ON DECEASED DONOR TRANSPLANTATION

Joke I. Roodnat[1], Mirjam Laging[1], Jacqueline Wetering[1], Marcia Kho[1], Judith Kal-van Gestel[1], Jan IJzermans[2], Willem Weimar[1]. *[1]Internal Medicine, Erasmusm MC, Rotterdam, Netherlands; [2]General Surgery, Erasmus MC, Rotterdam, Netherlands*

Background: In the past 10 years the number living donor (LD) transplantations increased considerably. We wondered about the influence of these programs on the population that remains dependent on a deceased donor (DD) transplantation.

Methods: This retrospective study includes all 1292 recipients of a renal transplant performed between 2000 and 2010 in the our centre; 501 with a DD and 791 with a LD.
Clinical and social variables were combined in our study. Clinical variables are: recipient age, gender, ethnicity, original disease, ABO blood type, PRA, type of pre-treatment, waiting time and transplantation year. Each recipient's post-code was linked to the CBS 2004 post-code area information data-base, to extract demographic information on: urbanization level, percentage non-Europeans in the area, income, and housing value. Chi square, ANOVA and uni- and multivariate-logistic regression analyses were performed.
Results: There were significant differences between the recipients of a LD versus those of DD kidney transplantation. In univariate logistic regression analyses most variables showed a significant influence on the chance of receiving LD kidney transplantation. In multivariate logistic regression analyses 10 variables remained significant. Predictors of LD transplantation were: lower PRA, ABO blood type A, recent transplantation year, pre-emptive transplantation or CAPD, younger age, female gender, European ethnicity, high housing value, high urbanization grade, and low percentage of non-Europeans in the area.





Conclusion: Non-participation of specific populations in the LD kidney transplantation programs influences the composition of the population that remains dependent on a DD transplantation. These populations are: ABO blood type O patients, highly sensitised patients, patients on haemodialysis, socially deprived patients, elderly, and non-European patients. It is very probable that the composition of the waiting list population will negatively influence the results of deceased donor transplantation.

O-086 WEIGHING UP THE EVIDENCE: DOES THE OBESE KIDNEY DONOR POSE TOO HIGH AN OPERATIVE RISK TO TAKE?

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Introduction: Demand for live-donor kidneys, coupled with national trends towards obesity, make it important to consider the expansion of the donor pool to include obese donors. Controversy arises from the possible increased risk for surgical complications and a concern that obesity may contribute to long-term renal disease. This study aims to delineate whether obesity is in fact associated with greater risk of perioperative and long-term complications in donors undergoing nephrectomy.
Methods: Data from 386 living-donor nephrectomies, utilizing the "mini-open" technique, were collected over five years at one of the United Kingdom's largest renal transplant units. Donors were stratified into quartiles by baseline body mass index (BMI), with 116 being obese (BMI >30 kg/m2). Extensive post-donation metabolic and renal function data, collected at 6-12 monthly intervals over a 5-year follow-up period, were analysed and compared to preoperative data. Perioperative endpoints and surgical complications were also reported.
Results: A high BMI (>35 kg/m2) was shown not to impact significantly on intraoperative endpoints including operative time and estimated blood loss. Postoperative complication rates were also not significantly different between groups, with pneumonia constituting the commonest complication across BMI categories. Long-term follow-up showed renal function and propensity towards hypertension and cardiovascular events not to be significantly different between groups. Major surgical complications, readmission, and reoperation rates were comparably low across BMI categories.
Conclusion: Our experience is that donor nephrectomy is safe in obese donors and does not result in higher rates of major perioperative complications. Long-term follow-up shows good outcomes for donors with elevated BMI. It would be prudent to re-evaluate obesity's position as an exclusion criterion if we are to successfully expand the organ pool.

Living kidney donation

O-087 DIFFUSION -WEIGHTED MRI-IMAGING REVEALS LONGITUDINAL FUNCTIONAL CHANGES IN THE REMAINING KIDNEY DURING THE FIRST YEAR AFTER LIVING DONOR NEPHRECTOMY

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Introduction: Diffusion weighted imaging (DWI) is a new functional MRI imaging method. We performed a prospective longitudinal study in living kidney donors and their recipients before and after transplantation. We hypothesized that diffusion parameters will change after nephron loss due to uninephrectomy.
Methods: 13 healthy kidney donors and their recipients were randomly enrolled. Donors and recipients underwent MR examinations at 7 days (D07), 3 months (M03) and 12 months (M12) after living donation and pre transplant only in donors. Clinical parameters, including serum creatinine, were used to calculate the glomerular filtration rate (eGFR) using MDRD formula. Coronal single shot EP-DWI was performed on a 3T MR scanner (Trio, Siemens) with 10 diffusion gradient b-values using respiratory triggering. DWI processing was performed I) without separating diffusion and perfusion contributions, yielding a "total" apparent diffusion coefficient (ADC_T), and II) separating diffusion and perfusion, yielding ADC_D (mostly determined by diffusion), and the perfusion fraction, F_P.
Results: Most importantly, ADC_D (and similarly ADC_T) rose in the remaining kidney at D07 after explantation and remained high at M03. At M12, ADC_D declined again in cortex, while it remained significantly elevated in medulla, demonstrating only a trend towards lower values (Fig.1a). The corticomedullary difference of ADC_D ($DADC_D$), which is present pre-transplantation, persisted until M03 and then vanished at M12 (Fig. 1b). F_P values showed a trend towards higher values, primarily in cortex. ADC values in medulla and the corticomedullary difference $DADC_T$ and $DADCT_D$ correlated significantly with eGFR ($p<0.002$). In the transplanted kidney all parameter remained remarkably stable during follow-up.

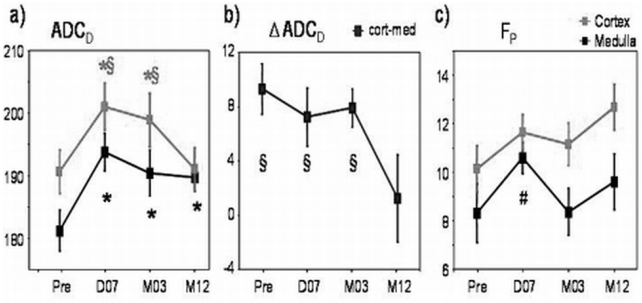

Fig.1: Time course of a) ADC_D, b) the corticomedullary difference in ADC_D, and c) F_P values from before (Pre) via D07 and M03 to month 12 (M12) after explantation.
*, §, and # denote significant changes compared to Pre, M12, and M03, respectively.

Conclusion: Increased diffusion parameters showed post-explantation compensatory changes of the remaining kidney, which might be induced by glomerular hyperfiltration. DWI measurements appear valuable to study pathophysiologic changes after renal transplantation.

O-088 HAND-ASSISTED RETROPERITONEOSCOPIC VERSUS STANDARD LAPAROSCOPIC DONOR NEPHRECTOMY: SINGLE BLIND, RANDOMISED CONTROLLED TRIAL

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Background: Laparoscopic donor nephrectomy (LDN) has become the preferred method to procure kidneys because of reduced surgical trauma and pain, shorter convalescence time and superior quality of life as compared with open approaches. However, safety have been debated. In theory, hand-assisted retroperitoneoscopic (HARP) combines the control, dexterity and speed of the hand-guided surgery with benefits of minimal invasive surgery, including retroperitoneal access and reduced surgical trauma. The objective is to determine the best approach for live donor nephrectomy to optimise donor's safety and comfort.
Methods: This multicenter, randomised controlled trial compares hand-assisted retroperitoneoscopic with standard laparoscopic donor nephrectomy. From July 2008 to September 2010, 190 consecutive donors were randomly assigned to left-sided HARP or left-sided LDN. Intra and post-operative data were prospectively collected and analysis on outcome was performed.
Results: Baseline characteristics were not significantly different. HARP donor nephrectomy resulted in shorter skin-to-skin time (median 162 vs. 190 minutes, $p < 0.001$), shorter warm ischemia time (2 vs. 4 minutes, $p < 0.001$), more blood loss (160 vs. 104 ml, $p = 0.063$), and less intra-operative complications (7% vs. 20%, $p < 0.001$). In the LDN group two conversions occurred, one to hand-assisted laparoscopic, and the other to open donor nephrectomy. One conversion to LDN occurred in the HARP-group. Total morphine requirement was not significantly different between HARP and LDN (11 vs. 12 mg, $p = 0.833$). Postoperative complications and hospital stay were not significantly different (both 8% and 3 days, $p = 0.135$). During follow-up estimated glomerular filtration rates in donors and recipients and graft- and recipient survival did not differ between groups.
Conclusion: Hand-assisted retroperitoneoscopic donor nephrectomy reduces operation and warm ischemia times, and provides better intra-operative safety. HARP is an important alternative for left-sided LDN.

O-089 RENAL AND CARDIOVASCULAR OUTCOMES FOLLOWING LIVING DONOR NEPHRECTOMY

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Introduction & Objectives: Knowledge of the long term health outcomes following living donor nephrectomy (LDN) are of great importance when counseling potential kidney donors. Using data from the national transplant database we report on the impact of LDN on renal function, cardiovascular disease and cardiac mortality in the UK LDN population.
Methods: Between 1 January 2001 and 31 December 2008, 4586 patients underwent a LDN in the UK. Pre and post operative data was collected prospectively by transplant units across the UK and entered into the National Health Service Blood and Transplant (NHSBT) database. This data was recovered in July 2010 with the approval of the NHSBT kidney-pancreas advisory committee.
Results: A measured pre-operative GFR and an estimated GFR after 1 year were available for 2929/4586 patients (63%). LDN resulted in a mean decrease in GFR from 103 ml/min/1.73m2 to 60 ml/min/1.73m2. 53% of patients with a pre-op GFR >60 ml/min/1.73m2 were found to have a GFR of <60 ml/min/1.73m2 following LDN. The mean GFR did not change significantly between year 1 and 5. Complete post-operative follow up data up to year 5 was available for only 784/4598 patients (17%). In patients with 5 year follow up, new onset hypertension was noted in 276/784 patients (35%), non fatal cardiac events reported in 8/784 patients (1%) and death from ischaemic heart disease reported in 1/4586 patients (0.02%).
Conclusion: Data from the UK national database shows that LDN results in a 42% decrease in the mean GFR. The GFR appears to remain stable for up to 5 years following donation. Over a 5 year period adverse cardiovascular events and cardiac mortality are rare, however high quality longer term follow up is required to establish the relationship between LDN, renal impairment and cardiovascular disease.

O-090 A COMPARISON OF OPEN AND MINIMALLY INVASIVE DONOR NEPHRECTOMY USING A STANDARDIZED DONOR MORBIDITY CLASSIFICATION

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Background: Live donor nephrectomy is a safe procedure with low rates of donor mortality [1]. Morbidity rates are more variable and may depend upon the procurement method. A standardised classification has been advocated for reporting donor peri-operative complications [2]. This study aims to compare donor morbidity after open and minimally invasive donor nephrectomy using this classification.
Methods: A retrospective study of our contemporaneous live donor database for the period January 1997 to March 2010 was performed. Donor peri-operative complications were graded according to the modified Clavien classification. Donor morbidity rates in each Clavien grade were compared between open and minimally invasive donor nephrectomy. Categorical data were analysed using Fishers exact test.
Results: Four hundred and sixteen live donor nephrectomies were performed. Complete data regarding peri-operative complications were available for 358 (86%) patients; 219 open donor nephrectomies (ODN; 112 anterior, 107 loin incision) and 139 minimally invasive donor nephrectomies (MIDN; 7 laparoscopic, 132 hand-assisted). Table 1 illustrates peri-operative complications in ODN and MIDN.

Table 1. Modifies Clavien classification donor morbidity in ODN and MIDN groups

Clavien Classification	ODN (n)	MIDN (n)	Odds ratio	P value (Fishers exact test)
Grade 1	74	33	1.67	0.04
Grade 2a	24	17	0.89	0.74
Grade 2b	5	9	0.34	0.06
Grade 2c	0	7	0	0.002
Grade 3	0	1	0	0.39
Grade 4	0	0	N/A	N/A

Conclusions: ODN is associated with increased risk of low grade Clavien classification donor morbidity. However, MIDN is not associated with increased risk of more severe (Clavien grade 3 or higher) donor morbidity.
This classification provides a method describing donor morbidity rates during patient counselling, and also allows comparison of different nephrectomy methods.
We recommend that studies of LDN should include a standardised classification of donor morbidity rates to allow more meaningful comparisons between published series to be made.
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2. Harper, J.D., et al., *Experience with 750 consecutive laparoscopic donor nephrectomies–is it time to use a standardized classification of complications?* J Urol. 183(5): p. 1941-6.

O-091 POSTOPERATIVE COMPLICATIONS IN 777 LIVING KIDNEY DONORS IN A SINGLE CENTRE

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Introduction: We assessed the complications in living kidney donors between 1968 and 2009.
Methods: Complications were scored retrospectively according to Clavien (Ann Surg 2004;240:205-213). We defined complication grade 1 and 2 as minor complication (MIC), ≥ grade 3 as major complication (MAC). Grading was performed on basis of the data of the control visits at 2 months after donor nephrectomy. Means (ranges) are given.
Results: Donor age: 48 years (19–79), females: 55%, BMI: 25 kg/m^2 (16-39). Till 2002 standard surgical method was the open nephrectomy (59% of all kidney donors), which thereafter gradually was replaced by laparoscopic nephrectomy (41% of all kidney donors). From 2004 onwards more than 90% of all nephrectomies were performed by laparoscopy. Conversion rate was 2,5%.
Complication rate was 25% in 1968-2002 (19% MIC, 6% MAC, n= 428, open nephrectomy), increased to 39% in 2003-2006 (32% MIC, 7% MAC, n=189, introduction of laparoscopy) and declined to 20% in 2007-2009 (19,5% MIC, 0,5% MAC, n=160, mainly laparoscopy).
MAC were pneumothorax and wound infections in 1968-2002, bleeding, wound infections in 2003-2006, one cerebellar infarction in 2007-2009.
In recent years MIC consisted mainly of infections and delayed recovery.

Conclusion: Complications continue to be a potential hazard to the donor. Introduction of laparoscopic nephrectomy was accompanied by a transiently increased complication rate. With longer surgical expertise complication rate declined considerably.

O-092 HAND-ASSISTED LAPAROSCOPIC DONOR NEPHRECTOMIES AND ANTERIOR EXTRAPERITONEAL DONOR NEPHRECTOMIES: WHO DOES BETTER?

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Background: Live kidney donation has evolved with laparoscopic and anterior extra-peritoneal donation developing as favoured methods. Enthusiasts advocate laparoscopic donation quoting improved recovery time and minimal complications. Adverse effects to the kidney from the pneumoperitoneum, specifically delayed graft function (DGF) require clarification. We compared outcomes in anterior both approaches.
Methods: A retrospective analysis was performed of donor nephrectomies (01/2003 to 04/2010; 268 patients) assessing outcomes in anterior extra-peritoneal and hand assisted laparoscopic (HALD) approaches. Operations were performed by Consultants and excluded paediatric recipients. The primary endpoint was DGF (dialysis $\geq$2 occasions.) Donor complications, length of stay and recipient Creatinine at 3 months were analysed.
Results: 108 patients donated via anterior extra-peritoneal approaches (Male: 63, Female: 45; mean age 45.3$\pm$1.2 (20-69)) whilst 158 patients underwent HALD (M:101, F:57, mean age 44.8$\pm$0.9 (19-71), p=NS). DGF was increased in the anterior approach compared to HALD donors (9/108 (8.3%) vs 5/158 (3.1%), p=0.06). Glomerular filtration rate was equivalent (53.25$\pm$2.76 and 53.4$\pm$1.68 respectively, p=0.94) Donor complications were equivalent (chest infections: 7.4% and 7.5%; urinary tract infections: 3.7% and 2.7%; blood transfusion requirement 2.7% and 2.5% respectively; all p=NS.) Wound infections were higher for HALD's (3.7% vs. 8.2%, p=0.14) and neuralgia in the extra-peritoneal approach (4.6% vs. 1.3%; p=0.09.) Length of stay was significantly shorter in HALD donors (3.7$\pm$0.18 vs. 4.4$\pm$0.27 days; p=0.01)
Discussion: Increased live kidney donation has heightened interest in potential procurement benefits with advocates for varying approaches developing. The adverse effects of the pneumoperitoneum on DGF appear unfounded. There is a tendency towards increased neuralgia with anterior approaches with equivalent renal function and other complications across both groups. Length of stay was shorter for HALD demonstrating potential economic benefits. Enthusiasm for both approaches is justified although patient preference undoubtedly targets approaches.

O-093 WEIGHT GAIN AFTER KIDNEY DONATION

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Introduction: Overweight (BMI 25-29.9 kg/m2) and obesity (BMI $\geq$ 30 kg/m2) in (candidate) living kidney donors are common. These are known cardiovascular risk factors and associated with loss of kidney function. In obese patients the risk of perioperative complications is increased. Therefore in our centre we urge such donors to lose weight. For obese donors attaining a BMI $<$30 is a prerequisite for donation.
In this study we investigate the periprocedural weight changes in living kidney donors. We hypothesize that weight gain after intentional weight loss for donation occurs frequently.
Methods: In a retrospective observational cohort of 63 live kidney donors: weight at start of screening, weight at admission for the donation and weight 1 year after donation were recorded.
Results: 63 donors were included (BMI $<$25 n =24, BMI 25-30 n=29, BMI $>$30 n=10). Mean age was 56.5 ($\pm$sd), 58,4 ($\pm$ sd) and 55.1 ($\pm$sd) year and the percentage of men was 25, 52 and 60 respectively. (p=0.06)
48% of donors managed to lose weight predonation, 73% gained weight after donation and 51% weighed more a year after donation than at the start of the screening. Of ten patients with a BMI$>$30 at presentation, 8 gained weight after the operation and 4 eventually weighed more than before the screening.
One year after donation: 76% of donors is overweight: BMI25-30 n=36, BMI$>$30 n=12.
Conclusion: Many donors who are either overweight or obese can lose weight before kidney donation. However in the year after donation weight gain is common. The current practice of weight loss prior to donation is possibly adequate to lower perioperative risk. However one should realize that predonation obesity will recur and may increase long term cardiovascular risk and risk of future decline in kidney function.

O-094 EXPANDED CRITERIA FOR LIVE DONOR NEPHRECTOMY

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Introduction & Objectives: Although a cut off age of 60 has been proposed by many programs as a limit for kidney donation, some centers are pushing this limit to people over 60. We herein present our series of live donor nephrectomy in people $>$ 60 years old. Furthermore we provide data on the related recipients.
Material & Methods: A retrospective analysis of the kidney transplant data base was performed at our institution for live donor nephrectomy and subsequent kidney transplantation. We defined three groups based on patient age: group 1 $<$ 50, group 2 50-60 and group 3 $>$ 60 year old. Peri-operative complications, hospital stay, delayed kidney function and preoperative and 12-month postoperative glomerular filtration rate (GFR) were analyzed.
Results: The analysis identified 192 live donor nephrectomies performed between 2000 and 2009. Group 1, 2 and 3 included 79 (41%), 73 (38%) and 40 (21%) donors respectively. There were no differences in major and minor complications and hospital stay between donors from the three groups (p=0.8). The mean preoperative and postoperative 12 month GFR in donors younger than 50 was 113, and 83 ml/min. It was 99 and 69 ml/min in donors between 50 and 60 year old and 94 and 58 ml/min in donors older than 60. (p=0.001). The mean 12 months postoperative GFR in recipient from donors from group 1, 2 and 3 was 67, 58 and 44 ml/min. (p=0.001). The acute rejection and acute tubular necrosis rates did not statistically differ between recipients from the three groups. Furthermore there was no kidney graft loss in recipients from older donors ($>$ 60).
Conclusions: The use of kidneys from older living donors appears to be acceptable in the elderly recipients. However, in younger recipients, unless considered extremely urgent, kidneys from younger donors should be recommended.

O-095 THE CHANGING DEMOGRAPHIC OF LIVE DONOR RENAL TRANSPLANTATION: A SINGLE UK CENTRES EXPERIENCE

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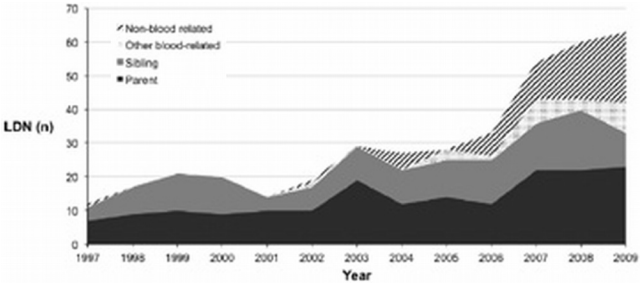
Background: Live donor transplant (LDT) activity is increasing in the UK and now accounts for one in three renal transplants [1]. Legislative changes permitting emotionally related, paired exchanges and altruistic donation have increased the potential donor pool [2]. However, blood group and HLA tissue type matching of "non-blood related" pairings is potentially less favourable. We sought to establish the changing demographics of our live donor programme.
Methods: A retrospective study of our contemporaneous live donor database for the period January 1997 to March 2010 was performed. Donor-recipient relationship was categorised as parent-child, sibling-sibling, other blood related or non-blood related. The proportion of each category was established for each year of the programme.
Results: Four hundred and sixteen LDTs were performed during the study period. The rate of LDT increased five-fold in 13 years (Figure 1). Eighty four percent of donor-recipient pairs were blood related (n=351; parent-child (n=203); sibling-sibling (n=137); other blood relative (n=11)). Sixteen percent of donor-recipient pairs were non-blood related (n=65; spouses (n=46); friends (n=17); altruistic donors (n=2)). Nine LDT were performed as part of a paired exchange programme. Non-blood related pairings accounted for 6% of the programmes activity prior to 2007 (n=13) compared to 12% after 2007 (n=42).



Conclusion: Reflecting national trends, LDT has increased five-fold at our centre over 13 years. Legislative changes and improved access to LDT have increased the proportion of non-blood related pairings. This may have implications for immunosuppressive protocols needed to overcome potentially blood group incompatible and unfavourable HLA mismatches.
References:
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O-096 HAND-ASSISTED LAPAROSCOPIC DONOR NEPHRECTOMY – DO WE NEED HAND PORTS?

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Background: Hand port devices (HPD) are used for Hand-Assisted Laparoscopic Surgery including Donor Nephrectomy (HALDN) allowing for decreased morbidity, shorter hospital stays and improved cosmesis. HALDN provides advantages over totally laparoscopic donation including tactile feedback, rapid control of bleeding by digital pressure, better exposure, rapid kidney removal and a shorter learning curve. HPD's, however, remain costly limiting adoption of the technique, particularly in the developing world with limited resources, resulting in alternative methods being preferred. We have developed experience in performing HALDN without a HPD ("device free") and we examined potential adverse effects to performing "device free" donor surgery.
Methods: A retrospective analysis was performed of left HALDN's utilising a standardised protocol over a 3 year period (2007-2010, 164 patients). 84 patients underwent device free HALDN whilst in 80 patients a HPD (Gelport™) was utilized. The primary endpoint was operation duration, with secondary endpoints of wound infections and incisional hernias.
Results: There was no difference in operation duration for the device free (98 minutes; range 43-215 minutes) and HPD group (94 minutes; range 36-180 minutes; p=0.37). A device was required in 3 (3.6%) patients in which device free approach was attempted. There was no difference in wound infection rates (0% vs. 2.5% respectively; p=0.24), all treated by conservative management. Incisional hernia incidence was also equivalent (2.4% vs. 1.4% respectively; p=1.00)
Conclusion: HALDN has revolutionised the process of renal donation, whilst device free approaches cause equivalent operating time and patient outcome. Fears of adverse operating conditions, due to pneumoperitoneum compromise proved unfounded. This approach has significant implications in both cost benefit and translation to developing units, as the cost of a Gelport™ is over €400 per single use. This ensures that this valuable source of potential donor organs is maximally utilized.

Liver / intestine miscellaneous

O-097 CHRONOTROPIC INCOMPETENCE ON DOBUTAMINE STRESS ECHOCARDIOGRAPHY IN PATIENTS WITH END-STAGE LIVER DISEASE

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Introduction: Dobutamine stress echocardiography (DSE) is an accepted screening test for preoperative cardiac risk assessment in candidates for liver transplantation (LT). However, many patients with end stage liver disease fail to achieve the target heart rate predicted for respective age and gender (chronotropic incompetence). The aim of this study is to examine the prevalence and causes of chronotropic incompetence in patients with ESLD.
Patients and Methods: A retrospective review of 111 patients who underwent a DSE between March 2009 and September 2010. The mean age was 55.1±8.4 years and 77 (69.3%) were men. Seventy-five (67.5%) patients had MELD score ≤15 and 9 (8.2%) patients had MELD score > 25. At the time of evaluation, 68 (61%) patients were on β-blockers, primarily for variceal bleeding prophylaxis.
Results: Overall, 80 (72.1%) patients completed the stress protocol and 41 (51.2%) patients achieved the target heart rate. Thirty-one (27.9%) patients could not complete the stress protocol and required early termination of test due to abnormal symptom or/and ECG findings. Overall, 17/68 (25%) patients with β-blockers achieved the target heart rate.

Predicted Maximal heart Rate	β-blocker (n = 68) n (%)	No β-blocker (n = 43) n (%)
Achieved target HR, ≥ 85%	17 (25)	27 (62.7)
Not achieved target HR, < 85%	30 (44.1)	9 (14)
≥ 80%	5 (7.3)	3 (6.9)
≥ 75%	11 (16.1)	6 (13.8)
> 65%	20 (29.4)	8 (18.6)
< 65%	11 (16.1)	0 (0)
Not completed the protocol	21 (30.9)	10 (23.3)

Eleven (9.9%) patients did not even achieve 65% of the predicted target heart rate, and notably all of them were on β-blockers. Thirty (73.1%) of 41 patients who achieved the target heart rate had MELD score ≤15 compared with 11 (26.9%) patients with MELD score > 15 ($p < 0.05$).

Predicted Maximal heart Rate	MELD ≤ 15 (n = 75) n (%)	MELD > 15 (n = 36) n (%)
Achieved target HR, ≥ 85%	30 (40)	11 (30.5)
Not achieved target HR, < 85%	26 (34.6)	13 (36.1)
≥ 80%	3 (4)	5 (13.8)
≥ 75%	10 (13.3)	7 (19.4)
> 65%	19 (25.3)	9 (25)
< 65%	6 (8)	5 (13.8)
Not completed the protocol	19 (25.4)	12 (33.3)

Conclusions: Chronotropic incompetence on DSE is frequent in patients with ESLD. In absence of any cardiac symptoms or/and ECG findings during DSE, a lower cut-off for target heart rate may be acceptable when patients are on β-blockers or/and MELD score >25 to avoid unnecessary further investigations. Large prospective studies are needed to support these findings.

O-098 INCIDENTAL PORTOPULMONARY HYPERTENSION DISCOVERED AT THE START OF LIVER TRANSPLANTATION, "*TO GO AHEAD OR TO LET GO...*"

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Background: Portopulmonary hypertension (PPHT) is the association of pulmonary hypertension (mean pulmonary artery pressure [mPAP] >25 mmHg) and portal hypertension with or without chronic liver disease. Moderate PPHT (mPAP >35 mmHg) is associated with higher morbidity/mortality and severe PPHT (mPAP> 45mmHg) is generally considered a contra-indication for Liver Transplantation (LTx). Moderate to severe PPHT may develop during the waiting time of LTx period. A retrospective analysis was done to review the short-term outcome of LTx in patients with incidental PPHT (e.g. diagnosed at the start of LTx and unkown at time of listing).
Methods: All medical records of patients with incidental PPHT were reviewed. Lab-MELD at time of LTx, mPAP immediately pre-LTx, post-LTx hospital stay, type/length of post-LTx medical treatment for PPHT and patient survival were analyzed (see Table 1).
Results: Between 2000-2011, 9/653 patients were diagnosed with moderate to severe PPHT at time of LTx induction. LTx was pursued in 7 patients. Of those, 6 had uneventful post-LTx recovery with spontaneous or medically assisted (vasodilators) resolution of PPHT; and 1 succumbed to complications of extra-corporeal membrane oxygenation. LTx was started but aborted in 1 due to hemodynamic unstability. LTx was not started in 1 who later received combined heart/lung/LTx.
Conclusion: The incidental discovery of a previously unknown moderate to severe PPHT at the start of LTX is a possibility that LTx teams should be aware of. PPHT has usually been seen as a contra-indication for LTx. However, favorable outcome in 6/7 recipients suggests that LTx should not necessarily be aborted in case of incidental PPHT.

Abstract O-098 – Table 1. LTx in patients with incidental PPHT

	Indication	labMELD	mPAP at induction (mmHg)	hospital stay (days)	Medical treatment post-LTx	Outcome	Follow-up (months)
♂/54yr	Post-ethyl cirrhosis	32	44	38	Spontaneous resolution	Alive and well	15
♀/37yr	Post-ethyl cirrhosis 26	26	40.6	42	Epoprostenol IV during 13 months	Alive and well	13
♀/62yr	HBV	31	46.7	92	Sildenafil PO during 9 months	Alive and well	12
♂/61yr	Post-ethyl cirrhosis and HCC	12	36.7	6	Extra-corporeal membrane oxygenation	†6d post-LTx	–
♀/67yr	Sarcoidosis	36	34.3	–	Aborted LTx	†1d post-LTx	–
♀/54yr	Post-ethyl cirrhosis	24	35.3	22	Spontaneous resolution	Alive and well	16
♀/53yr	Postethyl + HBV cirrhosis	19	37.3	19	Spontaneous resolution	Alive and well	55
♀/53yr	PBC	17	51.7	58	Sildenafil PO	Alive and well	6
♂/42yr	HCV	17	53.3	–	Listed for combined heart/lung/LTx	Alive and well	120

O-099 LIVER INMUNOPROTECTIVE EFFECT ON THE KIDNEY ALLOGRAFT IN SIMULTANEOUS LIVER AND KIDNEY TRANSPLANTATION

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Background: Simultaneous liver-kidney transplantation (SLK) has less incidence of renal graft rejection and inmunological graft lost against the receptors of an isolated renal transplantation (RT). In addition, a low rejection incidence and a good renal graft evolution have ben reported in cross-match (CM) positive (+) SLK patients. The low prevalence of immunological complications in high-risk immune ("HRI") SLK patients, suggests a liver's inmunoprotective effect on the kidney graft.
Material and methods: We present our experience in "HRI" SLK patients, defined as CM by cytotoxicity (CDC) post DTT + and/or "HRI" + pre transplantion (Tx). From May 1993 until December 2010, 58 SLK Tx were made (27 retransplanted patients), and eight patients had CM + pre Tx and other four patients had negative CM but positive "HRI".
Results: Of 12 "HRI" patients, 3 (25%) patients had graft dysfunction related to humoral acute rejection (HAR) during the first month after SLK Tx. Only one of these patients (33%) received Apheresis and Rituximab treatment with a good response. In the other two patients, HAR was resolved without specific treatment. None of 12 patients after 45±40 months follow-up, loss graft related to inmunological etiology. Six of 8 CM + pre Tx patients became negative post Tx.
Conclusion: High-risk inmune SLK patients have a low prevalence of immunological complications which suggests an inmunoprotector role of the liver on the kidney allograft in these patients.

O-100 EVOLUTION OF KIDNEY FUNCTION AFTER LIVER TRANSPLANTATION FOR ADULT POLYCYSTIC LIVER DISEASE AND INDICATIONS FOR COMBINED LIVER AND KIDNEY TRANSPLANTATION

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Background: Adult polycystic liver disease (PLD) is frequently associated with autosomal dominant polycystic kidney disease (ADPKD). Established indication for combined liver and kidney transplantation (CLKTx) is end stage renal failure. If renal insufficiency is less advanced, indications for combined kidney transplantation (KTx) are controversial. We reviewed our experience with isolated liver transplantation (LTx) and CLKTx in patients with PLD.
Methods: Between 1995-2008, 56 patients originating from 2 collaborating centers underwent LTx for PLD. 7 patients with isolated PLD received LTx alone. Of 49 patients with combined PLD and ADPKD, 31 underwent isolated LTx and 18 CLKTx. Among the 18 CLKTx recipients, 11 were dialysis-dependent pre-transplant whereas 7 had a creatinine clearance (CrCl) between 15 and 38 mL/min.
Results: Median follow up is 34 months (range, 26-167). 1 and 5-year patient and liver graft survival were 96% and 94%, and 96% and 90%, respectively. The 1 and 5-year kidney graft survival (death censored) is 100%. Of the 31 patients who underwent isolated LTx for combined PLD and ADPKD, 29% (n=9) developed terminal renal failure post-LTx. Their mean pre-LTx CrCl was 76 mL/min (range, 48-110). The mean pre-LTx CrCl in the 71% patients who display stable kidney function post-LTx was 78 mL/min (range, 47-153). Pre-LTx CrCl was not a significant factor for the development of renal failure after isolated LTx for combined PLD and ADPKD (p=0,835).
Conclusion: This series demonstrates that short- & long-term survival after LTx and CLKTx for PLD is excellent. In patients with clearly-proven & evolving renal impairment pre-transplant, CLKTx is the preferred option, anticipating the need for later KTx. In patients with preserved/mildly disturbed renal function, nephron sparing strategies are essential since evolution towards renal failure is seen in 29%, without clear prognosis factors.

O-101 LIVER TRANSPLANTATION (LTx) FOR TRANSTHYRETIN SYSTEMIC AMYLOIDOSIS DISORDERS: ANALYSIS FROM THE FAMILIAL AMYLOIDOTIC POLYNEUROPATHY WORLD TRANSPLANT REGISTER (FAPWTR)

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Background: Transthyretin (TTR) systemic amyloidosis disorders are treatable with Ltx. The FAPWTR was established in 1993 to assemble data on such patients.
Methods/Results: By December 2009, 1798 patients/1953 liver transplantations were reported to the FAPWTR from 72 centers in 19 different countries. Most transplantations were done in Portugal (n=866), France (n=216), Sweden (n=130) and Brazil (n=91). More than 45 different TTR-variants have been reported, the commonest being Val30Met (85%) followed by Ser77Tyr, Thr60Ala and Tyr114Cys. Gastrointestinal, cardiovascular and extraneurological manifestations appear more often in non-Val30Met than in Val30Met patients. 15% of the non-Val30Met patients underwent liver and heart transplantation compared to 0.1% of the Val30Met patients. Different countries show varying age at onset in Val30Met patients, with Brazil having the youngest patients and Sweden the oldest (32 years and 45 years, respectively). After Ltx, 80-90% of the ValMet30 patients reported stable or improved clinical symptoms compared to 60-65% in non-Val30Met patients. The overall 5-, 10- and 15-year patient survival is 79%, 70% and 64%, respectively. Most common cause of death is cardiac. Val30Met patients with a disease duration >7 years disclose inferior 5-year survival than patients with a duration ≤ 7 years (58.2% and 84.7%, respectively $p<0.001$). Results improve when analyzing patients transplanted in the last 5 years, but the 5-year survival still remains significantly better in patients with less than 7 years disease duration (72.1% and 88.7%, respectively $p<0.05$).
Conclusion: LTx is lifesaving in patients with TTR amyloidosis. Val30Met and non-Val30Met TTR mutations differ clinically. Cardiac related post transplant death is more common among these patients than patients transplanted due to chronic liver disease. FAPWTR data confirm the advantage of early transplantation but the long-term effects, especially for non-Val30Met patients, need further clarification.

O-102 INDOCYANINE GREEN CLEARENCE AS A TOOL TO PREDICT THE NEED OF LIVER TRANSPLANTATION IN PAEDIATRIC ACUTE LIVER FAILURE

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Bakground: Paediatric Acute Liver Failure (PALF) is a rare disease that results in death or the need of Liver Transplantation (LT) in nearly 50% of cases. The scoring systems aviables for the prognosis evaluation in adults are not able to predict survival without LT of paediatric patients. We aimed to assess the use of Indocyanine Green Plasma Disappearance Rate (ICG-PDR) as a tool to predict the evolution of patients affected of PALF.
Patients and Methods: All the patients enrolled were younger than 18 years without chronic liver disease, and present a prothrombin time (PT) higher than 15 sec or INR higher than 1.5 in the presence of Hepatic Encephalopathy (HE) or a PT higher than 20 sec or INR higher than 2 regardless of the HE. ICG-PDR were taken when the ALF diagnoses were performed and repeated every 24 hours until ALF resolution, death or LT. We calculated the Sensitivity (S), Specificity (E), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of ICG-PDR, KHC and Clychy s criteria.
Results: From January 2003 to July 2010, 68 patients were diagnosed of PALF. A total of 217 ICG-PDR were performed with a median ICG-PDR of 12.6%/min (r:6-26.95%/min). The median value of ICG-PDR was significantly lower in patients who suffered and irreversible liver injury compared with those who survived without LT (4.1%/min vs 20.5%/min respectively) (P 0.001). The S and PPV were higher for ICG-PDR than KHC and Clichy s criteria (S of 91.6%, 84% and 76% and VVP of 84.6%, 55% and 71% respectively)
Conclusion: ICG-PDR is an easy non-invasive tool that provides an accurate estimation of the need of LT in the setting of PALF under hemodinamic stability conditions.

O-103 CLINICAL RELEVANCE OF HLA-ANTIBODIES AFTER INTESTINAL TRANSPLANTATION

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Despite a negative crossmatch, intestinal transplant (ITX) recipients are capabale of mounting humoral immune reponses early after transplantation. The development of donorspecific HLA-antibodies (DSA) is associated with severe vascular graft injury and graft failure. We examined the development of HLA-antibodies in association with the clinical course and histopathological findings of 28 ITX recipients.
Between 06/2000 and 02/2011, 28 patients with a median age of 39.3±13.4 years received an isolated intestinal graft (n=18) or a multivisceral transplan-

tation (n=10). HLA-antibodies were screened regularly before and after transplantation. Panel reactive antibodies (PRA) of >10% HLA I or II were considered positive. In case of DSA, treatment was initiated with plasmaphereses and ivIG. In the event of DSA-persistence and/or treatment refractory rejection, rituximab and/or bortezomib were added.
14 patients showed HLA-antibodies after transplantation. 5 developed non-donor-specific (NDSA) HLA-antibodies, whereas 9 showed strongly positive DSA (20%-82%) with significant rejection episodes around the time of positive samples. Interestingly, 8 patients showed DSA within 6 months after transplantation, whereas one patient developed DSA 10 years after ITX. All but one patient, who was successfully treated with steroid pulse therapy alone, received plasmapheresis and ivIG. Rituximab was added in 7 patients at a dose of 375 mg/m with 1.6±0.9 applications/patient. One patient developed a rituximab-resistant antibody-mediated rejection and was successfully treated with bortezomib. PRA-values decreased with antirejection-therapy in 8 of the 9 patients. One patient died of a severe therapy-refractory cellular and humoral rejection.
Development of HLA-antibodies after ITX is often significantly associated with acute rejection episodes. Early diagnosis and therapy is necessary to prevent severe graft injury and graft loss. Combination therapies including rituximab are mainly used to control rejection. However, proteasome inhibitors like bortezomib may serve as a new treatment option in cases of persistent DSA-levels and associated refractory rejection.

O-104 VIABILITY OF THE HUMAN SMALL BOWEL GRAFT IN THE SCOPE OF INTESTINAL TRANSPLANTATION (ITX)

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Background: Results of ITX remain inferior compared with other transplant types. Pre-transplant graft viability is the key factor. The intestine is extremely sensitive to transplant-related damage. Effects of brain death (BD) on the intestine and the quality of intestinal grafts from donors deceased after cardiac death (DCD) are largely unknown. This descriptive study aims to assess intestinal graft viability and gain insight in pathophysiological damage mechanisms as a base for protecting strategies to improve ITX outcome.
Methods: Patient data, blood (before aortic-flush) and intestinal samples were collected during multi-organ-donation (MOD:BD/DCD) after aortic-flush. Living kidney donors provided control blood. Outcome parameters: histological Park score (0-8), Claudine-3 stain, IL-6 western blot (WB), serum lipopolysaccharide-binding-protein (LBP) and CRP.
Results: *Donor characteristics:* Sixty intestinal tissue samples were recovered from 33 donors.

Table 1. Donor descriptives

		DBD	DCD
Donors 2008–2009		22 (66%)	11 (33%)
BD Duration		653 min (420-948)	–
Ischemia Times	Mean WIT0	–	23 min
	Mean WIT1	–	30 min
	Mean CS time	298 min	210 min
	Mean total CIT	407 min	310 min
	Mean total ischemic time (TIT)	407 min (143–938)	355 min (187–1112)

Brain death (BD), Deceased after BD Donor (DBD), Deceased after Cardiac Death Donor (DCD), Warm Ischemia Time (WIT), Cold Storage (CS), Cold Ischemia Time (CIT).

Histology/structure:

Table 2. Intestinal Histology (Park score 0 (least damage)-8(most damage))

	Total Group	DCD	DBD	Difference DCD/DBD	Optimal Donor	Suboptimal Donor	Difference Sub/Optimal
Park score jejunum	3.9	4.1	3.8	p=0.617	3.4	3.9	0.815
Park score ileum	2.9	3.8	2.4	p=0.076	1.6	3.2	0.112
Difference jejunum /ileum	p=.032	p=0.734	p=0.017		p=0.058	p=0.162	

Deceased after Cardiac Death Donor (DCD), Deceased after Brain Death Donor (DBD), differences between DCD/DBD and Sub/Optimal Donors: Mann Whithney U test, Differences between jejunum and ileum Park score: Wilcoxon signed rank test.

Mean Park score was 3.9 (denuded villi) for jejunum and 2.9 (extensive epithelial lifting) for ileum. Ileum was less damaged than jejunum (p=.032). Optimal donors were selected by US OPTN criteria for potential intestine donation. Histology was unaffected by donor quality. There was a trend for better jejunum/ileum Claudine staining in optimal donors (.069/.062). SERUM MARKERS: CRP/LBP levels were higher for BDD and DCD donors compared to control (p=0.001,p=0.003/p=0.002,0.004). CRP/LBP levels did not differ between deceased donor types. WB: Jejunum Il-6 expression was higher in DBD vs. DCD (p=.046).
Conclusion: The intestinal graft endures substantial damage from BD and ischemia causing structural deterioration reflected by histology and the tendency for better Claudine-3 stain in optimal donors. Jejunum is more susceptible to structural damage (vs. ileum). Il-6 WB shows increased local jejunal inflammation in DBD vs. DCD. Increased serum CRP/LPB levels indicate systemic inflammation. Donor structure/barrier protective interventions could be a major step towards improvement of ITX.

O-105 COMPREHENSIVE SURGICAL INTESTINAL RESCUE PROGRAM IN ADULT PATIENTS: BOLOGNA EXPERIENCE

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Background:
Surgical approaches to complicated benign intestinal failure are worldwide accepted, especially in pediatric population. Intestinal transplant surgery is thought to rescue patients developing total parenteral nutrition complications. We report our experience about surgical intestinal rescue on adult population with intestinal failure.
Methods: An intestinal rehabilitation program started at our institution comprehensive medical rehabilitation, surgical bowel rescue, and transplantation. From 2000 and 2010, among 95 adult patients referred by our gastroenterologists for bowel rehabilitation,48,4% (45 adult patients) underwent 46 transplantations (34 isolated intestinal graft and 12 multivisceral); underlining disease were mainly represented by short bowel syndrome (SBS), Gardner's syndrome and intestinal pseudo-obstruction. 52,6% (50 patients) underwent surgical rescue consisting in bowel resection, adhesiolysis, stricturoplasty, liver transplant with porto-caval hemitrasposition (6 cases in 5 patients). Underlining disease were mainly represented by intestinal fistulas, stenosis or perforations, SBS, cocoon syndrome, complete portal thrombosis.
Results: After a mean follow up of 1529±1275 days, among transplant population, 23 patients (51%) are currently alive with a 1-3-5-years patient survival of 77%, 58% and 53% respectively; the 1-3-5 years graft survival is 73%, 56% and 51% respectively. After a mean follow-up of 901±404 days, among rescue population, 32 patients (82%) are actually alive (2 died and 5 lost at follow-up) and 75% is completly off TPN, 25% is on oral feeding with a TPN support. The 1 and 3 years survival rate is 100% and 83% respectively.
Conclusions: Deaths occurred mainly in transplant population: intestinal surgical rescue, when possible, is optimal.

O-106 YKL-40-GENE POLYMORPHISM IN THE DEVELOPMENT OF ACUTE CELLULAR REJECTION AFTER TRANSPLANTATION FOR HCV-INDUCED LIVER DISEASE

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Background: The development of end-stage liver and graft disease is suspected to be partially determined by an individual genetic background. YKL-40 is a chitinase-like protein, which is involved in matrix remodeling, influences the development of hepatic fibrosis. Aim of our study was to determine the prevalence of YKL-40-gene polymorphism after transplantation for HCV-induced liver disease and to describe their role in the incidence of acute cellular rejection (ACR), graft fibrosis development and antiviral treatment response.
Methods: 149 patients, who underwent liver transplantation for HCV-induced liver disease, has been genotyped for YKL-40 (rs4950928; G/C) by TaqMan Genotyping Assay and correlated to 616 post-transplant graft biopsies regarding inflammation, fibrosis and evidence for acute cellular rejection (ACR).
Results: No association of YKL-40-gemotypes was observed regarding mean inflammation grade (p=0.216) and antiviral treatment outcome (p=0.442). However, significant differences among YKL-40-genotypes could be detected in the mean post-operative duration of advanced (F3-4) fibrosis development in years: t(CC)=4.6 vs. t(CG/GG)=2.4; p=0.006 and ACR-incidence (CC=60.4% vs. 74.2%, CG=25.0% vs. 23.8% and GG=14.6% vs. 2.0%; p=0.009). Furthermore, lower ACR-rate was observed among recipients of grafts originating from the opposite donor gender (39.8% vs. 20.0%; p=0.012) and patients initially receiving dual immunosuppression (CNI+MMF vs. CNI; p=0.001).
Conclusion: Recipient genetic factors (YKL-40-polymorphism) and gender mismatch seem to be involved in the development of ACR after liver transplantation for HCV-induced liver disease. However, their causal relationship to ACR in HCV-positive graft recipients still remains unclear.

O-107 ABCG5/8 AND ABCB1 POLYMORPHISMS AS RISK FACTORS FOR ADVANCED FIBROSIS FOLLOWING LIVER TRANSPLANTATION

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Background: ATP-binding cassette (ABC) transporters are represented by a family of transmembrane proteins that facilitates the transport of different substrates, including immunosuppressive drugs, in an ATP dependent manner. These genes were slightly investigated following liver transplantation (LT).
Aim: To assess the influence of ABC-transporters polymorphisms on graft survival and to identify predictive factors of severe HCV recurrence after LT.
Methods: We prospectively genotyped ABCG5 (C1810G), ABCG8 (C1199A and C1895T), ABCB1 (C1236T, G2677T, C3435T) in 174 LT recipients (49 with recurrent hepatitis C after LT, 125 controls transplanted for other liver diseases) by PCR-restriction fragment length polymorphism assay.
Results: Analyses of single nucleotide polymorphisms (SNPs) of the above mentioned genes revealed no differences regarding the distribution of the genotypes between HCV and non-HCV patients. Graft survival in HCV patients was significantly lower compared to controls (45.2% vs 74.5% at 10 years, p=0.01). None of the genotypes of ABCG5/8 or ABCB1 genes influenced the graft survival following LT. In the univariate analysis ABCG8-1895C/C (p=0.03), ABCB1-3435T/T (p=0.01), ABCB1-2677G/T+T/T (p=0.04) and treatment with bolus corticosteroids for acute rejection episodes (p=0.01) were identified as predictors of fibrosis stage 3-4 in HCV recurrent hepatitis C. Independent predictors of severe HCV recurrence included ABCG8-1895C/C (p=0.03), ABCB1-3435T/T (p=0.01) and administration of corticosteroids bolus (p=0.01).
Conclusions: HCV LT recipients with ABCG8 and ABCB1 polymorphisms have a significantly higher prevalence of advanced fibrosis. Overall LT graft survival is not influenced by presence of ABC-transporters genes polymorphisms

O-108 A SCORE PREDICTING OUTCOME OF LIVER ALLOGRAFT RETRANSPLANTATION FOR HEPATITIS C

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Background: Cirrhosis induced by hepatitis C virus (HCV) is the leading cause of liver orthotopic transplantation (OLT) in the US. All the recipient will develop a recurrence of HCV infection. Re-OLT for HCV infection has shown worse results. Our goal was to design a score predicting outcome after second OLT for HCV.
Methods: Data were provided from the Scientific Registry of Transplant Recipients from 01.01.1990 to 31.01.2009. The patients receiving 2 OLT for HCV were included. Patients for whom interval between 2 OLT was < 30 days were excluded. Survival was measured from the date of second OLT. Univariate analysis of variables at 1st and 2nd OLT associated with survival was performed with Cox and Log-rank test, variables with $p < 0.15$ were considered for score assessment. Multivariate analysis was performed with a logistic regression. The score was its validation was developed with an Akaike test. $P < 0.05$ were considered significant.
Results: 1422 patients received a second OLT for HCV recurrence. Variables retained for the score were, at the second OLT: donor age (DnAge), pre-Tx serum creatinin (Creat), INR, pre-Tx serum albumin (Alb); at the first OLT: recipient age (RecAge); and the interval between 1st and 2nd OLT (Int). The score = (1.01*DnAge + 1.27*logCreat + 0.88*logInt + 1.14*INR + 1.01*RecAge + 0.85*Alb +2)*20. The score was split into 3 ranges including similar number of patients: Score I < 30, Score II 30-40, Score III > 40. At 3 years the Receiver Operating Characteristic area under curve was 0.6432, and the survival was: Score I = 65%, Score II=48%, Score III=31% (Log-rank < 0.0001).
Conclusion: Our score is discriminant to identify the few patients for whom a re-OLT for HCV infection will provide a prolonged survival.

Antigen presentation and T cells

O-109 CD8 T CELLS RECEIVE HELP FROM INDIRECT-PATHWAY CD4 T CELLS BY PROCESSING ALLOANTIGEN ACQUIRED FROM MHC I AND OTHER ALLOANTIGENS ON GRAFT CELLS

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Introduction: We investigated how indirect-pathway CD4 T cells that recognise processed alloantigen presented by recipient APCs provide unlinked help for cytotoxic CD8 T cells recognising intact MHC I on donor cells.
Methods & Results: TCR75 mice (B6.RAG1$^{-/-}$; monoclonal CD4 T cells recognising H2-K^{d} indirectly) acutely rejected BALB/c heart grafts (MST 15d) when reconstituted with CD8 T cells from WT but not MHC II$^{-/-}$ (39d) or H-2DMa mice (unable to process antigen; 55d), with corresponding CD8 T cell activation. Conversely, CD8 T cell activation and rejection of B6xBALB/c grafts (enabling additional "linked" help via direct allorecognition of both TCR75 CD4 and CD8 T cell target epitopes on donor APCs) was similar in all three groups. Female Mar recipients (B6.RAG2$^{-/-}$; monoclonal CD4 T cells recognising male H-Y peptide indirectly) also rejected male BALB/c grafts more rapidly if reconstituted with CD8 T cells from WT (MST 12d) rather than MHC II$^{-/-}$ (21d) or H-2DMa ($>$50d) mice, but B6xBALB/c grafts were rejected with a similar tempo in all three groups. In contrast, WT CD8 T cell-reconstituted Mar recipients challenged with male B6 APCs did not reject female BALB/c grafts despite activation of the Mar CD4 T cells. Effective help was thus only generated when H-Y and MHC I alloantigens were co-expressed on graft cells. Finally, flow cytometric analysis revealed acquisition of MHC II by activated CD8 T cells *in vivo* and *in vitro* upon culture with WT (but not MHC II$^{-/-}$) APCs.
Conclusion: Our data suggest that indirect pathway CD4 T cells provide help to allospecific CD8 T cells through recognition of MHC I-derived or other alloantigen that is internalised by CD8 T cells via the TCR and presented as processed allopeptide on acquired MHC II.

O-110 DIRECT-PATHWAY CD4 T CELLS ARE UNABLE TO PROVIDE HELP FOR CLASS-SWITCHED ALLOANTIBODY RESPONSES

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Introduction: Indirect-pathway CD4 T cells provide help for alloantibody production through cognate recognition of MHCII/allopeptide complexes on allospecific B cells. Doubt persists, however, as to whether naive or memory direct-pathway CD4 T cells can also provide, through linked recognition of T and B cell epitopes on donor APCs, "non-cognate" help for class-switched alloantibody production.
Methods and Results: T cell deficient but B cell-replete B6 TCR KO mice, when reconstituted with indirect-pathway Kd-peptide-specific TCR transgenic TCR75 CD4 T cells, mounted strong anti-Kd IgG responses against Balb/c heart grafts. In contrast, TCR KO recipients of bm12xBalb/c (F1) hearts did not develop anti-Kd alloantibody when reconstituted with ABM CD4 T cells (direct recognition of I-Abm12), despite the potential for ABM CD4 T cells and host B cells to interact via three cell clusters incorporating donor APCs. Similarly, BM chimeric TCR KO recipients of F1 allografts, in which ABM CD4 T cells are replenished from seeded ABM bone-marrow, did not mount an anti-Kd alloantibody response.
To examine the ability of memory direct-pathway CD4 T cells to provide help for generation of anti-MHCI alloantibody, TCRKO.ABM BM chimeric mice were primed with a bm12 skin graft (rejection time 10d) and subsequently received a second bm12 skin or an F1 heart allograft after 6 weeks. The second bm12 skin grafts rejected within 7 days, confirming the generation of a memory ABM CD4 T cell response, but no anti-Kd alloantibody was detected in recipients of F1 heart allografts which survived indefinitely.
Conclusions: Our results demonstrate definitively that help for anti-MHCI alloantibody responses cannot be provided by naive or memory direct-pathway CD4 T cells. Additionally our data confirm, without relying on MHCII KO models, that indirect pathway CD4 T cells can provide help for alloantibody production.

O-111 ANTIGENIC RECOGNITION PROPERTIES OF CD8+ REGULATORY T CELLS IN TRANSPLANTATION

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We recently reported that in a rat major histocompatibility complex (MHC) mismatched heart allograft model, treatment with CD40Ig, a chimeric molecule that blocks CD40L, leads to indefinite allograft survival mediated by CD8^{+}CD45RC^{low} Tregs. Although essential, the exact role of T cell receptor

(TCR)/MHC/peptide interaction in Treg activity is still unknown. We therefore characterize the allogeneic peptide(s) recognized and the TCR usage of the $CD8^+CD45RC^{low}$ Tregs.
Allogeneic peptide(s) were derived from polymorphic regions of donor MHC molecules. Sixty two overlapping peptides of 16 amino acids (aa) were tested in a coculture of Tregs with syngeneic pDCs (ratio 4:1). Moreover, the repertoire of the TCR of the $CD8^+$ Tregs was studied by flow cytometry analysis and sequencing the CDR3β region.
After six days of culture, two peptides in particular led to the activation of Tregs, as shown by the upregulation of the CD25 molecule (from 25.89% to 29.27% of CD25 expression). These activator peptides were characterized by prominent amino acids (aa) at rather central position, which could result in a large TCR repertoire diversity of the specific Tregs. We showed previously that $CD8^+CD45RC^{low}$ Tregs expressed a specific altered Vβ11 repertoire, with the same CDR3β length in all animals (9 aa). This upregulation was confirmed at the protein level, since 19.9±3.7% of Tregs from a CD40Ig-treated animal expressed the Vβ11 chain compared to 6.1±2.3% in naïve ones. Sequencing of 160 clones of Vβ11 TCRs from six long-surviving animals suggested a preferential use of a 9 aa long CDR3β and a particular Jβ region (Jβ1.6). Interestingly, conserved sequences were frequently found but no common clonotype was shared between animals, suggesting the private nature of the repertoire.
This study demonstrated that $CD8^+CD45RC^{low}$ Tregs recognize two allogeneic epitopes leading to a private TCR repertoire.

O-112 DONOR CD40 IS REQUIRED FOR THE Th17 RESPONSE TO DIRECT ALLOSTIMULATION

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Allo-reactive cytotoxic T lymphocytes (allo-CTLs) induce tubulointerstitial injury during kidney allograft rejection. CD40 is the only classical costimulatory molecule expressed on renal tubular epithelial cells (rTECs) under inflammatory conditions. Blockade of the CD40- and the CD28-pathways can induce long-term allograft survival. However, it is unclear whether CD40 blockade acts primarily on host or donor CD40. The aim of the study was to investigate the role of donor CD40 for direct T cell alloreactivity in vitro and in vivo.
Responder T cells were stimulated in vitro with allogeneic fully MHC-mismatched splenocytes. Proliferation was measured by thymidine incorporation. Cytotoxicity was tested by ^{51}Cr release against primary rTECs, which were prestimulated with IFN-β and –γ. Cytokine analysis of cocultures was performed by multiplex bead assay from supernatants and quantitative RT-PCR from isolated T cell subsets.
When CD8 and CD4 T cells were cocultured in the absence of donor CD40, proliferation, IFN-γ production, and cytotoxicity were reduced. IL-6 production and subsequent Th17 induction was completely abolished under these conditions. DCs were the main responsible stimulating cells in all assays. However, CD40 expression on target rTECs did not have any impact on cytotoxicity.
To assess the role of donor CD40 in vivo, fully MHC-mismatched skin and kidney grafts from WT and $CD40^{-/-}$ donors were performed. No difference was seen for skin graft rejection between WT and $CD40^{-/-}$ donors. In contrast, we found improved renal function and reduced intragraft IFN-γ expression for kidney allografts from $CD40^{-/-}$ donors.In summary, the absence of donor CD40 during allostimulation in vitro reduced all effector functions of allo-CTLs and abolished Th17 cell induction. Kidney graft function and inflammation were improved, when donor tissue did not express CD40. Thus, CD40 blockade might be therapeutically useful to prevent kidney allograft rejection.

O-113 TEMPORARY INHIBITION OF Trem-1 PROLONGS ALLOGRAFT SURVIVAL

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Background: Chronic allograft rejection remains the leading cause of death after the first post-transplantation year. During the multi-factorial process of allograft rejection, a pro-inflammatory role in TLR-dependent activation of the innate-immune system is inherited by "Triggering-receptor-expressed-on-myeloid-cells-1" (Trem-1). In this study we tried to elucidate the role of Trem-1 in the initial phase of chronic cardiac rejection influencing allograft survival.
Material and Methods: bm12 donor hearts were transplanted into wild type MHC-class-II-mismatched C57BL/6J recipient mice. Control mice received C56BL/6J donor hearts. Cytokine production in graft-infiltrating CD4+ cells was analyzed by ELISA. Localization of graft-infiltrating CD11b+Trem-1+ cells, as well as lymphocyte subsets, was determined with immunohistochemistry and flow-cytometry. Fibrosis and collagen deposition was determined by Masson's trichrome staining and qPCR. Following transplantation, mice were treated daily with Trem-1-inhibitory peptide LP17 or control peptide.
Results: Progressive allograft rejection of bm12-donor hearts with decreased organ function, severe vasculopathy and allograft fibrosis was evident 4 weeks after transplantation. Allograft rejection was associated with cellular infiltration and increased production of IL-17A and IFNγ. Further analysis of involvement of the innate immune system in chronic rejection showed a highly increased number of Trem-1+ antigen-presenting-cells in the allograft. Inhibition of Trem-1, leading to a reduced innate immune response, resulted in decreased vasculopathy, reduction of graft-infiltrating cells and prolongation of allograft survival. In addition, IL-17A and IFNγ production from CD4+ cells was significantly reduced following Trem-1 inhibition. Furthermore, temporary inhibition of Trem-1+ during early immune activation resulted in reduction of graft-infiltrating cells, limited pro-inflammatory cytokine production and led to continuous, long-term allograft survival.
Conclusion: Our results demonstrate an important role of Trem-1 during cardiac allograft rejection through the amplification of pro-inflammatory innate immune signalling. Blocking Trem-1+ antigen-presenting cells during early immune activation following transplantation resulted in prolongation of allograft survival.

O-114 TETRAHYDROBIOPTERIN PRECONDITIONING PROTECTS MURINE AORTIC ALLOGRAFTS FROM CHRONIC VASCULOPATHY

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Chronic allograft vasculopathy (CAV) is a major obstacle to long term graft survival and is thought to be associated with ischemia reperfusion injury (IRI). Herein we analyzed whether the attenuation of IRI by donor treatment with tetrahydrobiopterin (H4B) consequently attenuates CAV.
A fully MHC mismatched (BALB/c to C57BL/6) mouse cervical aortic transplantation model was used. Grafts were subjected to 24h cold ischemia time (CIT). Donor animals received either H4B (50mg/kg b.w.) or saline. Aortas without CIT and syngeneic animals (C57BL/6 to C57BL/6) served as controls. To determine IRI associated damage, ten hours following reperfusion glutathione tissue levels were measured and CD-31 immunohistochemistry were performed. Intimal hyperplasia was quantified by histopathology and immunohistochemistry (alpha-smooth muscle actin (α-SMA)), E-selectin, P-selectin and ICAM-1) 4 weeks following reperfusion.
Compared to controls CIT resulted in a significant reduction of glutathione tissue levels ($p<0.05$) whereas H4B-treatment leads to a significant restoration of glutathione tissue levels ($p<0.05$). Furthermore, reduced CD-31 expression following CIT was attenuated by H4B ($p<0.05$). Four weeks following transplantation prominent intimal hyperplasia was observed in the untreated group but not following pretreatment with H4B ($p<0.001$), which, by contrast, was comparable with syngeneic controls and grafts without CIT. These findings were confirmed by immunohistochemistry. Treatment with H4B elicited a significant reduction of α-SMA positive cells within the intima ($p<0.05$). Finally, reduced expression of E-selectin, P-selectin and ICAM-1 was detected in aortic grafts treated with H4B ($p<0.05$, $p<0.05$, $p<0.05$ respectively).
These data confirm that IRI strongly correlates with CAV development. A single time application of H4B before organ recovery might therefore represent a promising strategy in solid organ transplantation in order to prevent CAV.

O-115 CRITICAL DIFFERENCES OF THE IMMUNE RESPONSE WITH INCREASING AGE

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The characterization of age-dependent immune responses is gaining clinical significance. Chronological aging has been associated with an accumulation of memory T-cells, however, their function may be incapacitated. We postulated that allospecific memory T-cell responses are age-dependent.
Young (3mth) and old (18mth) C57Bl/6 recipients were sensitized with DBA2/J skin grafts. 4 wks later, memory T cells counts had increased in both old and young sensitized mice. Subsequently, DBA2/J cardiac allografts were transplanted into young and old sensitized C57Bl/6 mice. In the absence of sensitization, the tempo of cardiac rejection was recipient age-dependent and significantly delayed in old compared to young non-sensitized recipients (6.8±0.7

vs 10.0±1.3 days, p<0.0001). Sensitization accelerated rejection in an age-independent way. The temp of rejection was comparable in young and old sensitized recipients (4.9±0.1 vs 5.6±0.6 days, P=ns.). Alloreactivity (IFN-γ production) and proliferative capacity (CFSE assay) were comparable in both sensitized old and young recipients. Adoptive transfer of CD3+ T cells isolated from either young or old sensitized animals into RAG1 knockout recipients results in rejection of DBA skin grafts by 11 days in both groups, indicating an age independent memory T cell function in vivo. Next, we separated memory and naive T-cells by CD-62L beads and evaluated their function. Prior to sensitization, the IFN-γ production of CD-62L- memory T-cells was compromised in both young and old animals indicating that the primary alloresponse is driven by naïve T-cells. However, following sensitization alloresponsiveness was mediated predominantly by the memory T-cell population.
Increasing recipient age delays the tempo of acute rejection and is associated with a compromised primary T-cell response. Sensitization accelerates rejection in an age-independent fashion associated with a potent and well preserved alloresponsiveness of older memory T-cells. Those data have important clinical implications on age-adapted immunosuppression.

O-116 SYSTEMIC EFFECTS OF LIVER REGENERATION ON BETA CELLS IN DIABETIC RAT: IN VIVO AND IN VITRO STUDIES

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Background: Liver regeneration induces the release of humoral factors in situ and in blood circulation possibly contributing to pancreatic islet engraftment. This work studies the systemic effect of liver regeneration on the native pancreas in normal rats and in vitro by examining the outcome of rat RINm5F beta cells, grown in standard medium supplemented with normal or diabetic hepatectomized (Hx) rat serum.
Material and methods: 2/3 partial hepatectomy was performed in normal and streptozotocin induced diabetic Lewis rats (n=6). Pancreas from control, SHAM or Hx normal rats were weighted one, two or three days after surgery, islets size examined by insulin staining. RINm5F β cells were grown in RPMI medium containing 10% serum from Hx rats. After 24 h, cell viability and count were evaluated by CellTiter and cell counter.
Results: The ratio pancreas/body weight was higher in Hx rats compared to SHAM, 3 days after surgery: 0.58 vs 0.39 (p=0,057). Proportion of small size islets (<10 000 μm2) increased significantly from 67% to 92% (p <0.05). By comparison with the serum obtained from Sham animals at day two or three after surgery, Hx normal rat serum promoted a significant increase in Rinm5F cell viability: 126% vs 94% (p=0,005) 121% vs 94% (p=0,006) respectively and cell proliferation measured at day 3 increased accordingly: 4,62 vs 3,57 10^5 cells/ml (p=0,036). Conversely, Hx diabetic rat serum withdrawn 3 days after hepatectomy had an opposite effect with significant decrease in cell viability: 79 vs 95% (p=0,036).
Conclusion: Liver regeneration induces systemic effects on pancreatic islets and increases their viability in normal rat but not in diabetic rat. These data suggest impairment of islet engraftment in liver during diabetes.

O-117 REGULATORY T AND Th17 T CELL RESPONSES IN THE CONTROL OF AUTOANTIBODY-MEDIATED ALLOGRAFT VASCULOPATHY

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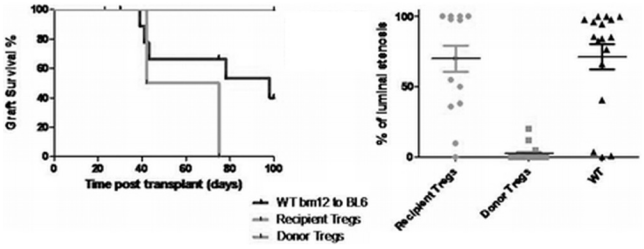
We have recently reported a role for autoantibody in the development of mouse heart graft vasculopathy that was dependent upon donor CD4T cells within the graft. Regulatory T cells are important in protection against autoimmunity but in the presence of IL-6, their differentiation is instead skewed to a Th17 response. Here we examine the inverse reciprocal arrangement between the development of Th17 and Tregs in the development of autoantibody and allograft vasculopathy (AV).
The contribution of Tregs and Th17 T cells to AV and autoantibody development was studied in an MHCII-mismatched mouse model of heart transplantation, by treating recipients with anti-CD25 or anti-IL-17 antibody and by adoptive transfer post-transplant of naturally occurring $CD25^+$Tregs (nTregs) (from either donor or recipient). Graft survival was assessed by daily palpation and autoantibody production assayed by indirect-immunofluorescence of nuclear-antigen-expressing HEp-2 cells.
Anti-IL-17 treatment influenced neither development of autoantibody nor progression of AV. In contrast, depleting recipient Tregs markedly exacerbated autoantibody production and accelerated rejection (MST 20d vs. WT 95d). Grafts from CD4-T cell-depleted donors provoked significantly less autoantibody and survived longer (MST 32d, p<0.01) than unmanipulated hearts in the Treg-depleted recipients, indicating that accelerated rejection following Treg depletion is partly due to exacerbation of humoral autoimmunity.
Surprisingly, whereas adoptive transfer of recipient nTregs influenced neither autoantibody production nor AV development, transfer of donor nTregs abrogated autoantibody and significantly reduced AV.



Our results demonstrate a previously unrealized mechanism whereby Tregs contribute to graft survival by preventing effector autoantibody responses. We hypothesise that donor nTregs are more effective at preventing autoimmunity because they recognise the same target ligand (MHC class II on host autoreactive B cells) as is recognised by the donor, helper $CD25^{-ve}$ CD4 T cells that are passengers within the heart graft.

O-118 MIGRATION INTO THE INJURED LUNG INDUCES LOSS OF CD200 ON NK CELLS: AIR-LIQUID IN VITRO MODEL

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Background: NK cells routinely infiltrate the transplanted lung as part of immune surveillance, yet during acute rejection, the NK cell induces widespread immune activation. The NK cell can regulate the immune response via CD200 expression; ligation of which inhibits DC maturation, T-cell/marcophage degranulation and pro-inflammatory cytokine secretion. We hypothesised that downregulation of NK cell CD200 would occur in response to tissue injury.
Methodology: NK migration into the *in vitro* lung was assessed using a bi-olayer air-liquid alveolus model consisting of pulmonary capillary endothelial cells (mounted below) and alveolar epithelia (mounted above) a 3um transwell layer. PBMC from lung transplant recipients (N=5) were cultured in media on the capillary side and allowed to migrate into the alveolar layer in response to injury (heat-stress). NK cells were characterised using markers of activation (CD69, CD161 and NKG2D), cytotoxicity (CD107a) and regulation (CD200 and CD200R). Transmigration between injured and control cell layers were compared.
Results: NK cell CD200 expression decreased following migration into the injured lung compared with controls (p=0.043). CD69 was upregulated on the capillary layer (p=0.034; 4642±1463 vs 3072±524) and CD161 was upregulated on the alveolar layer (p=0.043; 5729±1695 vs 10811±1189), irrelevant of tissue injury. No significant differences were observed with CD107a or CD200R.
Conclusions: Diapedesis from the vascular bed to the healthy alveolus has no impact on CD200 expression. However, diapedisis to the injured alveolus results in the downregulation of CD200, indicating that NK cell immunoregulatory capacity is lost. This would prevent inhibitory signals to CD200R immune cells including T cells, DC, and macrophages, and allow allogeneic responses to occur.

O-119 REGULATORY T CELL FUNCTION OF SOTRASTAURIN TREATED KIDNEY TRANSPLANT PATIENTS

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Background: The novel immunosuppressant sotrastaurin is a potent and selective inhibitor of protein kinase C isoforms that are critical in signaling pathways downstream of the T cell receptor (TCR). The TCR triggered intracellular signaling pathways are different for effector and $FoxP3^+$ regulatory T cells (Tregs). Therefore, inhibition of PKCθ may have a differential effect on the function of effector T cells and Tregs.
Methods: The frequency, function and phenotype of peripheral $FoxP3^+CD4^+CD25^{high}$ Tregs and T effector cells were analyzed in kidney allograft patients treated with basiliximab, everolimus, steroids and sotrastaurin (n=14) or cyclosporine A (CsA) (n=7). The IC_{50} for sotrastaurin and CsA was determined in proliferation assays of alloantigen-activated PBMC's from blood bank donors (n=38) using dose response curves.

Results: Phenotypical analysis of blood samples from sotrastaurin and CsA treated patients showed that the absolute numbers of lymphocytes, B-cells, NK-cells and of regulatory FoxP3$^+$CD4$^+$CD25^{high} T cells were comparable between the two immunosuppressive regimens for at least 1 year. Functional studies showed that sotrastaurin effectively inhibited effector T cell proliferation. The IC_{50} to inhibit alloactivated T cell proliferation was 90 nM (45 ng/ml) for sotrastaurin and 30 nM (37 ng/ml) for CsA. In contrast, Treg function was not affected by sotrastaurin. In the presence of in vitro added sotrastaurin (50 ng/ml) Tregs suppressed the proliferation of alloactivated T effector cells at a 1:10 ratio by 52% (median) vs 53% in the absence of the drug. This potent Treg function was also found in cells of patients treated with sotrastaurin. In coculture experiments the Tregs at 6 months inhibited the anti-donor response in MLR by 66%, which was comparable to pre-transplantation (82%).
Conclusion: Sotrastaurin is a potent inhibitor of alloreactivity. In patients treated with sotrastaurin the function and phenotype of Tregs remained intact after kidney transplantation.

O-120 THE IMMUNOLOGICAL TOLERANCE EFFECTS OF DONOR SPECIFIC REGULATORY T CELLS THROUGH INTERFERON-GAMMA INDUCIBLE INDOLEAMINE 2, 3-DIOXYGENASE (IDO)$^+$ PLASMACYTOID DENDRITIC CELL

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Background and Aims: We previously reported that Donor-specific transfusion (DST) has a great potential to induce immunological tolerance by generating regulatory T cells (Treg) through the regulation of indoleamine 2, 3-dioxygenase (IDO, ATC 2009); however, its mechanism is still unclear. In this study, we herein show that Treg mediated by dendritic cell (DC) which induced by donor-derived CD11b$^+$cell play important roles in donor-specific immunological tolerance in DST rodent model.
Materials and Methods: Skin grafts (donor; H2^d Balb/c) were transplanted into recipients (H2^k C3H/He) seven days after the infusion of donor-derived whole splenocytes, isolated MHC classII$^+$CD90$^-$ cells, CD19$^+$CD45R$^+$cells, CD3$^+$cells, CD3$^-$CD19$^-$ cells, CD11c$^+$cells, and CD11b$^+$cells without immunosuppression. Graft survivals, FACs analysis and IDO immunohistological analysis were investigated.
Results: Skin graft survivals were significantly prolonged in recipients of whole splenocytes (22.2±3.3days, $P<0.001$), MHC classII$^+$ CD90$^-$ cells (20.5±3.0 days, $P<0.05$), CD3$^-$CD19$^-$ cells (22.4±4.2 days, $P<0.01$) when compared to control group (10.4±1.5 days). CD11b$^+$ cells infusion resulted in significant prolongation of graft survivals ($P<0.01$) as compared to CD11c$^+$cell infusion. Foxp3$^+$CD4$^+$CD25$^+$Tcells were significantly increased in recipients (spleen, $P<0.001$. LNs, $P<0.05$) by donor splenocytes infusion and CD11b$^+$ cells infusion (Spleen: $P<0.01$, LNs: $P<0.01$), and peaked at Day 7. MLR showed donor-specificity ($P<0.001$). Secretion of IL-10 and IFN-g were increased ($P<0.01$). High IDO expression was observed in CD11b$^+$ cells infused recipients' spleen. The graft survivals of IDO antagonist (1MT) given DST mice were not prolonged. According to FACs analysis, these DCs were considered as plasmacytoid DC.
Conclusions: Our data showed that generating donor-specific Treg through IFN-g inducible IDO expression by plasmacytoid DC is a key factor in induction of donor-specific tolerance by DST.

Allocation

O-121 OUTCOME OF PATIENTS WITH A MELD ≥25, GIVEN REGIONAL PRIORITY FOR LIVER TRANSPLANTATION

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Background: Since August 2006, three UK transplant centres agreed to give regional priority for transplantation to all patients reaching a MELD≥25 ("top-band"). This system offers the first available liver in the three retrieval zones to the patient who is listed within the top-band system, to reduce waiting list time and mortality. This study aims to review the impact of this policy from a single centre perspective.
Methods: We reviewed the prospectively collected transplant unit and the NHSBT records between August 2006 and December 2010.
Results: During the study period, 326 patients were registered electively for liver transplantation in our centre. Of these, 72 (22%) reached the top-band criteria. 39 patients were registered with a MELD≥ 25 (range: 25-49) whilst 33 became top-band after a median of 147 days on the list (initial registration MELD 11-23). 16 (22.2%) patients registered as top-band died on the transplant waiting list. The median waiting time from registration to transplantation was 11.5 days (range 0-78). In comparison, the time to transplantation for all other elective transplants was 95 days.
During this period, we performed 243 elective transplants, of which 55 (22.6%) were top band. Intention-to-treat survival from registration, in top-band patients was 60% at 1 year, and 45% at 3 years. Survival post-transplant in patients transplanted in the top-band was 75.3% at one year and 62.8% at 3 years. During same period, survival in the elective, non-top band, transplant recipients was 89% at 1 year and 81% at 3 years.
Conclusion: The introduction of a regional priority for patients with a MELD≥ 25 has provided a faster access to transplantation with a good outcome. Despite that, these patients still have high waiting list mortality.

O-122 LIVER TRANSPLANTATION: WHO SHOULD OR SHOULD NOT DIE? A NOVEL SCORE TARGETING JUSTICE AND UTILITY IN THE MELD ERA

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Background data: The MELD score is used in many countries to allocate liver grafts to the sickest patients on the waiting list. Several authors have reported unacceptable mortality in recipients with a MELD score ≥30. Therefore, a challenging task is to predict outcome in high MELD recipients. We developed a new and simple score system (balance-of-risk, BAR score) by combining worldwide readily available pre-transplant donor and recipient factors, and compared this new score with other prediction systems.
Methods: Using an UNOS database, we first performed a risk analysis in adult (≥18yr) recipients of OLT in the USA between March 2002 and September 2010 (n=37,255). We excluded DCD liver transplants, living donor, and partial or combined liver transplants. Next, we calculated a risk score based on logistic regression factors, and validated this score using our own OLT database from January 2003 to October 2010 (n=233). Finally, we compared the new score with other prediction systems including DRI (donor-risk-index), SOFT (survival-outcome following-liver-transplantation), D-MELD (donor-age combined with MELD), and MELD score alone.
Results: We identified the six strongest predictors of post transplant survival: recipient MELD score, cold ischemia time, recipient age, donor age, previous OLT, and life support dependence prior to transplant. The new balance of risk (BAR) score stratified recipients best in terms of patient survival in the UNOS data, as in our European population.
Conclusions: The BAR system provides a new, simple and reliable tool to detect unfavorable combinations of donor and recipient factors, which is readily available prior to decision making of accepting or not an organ for a specific recipient. This score may offer great potential for better justice and utility, as it revealed to be superior to recent developed other prediction scores.

O-123 www.D-MELD.com. THE ONLINE PROGNOSTIC CALCULATOR TO OPTIMIZE DONOR-RECIPIENT MATCH

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Optimization of donor-recipient match represents one of the major challenges in liver transplantation. The variable donor organ quality and recipient liver disease severity explain the various types of match adopted. Sometimes the

match or the mismatch is purely the results of chance. Nevertheless, in the majority of cases surgeons and hepatologists can take the opportunity to combine organ and recipient on the basis of specific risk assesment methods.
In order to develop an algorithms able to guide organ allocation and avoid futile match (life-expectancy <50% at 5 years) a database was created and filled with data from 5946 liver transplants performed in 21 Italian Centers during the 2002-2009 period. A web-based prognostic calculator was developed using D-MELD (donor age x biochemical MELD) and other prognostic factors. The calculator is available online at the web address www.d-meld.com (ESOT password: D-MELD123).
Using logistic regression at 3-years the following significant prognostic factors were identified: D-MELD >1628 (OR=2.03; 95%CI 1.44-2.85), recipient age (OR=1.015; 95%CI 1.002-1.028), HCV (OR=1.42; 95%CI 1.11-1.81), HBV (OR=0.69; 95%CI 0.51-0.93), re-transplant (OR=1.82; 95%CI 1.16-2.87). Cox regression analises performed at 1-90 months confirmed the results.
For a given donor (expressed by donor age) and for 3 potential recipients (expressed by values of biochemical MELD, recipient age, HCV, HBV, portal thrombosis, re-transplant status), the web-site calculates the patient survival at 90 days, 1 year, 3 year and allows the detection of futile matches.

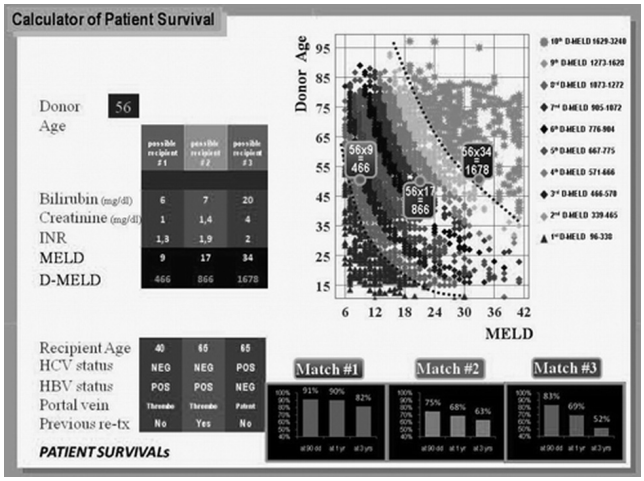

Figure 1

This innovative approach allows the identification of the best patient for each referred donor and avoids futile matches. Use of the *d-meld.com* web-site could help hepatologists, transplant surgeons and transplant coordinators in the everyday practice of matching donor and recipients.

O-124 LOW TITRE ABO-INCOMPATIBLE DECEASED DONOR RENAL TRANSPLANTATION IN PAEDIATRIC PATIENTS: A NEW APPROACH TO LONG WAITING LISTS

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Background: Outcomes for ABO-incompatible (ABOi) living donor renal transplantation are comparable to ABO-compatible transplantation. ABOi deceased donor renal transplantation is limited by its unplanned nature, restricting time for desensitisation. Adult ABOi living donor renal transplantation has been performed safely with minimal or no desensitisation in recipients with low antibody titres.
Methods: Anti-A and anti-B antibody titres (total immunoglobulin load) were measured in all paediatric patients active on the deceased donor renal transplant waiting list from 2 United Kingdom renal transplant centres, using DiaMed gel cards. Blood group O patients on the waiting list for >200 days were classified as long waiters.
Results: 24 paediatric patients (mean age 10 (range 3-17); M:F 18:6) were included, with a mean waiting time of 638 days (range 31-1410).
In total, 8/24 (33.33%) had antibody titres of 8 or lower (7 anti-B and 1 anti-A); a further 3 (12.5%) had antibody titres of 16 (2 anti-B and 1 anti-A). Therefore 11 (45.83%) patients were potentially suitable for a low-titre ABOi transplant.
This would reduce the average waiting time for these 11 patients by 54 days. No patient below the age of 5 had an antibody titre greater than 64: 3 out of these 6 had an anti-B antibody titre of 4.

Table 1. Paediatric patients on the deceased donor renal transplant waiting list

Blood group	Mean waiting time in days (95% CI) (National waiting list)	Number in in study population	Mean waiting time days (study population)
O	340 (251–429)	19	509
A	174 (99–249)	2	2180
B	320 (179–461)	2	260.5
AB	125 (0–332)	1	335

Mean National waiting times courtesy of NHS Blood and Transplant.

Table 2. Blood group of paediatric patients with low titres

Blood group of patient	Low antibody titre group	Resulting, equivalent phenotype	Number
O	B	B	7
O	A	A	2
A	B	AB	2
B	A	AB	1

Of the long waiters, 5/15 (33.33%) had antibody titres of 8 or lower (4 anti-B and 1 anti-A); a further 2 (13.33%) had titres of 16 (both anti-B).
Conclusions: A large proportion (45.83% in the total study population) of paediatric patients have low anti-A or anti-B antibody titres. We suggest a change in organ allocation practice, to allow low-titre ABOi deceased donor renal transplantation. This should reduce the average waiting time significantly, thereby reducing renal replacement therapy related morbidity and mortality.

O-125 DONOR SCORING SYSTEM FOR HEART TRANSPLANTATION AND THE IMPACT ON PATIENT SURVIVAL

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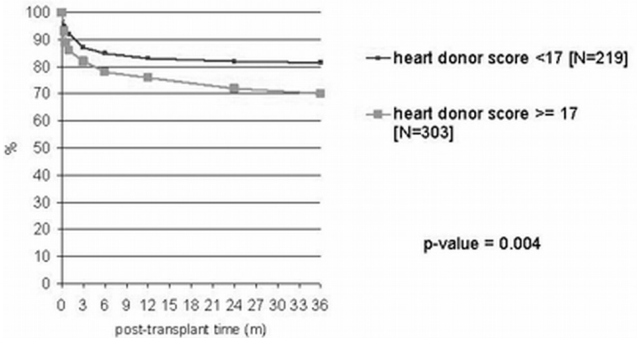
Background: The aim of this study was to design and validate a heart donor score that reflects experts' perceived risk of allograft failure.
Methods: All heart donors reported to Eurotransplant from January 1, 2005 to December 31, 2008 [N=4110] were used to create a donor score. Based on observed discard rates and using multivariate regression, points were assigned for the following donor factors: age, cause of death, body mass index (BMI), diabetes mellitus (DM), duration of ICU stay, compromised history (drug, abuse, sepsis, meningitis, malignancy, HbsAg+ or Anti-HCV+), hypertension, cardiac arrest, echo cardiography, coronarography, serum sodium and noradrenaline and dopamine/dobutamine dosages. The donor score was obtained by adding all points. All heart donors reported to Eurotransplant in 2009 were used to validate the score [N=885].
Results: All donor factors except BMI, DM and duration of ICU stay, significantly predicted discard. Based on the median value of the score, donors were classified into standard criteria donors (SCD): 16 points or less and extended criteria donors (ECD): 17 points or more. In the validation set discard rates were significantly different for ECD: 35% *vs.* SCD: 7% (p<0.0001). In addition the heart donor score was significantly associated with 3-year survival: ECD: 81.5% *vs.* SCD: 70.0% (p=0.004).

Patient survival rates by heart donor score, all heart transplants performed in 2005 in Eurotransplant N=522



Conclusions: The heart donor score accurately reflects the likelihood of organ acceptance and predicts long term patient mortality, its application at time of donor reporting may facilitate donor risk assessment and allow more appropriate matching of extended criteria donor hearts.

O-126 REVISED NATIONAL HLA MATCHING CRITERIA IMPROVE ACCESS TO TRANSPLANT FOR PATIENTS FROM ETHNIC MINORITIES

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Background: HLA matching is often included in kidney allocation algorithms, but this policy can disadvantage patients with rare HLA types. The 2006 UK national kidney allocation scheme (NKAS) favours well matched patients but is designed to improve equity of access to transplant.
The HLA matching criteria have been redefined so that rare (<2% frequency in the donor pool) HLA specificities are assigned to more common, related specificities. 3 HLA-A, 16 HLA-B and 4 HLA-DR specificities have been reassigned. For example, HLA-A36 occurs in 0.05% UK donors but by assigning A36 to the serologically-related specificity, HLA-A1, HLA-A36 patients have access to HLA-A1 donors, which represent 18% of the donor pool. HLA-A36 positive donors are still matched to HLA-A36 patients.
Methods: The allocation scheme was phased in, but has been fully implemented since April 2009.
Results: Recent monitoring shows that since April 2009, reassigned HLA antigens have been a factor in patient selection in 199/1693 (11.8%) kidney transplants. A single reassigned HLA-DR specificity was most likely to have influenced allocation (70% occasions). Antigen reassignment was of benefit to patients who are calculated to be difficult to match (p=0.002). The proportion of transplants involving reassigned specificities varied significantly according to patient ethnicity; 8% transplants in White patients in contrast to 15%tranplants in Asian patients and 43% in Black patients (p<0.0001). Transplants in ethnic minority patients have also increased from 17% to 25.4% of transplants nationally.
Conclusion: While retaining HLA matching in kidney allocation, the UK 2006 NKAS strives to achieve equity of access to transplant for difficult to match patients by assigning rare antigens to more common, related specificities. This approach increases access to transplant for ethnic minority and other difficult to match patients.

O-127 THE 2006 NATIONAL KIDNEY ALLOCATION SCHEME – THE FIRST FOUR YEARS

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Introduction: The 2006 National Kidney Allocation Scheme (2006 NKAS) was introduced in the UK to improve equity of access to kidney transplantation while incorporating evidence-based factors to ensure good transplant outcomes. Kidneys from over 600 donors after brain death per year are allocated to over 7000 waiting patients.
Methods: The scheme prioritises all 000 HLA-A,B,DR mismatched (mm) patients and other well matched paediatric patients (100/010/110 HLA-A,B,DR mm). 75% of kidneys are allocated to remaining patients. Priority is primarily determined by waiting time, and age combined with HLA mm (to ensure well matched transplants for younger patients).
Results: In the first 4 years of the 2006 NKAS, the proportion of patients on the list for over 5 years dropped from 16% to 10%, with significant transplant centre variation in waiting times much reduced. The HLA matching policy maximises 000 mm while avoiding poorly matched grafts altogether and facilitates retransplantation in younger patients by prioritising well matched transplants for younger patients: 71% Levels 1&2 mm (000 mm & 100/010/110/200/210 mm) in 18-29 year-olds compared with 35% in patients over 60 years. The excess of HLA-DR homozygous patients on the list has been eliminated (from 17% of the list to 14%). One year graft and patient survival rates are comparable with those seen previously (p=0.8 and 0.2, respectively).
Conclusions: The 2006 NKAS has been successful in improving equity of access to kidney transplantation in the UK. An excess of long-waiting patients on the list has been much reduced while well-matched grafts have been achieved for those in whom it is important.

O-128 A NEW ALLOCATION MODEL FOR LIVER TRANSPLANTATION COMBINING URGENCY BASED PRIORITIZATION WITH DONOR–RECIPIENT MATCHING: A PROSPECTIVE CONTROLLED STUDY

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Background: The adoption of donor–recipient matching in liver transplantation (LT) has the potential to optimize post-LT outcome ensuring allocation equity for patients in the waiting list (WL).
Methods: Primary endpoint: 2-year intention-to-treat survival. Study design: "non inferiority" analysis comparing a prospective cohort of adult patients with chronic liver disease enlisted for primary LT versus an historical cohort of patients with similar inclusion criteria.
01/07/2006-31/12/2009: WL for LT was divided in 2 sub-lists. Cirrhotic patients classified according to MELD score (MELD list); patients with hepatocellular carcinoma (HCC) with MELD < 20 and other exceptions organized according to a specific score (NON MELD list). Allocation algorithm (donor-recipient match): optimal graft assigned to the first MELD candidate; suboptimal graft allocated to the first NON MELD patient. 01/01/2003-30/06/2006: single WL arbitrarily ordered according to generic rules of urgency disease severity and time on the WL.
Results: Three-hundred thirty patients were enrolled in the study group, whereas 326 in the control group. The new prioritization model favoured patients most in need in terms of cirrhosis severity and presence of HCC: the median MELD score in enlisted (14 vs 13, p<.01) and transplanted (20 vs 15, p<.01) patients, and the proportions of enlisted (35% vs 26%, p<.01) and transplanted (49% vs 32%, p<.01) HCC patients, in fact, were significantly higher in the study than in the control group.
The 2-year intention-to-treat survival rates were similar in the study and in the control group overall (80% vs 82%, p=ns), and both in non HCC (82% vs 83%, p=ns) and HCC patients (75% vs 80%, p=ns).
Conclusions: We prospectively validated a prioritization model based on objective criteria favouring patients most in need without impairing overall LT outcome.

O-129 LABORATORY MELD-BASED ALLOCATION OF LIVER GRAFTS: IS THE "SICKEST FIRST" PRINCIPLE JUSTIFIED?

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Limited supply with increased demand for liver grafts and increased waiting list mortality has led many countries to prioritize Liver Transplant (LT) candidates according to their Model for End-stage Liver Disease (MELD) score (creatinine, INR, bilirubin). This "sickest first" allocation is questioned because LT in (very) high MELD patients may cause unacceptable mortality, particularly early post-LT, resulting in the futile use of scarce grafts and resources.
Aim: We sought to determine the impact of Lab-MELD on short term patient/graft survival and hospital stay post-LT.
Methods: We analyzed data of all patients transplanted between 01/2006-09/2010 and excluded LT for acute liver failure, multiorgan-, and re-LT. Lab MELD was calculated immediately pre-LT. We categorized patients according to Eurotransplant MELD classes (6-19; 20-24; 25-29; 30-34; 35) and analyzed 3-12mo patient/graft survival & length of ICU/hospital stay.
Results: During the study period, 221 isolated first LT for non-acute liver disease were performed [post-ethyl cirrhosis (42%), viral infections (17%), cholestatic disease (12%), metabolic disease (11%), other indications (18%)]. HCC was present in 36% of cases. Mean recipient age 55.7 (SD 13.8y). Mean Lab-MELD 16.5 (SD 8.8). MELD was associated with 12mo patient/graft survival (proportional hazard regression p 0.03/0.04). Above MELD 19, patient/graft survival did not differ among the various MELD classes (Log-rank/Bonferroni correction). ICU/hospital stay was prolonged by a factor 2 to 3 in patients with MELD>19 (Kruskal-Wallis p<0.001).

Patient Survival/length of stay according to MELD class

	3 month Patient Survival (%)	12 month Patient Survival (%)	ICU stay (days)	Hospital stay (days)
MELD 6-19 (n=155)	96	95	3 (1–57)	18 (5–134)
MELD 20-24 (n=26)	88	80	9,5 (1–94)	30,5 (13–109)
MELD 25-29 (n=20)	90	65	8,5 (1–54)	33 (3–240)
MELD 30-34 (n=12)	100	78	7 (2–39)	43 (14–72)
MELD ≥35 (n=8)	88	87	8 (4–39)	33 (21–167)

Conclusion: MELD has no impact on early post-LT mortality or graft loss; however it does affect 1y patient/graft survival. Higher MELD implies higher resource utilization. Nevertheless, MELD-based allocation seems justified considering the favorable results reached even in (very) high MELD recipients and the extremely dismal prognosis without LT. Long term data are warranted.

O-130 ALLOCATION OF MARGINAL KIDNEYS: KARPINSKY SCORE 4 FOR SINGLE (SKT) OR DUAL (DKT) TRANSPLANTATION?

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Background: DKT is a recognized strategy for implementing the utilization rate of kidneys which would be otherwise discarded. Pretransplant histological evaluation (Karpinsky score, KS) is one of the allocation criteria for SKT or DKT: DKT if KS>3 and <7 according to Remuzzi (JASN, 1999). In our Center DKT is performed from 1999: Remuzzi score was adopted up to 2005 when we decided to extend also to score 4 the SKT. We retrospectively compare results and outcomes in KS4 used for SKT or DKT (1999-2009).
Methods and Materials:

Demographics

	SKT (n=30)	DKT (n=16)	p
F/up (months)	42±30	66±29	–
Recipient age	59±8	57,5±6	ns
Donor age	66±7	71±9	ns
CIT (h)	19±6	20±4	ns
Cockroft-Gault (ml/min)	82±18	69±18	0,006

Results:

Clinical results

	SKT	DKT	p
DGF	20%	31%	0,05
Acute rejection	17%	19%	ns
sCrs (mg/dl) 1 yr	2±0,6	1,7±0,6	0,048
sCrs 3 yrs	1,9±0,5	1,6±0,5	ns
sCrs 5 yrs	1,9±0,7	1,5±0,2	ns
Proteinuria (g/24h) 1 yr	0,4±0,5	0,1±0,2	0,01
Proteinuria 3 yrs	0,4±0,5	0,4±0,5	ns
Proteinuria 5 yrs	0,3±0,2	1,3±1,4	ns
Graft losses	3 (10%)	5 (31%)	ns
Deaths	3 (10%)	2 (12%)	ns

No significant difference was found in patient (90% at 5 years for both groups) and organ survival rates at 1, 3, 5 years (DKT: 93%, 87%, 78%; SKT: 83%, 83%, 77%; p=0,8).
Conclusions: Outcomes of these groups are similar. No difference in graft and patient survival rates is found. When KS4 is allocated in SKT a worse trend in sCr is noted, as it could be expected. However, in a setting of profound disparity between supply and demand, we suggest the KS4 for SKT as an acceptable strategy to reduce this gap.

O-131 PAIRED LIVING KIDNEY DONATION IN THE UK

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Introduction: A national programme for kidney paired donation (KPD) started in the UK in 2007. Matching is carried out 3-monthly, identifying all possible exchanges involving two or three incompatible donor-recipient pairs.
Methods: Data on enrolled and transplanted pairs are summarised. Univariate transplant survival rates (time to transplant failure or death) are investigated.
Results: By January 2011, 465 patients had enrolled in the scheme: 63% were HLA incompatible [HLAi] (including 16% also ABO incompatible [ABOi]); the remainder were ABOi only. Half were spouse/partner couples.
Recent matching runs have included up to 186 possible recipients and identified between 6 and 36 possible transplants, with an increasing proportion actually proceeding.
It is difficult to identify potential transplants as 50% of patients in recent matching runs had calculated HLA antibody reaction frequencies of 95-100%. Despite this, almost 200 possible transplants were identified, half not proceeding for reasons including positive crossmatch (38%) or ill-health of donor or recipient (24%).
In total, 80 recipients (17% of enrolled patients) received a KPD transplant by January 2011, including 8 three-way exchanges. Pairs most successful are A donor/B recipient and vice-versa ABOi pairs (approx 30% transplanted), and HLAi pairs with low/moderate levels of sensitisation (approx 30%).
One year transplant survival (94%, 95% CI 83-98%) is comparable with other specified living donations (including directed donations to highly sensitised (91%, 95% CI 86-95%) and ABOi (89%, 95% CI 83-93%) recipients).
Conclusions: KPD has been introduced successfully in the UK, allowing two- and three-way exchanges, and now accounting for 3% of all living donor kidney transplants. Domino (chain) paired donation will commence in 2011 and most altruistic non-directed donors (26 in 2010) will donate to the scheme rather than to the deceased donor list as currently, increasing markedly the efficacy of the scheme.

O-132 HIGH MELD SCORES ARE NOT ALWAYS ASSOCIATED WITH POOR OUTCOME AFTER LIVER TRANSPLANTATION: SINGLE CENTRE EXPERIENCE WITH 51 PATIENTS WITH LAB-MELD > 30

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Background: Currently, in Germany, mean Lab-MELD needed to get a liver offered is > 35, but this is associated with a postoperative mortality after liver transplantation > 50%. (Weissmueller Transplant International 2011).
Patients & Methods: Retrospective analyses of 51 consecutive Liver Transplantation (LT) in pts with decompensated chronic liver disease and Lab-MELD ≥30 in the timeframe January 2007 - December 2010.
The recipients (mean age 49±11 years) were divided in 2 Groups: G1 (MELD 30-35; n=24) and G2 (MELD 36-40; n=27).
Results: see Table 1

Table 1

	Overall (n=51)	G1 (n=24)	G2 (n=27)
STATUS pre-LT			
Lab-MELD	36±4	33±2	39±1
ICU-Stay days	63% (9±8)	50% (7±6)	74% (11±9)
Hemodialysis	43%	21%	63%
Ventilation	26%	16%	33%
Vasopressor	27%	16%	37%
ECD-Donor >1	18%	21%	15%
LT-Procedure			
Whole	47(92%)	23 (96%)	24 (89%)
Split	4 (8%)	1 (4%)	3 (11%)
OP-Time (min)	370±96	362±97	378±97
OP-Morbidity <3 Mo			
Medical Complications			
– Infection	53%	50%	56%
– Pulmonary	47%	42%	52%
– Cardiovascular	20%	13%	26%
– Neurological	20%	18%	21%
– Metabolic	10%	13%	7%
Surgical Complications			
– Bleeding	21%	17%	25%
– Biliary	21%	21%	22%
– Vascular	8%	4%	11%
Outcome			
ICU-Stay (days)	20±18	16±15	23±22
Hospital-Stay (days)	45±30	33±23	51±29
Graft Survival			
– Early	94%	92%	96%
– Late (2 years)	92%	88%	96%
Pat. Mortality			
– Early	6% (n: 3)	0%	11% (n: 3)
– Late	6% (n: 3)	8% (n: 2)	4% (n: 1)

Conclusion: LT in Patient with MELD score > 30 can reach excellent results thanks to accurate recipient selection, good graft quality (split grafts included), short operative time and optimal ICU-management.

Kidney (ABO and antibody incompatible transplants and immunosuppression)

O-133 THE CLINICAL STUDY OF IMMUNOSUPPRESSION PROTOCOL IN ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION

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Background: Immunosuppression therapy for ABO-incompatible kidney transplantations have previously been more rigorously practiced than ABO-compatible. However, we found that patients treated with this regimen developed a high rate of complications. In our search for optimal immunosuppression, we have evolved and made two major changes in the induction period immunosuppressive therapy in ABO-incompatible kidney transplantation. We have been comparable with two induction period immunosuppressive therapy.
Methods: Sixty-one kidney recipients were enrolled in our study comparing the two protocols. GroupI (n=19) patients received a standard immunosupressants (a standard-dose calcineurin-inhibitor (CYA or FK), MMF, and a standard-dose

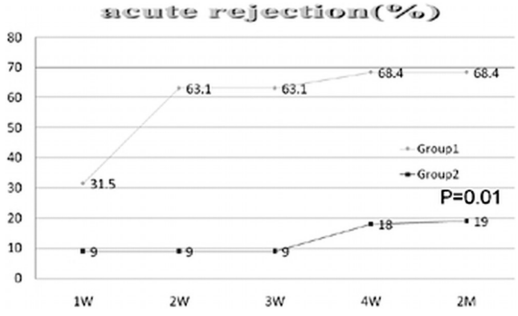
steroid with splenectomy during April, 1996-August, 2004. Group II (n=43) patients were treated with a low-dose calcineurin-inhibitor with MMF, an early low-dose steroid and Basiliximab induction with Rituximab without splenectomy during September, 2004-December, 2010.
Results: Patient (94.7% versus 100%) and graft survival (68.4% versus 97.6%) at 3year were no different between the groups. Patients of Group II experienced significantly less acute rejections than those in Group I (68.7% versus 19%, P=0.01). Group I patients had three AMR (antibody-mediated rejection) episodes (15.7%), whereas alone AMR episode in Group II (2.3%). Group I had developed acute rejection in the early period (within 2weeks) after transplantation in comparison Group II. Allograft function and incidence of adverse events and infections were similar between the two groups. However the early low-dose steroid protocol Group (GII) patients experienced significantly less peptic ulcer and PTDM (post-transplant DM).

Patient and graft survival

	Group 1 (n=19)	Group 2 (n=42)	P-value
Patient survival (3 years)	18/19 (94.7%)	42/42 (100%)	0.39
Allograft survival (3 years)	13/19 (68.4%)	41/42 (97.6%)	0.05
Acute rejection	13/19 (68.4%)	8/42 (19%)	0.01
Acute AMR*	3/19 (15.7%)	1/42 (2.3%)	0.101
Anti-blood antibody	1:16 (2–32)	1:64 (8–512)	0.05

Patients characteristics

	Group 1 (n=19)	Group 2 (n=42)	P-value
Time	1996.4–2004.8	2004.9–2010.12	NS
Sex	Men 17, Female 2	Men 34, Female 8	NS
Mean age	34.2±9.7 (19–55)	44.0±16.0 (12–68)	NS
Mean time on HD (year)	4.2±3.2 (0.7–18)	2.7±4.7 (0–18)	NS
ABO type	A 9, B 10	A 20, B 18, AB 2	NS



Incidence of acute rejection.

Conclusion: We have be able to improve graft survival and reduced complications, through we have evolved in the induction period immunosuppressive therapy in ABO-incompatible kidney transplantation.

O-134 SUCCESSFUL OUTCOMES AFTER MINIMISING ANTIBODY MODULATION IN BLOOD GROUP INCOMPATIBLE KIDNEY TRANSPLANTATION

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Background: Blood group incompatible kidney transplantation (ABOi) has become increasingly frequent. Conventional protocols include rituximab, ivIG, and pre- and post-operative immunoabsorption. We modified this by tailoring our approach according to antibody titre, allowing minimisation of this regimen.
Methods: 52 patients underwent ABOi transplantation and were compared with 114 recipients of blood group compatible transplants (ABOc) from the same period. IvIG was used only during the first two years of the programme and for combined ABOi and HLA incompatible (HLAi) transplants. Rituximab was used routinely for the first two years, then only if baseline antibody titres were 1 in 8 or above. Antibody removal was used only if baseline titres were 1 in 8 or above and post-operative antibody removal was used in the first 9 patients only.
Results: Mean follow-up was 719 days for ABOi transplants and 1111 days for ABOc transplants. Of 52 ABOi patients, 8 were given no Rituximab, 38 had no ivIG, 11 had no pre-operative antibody removal and 43 had no post-operative antibody removal. Four patients had combined ABOi and HLAi transplantation. Overall graft survival at follow-up was 96.2% for ABOi and 97.4% for ABOc recipients (logrank p=0.633). Two patients died in the ABOi group and one in the ABOc group. For those with at least one year of follow-up, 12 month death-censored graft survival was 97.6% for ABOi (n=40) and 97.4% for ABOc (n=114) transplants (logrank p=0.957), acute T cell mediated rejection was 20% v 18% (p=0.92) and AMR was 2.5% v 3.5% (p=0.84) respectively. For 22 ABOi patients with 3 years' follow-up, graft survival was 100%, whilst for 56 ABOc patients it was 96.4% (p=0.92).
Conclusions: Tailoring antibody modulation according to baseline titre allows minimisation of therapy and maintains excellent outcomes.

O-135 THE INCIDENCE OF ACUTE ANTIBODY MEDIATED KIDNEY TRANSPLANT REJECTION UNDER MODERN IMMUNOSUPPRESSION: COMPARISON OF TACROLIMUS MONOTHERAPY WITH TACROLIMUS/MMF STEROID-FREE MAINTENANCE REGIMES

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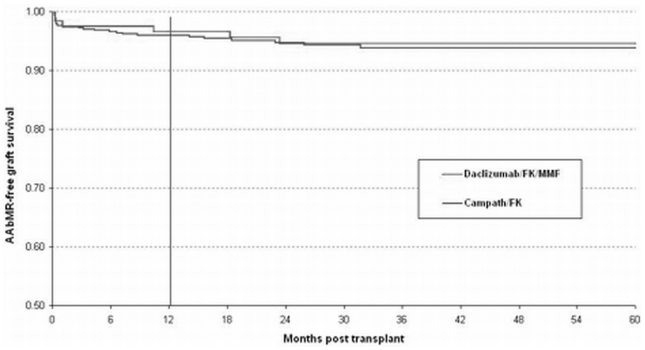
Background: The availability of sensitive assays for anti-HLA antibody detection and quantification, combined with new understanding of the histopathological correlates of antibody mediated rejection (AbMR) in renal allografts, combined with increasing awareness of, and interest in, this pathway to rejection has led to an apparent increase in the reported incidence of acute antibody mediated rejection (AAbMR). It is not known whether different modern immunosuppressive agents are associated with different levels of risk for AAbMR, although regimes involving no maintenance immunosuppression, or mTor inhibitor maintenance monotherapy have been reported to suffer a high risk of this lesion.
Methods: We retrospectively compared the incidence of acute *de-novo* antibody-mediated rejecion (diagnosed by a combination of light microscopy, C4d staining, and the presence of donor-specific HLA antibodies (dsAb) detected by Luminex single-antigen bead binding) in 504 kidney transplant recipients who had recieved Campath1-H induction followed by Tacrolimus maintenance monotherapy, and 120 recipients transplanted under Daclizumab induction with Tacrolimus/MMF combination maintenance during the era of availability of Luminex dsAb screening. All patients recieved a steroid-sparing regime of 7-days oral steroids at the time of tranplantation with steroid cessation at day 8. Patients with pre-existing dsAb were not included in this analysis.
Results: AAbMR rejection-free graft survival (censored for death-with-function and other causes of graft loss) at 5 years was 93.8% in the Campath/Tacrolimus group, and 94.5% in the Daclizumab/Tacrolimu/MMF group. There was no significant difference in the incidence of AAbMR (by log-rank analysis) at any of the time points.

Cumulative incidence of Acute Ab mediated rejection

Regime	1 year	2 years	5 years
Campath/FK	4.0%	6.2%	6.2%
Daclizumab/FK/MMF	3.4%	5.5%	5.5%



Conclusion: AAbMR due to de-novo dsAb remains a rare, usually early, complication of renal transplantation, with no evident difference in frequency between these two steroid-free maintenance regimes (with and without MMF).

O-137 A MULTICENTRIC PROTOCOL USING Glycosorb® IMMUNADSORPTION FOR AB0-INCOMPATIBLE KIDNEY TRANSPLANTATION: THE SWISS EXPERIENCE

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Background: In order to expand the kidney donor pool we have developed

a national protocol for AB0-incompatible kidney transplantation in Switzerland. The protocol consists of a standardized immunosuppression and perioperative Glycosorb® immunoadsorption to reduce circulating anti-A/-B antibodies.
Methods: Patients qualifying for AB0-incompatible kidney transplantation received Rituximab® one month before transplant and standard immunosuppression with tacrolimus, mycophenolate mofetil and steroids. Perioperative immunoadsorption was performed using Glycosorb® columns. Graft survival, patient survival, kidney function, rejections and anti-A/-B antibody titers were assessed.
Results: A total of 59 patients (12 females and 47 males) were transplanted within a period of 5 years in 5 Swiss transplant centers. The mean follow up was 22 months and the mean recipient age was 52 years. The median number of immunoadsorptions performed prior to transplantation was 5 (range 3-16), and the antibody titer at the time of transplantation was less than 1:8. Only 8 (13.5%) patients needed immunoadsorption after transplantation (median number of immunoadsorptions after transplantation: 0; range 0-11). All centers successfully performed regular column reuse (1-3 columns per patient). The patient survival rate was 98.3% and the overall graft survival rate was 96.6%. One graft had to be removed due to emphysematous pyelonephritis two months after transplantation, and one patient died due to E. coli sepsis. Kidney graft function was excellent with a mean serum creatinine level of 126 μmol/l (SD 36.2) one year after transplantation. We observed 11 biopsy proven rejections (18.6%) at mean interval of 7.4 months after transplantation.
Conclusion: We have established a national protocol using Glycosorb® immunoadsorption in AB0-incompatible kidney transplantation, which is efficient and safe. Only a minority of patients needed postoperative immunoadsorption. Column reuse proved to be efficient.

O-138 SUCCESSFUL PREVENTION OF CHRONIC AMR IN DSA-POSITIVE KIDNEY TRANSPLANTATION BY RITUXIMAB-BASED PRECONDITIONING

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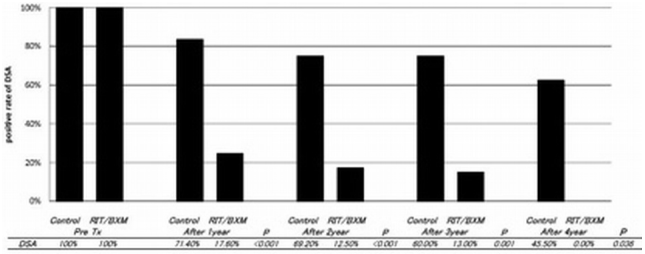
Background: Donor specific anti-HLA antibody (DSA) is a well known immunological risk factor for acute and chronic antibody mediated rejection (AMR) and eventually causes graft loss in most cases. The objective of this study is to evaluate our new preconditioning regimen including rituximab (RIT)/basiliximab (BXM) in DSA-positive kidney transplant recipients in the long term.
Material and methods: Between 2001 and 2009, 113 DSA-positive recipients determined by Luminex single-bead method underwent living kidney transplantation at our institution. Eighty two patients were treated with a preconditioning regimen consisting of a single low dose of RIT and BXM (RIT/BXM group). Maintenance immunosuppression consisted of tacrolimus, mycophenolate mofetil and methylprednisolone. Thirty one DSA-positive recipients who were not treated with RIT/BXM were employed as a control group.
Results: Five year graft survival rate was 98% in the RIT/BXM group and 88.9% in the control group (p=0.035). The incidence of acute and chronic AMR were significantly reduced in the RIT/BXM group compared to the control group (acute AMR: 6% vs. 26%, p=0.008, chronic AMR 12% vs. 48%, p<0.001, respectively). The incidences of DSA positivity were significantly less in the RIT/BXM group compared to the control group during the follow up period.



Conclusion: RIT/BXM induction therapy seems to be a very effective and safe treatment option for DSA-positive transplant recipients.

O-139 PRELIMINARY RESULTS FROM A PROSPECTIVE MULTICENTER STUDY REVEAL THE EARLY APPEARANCE OF *DE NOVO* ANTI-HLA ANTIBODIES FOLLOWING RENAL TRANSPLANTATION

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Background: Long term survival of transplanted organs has only marginally improved despite the development of novel immunosuppressive agents. This may due to the detrimental role of elicited donor (DSA) and/or non-donor specific (NDSA) anti-HLA antibodies.
Methods: First-transplant kidney recipients (n=189) with no anti-HLA antibodies at transplantation were included in a prospective, multicenter clinical study. Anti-HLA class I and class II antibodies were monitored throughout the study using either flow cytometry or Luminex (kits kindly donated by One Lambda, USA). Clinical parameters for renal function as well as histological and immunological evaluations are being analysed throughout the study.
Results: The current median enrolment time is 18 months (range 1-45 months). To date, 37 patients have developed antibodies between 1 and 39 months post-transplantation. Notably, 19.7% and 31% patients developed *de-novo* anti-HLA antibodies within 12 and 24 months post-transplantation, respectively. Both DSA and NDSA class I or class II antibodies were observed in 45% positive patients while 50% showed only NDSA and 1 patient presented DSA specificities only. At the time of *de-novo* antibody detection creatinine levels ranged from 61 to 418 μmol/l (138.7±67.6). The total mean mismatch number in patients with or without elicited antibodies was 4.1±1.25 and 3.6±1.0 (p=0.051), respectively.
Conclusion: Our study shows that a considerable number of patients elicit an early *de novo* anti-HLA antibody response following renal transplantation. Interestingly, NDSA appear to be present in all cases. Given that *de novo* anti-HLA class I and class II antibodies are associated with reduced long term allograft survival, routine antibody monitoring and specific intervention strategies with the aim of modulating both donor and non-donor specific immune responses, may become necessary in order to improve long term results.

O-140 ACUTE REJECTION IN RENAL TRANSPLANTATION RECIPIENTS TREATED WITH TACROLIMUS PROLONGED RELEASE- AND IMMEDIATE RELEASE-BASED THERAPY – THE OSAKA STUDY (OPTIMIZING IMMUNOSUPPRESSION AFTER KIDNEY TRANSPLANTATION WITH Advagraf®

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Background: The incidence of and time to first acute rejection and biopsy confirmed acute rejection (BCAR) and severity of BCAR in renal recipients treated with tacrolimus prolonged release (QD) and immediate release (BID) were compared in an international, multicentre, open-label, parallel group study.
Methods: Subjects (n=1251) were randomised 1:1:1:1 to receive tacrolimus BID 0.2mg/kg/day (Arm 1), tacrolimus QD 0.2mg/kg/day (Arm 2), tacrolimus QD 0.3mg/kg/day (Arm 3), all with MMF and corticosteroids for 24 weeks, or tacrolimus QD 0.2mg/kg/day with MMF, basiliximab and corticosteroids given only perioperatively (Arm 4). Severity of BCAR was graded using the Banff '97 criteria (Racusen, L.C. et al. Kidney International 1999; 55: 713–723).
Results: Rates of BCAR were low, ranging from 10.3–16.1% (Table 1) and there was no significant difference between treatment arms in the incidence

of and time to first incidence of BCAR (p=0.244). The severity grade of BCAR was comparable across treatment arms.

Table 1. Incidence and severity grade of BCAR

	Arm 1, n (%) (n=309)	Arm 2, n (%) (n=302)	Arm 3, n (%) (n=304)	Arm 4, n (%) (n=283)
BCAR	42 (13.6)	31 (10.3)	49 (16.1)	36 (12.7)
Severity:				
Grade 1 (mild)	24 (7.8)	13 (4.3)	28 (9.2)	14 (5.6)
Grade 2 (moderate)	18 (5.8)	15 (5.0)	19 (7.2)	18 (6.4)
Grade 3 (severe)	0	3 (1.0)	2 (0.7)	2 (0.7)

Conclusion: Tacrolimus BID or QD initiated at the lowest recommended dose, without induction therapy, resulted in similar very low rates of BCAR. There was no benefit from a higher starting dose of tacrolimus QD.

O-141 EFFICACY AND SAFETY OF TACROLIMUS PROLONGED RELEASE AND IMMEDIATE RELEASE IN *DE NOVO* RENAL TRANSPLANTATION – THE OSAKA STUDY (OPTIMIZING IMMUNOSUPPRESSION AFTER KIDNEY TRANSPLANTATION WITH Advagraf®

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Background: Tacrolimus prolonged release (QD) and immediate release (BID) were compared in a large international study in *de novo* kidney transplant recipients.
Methods: 1251 patients were randomised 1:1:1:1 to starting doses of tacrolimus BID 0.2 mg/kg/day (Arm 1), tacrolimus QD 0.2 mg/kg/day (Arm 2), tacrolimus QD 0.3 mg/kg/day (Arm 3), all with MMF and corticosteroids for 24 weeks, or tacrolimus QD 0.2 mg/kg/day with MMF, basiliximab and corticosteroids given only perioperatively (Arm 4). Efficacy failure (graft loss, biopsy confirmed acute rejection [BCAR] or graft dysfunction [eGFR $<$40 mL/min/1.73m^2] at 24 weeks) was the primary composite endpoint. A 12.5% non-inferiority margin was pre-defined.
Results: Mean donor age was 51.5 years; ~50% were extended criteria donors. Arm 2 was non-inferior to Arm 1 (95% CI: –12.2, 9.0%). Tacrolimus QD 0.3 mg/kg/day offered no efficacy benefit. The main driver for "efficacy failure" was graft dysfunction; BCAR rates were low (Table 1). Arm 4 was associated with lower mean eGFR but a lower incidence of diabetes (2.8% vs 6.4%, 4.9% and 4.6% in Arms 1, 2 and 3) and improved lipid profiles. Adverse events were ~94% in each arm; ~60% were mild or moderate in intensity.

Table 1. Efficacy and serum lipid parameters

	Arm 1	Arm 2	Arm 3	Arm 4
Efficacy (PPS)				
BCAR	13.6%	10.3%	16.1%	12.7%
Efficacy failure	40.6%	42.2%	44.2%	48.2%
Mean eGFR (mL/min) (24 weeks)	48.3±1.09	45.7±1.10	45.9±1.09	41.7±1.13
Least squares mean change mmol/L (SAF): Change – baseline to Week 24				
Total cholesterol	0.19	0.21	0.18	–0.12*
LDL-C	0.18	0.196	0.11	0.04*
Triglycerides	–0.32	–0.15	–0.04	–0.52*

*p$<$0.05; PPS = per protocol set; SAF = safety analysis set.

Conclusion: Tacrolimus QD 0.2 mg/kg/day was non-inferior to the same starting dose of tacrolimus BID in *de novo* renal transplantation. Tacrolimus QD at 0.3 mg/kg/day did not improve efficacy. Efficacy failure rates reflected the eGFR set for graft dysfunction and the high proportion of extended criteria donors. Steroid avoidance offered metabolic advantages but possibly inferior renal function.

O-142 EFFICACY AND SAFETY OF 24 MONTHS IMMUNOSUPPRESSION WITH CONCENTRATION-CONTROLLED EVEROLIMUS AND REDUCED CYCLOSPORINE IN *DE NOVO* RENAL TRANSPLANT RECIPIENTS

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Background: Everolimus (EVR), an mTOR inhibitor provides synergistic effects with cyclosporine (CsA) in renal transplant recipients (RTxR).
Methods: A2309 is a 24-month (M) randomized, multicenter, open-label study in 833 *de novo* RTxR comparing two targets of EVR exposure (C0 3-8ng/mL or C0 6-12ng/mL) with reduced CsA concentration versus a control group receiving enteric-coated mycophenolate sodium (MPA) 1.44g/day with standard CsA concentration. At 24M, study endpoints were composite efficacy failure (incidence of BPAR, graft loss, death, or loss to follow-up), renal function and safety comparisons between EVR groups and MPA control group.
Results: Donor and recipient characteristics were comparable between treatment groups. The primary endpoint (non-inferiority of composite efficacy failure) was met at 12M as previously described. From 12M to 24M, a 60% reduction in mean CsA concentrations was maintained between the EVR 3-8 and 6-12ng/mL groups (52±51 and 50±41ng/mL) and the MPA control group (135±64ng/mL). At 24M, incidence of efficacy failure parameters was higher in the EVR 3-8ng/mL group vs. MPA in the ITT population (rate difference 5.4%; 95% CI [-2.2, 13.0]) but similar in the on-treatment population (rate difference -0.7%; 95% CI [-8.1, 6.6]). More severe BPAR (≥IIA) rates were identified in the MPA vs. EVR groups (Table).

Table 1. Incidence rates (n [%]) of efficacy endpoints at 24 months (ITT population)

	EVR 3–8 ng/mL (N=277)	EVR 6–12 ng/mL (N=279)	MPA 1.44 g (N=277)
Primary composite efficacy failure	91 (32.9)	75 (26.9)	76 (27.4)
Death	9 (3.2)	10 (3.6)	8 (2.9)
Graft loss	16 (5.8)	17 (6.1)	11 (4.0)
Death or graft loss	23 (8.3)	26 (9.3)	18 (6.5)
Loss to follow-up	21 (7.6)	14 (5.0)	12 (4.3)
Treated BPAR (n patients)	55 (19.9)	42 (15.1)	53 (19.1)
treated BPAR events $<$IIA	38 (13.7)	30 (10.8)	35 (12.6)
Treated BPAR events ≥IIA	14 (5.0)	13 (4.6)	22 (7.9)
Missing BPAR grading	6 (2.2)	4 (1.4)	4 (1.4)
On-treatment primary composite*	72 (26.1)	56 (20.1)	74 (26.7)

*On-treatment analysis excludes efficacy events that occurred $>$30 days after study drug discontinuation.

At 24M, the incidence of serious AEs was higher in the EVR 6-12ng/mL group (69%), but comparable between EVR 3-8ng/mL and MPA groups (64 vs. 62%). Renal function at 24M was comparable with a GFR of 52, 49 and 51 mL/min/1.73m^2 in the EVR 3-8ng/mL, 6-12ng/mL and MPA groups.
Conclusion: This analysis at 24M confirms that targeting EVR C0 between 3-8 ng/mL allows for a 60% reduction in CsA concentration as compared to MPA while maintaining efficacy and safety in RTxRs.

O-143 EVEROLIMUS (EVE) IN MAINTENANCE RENAL TRANSPLANT RECIPIENTS CONVERTED FROM CALCINEURIN INHIBITORS (CNI) IMMUNOSUPPRESSION TREATMENT

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Introduction: Everolimus is a proliferation signal inhibitor utilized as immunosupressant in combination with CNI in renal transplantation. The present paper reports our experience with renal grafted patients treated with CNI who were switched to EVE in order to avoid CNI nephrotoxicity.
Methods: From february 2005, 120 cadaver kidney grafted patients (82 men and 38 women) were switched from CNI to EVE. The age of the patients was 56±15 years, with 8.5±6 years (1-23) followup period. The immunosuppression treatment was based on Prednisone, Mycophenolate Mophetil/Sodium Mycophenolic Acid and Tacrolimus/Cyclosporine. CNI was stopped according to an abrupt conversion protocol. EVE was started at 1.0 mg day divided in two doses and blood concentration was maintained between 3-5 ng/ml.
Results: At 2 year patient and graft survival was 100% and there were not acute rejection episodes. Blood presure shows no changes and there were not changes in glucose metabolism. Lipids increased slightly during the first three months after EVE treatment. Proteinuria apear in patients with previos glomerular lesions. GFR improved in 55% of patients.

Conclusion: In conclusion, conversion from CNI to EVE in renal transplant patients is safe and affect slightly to lipids metabolism.

O-144 A ONE YEAR PROSPECTIVE, RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF INTRAVENOUS IBANDRONATE ON BONE LOSS FOLLOWING RENAL TRANSPLANTATION

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Background: Loss of bone mass is common after kidney transplantation. However the efficacy and safety of treatment in these patients have not been well studied.
Methods/Materials: We randomised 129 patients with early stable renal function ($\leq$ 28 days post-transplantation, GFR $\geq$ 30 ml/min) in this double-blind, placebo-controlled trial. They received ibandronate 3 mg iv every 3 months or placebo. All received active vitamin D3 0.25μg/d and calcium 500 mg b.i.d. for 52 weeks.
Results: 66 patients were randomized to ibandronate (1 drop-out) and 63 to placebo (5 drop-outs). The primary end-point, change in bone mass density (BMD) for lumbar-spine (L2-L4) from baseline to 12 months was not different, +0.017 g/cm^2 (+1.5%) for ibandronate versus +0.004 g/cm^2 (+0.5%) for placebo (p=0.28), but significantly higher with ibandronate in total femur +0.011 g/cm^2 (+1.3%) vs –0.007 g/cm^2 (-0.5%), p=0.01 and in ultradistal radius +0.002 g/cm^2 (+0.6%) vs –0.008 g/cm^2 (-1.9%), p=0.039. Markers of bone formation were reduced by ibandronate: P1NP -13.1 μg/L vs +21.6 μg/L, p$<$0.001; osteocalcin -5.5 U/L vs +1.1 U/L, p$<$0.001 and BALP -0.5 U/L vs +1.7 U/L, p=0.001. A marker of bone resorption, NTX, was reduced both with ibandronate -12.5 NM-BCE and placebo, -6.2 NM-BCE, p=0.056. The adverse event profile was similar in both groups, most frequent were transplant rejections (reported by n=18 patients for ibandronate vs 22 for placebo), and CMV infections (n=14 vs 21, respectively). Elevated serum creatinine at 12 months was reported for 5 patients receiving ibandronate and in 10 receiving placebo.
Conclusion: Active vitamin D3 and calcium alone generally prevented bone loss in the first year after renal transplantation. Ibandronate appeared safe and further improved BMD in the femur and ultradistal radius but not in the lumbar spine.

Infection in transplantation

O-145 IMPROVED LONG-TERM RENAL ALLOGRAFT SURVIVAL IN PREEMPTIVE VALGANCICLOVIR THERAPY COMPARED TO VALACYCLOVIR PROPHYLAXIS FOR CYTOMEGALOVIRUS: RESULTS OF RANDOMIZED CONTROLLED TRIAL

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Background: Both preemptive therapy and universal prophylaxis are used to prevent cytomegalovirus (CMV) disease after solid organ transplantation. Randomized trials comparing both strategies in the long-term are sparse.
Methods: Renal transplant recipients at risk for CMV (D+/R-, D+/R+, D-/R+) were randomized to 3-month prophylaxis with valacyclovir (2g q.i.d., n = 34) or preemptive therapy with valganciclovir (900mg b.i.d. for a minimum of 14 days, n = 36) for significant CMV DNAemia ($\geq$2000 copies/mL by quantitative PCR in whole blood). Data at month 12 were reported previously (AJT 2008). All patients were prospectively followed up to 4 years. Protocol biopsy was performed in all patients at 3 years post-transplant.
Results: No CMV disease beyond 1 year was detected. Despite a lower risk of biopsy-proven acute rejection in the valacyclovir group during the first year post-transplant, preemptive group patients showed better 4-year allograft survival compared to universal valacyclovir prophylaxis (92 vs. 74%, P = 0.049). Moreover, a trend toward improved renal function assessed by serum creatinine (143$\pm$58 vs. 162$\pm$65 μmol/L, P = 0.072) and decreased incidence of moderate or severe interstitial fibrosis and tubular atrophy in protocol biopsy at 3 years post-transplant (19 vs. 38%, P = 0.222) was noted. Significantly higher intrarenal mRNA expression of pro-fibrogenic cytokines was observed in the valacyclovir group.
Conclusion: In renal transplant recipients preemptive valganciclovir therapy compared to universal valacyclovir prophylaxis for prevention of CMV disease is associated with improved long-term graft survival and slower progression of interstitial fibrosis and tubular atrophy.

O-146 CMV VALGANCICLOVIR PROPHYLAXIS VS. PREEMPTIVE THERAPY FOLLOWING KIDNEY TRANSPLANTATION: 2-YEAR-DATA FROM THE VIPP-TRIAL

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Background: In moderate risk (D- or D+/R+) renal transplant (Tx) patients (pts) prophylaxis may be the therapy of choice for preventing CMV.
Methods: RCT to determine if R+ renal Tx pts had a higher rate of active CMV infection/disease when treated preemptively vs. pts receiving prophylaxis; & if this correlates with chronic graft impairment & long-term graft/patient survival. Prophylaxis: 2x450 mg valganciclovir (VAC)/d (adj. for renal function) for 100d post-Tx. Pts were monitored with a quant. CMV PCR test and positive pts received 2x900 mg VAC/d followed by secondary prophylaxis with 900 mg VAC/d for 28 d. Pts are followed for 5 yrs; 24-month-data are presented.
Results: In 01/11, data of 201 pts were analyzed: 99 (prophylaxis) and 101 (preemptive). At 24 months tolerability was good. 141 episodes of acute graft rejection (Table) occurred in 82 pts: 43 (prophylaxis), 39 (preemptive). Active CMV infection was significantly higher with preemptive therapy (36.0% vs. 10.3%, p$<$0.0001), and most CMV infection was seen for D+/R+ pts receiving preemptive therapy (51.3% vs. 14.4%, p$<$0.0001). Similarly, D+/R+ pts with preemptive therapy had the highest rate of CMV disease (20.5% vs. 4.4%, p=0.0016). Renal function was similar for both, but D+/R+ pts under a preemptive regimen with CMV infection vs. prophylactic treatment pts had a slightly lower GFR at 24 months (57.95 22.85 ml/min vs. 60.57 22.83 ml/min). Graft loss occurred for more preemptive pts (n=8, 5.3%) vs prophylaxis (n=4, 2.7%, p=0.3782).

Episodes of acute rejection during the 24-month-study period, according to treatment and CMV donor status

Donor CMV status	Prophylaxis (%)	Preemptive (%)	p-value
D–	24 (17.0%)	29 (20.6%)	0.6043
D+	41 (29.1%)	47 (33.3%)	0.8665
All	65 (46.1%)	76 (53.9%)	0.3053

Conclusion: At 2 yrs post-Tx, VAC prophylaxis was associated with a sustained & significant reduction of incidence of active CMV infection, especially in D+/R+ pts. The ongoing follow-up will determine if the observed numerical advantage for graft survival in R+ recipients exposed to VAC prophylaxis may develop into a robust clinical difference.

O-147 CYTOMEGALOVIRUS AND BK VIRUS INFECTIONS ARE LESS FREQUENT WITH EVEROLIMUS VERSUS MYCOPHENOLATE IMMUNOSUPPRESSION: 24-MONTH UPDATE FROM THE 2309 STUDY IN DE NOVO RENAL TRANSPLANT RECIPIENTS

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Background: Cytomegalovirus (CMV) and BK virus (BKV) infections are associated with acute and chronic renal impairment. Studies have shown that the incidence of CMV infections is lower in de novo renal transplant recipients (RTxR) receiving everolimus (EVR).
Methods: A2309 is a 24-month (M) randomized, multicenter, open-label study in 833 de novo RTxR comparing 2 target doses of EVR (C0 3-8 or 6-12 ng/mL) with reduced CsA exposure vs. a control group receiving enteric-coated mycophenolate sodium (MPA) 1.44g/day with standard CsA exposure. At 24M, study endpoints were composite efficacy (incidence of BPAR, graft loss, death or loss to follow-up), renal function and safety comparisons between EVR groups and MPA. All RTxR received basiliximab induction and steroids as per center practice. CMV prophylaxis was used in all high risk patients (donor positive/recipient negative). CMV infection details were captured in specific CMV event forms as per study protocol and BKV infections were reported as per local center evaluations.

Results: At 24M, both EVR groups were comparable to MPA for efficacy, renal function, and overall safety. Incidence of treated BPAR was similar between EVR 3-8 and MPA (19.9% vs. 19.1%) and lower for EVR 6-12ng/mL (15.1%). The pattern of steroid use was similar for all 3 groups. The incidence of CMV infections, CMV events of all categories and BKV infections was lower in both the EVR groups vs. MPA (Table 1). Use of antiviral prophylaxis did not reduce the incidence of CMV infections if the donor and/or recipient were seropositive at baseline.

	EVR 3–8 ng/mL (N=274)	EVR 6–12 ng/mL (N=278)	MPA 1.44 g (N=273)
Infections reported as adverse events (AE) at M24 (%), safety population			
Total AEs	98.9	99.3	98.9
Total infections	67.9	69.8	76.2
– Bacterial	28.5	32.0	28.6
– Fungal	6.2	6.8	6.2
– Viral	10.6	8.3	24.9
thereof BKV infection	0.7	1.4	4.8
thereof CMV infection	1.5	0.4	9.2
CMV events reported in the CMV case report form			
CMV syndrome	1.5	1.8	5.1
CMV lab evidence/antigenemia	1.1	2.2	6.6
CMV disease (organ involvement)	0.7	1.1	2.9

Conclusion: The lower incidence of CMV and BKV infections seen in *de novo* RTxR on EVR therapy may translate into an additional benefit of EVR versus standard therapy.

O-148 EFFECT OF EVEROLIMUS AND DONOR/RECIPIENT SEROLOGY ON CYTOMEGALOVIRUS INFECTION IN HEART TRANSPLANT RECIPIENTS: A SUBANALYSIS OF THE RANOMIZED TRIAL A2310

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Cytomegalovirus (CMV) is the most common infectious agent affecting heart transplant (HT) recipients. Although donor/recipient (D/R) serology is essential in stratifying the risk for CMV, with D+/R– patients at highest risk for clinically relevant infections, immunosuppression additionally affects CMV development. However, the interplay between immunosuppressive regimens and serological risk for CMV is unexplored.

We analyzed 12-month occurrence of CMV infection and syndrome/disease in patients enrolled in A2310 (NCT 00300274). This is a 24-month, multicenter, randomized, open-label study comparing two everolimus arms (target C0 3-8ng/mL or 6-12ng/ml) with reduced cyclosporine to mycophenolate mofetil (MMF) with standard cyclosporine for efficacy, renal function and safety. High everolimus arm was prematurely interrupted because of excess mortality, and is not included in this analysis. CMV prophylaxis was recommended for D+/R- patients.

547 patients comprised the safety population included in this analysis: 279 received everolimus and 268 MMF. Everolimus was associated with lower incidence of CMV infections (8.2% vs. 20.5%, P<0.001), and of CMV syndrome/disease (3.4% vs. 12.5%, P<0.001). As expected, the 108 D+/R– patients were overall more frequently affected by CMV infection and syndrome/disease than the 280 R+ patients (all P<0.05). However, serology impact persisted only in MMF patients, with 22.4% D+/R– vs. 9% R+ with CMV syndrome/disease (P=0.02). Conversely, CMV syndrome/disease occurred in 5.1% of everolimus treated D+/R–, and in 2.7 of R+ (P=0.4; Figure 1).

Everolimus with low-dose cyclosporine reduced about three times the risk for CMV infection and syndrome/disease as compared with standard cyclosporine and MMF. Everolimus remarkably mitigated CMV risk in naïve recipients, who confirmed a high risk for CMV syndrome/disease only in the MMF arm. These data raise the hypothesis that everolimus effect on CMV is independent from recipient's CMV-specific immunity.

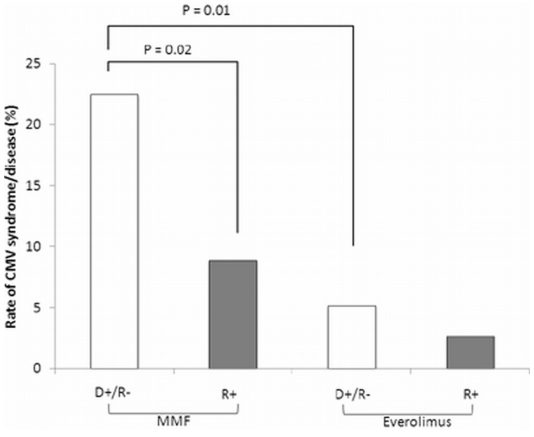

Figure 1

O-149 PRIMARY CMV INFECTIONS AND HHV-6 REACTIVATIONS IN LIVER TRANSPLANT RECIPIENTS RECEIVING VALGANCICLOVIR PROPHYLAXIS

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Background: Cytomegalovirus (CMV) and human herpesvirus-6 (HHV-6) infections are common after liver transplantation. HHV-6 reactivations are usually asymptomatic, but hepatitis or graft dysfunction may occur. Based on *in vitro* studies, the antivirals effective against CMV, have also activity against HHV-6. The efficiency of valganciclovir prophylaxis, given to CMV risk patients (D+/R-), was investigated in preventing primary CMV infections and HHV-6 reactivations.

Methods: Of 196 adult liver transplant patients 32 belonging to CMV high-risk group received antiviral prophylaxis (valganciclovir 900 mg/d and/or i.v. ganciclovir 5mg/kg/d) up to 3 months after transplantation. The patients were monitored for CMV by quantitative PCR and HHV-6 reactivations diagnosed by HHV-6 antigenemia test using monoclonal antibodies against HHV-6B and HHV-6A. Tissue invasive CMV and HHV-6 were demonstrated in biopsies by immunostaining.

Results: During antiviral prophylaxis, no break-through CMV infections were recorded. On the contrary, HHV-6 antigenemia was detected in 12/32 (37%) patients mean 12 days (range 7-22 days) after transplantation. All reactivations were caused by HHV-6B. In three patients HHV-6 antigens were detected in the liver biopsy during graft dysfunction. After cessation of valganciclovir prophylaxis 12/32 (37%) patients developed primary CMV infection mean 181 days (range 95-365 days) post transplantation. Two low level CMV infections were asymptomatic and not treated, 6 infections were successfully treated with valganciclovir, and 4 severe CMV diseases with intravenous ganciclovir. No intragraft CMV infection was found, but one patient developed gastrointestinal CMV. No patient or graft was lost due to CMV or HHV-6.

Conclusions: During valganciclovir prophylaxis, no break-through CMV infections were recorded but HHV-6 reactivations were common, infecting the transplant in three cases. After prophylaxis, primary CMV infections were frequent, but successfully treated with valganciclovir/ganciclovir. Antiviral prophylaxis did not prevent HHV-6 reactivations, but delayed primary CMV infections.

O-150 CMV SPECIFIC IMMUNE RECONSTITUTION IN HTx PATIENTS TREATED WITH PRE-EMPTIVE STRATEGY: LIGHTS AND SHADOWS

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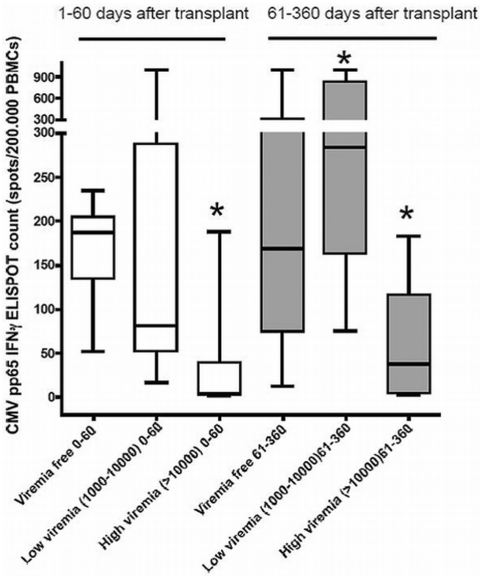
Background: CMV is the most prominent pathogen affecting Heart Transplantation Patients (HTxs). T-cells play a crucial role in controlling Cytomegalovirus (CMV) viremia. The aim of this study is to determine how virus exposure and antiviral pre-emptive treatment affect T-cell recovery after transplantation.

Patients and methods: We recruited 48 CMV R+ patients in a cross-sectional study. We evaluated CMV-DNAemia and CMV specific T-cell response (IFN-gamma ELISPOT test) at the time-points: within 60 days, between 60 and 100 days and after 100 days post-transplant. All HTxs were managed with pre-emptive therapy.

Results: As shown in figure 1, within 60 days after transplant, HTxs not experiencing-post transplant viremia had a median 161 ELISPOT count, while HTxs experiencing moderate viremia (1000-10000) had a median of 91 ELISPOT and patients experiencing overt viremia (>10000) post transplant displayed low (5) median ELISPOT.

From 61-360 days after transplant, HTxs not experiencing viremia continue

to display ELISPOT median of 170 spots, while moderate viremia is statistically associated to an increased CMV specific T-cell response (median 283 ELISPOT). Once more, high overt viremia, after 60 days after transplant, is always statistically associated with a depression in CMV specific T-cell response (median 38 ELISPOT).



Conclusion: The data show that pre-emptive strategy favors the specific CMV immune reconstitution in about 80% of HTx, while the remaining 20% continue to display a low response in spite of higher CMV viremias. The early identification of HTx displaying inefficient antiviral reconstitution may be helpful since this group may find benefit from the antiviral prophylaxis strategy.

O-151 A NOVEL CELL EFFECTOR IN THE ANTIBODY MEDIATED MICROCIRCULATION LESIONS

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CMV infection has been associated with adverse renal allograft outcome, and with a major specific $\gamma\delta$ T cell response. Here we showed that the CMV-induced $\gamma\delta$ T cells participate to the anti-HLA donor specific antibodies (DSAs) mediated lesions.

After extensive phenotyping of CD16 positive circulating lymphocytes, we generated CD16+ Vδ2neg $\gamma\delta$ T cell lines and tested their activity in a model of allogenic antibody dependent cell cytotoxicity -ADCC. Immunofluorescence staining with the anti-endothelial CD31 and anti pan $\gamma\delta$ monoclonal antibodies (mAbs) was then performed on graft biopsies (N=23). Finally, $\gamma\delta$ T cells and estimated glomerular filtration rate (e-GFR) were analyzed in two retrospective cohorts, the first one in kidney transplant recipients (KTR) with DSA (N=21); the second one in non-HLA immunized KTR (N=162).

After CMV infection: i) in vivo, we observed that the CD16+ lymphocyte compartment was equally composed of Vδ2neg $\gamma\delta$ T and NK cells.

ii) in vitro, we demonstrated the ability of the CD16+ Vδ2neg $\gamma\delta$ T cells to induce ADCC by incubating them with DSA and human cell lines, phenotyped for their HLA molecules.

iii) ex vivo in graft biopsies, $\gamma\delta$ T cells were found more frequently in peritubular capillaries and glomeruli of antibody mediated acute rejections - AMAR - (34.8/mm2 and 2.57/mm2, respectively) than those of cell mediated rejection -CMAR- (3.8/mm2 and 0.07/mm2; p=0.02 and p=0.0009 respectively) in CMV infected patients; whereas in CMV naive patients, the rate of $\gamma\delta$ T cells was low in peritubular capillaries and glomeruli in both CAMR and AMAR.

iv) In KTR with DSA, high percentage of CMV-induced $\gamma\delta$ T cells were associated with a poor e-GFR, unlike in HLA non-immunized patients.

In DSA-immunized patients, the association between CMV infection and poor graft outcome could partly be explained by CD16+ $\gamma\delta$ T cells ADCC.

O-152 RENAL PATIENTS SHOWED A POOR RESPONSE TO THE 2009 PANDEMIC NEW INFLUENZA A H1N1 VIRUS VACCINE

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Background: We assessed safety and efficacy of available 2009 pandemic influenza A H1N1 vaccine in immunosuppressed renal patients.

Methods: We prospectively evaluated seroconversion, predictors of response and vaccine safety in renal allograft recipients and patients on hemodialysis. Hemagglutination inhibition tests to detect serum antibodies against Influenza A-H1N1 new virus [strain A/California/7/2009 (H1N1)v] were performed in 79 transplant patients, 48 hemodialysis patients and 15 healthy workers before and one month after vaccination. All controls and 88/127 renal patients were vaccinated. Seroconversion was considered when at least 2 dilutions increase in titer was observed.

Results: We excluded 19 individuals who showed seroprotection ($\geq$1/40) against the novel H1N1 in the initial sample. The efficacy rate in the 96 vaccinated individuals was 43.7% (42/96 seroconverted compared to 4/27 non-vaccinated patients, p=0.007). For subgroups, efficacy rate was 41.8% in kidney transplant patients (p=0.039 compared to 2/16 non-vaccinated), 33.3% in hemodialysis patients (p=0.450 compared to 2/11 non-vaccinated) and 81.8% in healthy controls. Healthy controls showed a significant better response to vaccine than transplant (p=0.021) and dialysis patients (p=0.012). For the transplant subgroup, longer time after transplantation (92$\pm$87 vs 45$\pm$51 months, p=0.028), higher proteinuria levels ($p<0.05$) were associated to seroconversion, but no influence was found for age, gender, renal function, albumin, hemoglobin, leucocytes, ferritin, PTH, vitamin D, immunosuppressive drugs and levels. In the hemodialysis subgroup, younger age was associated to response (55.7$\pm$20.8 vs 71.6$\pm$10.1 years, p=0.042), but not other specific variables including KT/V or time on dialysis. No serious adverse events were reported, and kidney function was stable.

Conclusion: The novel influenza A 2009 H1N1 vaccine proved to be safe in renal patients, though the administration of a single dose of adjuvanted vaccine induced a poor response in these patients

O-153 SAFETY AND EFFICACY OF AN INACTIVATED, UNADJUVANTED VACCINE AGAINST THE NOVEL INFLUENZA A VARIANT (H1N1v) IN RENAL TRANSPLANT RECIPIENTS (INSERM C09-32, TRANSFLUVAC STUDY)

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Background: New immunization strategies are needed in transplant patients who weakly respond to vaccines. The goal was to assess the efficacy and safety of two injections of the non-adjuvanted monovalent A H1N1v 2009 vaccine in a renal transplant population at the time of the 2009 Flu pandemic.

Methods: 121 recipients under triple immunosuppressive regimen were immunized against influenza H1N1v in a prospective single-arm study. Patients received 2 injections (day 0, day 21) of an inactivated, non-adjuvanted H1N1v vaccine. The primary endpoint was the assessment of the humoral immunity by hemagglutination-inhibition (HI) assay on days 21, 42 and 182.

Results: The seroprotection rate (antibody titer 1/40) was 19% (95% CI: 1328) at day 0 (n=119), 53% (95% CI, 4362) at day 21 (n=118), 60% (95% CI: 5169) at day 42 (n=116) (p=0.013; day 42 vs. day 21) and 56% (95% CI, 4665) at day 182 (n=113). The seroconversion rate was 24% (95% CI: 1632) after the first injection and 32% (95% CI: 2442) after the second injection. The geometric mean fold rise was 3.7 (95% CI: 2.84.9) after the first injection and 4.6 (95% CI: 3.56.0) after the second injection. Creatinine clearance remained unchanged throughout the study. No rejection episodes related to vaccination were observed. No change in Donor specific antibodies was recorded throughout the study period.

Conclusions: H1N1v vaccine application was safe in transplant patients. The humoral response remained low. The immunogenicity increased after the second injection reaching levels usually observed in the non-immunosuppressed

elderly population. These results confirm that immunizing transplant patients against influenza remains a challenge and requires new strategies to be explored (dose increase, additional injections, intradermal route).

O-154 MONITORING OF PERIPHERAL BLOOD NATURAL KILLER CELLS TO IDENTIFY HEART TRANSPLANT RECIPIENTS AT RISK OF INFECTION

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Background: Infection remains a source of mortality in heart recipients. We assessed whether monitoring of NK-cells could prove useful when identifying patients at risk of infection.
Methods: We prospectively studied 133 consecutive heart recipients over a 12-month period. Severe infections that required intravenous antimicrobial therapy was the primary outcome. Superficial incisional surgical site infection, catheter-related infections were not considered infectious episodes in this study. As for immunosuppressive treatment, patients received induction therapy with the interleukin (IL) 2 receptor antagonist daclizumab (n=108 [93.1%]) or basiliximab (n=5 [4.3%]). Maintenance immunosuppression included mycophenolate mofetil, prednisone, and either cyclosporine (n=35, 30.2%) or tacrolimus (n=79, 68.1%), depending on the side effects. Total counts and percentages of NK-lymphocyte subsets (CD3–CD56/CD16+) were analyzed by four-color flow cytometry whole blood.
Results: Forty-eight patients had at least one episode of severe infection. Patients with severe infection (n=48) disclosed lower NK absolute counts (day-7 after transplantation [28 vs 57, P=0.021]), 3 months [96 vs 168 cells/uL, P=0.002], 6 months [127 vs 183 cells/uL, P=0.011] and 1 year [154 vs 254 cells/uL, P=0.014]). Patients with bacterial infections (n=27) disclosed lower NK absolute counts (day-7 [22 vs 52 cells/uL, P=0.040]). Patients with CMV infection (n=22) disclosed lower NK percentages (1 year [7 vs 14, P=0.006]), lower NK-cell absolute counts (day-30 [80 vs 117 cells/uL, P=0.05], 3 months [96 vs 151 cells/uL, P=0.016] and 1 year [133 vs 234 cells/uL, P=0.043]). In Cox regression analysis we found an association between the risk of developing an infection and lower day-7 absolute NK-cell count (per decrease of 10 cells/uL, RH 1.24, P=0.011).
Conclusion: Data suggest that monitoring including NK-cell testing is useful when attempting to identify the risk of infection in heart recipients.

O-155 EARLY URETERIC STENT REMOVAL REDUCES URINARY TRACT INFECTION IN KIDNEY TRANSPLANT RECIPIENTS, A RANDOMIZED CONTROLLED TRIAL (EUREKA)

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Background: Duration of retaining ureteric stent after kidney transplantation was still controversy. Short duration of ureteric stent may reduce urinary tract infection (UTI) after kidney transplantation. This study aims to determine benefits and risks of early versus routine stent removal in kidney transplantation.
Methods: Single-center parallel randomized controlled, open label, trial. Randomization was computer-generated block of 4, allocation concealment by sealed opaque envelops. 80 patients who underwent kidney transplantation at a University-based hospital in Thailand from April2010- January2011 were enrolled. Patients were randomized to early ureteric stent removal (8 days) or routine ureteric stent removal (15 days) after kidney transplantation. The primary outcome was rate of UTI during postoperative to 1 week after discharge. Chi-square or Fisher's exact was used to compare the proportion of UTI between groups.
Results: 65 patients (57% living donor) fulfilled the randomized criteria (early remove n=32; routine remove n=33). By intention to treat analysis, incidence of UTI in early stent removal was less than routine stent removal group (12/32, 37.5% VS 24/33, 72.7%; Risk reduction 35.2%; 95%CI 12.5 to 57.8%, P=0.004). The benefit of early ureteric stent removal is demonstrated mostly in living donor subgroup. Incidence of UTI was significantly associated with the duration of stent retention. Incidence of urologic complications was not different in both groups.
Conclusions: Shortening the duration of ureteric stent in kidney transplant recipients from 15 to 8 days is safe. This approach helps to reduce incidence of UTI particularly in living kidney transplantation. (Funded by Thai Transplant Society; Trial registration ACTRN12610000310066)
Key Words: kidney transplantation, ureteric stent, urinary tract infection, urologic complication

O-156 POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD): CHARACTERISTICS AND OUTCOME IN A BELGIAN UNIVERSITY HOSPITAL

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Background: PTLD is a life-threatening complication of all types of transplantation (Tx).
Methods/Materials: Retrospective analysis of medical records of all patients diagnosed with PTLD between January 1989 and December 2010 at the University Hospitals of Leuven, aiming to obtain information about incidence, pretreatment characteristics, treatment and outcome.
Results: 140 biopsy proven PTLD cases were included. Overall incidence was 2%. Highest incidence was reported in heart-lung Tx (7.5%), followed by heart (4.9%), lung (2.9%), liver (2.67%), stem cell (1.4%), kidney (1.3%) and intestinal Tx (0%). Most PTLD were monomorphic (83.6%), with diffuse large B cell lymphoma (DLBCL) being the most frequent subtype. 66.2% of the cases were EBV positive. The majority of cases (70.7%) occurred $>$ 1 year post-Tx. At diagnosis immunosuppressive therapy included calcineurin inhibitors (92%), antimetabolites (71%) and low dose steroids (71%). Reduction of immunosuppression (RIS) was performed in 88.5%. Other first line treatment modalities included rituximab (53%), chemotherapy (28%), surgery (12%) and radiotherapy (7%). Following first line therapy overall response rate was 68.5% (53.5 CR, 15% PR). At last follow up 43% of the patients were alive whereas 10.7% of the patients lost their graft during follow up. In multivariate analysis higher age at diagnosis, hypoalbuminemia and elevated LDH were associated with poor overall survival.
Conclusion: Overall PTLD incidence was 2%. As expected most cases were DLBCL, presented with advanced stage and had a poor outcome. 66.2% were EBV positive. Except for RIS, treatment was very heterogeneous. Contrary to data from the literature the majority of cases occurred late, whereas rituximab therapy was not associated with higher response rates. Although the prognostic role of the international prognostic index (IPI) score in PTLD has been questioned, we were able to confirm its value in our analysis.

Kidney (DCD / ECD)

O-157 BELGIAN EXPERIENCE OF DCD KIDNEY TRANSPLANTATION

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Background: Donation after cardiac death (DCD) was (re)introduced in Belgium in 2000 to expand the pool of kidney grafts. We reviewed the Belgian experience of DCD kidney transplantation (KTx) and compared short and long term graft and patient survival between machine perfusion (MP) and cold storage (CS) preservation.

Methods: We reviewed all DCD KTx performed in Belgium between 01/2000 and 12/2009. Donor and recipient data were collected from Eurotransplant and all 6 Belgian KTx centers.
Results: During the study period, 287 DCD KTx were performed (13% of all deceased KTx). Median follow up was 34 (8-130) months. Kidneys were stored by CS (n=135) or MP (n=152). The incidence of delayed graft function (DGF) was 10% lower in MP compared to CS kidneys (p=0.07), despite longer cold ischemia time (CIT) [17.9 (4.30-30.8) h *versus* 13.8 (3.5-26.7) h; p<0.001)) and anastomotic time [34 (20-70) min *versus* 31 (11-71) min; p<0,001) and more uncontrolled DCD donors (10.5% *versus* 3%) in MP kidneys. In multivariate analysis, MP reduced the risk of DGF (Odds ratio 0.30 (0.14-0,66); p=0,003). CIT was also an independent risk factor of DGF (Odds ratio 1.14 (1.05-1.23);p<0.001). The 1, 3 and 5-year patient/censored graft survival were comparable between MP and CS (97%, 96%, 92%/97%, 93%, 93% MP *versus* 96%, 92%, 81%/93%, 89%, 78% CS; log rank 0,06/0,20).
Conclusion: DCD KTx in Belgium is associated with excellent short and middle term results. In this Belgian patient cohort and in line with previous studies, MP decreases the risk of development of DGF whereas CIT increases this risk. In addition, our data strongly suggest that the impact of CIT on DGF is reduced by MP.

O-158 DCD KIDNEY TRANSPLANTATION: LONG-TERM RESULTS OF UNCONTROLLED VERSUS CONTROLLED DONORS

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Background: There is a general reluctance to use kidneys from uncontrolled donors after cardiac death (DCD) for transplantation because of the relatively high incidence of primary non-function (PNF) and delayed graft function (DGF). Therefore, we compared the initial and long-term graft function and the survival of kidneys between uncontrolled and controlled DCD donors.
Methods: From January 1981 to January 2008, 523 DCD kidneys were procured in the Maastricht region of which 173 were discarded. 334 DCD kidneys (128 uncontrolled and 206 controlled) were transplanted in the Eurotransplant region and completed follow-up. We studied the short and long-term graft function and the graft survival after transplantation.
Results: The incidence of PNF and DGF in both uncontrolled and controlled DCD kidneys is relatively high (PNF: 22% vs.21%, p = 0.81, and DGF: 79% vs. 71%, p = 0.20, respectively). Graft function assessed with estimated glomerular filtration rate (eGFR) at year 1 after transplantation is 40±16 vs. 42±19 mL/min/1.73m^2, p = 0.55, with a yearly decline thereafter of 0.67±3 vs. 0.70±7 mL/min/1.73m^2/year, p = 0.97. Furthermore, the long-term graft and recipient survival at ten years after transplantation do not differ between uncontrolled and controlled DCD kidneys: 52% vs. 46%, p = 0.68 and 61% vs. 60%, p = 0.76, respectively.
Conclusion: This study demonstrates that the initial function and long-term outcome of uncontrolled DCD kidneys is comparable to the outcome of controlled DCD kidneys. In both groups, careful selection of both donor kidneys and recipients is mandatory to reduce the risk of PNF. These results justify expansion of the donor pool with uncontrolled donors to reduce the still growing waiting list for renal transplantations and may stimulate implementation of uncontrolled DCD kidney donation programmes.

O-159 KIDNEY GRAFT QUALITY AFTER DONATION FROM UNCONTROLLED DECEASED DONORS AFTER CARDIAC ARREST

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Kidney grafts from uncontrolled deceased donors after cardiac arrest (uDDCA) have recently been used in France to counteract organ shortage. The quality of these kidneys remains debatable. The aim of our study was to compare the outcomes and the quality of uDDCA kidneys with that of kidneys from optimal donors such as simultaneous kidney and pancreas (SPK) donors and extended-criteria donors (ECD).
27 kidney grafts from uDDCA (mean donor age, 41) were compared with 24 kidney grafts from SPK donors (mean donor age, 26), and 30 kidney grafts from ECD (mean donor age, 66). All three patient groups were non-immunized and received the same induction and maintenance immuossupressive therapy.
The quality of the grafts was assessed by renal function and histology. GFR was estimated by MDRD formula (eGFR) at M1 (n=80), M3 (n=80), M6 (n=79), M12 (n=74), M24 (n=70) and M36 (n=51) and measured by inuline clearance (mGFR) at M12 (n=66) and M36 (n=46). Interstitial fibrosis (IF) and vascular lesions were analyzed in systematic kidney biopsies at M3 (n=54) and M12 (n=50) with the Banff 2007 classification. IF was quantitatively measured by colour image analysis.
Kidney graft quality from SPK group was always superior than the two others groups. In the short term, DGF in the uDDCA group was significantly higher than in the ECD group (Table 1).

Table 1. Early outcome

	uDDCA	ECD	p uDDCA vs ECD
PNF (%)	0	0	NA
DGF (%)	81.5	27.6	<0.0001
Mean (sd) HD session	4.7 (3.9)	0.7 (1.4)	<0.0001
Mean (sd) time of HD (days)	15.6 (13.0)	2.8 (5.9)	<0.0001
Mean (sd) time of renal function recovery (days)	17.8 (9.2)	5.0 (5.2)	<0.0001
Clinical rejetion n (%)	5 (18.5)	7 (23.3)	0.62
Subclinical rejection n (%)	2 (7.4)	3 (7.5)	0.71
Borderline changes n (%)	12 (44.4)	8 (26.6)	0.15

PNF = Primary non function, DGF = Delayed graft function, HD = Hemodialysis.

In the uDDCA group renal function was initially poorer but improved during the first year.
However on the long term, renal function and interstitial fibrosis was not different in uDDCA vs ECD group (Table 2).

Table 2. Kidney graft function and histology

Mean (sd)	uDDCA	ECD	p uDDCA vs ECD
e GFR M1	23.4 (8.7)	40.2 (16.0)	<0.001
e GFR M3	38.9 (11.7)	39.5 (17.0)	0.96
e GFR M6	41.7 (12.6)	41.5 (16.5)	0.88
e GFR M12	45.2 (13.0)	45.2 (15.4)	0.97
e GFR M24	45.2 (13.8)	45.0 (20.9)	0.97
e GFR M36	44.1 (14.1)	37.4 (10.4)	0.13
m GFR M12	44.3 (13.0)	40.2 (14.6)	0.31
m GFR M36	41.2 (12.3)	33.7 (11.2)	0.09
IF score M3	30% (9)	28% (12)	0.52
IF score M12	36% (13)	33% (14)	0.47

e GFR: estimated GFR accoring to simplified MDRD formula; m GFR: GFR measured by inulin clearance; IF score: Intersitial fibrosis score obtained by colour image analysis.

Conclusion: Our study suggests that the quality of kidneys from uDDCA donors is similar to that of ECD and that these kidneys should be attributed to the same recipient population.

O-160 DONOR KIDNEY DISEASE AND TRANSPLANT OUTCOMES FOR KIDNEYS DONATED AFTER CARDIAC DEATH

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Donation after Cardiac death (DCD) is becoming increasingly common and provides kidneys with comparable outcome to heart-beating (DBD) kidneys. Increasingly marginal DCD kidneys from elderly donors (60+) have been used and long term outcomes are not yet known. Histopathological scoring systems for marginal DBD kidneys based on the presence of chronic damage have not been validated for DCD kidneys. Here we report how baseline damage impacts on outcomes of DCD kidneys.
Outcomes of all first time and single-kidney DCD (213) and DBD (100) transplants performed at our centre between 2006 and 2010 were analysed. Time zero biopsies were performed routinely and were scored histopathologically according to the presence of glomerular, tubular, parenchymal and vascular disease (0-3 for each component) as described previously by Remuzzi et al. Multivariate analysis was performed to assess the effect of a number of donor variables (age, sex, type [DCD vs DBD], hypertension, smoking, cold ischaemic time and HLA mismatch level) on outcome.
DCD kidneys scoring 4-6 had poorer graft survival than DCD kidneys scoring 0-3 though acceptable graft survival rates were achieved. DCD Kidneys with donor age >55 and score 4-6 appear to have poorer graft survival with only 40% of grafts surviving past 3 years. Multiple regression analysis showed that the effect of baseline score on outcome remained after controlling for donor age (Table 1).

Table 1. Multiple regression analysis of donor age and global score vs 90 day eGFR

Variables (n=114)	Range	Estimate	Standard Error	P-value
Donor Age (years)	14– 82	–0.35	0.11	0.001
Global Score (0-12)	0–6	–2.00	1.00	0.047

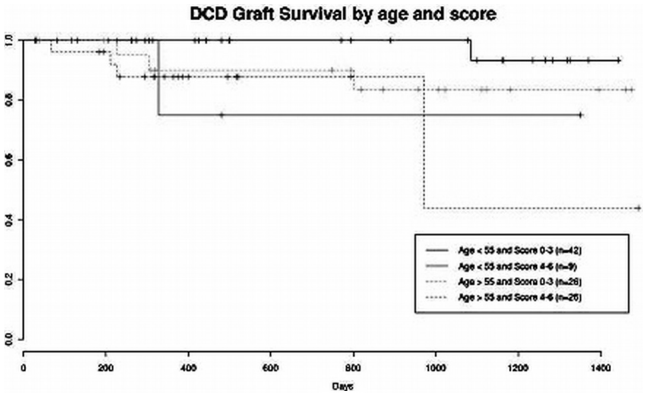

Figure 1. Graft survival of DCD kidneys grouped by global score and donor age.

Results for single DCD kidneys with moderate scores (4-6) are poorer than for low scores (0-3), but overall, the results for this cohort are acceptable. Multiple regression analysis revealed that both donor age and baseline score are independent predictors of outcome thus combination of an old donor and moderate score should be considered carefully.

O-161 PRETRANSPLANT EX VIVO REPAIR OF DENUDED TUBULE EPITHELIUM IN AN UNCONTROLLED PORCINE DCD KIDNEY MODEL

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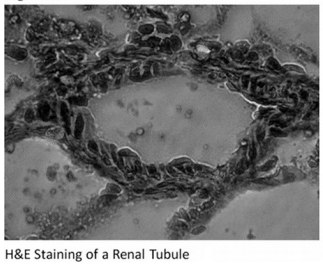
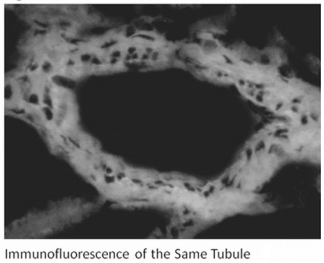
Background: The ability to repair warm ischemically (WI) damaged renal tubule epithelium prior to transplantation would provide expanded donor criteria to include uncontrolled DCD (uDCD) patients. We have previously demonstrated that an ex vivo acellular, near-normothermic perfusion can resuscitate oxidative metabolism after as much as 2-hours of WI sufficiently for there to be new synthesis that restores cytoskeletal integrity. However, during the period of ex vivo perfusion, the tubule epithelium is not regenerated because rather than repair, cell replacement is needed. We now describe the ability to deliver progenitor renal epithelial cells (REC) during ex vivo perfusion to the renal tubule epithelium with homing to the sites of damage.
Methods/Materials: Human REC were labeled with PKH26 red fluorescent cell linker. Porcine kidneys were damaged by 60-minutes of postmortem WI. The damaged kidneys were then flushed and placed on exsanguinous metabolic support perfusion at 32°C to restore oxidative metabolism, normalize perfusion pressures and vascular flow rates. 5.0×10^7 labeled human REC were then infused into the renal artery at the rate of 5.0×10^5 per minute. The perfusion was continued for an additional 5-hours.
Results: During the administration of the REC there were no adverse vascular reactions. More than 90% of the fluorescently labeled human REC were taken up by the kidney and could be detected predominantly in the tubules of the outer medulla (Figure 1 & 2).

Figure 1



H&E Staining of a Renal Tubule

Figure 2



Immunofluorescence of the Same Tubule

Conclusions: These results demonstrate the ability to deliver REC to the renal parenchyma during an ex vivo perfusion with homing to the site of damaged tubule epithelium and the corresponding ability to quantify the number of REC retained within the renal tissue. The ability to target progenitor cell delivery provides opportunities for enhanced ex vivo repair of ischemically damaged kidney allografts.

O-162 RENAL TRANSPLANTATION USING DCD DONORS: DOES CIT MATTER?

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Background: Kidney transplantation using cardiac death donors (DCD) has risen in the UK over the last decade. Concerns have been expressed about organ quality but recent data suggests comparable outcomes to brain dead donors and NHSBT plan to allocate DCD kidneys nationally. This study compares paired outcomes of the second kidney transplanted with the first from matched donors as poorer outcomes may be predicted with prolonged cold ischaemic times (CIT).
Method: We retrospectively reviewed paired recipient outcomes from single donors between 2002-2010 in 5 centres. Recipient data assessed was age, gender, CIT, delayed graft function (DGF), primary non function (PNF), graft survival at 3 and 12 months.
Results: 133 kidney pairs had data available for analysis. Group A was implanted first and group B was second. Donor creatinine at retrieval was 89.8 ± 47 μmol/l (mean $\pm$ SD) and primary warm ischaemia time was 19 minutes. Mean difference in CIT was 5 hours and 22 minutes. (Group A: 15:39 hours vs group B: 21:01 hours) ($p < 0.0001$). There were no significant differences between groups for recipient age, gender, co-morbidity. 5 cases of PNF were seen. (2 vs 3). DGF rates were higher with prolonged CIT though it was not statistically significant (69 vs 85 cases, p= 0.062). There was no difference in creatinine at 3 months (153 ± 60 vs 162 ± 75 μmol/l, $p>0.05$) or 12 months (140 ± 61 vs 162 ± 82 μmol, $p>0.05$).
Discussion: Transplantation of the second kidney does not result in higher PNF rates nor creatinines at 3 and 12 months compared to the first kidney from the same donor. DGF rates are higher though not statistically significant. These results support national allocation of DCD kidneys although the potential impact of prolonged CIT on DGF warrants further investigation.

O-163 KIDNEYS FROM DONORS AFTER CARDIAC DEATH (DCD) PROVIDE SIMILAR ALLOGRAFT FUNCTION AT 1 YEAR COMPARED TO KIDNEYS FROM DONORS AFTER BRAINSTEM DEATH (DBD)

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Background: The use of DCD kidneys is increasing due to growing demand. Here, we compare graft and patient outcomes between DCD and DBD transplants performed in our unit.
Methods: Observational analysis of all patients who received a DBD or DCD kidney in our unit during a 5-year period. DCD recipients received ATG induction, DBD recipients basiliximab. Lower trough tacrolimus level was aimed for the DCD patients (4-7 μg/l). Kaplan-Meier (K-M) estimates were used to assess graft survival.
Results: Over 5 years, 226/514 (44%) patients received a DBD and 80/514 (15.5%) DCD kidney. Median follow-up time was 30 and 40 months for DCD and DBD recipients respectively. Baseline characteristics are shown in table 1.

Table 1. Baseline characteristics

	DCD	DBD	p
Recipient age in years (median)	51.5	51	0.2†
Males %	68	64	0.5‡
Diabetics %	11	8	0.3‡
First transplants %	91	77	0.005‡
Dialysis duration in months (median)	32	33	0.8†

†Mann-Whitney U test, ‡Chi-square test.

Median recipient age (DCD 51.5 [19-72] vs DBD 51 [18-78] years, p=0.2) and median donor age (DCD 47 [17-68] vs DBD 52 [2-78] years, p=0.2) were similar.

Table 2. Summary of results

	DCD (n=80)	DBD (n=226)	p
Mismatch number (median)	2	3	<0.001†
Cold isch. time in hours, median (range)	13	15	<0.001†
Delayed graft function	74%	27%	<0.001‡
Length of hospital stay post-transplant (days)	12	9	<0.001†
Acute rejection within 1 year	9%	23%	<0.001‡
Median eGFR at 1 year	50	51.5	0.6†
One year graft survival	92%	90%	0.7‡
One year patient survival	95%	96%	0.9‡

†Mann-Whitney U test, ‡Chi-square test.

Median cold ischemic time (CIT) was significantly shorter in the DCD group (13 [5-27] vs. 15 [6-32] hours, $p<0.001$). There was significantly higher incidence of DGF in the DCD group (74% vs 27%, $p<0.001$). There was one instance of primary non-function in DBD group and none in DCD group. Biopsy proven

acute rejection was less common in the DCD group. Median eGFR at 1 year were similar in both groups. K-M estimates showed a similar probability of allograft survival in both groups (log-rank test, p=0.5) (Figure 1).

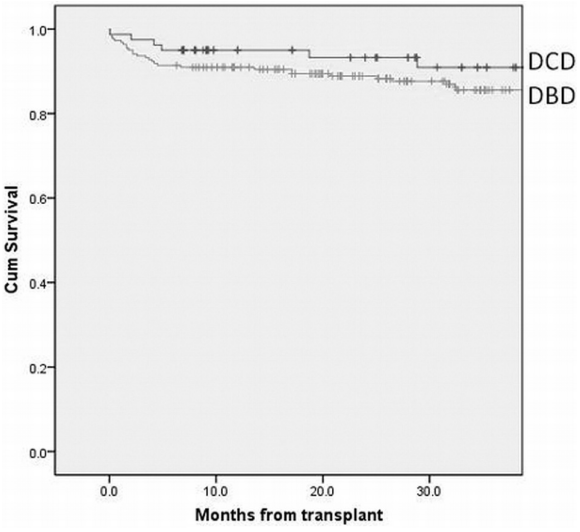

Figure 1

Conclusions: In this analysis, patient and graft survival, and eGFR at 1 year were similar for DBD and DCD recipients. The observed increase in DGF among DCD patients did not influence 1 year outcomes, but did contribute to longer hospitalization post-transplantation.

O-164 EXTRACORPORAL NORMOTHERMIC ABDOMINAL PERFUSION "IN SITU" FOR RESUSCITATION KIDNEY IN UNCONTROLLED DONORS WITH ONE HOUR WARM ISCHEMIC TIME

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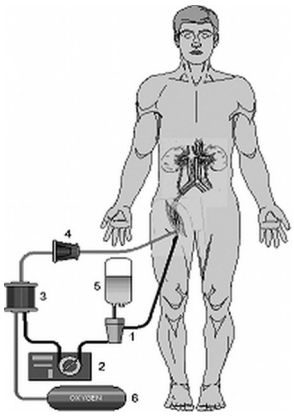
The availability of brain death donors is restricted by many factors. Use of uncontrolled donors after cardiac death could be a promising perspective, but the limiting factor in uncontrolled donation after cardiac death is the warm ischemic time. In 2009-2010 we implemented new model of kidney donation from uncontrolled donors with warm ischemic (asystolic) time (WIT) from 45 to 91 minutes. The explantation team arrived in donor's hospital after cardiac arrest. The developed device was adapted for treatment of ischemically damaged kidney inside the body of donor. by normothermic extracorporal perfusion with leukocyte depletion before procurement, providing restoration of kidney after ischemic damage.
After getting a permission of administration, asystolic donors were transferred to an operating room by hospital staff. A perfusion contour included the following components: leukocyte filter; mechatronic perfusion module; portable source of oxygen, oxygenator and venous reservoir; extracorporal perfusion system tubes. Procurement team inserted three-luminal double balloon through the femoral artery in aorta for isolated perfusion of abdominal basin, and vena cava was drained through the femoral vein. Then the vascular ports were connected to the perfusion contour and the normothermic extracorporal perfusion (NECP) of isolated abdominal region in situ with membrane oxygenation (MO) and leukocyte depletion (LD)commenced. There were 14 donors with unexpected cardiac arrest, the WIT is 77.1±3.6 min. In 9 out of 28 kidney recipients, graft function was recovered immediately. All kidney grafts are functioning and to the end of the first year after transplantation, the average creatinine is 137.9±0.013 mkmol/L.



Main results of transplantation of resuscitated kidney

Characteristics	Results
Immediate function	9 (32,1%)
Delayed graft function	19 (67,8%)
Creatinine at 90 days, mkml/l	0,133±0,013
Creatinine at 1 year, mkmol/l	0,137±0,013
Duration of hospital stay, days	35,3±2,9

Seemingly NECP with MO and LD is not only a supporting, but resuscitating, treatment, and rehabilitation procedure for donor organs after long warm ischemic time.In that way it could be promissory practice for expanding donors' pool.

O-165 RESULTS OF UNCONTROLLED DCD MAASTRICHT CLASS II KIDNEY TRANSPLANT (KT) WITH PULSATILE PERFUSION (PP) MACHINE PRESERVATION

David Paredes[1], Angel Ruiz[1], Camino Rodriguez-Villar[1], Marta Alberola[1], Lluis Peri[2], Blanca Miranda[1], Antonio Alcaraz[2], Frederic Oppenheimer[3].
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Introduction: In our center, DCD has been increasing to become 52% of present procurement activity. Those grafts could have increased Discard Rate (DR), DGF and PNF. The objective was to study the effect of PP for kidney preservation.
Methods: A prospective cohort study on DR and transplant outcomes of 118 DCD kidneys preserved with PP (RM3 Waters Medical Systems) were compared with a cohort of 91 DCD kidneys preserved in Cold Storage (CS). All donors undergone ECMO-Normothermic-Recirculation as the preservation technique before organ retrieval.
Results: Since January 1999 to December 2009, 209 DCD kidneys were retrieved. PP and CS groups were not different in gender and age, but were different in donors >60 years (28,7% vs 14,3% p=0,04), stroke as a cause of death (12,6% vs 6,1% p=0,017), presence of HBP (22,7% vs 4,9% p=0,017) and DM (8% vs 4,7% NS). DR showed a significant reduction from 57% to 30% ($p<0.05$) between PP and CS. Transplant results were analyzed only for KT performed in our center (PP=63 and CS=49). No PNF was found in PP, while 3 cases occurred in CS. Mean CIT was not different between groups (13,4 vs 12,6h). Comparing PP with CS, a significant reduction in DGF (60,9% vs 79,5% p=0,02); hospital stay (20,35 days vs 32,81 days p=0,001) and dialysis sessions (3 vs 7,3 p=0,051) were observed. No difference was observed at 5 years graft survival (80,3% vs 82,3% p=0,052).
Conclusions: Even considering that PP group was worst in some risk factors, PP preservation reduced kidney DR and improved KT outcomes. The absence of PNF and reduced DGF, hospital stay and dialysis sessions represent a direct benefit for the patient. In turn, these factors financially balance the costs of PP.

O-166 IMPACT OF THE COMPLEMENTARY EVALUATION OF ECD AND DCD KIDNEYS COMBINING KIDNEY BIOPSY AND PULSATILE PERFUSION (PP) MACHINE

David Paredes[1], Angel Ruiz[1], Camino Rodriguez-Villar[1], Marta Alberola[1], Lluis Peri[2], Blanca Miranda[1], Antonio Alcaraz[2], Frederic Oppenheimer[3].
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Introduction: Donors after Brain Death with Extended Criteria (ECD) and uncontrolled DCD have become 60% of our current activity, with a high risk of Discard Rate (DR), PNF and DGF. The objective is to evaluate PP preservation as a complementary assessment to pre-transplant Kidney Biopsy Score (KBS).
Methods: Since 2004, PP (RM3-Waters Medical Systems) was added to KBS to evaluate DCD and ECD kidneys. Criteria for single KT were KBS<4 and

RR<0.4 and RF>70ml/min. A prospective cohort of high risk kidneys in terms of DR, PNF, DGF and transplant outcomes is described.
Results: From 419 kidneys retrieved, only 158 cases have KBS and PP (DCD 52/ECD 106). The Pearson Index Correlation between KBS and PP were 0.234 ($p<0.05$). 14 kidneys met neither KBS nor PP criteria and were discarded. 99 kidneys which met both criteria were transplanted: None PNF, DGF 30%, Hospital Stay > 10 days (HS) 46% and Graft Survival (GS) 88.7%. In 45 cases both criteria were not coincident: from 25 with good PP (RR=0.25) but high KBS (X: 4.7), 17 kidneys with KBS=4 were grafted: DR 32%, none PNF, DGF 43%, HS 60.7% and GS 82.3%. From 20 with accepted KBS (X=2) but high RR (X=0.72) only 6 with RR=0.51 were transplanted: DR 70% and evolution: none PNF, DGF 60%, HS 72% and GS 82.3%.
Conclusions: After using PP criteria, 14.6% more kidneys were transplanted. KT with both normal parameters has better results. In case of discrepancy, a good PP and KBS<5 compared with KBS<4 and high PP had a reduced DR (32% vs 70%) and less DGF (43% vs 60%). ECD and DCD kidneys require a step-wise quality evaluation based in the complementary evaluation capacity of PP for organ acceptance.

O-167 KIDNEY INJURIES DURING ORGAN PROCUREMENT IN DONATION AFTER CARDIAC DEATH DONORS

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Introduction: Injuries during kidney procurement for organ transplantation are relatively uncommon but they can result in significant morbidity even if the injury is recognised and repaired. During the last 10 years, kidneys retrieved/transplanted from Donors after Cardiac Death (DCD) have significantly increased. To optimize their use there has been an urgent need to minimize both warm and cold ischaemia which often necessitates less dissection and more rapid removal. Together this combination can make kidney procurement from DCD's a more challenging operation.
Aim: To compare the rates of kidney injury during procurement from DCD and DBD organ donors.
Results: A total of 13260 kidney procurements were performed in the UK over a 10 year period (2000 to 2010). Data was retrieved from a prospectively maintained National Health Service Blood and Transplant (NHSBT) database. Median donor age was 47 years (range 0-85), median donor BMI was 25 (range 7-69). Injuries occurred in 903 procedures (6.8%). Capsular, ureteric and vascular injuries occurred in 1.7%, 0.7% and 4.8% procurements respectively. 12372 (93.3%) kidneys were retrieved from DBD donors and 888 (6.7%) from DCD donors. The rates of kidney injury were significantly higher when retrieved from DCD donors (11.4% vs 6.8%, $p<0.001$). Capsular, ureteric and vascular injuries were all significantly more frequent ($p=0.002$, $p<0.001$ and $p=0.034$ respectively). Those discarded due to significant injury was more common after DCD donation ($p=0.002$). Multi-variate analysis (binary logistic regression) demonstrated procurement injuries were significantly associated with DCD donors ($p=0.029$) as well as donor age, donor BMI and male gender.
Conclusion: This is the first study analysing the occurrence of injuries during DCD kidney procurement. We conclude that procurement from DCD donors leads to higher rates of injury to the kidney and are more likely to be discarded.

O-168 DELAYED GRAFT FUNCTION LONGER THAN ONE WEEK IS THE CRITICAL RISK FACTOR FOR LONG TERM GRAFT SURVIVAL OF KIDNEY TRANSPLANTATION FROM DONORS AFTER CARDIAC DEATH

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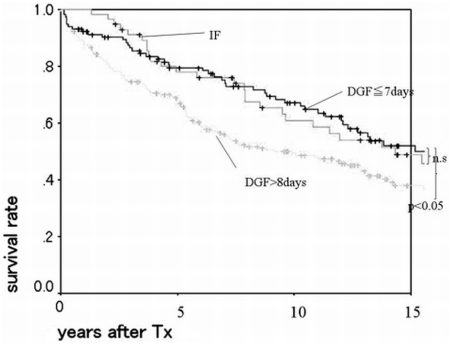
Donors after cardiac death (DCD) have become an important source of renal transplants to alleviate the shortage of renal grafts, although DCD kidneys often have complications associated with a delayed graft function (DGF). DGF predisposes the graft to both acute and chronic rejection, indicating that DGF is a crucial risk factor for graft survival. The purpose of this study is to investigate the impact of the DGF to graft survival after kidney transplantation from DCD. Since 1979, 523 kidneys were retrieved from DCD at our centerther using *in situ* regional cooling technique. 411 grafts transplanted since 1983 through 2009 were enrolled in this study. The age of the donors and recipients ranged from 0.8 to 75 (mean; 46.2) and from 7 to 65 (mean; 41.2). The warm ischemic time ranged from 1 to 71 minutes (mean; 12.0). For the statistical analysis, Kaplan-Meier method and Log-rank test were used. Following renal transplants, primary non function was noted in 27 (6.5%), immediate function (IF) was 57 (13.9%), DGF≤7days was 116 (28.2%) and DGF>8days was 211 (41.3%). The 1, 3, 5, 10 and 15 year graft survival rates in IF and DGF≤7days were 100% vs 92.2%, 91.1% vs 86.5%, 78.0% vs 79.5%, 60.8% vs 67.2% and 48.9% vs 51.9%, respectively. There were no difference between two groups. The 1, 3, 5, 10 and 15 year graft survival rates in DGF>8days were 88.1%, 74.5%, 68.4%, 49.3% and 38.1%, respectively. The difference in the graft survival betweeen IF, DGF≤7days and DGF>8days were significant ($p<0.05$).



The renal grafts retreaved from DCD and the duration of DGF longer one week revealed significantly poor long-term graft survival compred to IF and the DGF≤7days.

Tuesday, 6 September 2011

Islet / cell transplantation

O-169 BOTH ALLOANTIBODIES AND AUTOANTIBODIES ARE A SIGNIFICANT BARRIER TO PRESERVATION OF ISLET CELL FUNCTION AFTER TRANSPLANTATION

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Background: HLA antibodies have been shown to have a deleterious effect on most organ transplants and donor specific HLA antibodies (DSA) could be a major cause of function loss in islet cell transplant recipients. In addition anti-GAD (GADA) and anti-IA2 (IA2A) autoantibodies (AutoAb) may also lead to poor outcomes in islet recipients. Herein we describe the association between antibodies (allo- and auto-) and outcomes in islet cell transplant patients.
Methods: Islet cell transplanted patients (n=44, 36 ITA and 8 IAK) between 2001 and 2010 were studied. Sera (n=385) were serially collected from the date of transplant and tested for DSA (via single antigen beads), GADA and IA2A.
Results: The median survival of transplant from first islet cell infusion (Tx) was 441±244 days. Insulin independence, partial function and early graft loss were achieved in 48% (21/44), 27% (12/44) and 25% (11/44) of recipients, respectively. Regarding DSA, 29% (13/44) of the patients became positive post-transplant (isotype: 4/13 IgG, 4/13 IgM, 5/13 IgG and IgM). Regarding AutoAb, 25% (11/44) showed a significant rise post-transplant (7/11 GADA, 4/11 GADA and IA2A). The median time of Ab appearance was 14±3, 28±2 and 189±59 days post Tx for AutoAb, DSA IgM and DSA IgG, respectively. The probability to develop DSA was associated with the number of donor and mismatches and with the absence of calcineurin inhibitor treatment while the probability to develop AutoAb was associated with the absence of rapamycin treatment. Of major importance it was found that development of DSA and/or AutoAb was significantly associated with loss of islet function ($p<0.005$).
Conclusion: These data suggest that monitoring DSA and AutoAb in islet cell transplant patients is important and that treatment to remove these antibodies may benefit outcomes.

O-170 CELLULAR IMMUNE REACTIVITY AGAINST DONOR-ANTIGEN CORRELATES WITH THE OUTCOME AFTER CLINICAL ISLET TRANSPLANTATION: TOWARD A BETTER POST-TRANSPLANT MONITORING

Stéphanie Lacotte[1], Sylvie Ferrari[2], Sophie Borot[1], Jean Villard[2], Sandrine Demuylder-Mischler[1], Philippe Morel[1], Gilles Mentha[1], Thierry Berney[1], Christian Toso[1]. *[1]Abdominal and Transplant Surgery, Department of Surgery, Geneva University Hospital, Geneva, Switzerland; [2]Immunology and Transplant Unit, Geneva University Hospital, Geneva, Switzerland*

Improved post-transplant monitoring and on-time detection of rejection and/or recurrence of autoimmunity would improve islet transplantation outcome. The aim of this study was to highlight the efficiency of immune assays to detect alloreactivity following transplantation and to demonstrate a correlation with clinical outcome.

Peripheral blood mononuclear cells were isolated from 14 islet recipients. Mixed lymphocyte culture used recipient cells, and donor or third party cells. The level of immune reactivity was assessed as the release of IFNγ (ELISpot), cell proliferation (FACS analysis) and cytokine quantification (Luminex). Clinical outcome was assessed with the beta-score (Ryan et al, 2005).

Clinical outcome correlated with the number of IFNγ-secreting cells following incubation with donor cells (-0.485, p=0.007, Spearman), but not with third party cells (0.6, p=0.84). Similarly, a high number of donor-specific proliferating cells was associated with a low beta-score (-0.505, p=0.006). Both IFNγ-ELISPOT and proliferation were accurate in predicting outcome (ROC curve AUC=0.77 and 0.83 respectively). The cell subset distribution was similar in IFNγ-producing cells than in the whole population of peripheral mononuclear cells. Regarding phenotype of proliferating cells, CD4-expressing T lymphocytes (CD3+CD4+) and NK cells (CD3-CD56+) were the main Ki67+ cells (24.6±2.7% and 17.3±2.1% respectively). The proliferation of CD8-expressing T lymphocytes was less intense (6.8±1.2%). Patients with the worse islet function (beta-score <4) showed increased levels CD4+Ki67+ cells (37.6% vs 16.6%, p=0.0001). No significant differences were observed in CD8+Ki67+ cells and CD56+Ki67+ cells (9.8% vs 5.1% and 15.6% vs 17,5%, p>0.5).

We demonstrate that cellular immune reactivity against donor cells correlates with post-transplant islet function. Phenotype of cell-subsets and cytokine profile may help to establish immune profile of islet-transplanted patients. These assays could be of substantial help in the management of islet-graft recipients.

O-171 IMPACT OF THE NUMBER OF INFUSIONS ON TWO-YEAR METABOLIC RESULTS OF ISLET-AFTER-KIDNEY TRANSPLANTATION IN THE GRAGIL NETWORK

Sophie Borot[1], Nadja Niclauss[1], Anne Wojtusciszyn[3], Coralie Brault[2], Yannick Muller[1], Laurianne Giovannoni[1], Géraldine Parnaud[1], Raphael Meier[1], Laurence Kessler[4], Charles Thivolet[5], Alfred Penfornis[7], Emmanuel Morelon[8], Lionel Badet[9], François Bayle[10], Luc Frimat[11], Christian Toso[1], Philippe Morel[1], Domenico Bosco[1], Cyrille Colin[2], Pierre Yves Benhamou[6], Thierry Berney[1]. *[1]Islet Isolation and Transplantation Center, Geneva University Hospitals, Geneva, Switzerland; [2]Pôle Informartique Médicale Evaluation Recherche, Hospices Civils de Lyon, Lyon, France; [3]Diabetology, Montpeller University Hospital, Montpeller, France; [4]Diabetology, Strasbourg University Hospital, Strasbourg, France; [5]Diabetology, Hospices Civils de Lyon, Lyon, France; [6]Diabetology, Grenoble University Hospital, Grenoble, France; [7]Diabetology, Besançon University Hospital, Besançon, France; [8]Nephrology and Transplantation, Hospices Civils de Lyon, Lyon, France; [9]Urology and Transplantation Surgery, Hospices Civils de Lyon, Lyon, France; [10]Nephrology and Transplantation, Grenoble University Hospitals, Grenoble, France; [11]Nephrology and Transplantation, Nancy University Hospitals, Nancy, France*

Background: Insulin independence after islet transplantation is generally achieved after multiple islet infusions. However, single infusions would allow increasing the number of islet transplant recipients. The aim of this study was to evaluate the results of IAK transplantation in type 1 diabetic patients according to the number of islet infusions.

Methods: Islets were isolated at the University of Geneva and shipped and transplanted between 2004 and 2010 into French patients from the Swiss-French GRAGIL network, on the "Edmonton" immunosuppression protocol.

Results: Nineteen patients were transplanted with 33 preparations isolated from 36 donors. Fifteen patients reached 24 months follow-up after the first islet infusion: 8 subjects were single graft recipients (Group 1) and 7 were double graft recipients (Group 2). Single graft recipients received 5,312 IEQ/kg (5,186-6,388) vs 10,564 (10,054-11,375) for double graft recipients. Insulin-independence was achieved in 5/8 Group 1 subjects vs 5/7 in Group 2 subjects. Insulin-independence duration was 4.7 months (3.1-15.2) in Group 1 vs 19 months (9.6-20.8) in Group 2. At 24 months post-transplant, insulin doses were reduced by 58% in Group 1 (31-80) vs 97% (60-100) in Group 2, HbA1c was 6.5% (5.9-6.8) in Group 1 vs 6.2% (5.9-6.3) in Group 2, and basal C-peptide was 0.9 ng/mL (0.4-1.4) in Group 1 vs 1.8 (1.2;2.4) in Group 2.

Conclusions: One infusion achieves good glycemic control and sometimes insulin-independence. However, patients with 2 infusions remain insulin-free longer, and have lower HbA1c and better graft function 24 months after the first transplant.

O-172 CONTINUING IMPROVEMENT OF ISLET TRANSPLANTATION EFFICACY OUTCOMES FROM THE COLLABORATIVE ISLET TRANSPLANT REGISTRY 1999-2010

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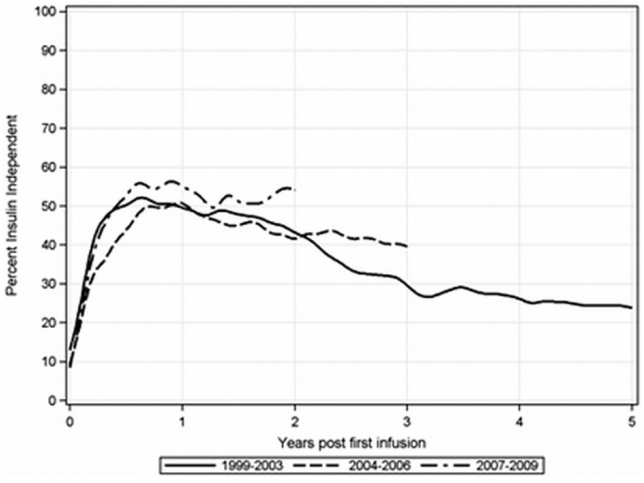
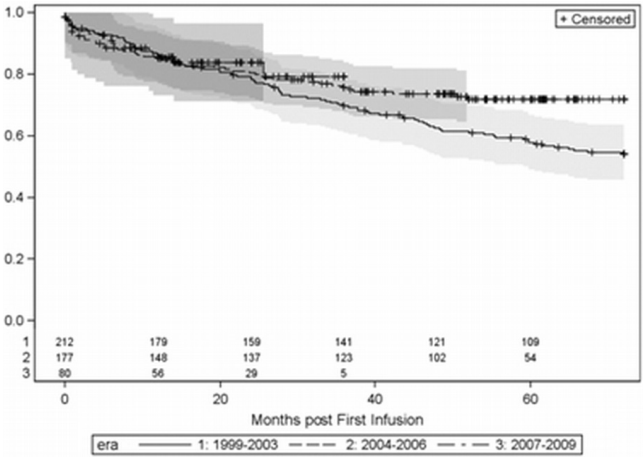
Background: Allogeneic islet transplantation (AIT) is an evolving therapy for the treatment of glycemic instability and severe hypoglycemia in T1D. The goal is to balance clinical benefit against undesirable effects of immunosuppression. Any secular improvements of islet transplantation outcomes would substantially contribute towards benefit.

Methods: Accruing data from the NIDDK and JDRF-sponsored Collaborative Islet Transplant Registry from 1999 continue to be monitored for secular trends in efficacy as well as safety. Prevalence of insulin independence>14 days (II) and survival estimates of sustained C-peptide >0.3 ng/mL were analyzed over the eras 1999-2003 (1), 2004-2006 (2) and 2007-2009 (3) with follow-up ≥1 year.

Results: Of N=209 era 1, 183 era 2, and 98 era 3 AIT recipients with CITR consent as of November 2010, era 2 exhibited 42% II at 3 years compared to 30% in era 1 (p=0.05), and 55% of era 3 exhibited II at 2 years compared with 43% in eras 1-2 (p=0.11), based on all follow-up including multiple infusions.



Kaplan-Meier estimates of complete loss of islet graft were 20% by year 3 in era 1 compared with 23% and 30% in eras 2 and 3, and 29% by 5 years in era 2 compared to 42% in era 1 (log-rank p=0.044, 92 events of N=212 in era 1, 47 events of N=177 in era 2, and 13 events of N=86 in era 3).



Conclusions: The efficacy of islet transplantation measured by these primary

outcomes has notably improved over 10 years of AIT from CITR. These overall results, which are unadjusted for any covariates, reflect evolving immunosuppression regimens and site-specific protocols. Importantly, current management practices yield sustainable insulin independence of at least 50% over two years and 80% graft retention over two years.

O-173 MORPHOLOGIC ANALYSIS OF HUMAN ISLETS TRANSPLANTED INTO THE LIVER AFTER 14 YEARS INSULIN INDEPENDENCE

Yannick D. Muller, Domenico Bosco, Philippe Morel, Johannes A. Lobrinus, Géraldine Parnaud, Laurianne Giovannoni, Raphael Meier, Sophie Borot, Nadja Niclauss, Christian Toso, Thierry Berney. *Department of Surgery, Cell Isolation and Transplantation Center, Geneva, Switzerland*

Long term insulin independence after islet of Langerhans transplantation is an achievable objective although rarely successful. Here we assessed the morphology and vascularization of islets transplanted in the liver, as compared to native pancreatic islets, in a type 1 diabetic subject deceased of cerebral hemorrhage after 14 years insulin independence. The patient remained free of insulin, with normal glycated hemoglobin (5.7%) and basal C-peptide level of 778ng/mL at the time of her death. Importantly, insulin positive islets were absent in the pancreas, ruling out a possible involvement of native β-cell regeneration in endogenous insulin secretion. We then analyzed 143 islets in the central and peripheral parts of the left and right lobes of the liver after double immunofluorescence for insulin and glucagon. Mean islet diameter was 40.1μm (min:10.7μm, max:176.1μm) and β-cell:α-cell ratio was 84:16. β-cells had a core location, whereas α cells had a mantle location. CD34 analysis revealed that only exceptional vascular channels penetrate into the core of the islets. Three dimensional analysis using consecutive serial sections suggested that the islets lost their initial round and compact morphology and further split up into small size islets constituted by one epithelial band. Overall, this case report demonstrates that allogeneic islets of Langerhans implanted inside the liver can survive several years to achieve long term insulin independence. Significant remodeling seems to occur over the years, as suggested by the very small size of islets implanted in the liver.

O-174 AUTOLOGOUS PANCREATIC ISLET TRANSPLANTATION (IAT) AS ENDOCRINE TISSUE RESCUE IN PATIENTS UNDERGOING COMPLETION PANCREATECTOMY BECAUSE OF SURGICAL COMPLICATION AFTER WHIPPLE RESECTION

Giampaolo Balzano, Rita Nano, Paola Maffi, Raffaella Melzi, Alessia Mercalli, Cristina Ridolfi, Federica Merlini, Paola Magistretti, Antonio Secchi, Valerio Di Carlo, Lorenzo Piemonti. *Diabetes Research Institute, San Raffaele Scientific Institute, Milan, Italy*

Objective: We tested the safety and feasibility of IAT in patients (pts) undergoing completion pancreatectomy because of pancreatic anastomosis leakage.
Method: Between November 2008 and September 2010, 8 pts were recruited (4M/4F, age 52±17 yrs). Six of 8 were cancer-bearing pts (#1 and #7 Vater's papilla carcinomas; #2 neuroendocrine carcinoma of ampulla; #3 focal chronic pancreatitis; #4 ductal carcinoma; #5 and #6 common bile duct carcinomas; #8 pseudopapillary solid tumor). Total pancreatectomy (#8 subtotal) was performed 18±9 days after Whipple resection because of anastomosis leakage. Islets were obtained and purified by the pancreatic remnant (64±18g) as for allogenic transplantation.
Results: Isolation was possible in 7/8 pts (#5 failed for pancreas caseous necrosis). Mean islet yield was 2,746±957 IEQ/g of tissue, resulting in transplantation of 1,966±1,090 IEQ/kg. Islets were transplanted within 24h by percutaneous transhepatic intraportal infusion (#1 by cannulating portal vein during pancreatectomy). Due to the presence of preexisting portal thrombosis, islets were infused into iliac crest in #3. Three pts had IAT-related complications solved without any intervention including portal vein thrombosis (#1, #4) and perihepatic hematoma (#2). Patient #3 died at day 5 for IAT unrelated fatal bleeding. All the other patients are still alive (median follow-up: 299 days). Pts #2 (2,157 IEQ/kg) and #8 (4,570 IEQ/kg) gained (at day +118 and +1) and maintained insulin free regimen until last observation (day +746 and +154). Pts #1, #4, #6, #7 showed transplant partial function. At six month follow up C-peptide, insulin requirement and HbA1c were 0.86±0.66ng/ml, 0.27±0.19UI/kg/day and 7.2±0.6%, respectively. No symptomatic hypoglycemia or hepatic recurrences of pancreatic disease were recorded during the follow-up.
Conclusion: IAT is a safe and feasible procedure to improve glycemic control after completion pancreatectomy.

Improving preservation in transplant organs

O-175 CYSTATHIONINE γ-LYASE IS AN ENDOGENOUS MODULATOR OF OXIDATIVE STRESS

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Cystathionine γ-lyase (CSE) is the main H_2S-producing enzyme in the mammalian cardiovascular system, and mice deficient in CSE develop hypertension. We have previously shown the highly beneficial effects of exogenous H_2S-treatment on renal and hepatic ischemia/reperfusion injury (IRI). In this study, we investigated whether endogenous H_2S-production has a beneficial role in the response to IRI, and whether in-vito overexpression of CSE would reduce ROS production.
Male C57BL/6 wildtype (WT) or $CSE^{-/-}$ were subjected to 30 minutes of bilateral renal ischemia. After 24h kidneys and plasma were collected for analysis. Renal H_2S production rate in untreated WT and $CSE^{-/-}$ mice was measured. Human embryonic kidney 293 (HEK293) cells were transfected with a pIRES2-EGFP-CSE plasmid to overexpress CSE. An in-vitro model of Antimycin A induced ROS-production was used to measure the effects of CSE overexpression or different concentrations of NaHS on mitochondrial superoxide production (MitoSOX probe) or general intracellular ROS (using dihydroethidine (DHE)).
Renal IRI caused considerable mortality in $CSE^{-/-}$ mice, while WT mice showed no deaths post reperfusion (64% vs. 100% survival, p=0.01). In addition, tubular necrotic area in the renal cortex of $CSE^{-/-}$ animals was 60% higher compared to WT ($p<0.01$). This coincides with a 91% reduced renal production of H_2S in the $CSE^{-/-}$ mice ($p<0.01$). In-vitro results showed that increasing concentrations of NaHS could concentration-dependently reduce Antymicin induced mitochondrial superoxide production and cytoplasmatic ROS. In addition, CSE overexpression significantly reduced MitoSOX (75%) and DHE (49%) fluorescence after Antimycin treatment when compared to mock-transfected cells ($p<0.001$).
Our results show that endogenous H_2S-production by CSE has a role in the response to renal IRI. This is likely mediated in part by the effects of H_2S on ROS, possibly by direct scavenging, by increasing the availability of glutathione or by directly affecting mitochondrial ROS production.

O-176 $HEMO_2LIFE^{®}$, A DEDICATED OXYGEN CARRIER, OPTIMIZES STATIC KIDNEY PRESERVATION

Raphael Thuillier[1,3], Delphine Dutheil[2], Vanessa Mallet[1], Trieu Minh Tri Ngo[1], Geraldine Allain[1], Morgane Rousselot[2], Magali Denizot[2], Jean Michel Goujon[1], Frank Zal[2], Thierry Hauet[1,3,4]. *[1]InsermU927, Faculte de Medecine et Pharmacie Poitiers, CHU Poitier, Poitiers, France; [2]Hemarina SA, Morlaix, Finland; [3]FLIRT, (Federation pour L'etude de l'Ischemie Reperfusion en Transplantation), Poitiers, France; [4]Plateforme IBiSA, INRA le Magneraud, Surgeres, France*

Background: Static preservation is currently the most widely used organ preservation strategy. Optimizing this method through the addition of specific compounds aimed at ischemia reperfusion injury pathways shows promises in terms of outcome. We tested a compound aimed at the heart of ischemia: oxygen deprivation.

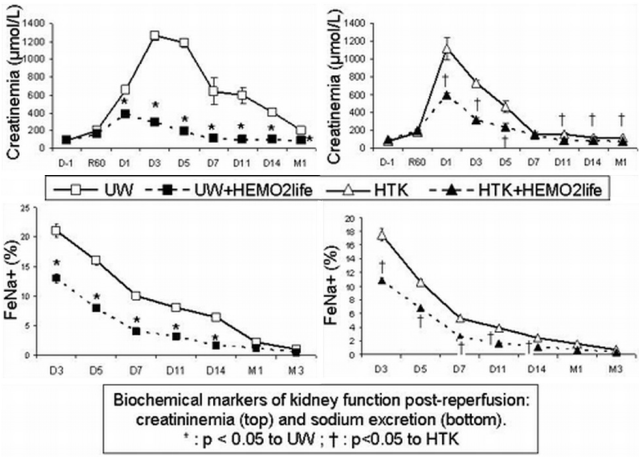

Figure 1. Function recovery.

Methods: $HEMO_2life^®$ is based on a marine protein (HEMARINA-M101), an extracellular hemoglobin from a marine invertebrate characterized by a high oxygen affinity and its capacity to function at very low temperature. We tested the addition of this molecule to either University of Wisconsin solution (UW) or Custodiol (HTK) in a highly reproducible pig kidney autotransplantation model.
Results: Early follow up demonstrated a clear superiority of $HEMO_2life^®$ - supplemented solutions, lowering the peak of serum creatinine and increasing the speed of function recovery (Fig. 1). On the longer term, supplementation with $HEMO_2life^®$ reduced kidney inflammation levels and maintained structural integrity, particularly in the UW group. At the end of the 3 month follow up, $HEMO_2life^®$ supplementation proved beneficial in terms of survival and function, as well as slowing the advance of interstitial fibrosis.

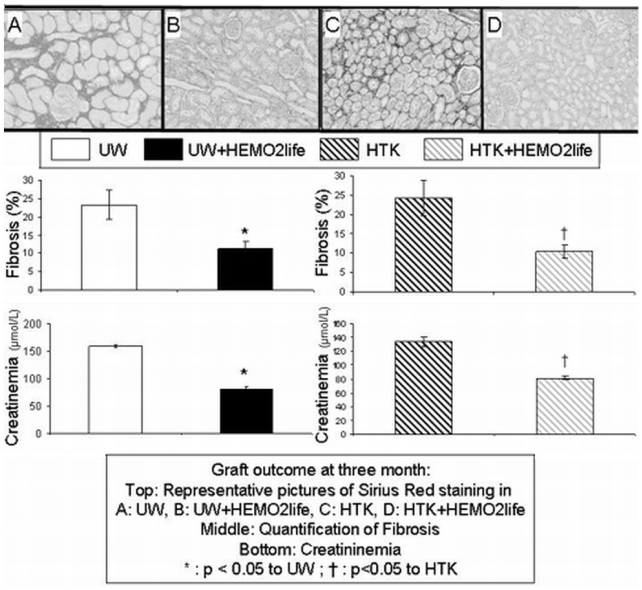

Figure 2. Chronic outcome.

Conclusions: Supplementation of static preservation with dedicated molecules is a simple and rapidly translatable strategy to significantly improve kidney graft outcome. $HEMO_2life^®$ is an interesting candidate as it was designed by evolution to allow survival in hypoxia and hypothermia, properties which show remarkable benefits in our transplantation model.

O-177 IMPROVED SHORT AND LONG TERM FUNCTION OF DCD KIDNEYS WITH OXYGENATED MACHINE PERFUSION

Raphael Thuillier[1,3], Geraldine Allain[1], Benoit Barrou[1,3,4,5], Henri Leuvenink[3,6], Thierry Hauet[1,2,3]. *[1]insermU927, Faculte de Medecine et Pharmacie Poitiers, CHU Poitier, Poitiers, France; [2]Plateforme IBiSA, INRA Le Magneraud, Surgeres, France; [3]FLIRT, (Fédération pour L'étude de l'Ischemie Reperfusion en Transplantation), Poitiers, France; [4]Service d'Urologie et Transplantation, Hôpital Pitié Salpétrière, Groupe Hospitalier Universitaire Est, Paris, France; [5]Université Pierre et Marie Curie, Paris, France; [6]Department of Surgery, University Medical Center of Groningen, Groningen, Netherlands*

Background: Organ preservation by machine perfusion (MP) is a superior preservation strategy compared to cold storage. The constant delivery of preservation fluid might be one of the mechanisms for superiority. We hypothesized that active oxygenation during MP would further improve preservation capacity by improving supporting kidney metabolism.
Methods: In a porcine model of deceased after cardiac (DCD) donor transplantation, kidneys were subjected to 60 min warm ischemia followed by preservation by MP on the Kidney Assist Machine with oxygen (Kidney-AssistO2) or without oxygen (Kidney-Assist). Endpoints were diuresis, serum creatinine, sodium uptake and proteinuria.
Results: Analyses post reperfusion (Fig. 1) showed that diuresis was recovered as early as day 1 in both groups. Post transplant serum creatinine peaked earlier in the oxygenated group, and showed a faster recovery, with lower levels reached at day 14. Sodium re-uptake also tended towards faster recovery in the Kidney-AssistO2 group.
Chronic function analysis confirmed the superiority of oxygenated perfusion (Fig. 2), in terms of lower serum creatinine and proteinuria.

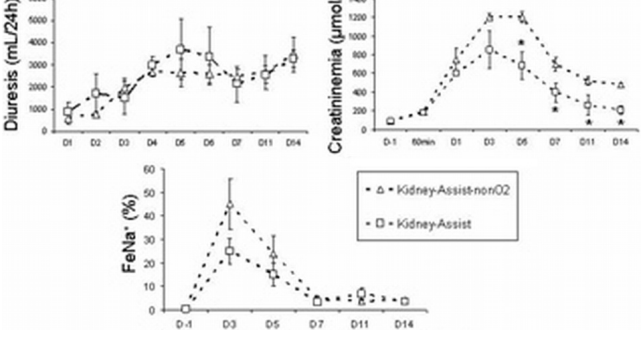

Figure 1. Early graft function in transplanted pigs. *p<0.05.

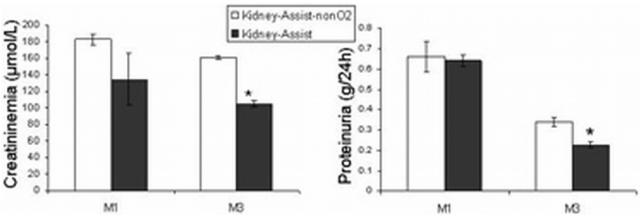

Figure 2. Chronic function in transplanted pigs. *p<0.05.

Conclusions: Further analyses are ongoing, examining the effects of oxygenation on inflammation and fibrosis. Here, we demonstrate a clear superiority of oxygenated MP over standard MP indicating that use of oxygenated MP may further improve outcome of DCD kidney transplantation.

O-178 REVERSE SIGNALING THROUGH CD137 LIGAND IN TUBULAR EPITHELIAL CELLS IS CRITICAL FOR RENAL ISCHEMIA-REPERFUSION INJURY

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Even though CD137/CD137 ligand (CD137L) interactions are implicated in a variety of immunological processes, the extent to which they contribute to acute inflammation is unknown. We used a mouse model of acute kidney ischemia-reperfusion injury (IRI) to test the hypothesis that CD137/CD137L interactions are involved in the early phase of kidney inflammation that is caused by IRI. Deficiency of CD137 in hematopoietic cells but not in non-hematopoietic cells attenuated neutrophil infiltration in acute kidney IRI in mice. We further demonstrated that the presence of CD137 on NK cells was sufficient to completely restore renal IRI in CD137-deficient mice. Moreover, subcapsular transplantation of CD137L-expressing wild-type (WT) tubular epithelial cells (TECs) into CD137L-deficient mice also recovered their disease severity to the extent as seen in WT mice. These results suggested that interactions between CD137 on NK cells and CD137L on TECs are critical for kidney acute IRI in mice. The observation that whileanti-CD137 mAb inhibited renal IRI, CD137-Fc has the opposite effect indicated that CD137L signaling in TECs is important for the initiation of renal IRI. Indeed, in vitro studies demonstrated that CD137 on NK cells triggered CD137L signaling in TECs that led to their apoptosis and secretion of chemokines involved in neutrophil chemotaxis. This increased production of chemokines occurred through the activation of p38, ERK, JNK and the transcription factor C/EBP but not NF-κB. Our results identify a previously unrecognized innate pathogenic pathway for renal IRI that involves the NK cell-TEC-neutrophil axis and that, in this axis, CD137/CD137L interactions have the causal contribution of epithelial dysregulation to renal IRI. Therefore, the CD137 reverse signaling pathway in parenchymal cells such as TECs might be a good target to block the initial stage of inflammatory diseases, including renal IRI.

O-179 A THERAPY TO MAKE BORDERLINE GRAFTS SUITABLE: CURCUMIN SUPPLEMENTATION OPTIMISES FUNCTION RECOVERY AND OUTCOME

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Background: Kidney transplantations from deceased after cardiac arrest donors (DCD) suffer from ischemia reperfusion injury (IRI) and show increased occurrence of delayed graft function (DGF) and lower long term survival. Oxidative stress and NF-κB activation are well described elements of IRI. We evaluated the benefits of supplementing the current preservation protocol with curcumin, a potent antioxidant and NF-κB inhibitor.
Methods: We used an autologous DCD kidney transplantation model in Large White pigs. Kidneys undergo warm ischemia for 60 min before being preserved at 4°C for 24 hours using UW supplemented with Heparin (UW group). Cyclodextrin-complexed curcumin, a novel water soluble formulation, was added to the preservation solution (Curcumin group).
Results: Curcumin supplementation greatly improved recovery of function: animals resumed urine production two days before controls, serum creatinine started recovering at day 5 (vs. day 11) and reached stable levels by day 11, while controls did not reach similar levels by day 30 (Fig. 1). Animal survival was critically improved for the curcumin group (83.3% vs. 29% in controls, $p<0.05$) (Fig. 2).
At 3 month, chronic fibrosis was well defined in UW (Fig. 3, 55.8%) while it remains limited in Curcumin animals (7.1%). RtqPCR analysis showed protection in Curcumin group from the upregulation of several pro-lesion pathways such as inflammation, EMT and fibrosis).

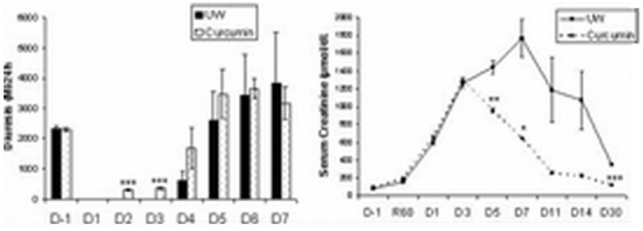

Figure 1. Graft function evaluation.

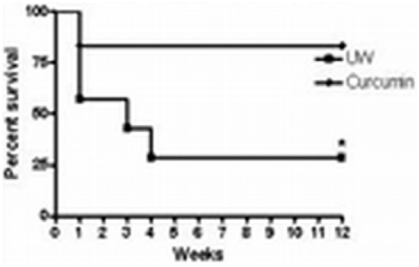

Figure 2. Animal survival.

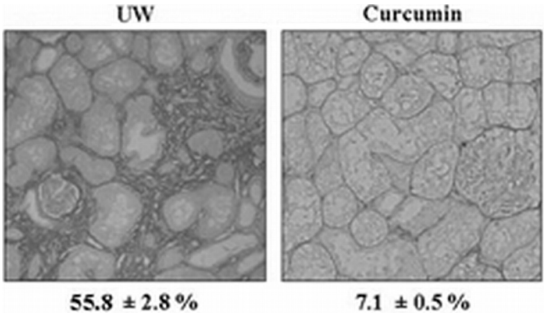

Figure 3. Graft fibrosis at 3 months.

Conclusion: Curcumin supplementation of UW in a pre-clinical transplantation model with stringent ischemia reperfusion injury rescued kidney grafts from an unfavourable prognosis. Early graft function was recovered faster and survival was substantially improved. The mechanism of action is currently being investigated through in vitro and ex vivo models. As curcumin has proved well tolerated and nontoxic, this preservation strategy shows great promise for translation to clinical kidney transplantations particularly as it requires little addition to current protocols.

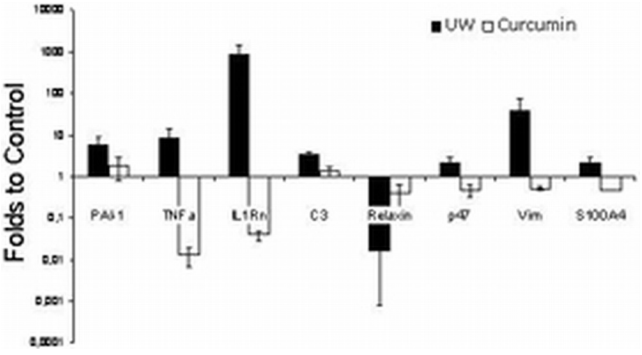

Figure 4. Q-PCR at 3 months.

O-180 EXPERIMENTAL ACUTE KIDNEY INJURY CAN BE MODIFIED BY SEX-SPECIFIC PHARMACOLOGIC TARGETING OF CYP-DEPENDENT EICOSANOIDS

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In renal transplantation female recipients have a better graft and patient survival compared with males. Recently we found that pharmacologic inhibition of the arachidonic acid (AA) metabolite 20-HETE protected against experimental acute kidney injury in male rats. We hypothesized that sex-specific differences in the balance of CYP450-dependent AA metabolites aggravate renal I/R-I in males but mediate protection in females.
To test this hypothesis, we used newly developed synthetic agonists and antagonists specifically amplifying or blocking the biological activities of 20-HETE or EETs and analyzed their effects on the severity of renal I/R-I in uninephrectomized male and female Lewis rats. Ischemia was induced through 45 min of left renal artery clamping, 5 min after intrarenal injections of drug or vehicle. Two days after reperfusion, vehicle treated males developed severe I/R-I as indicated by significantly reduced creatinine clearance as well as severe tubular necrosis and inflammatory cell infiltration. Pretreatment with 20-HETE antagonist or EET agonist significantly improved loss of function and protected against tubular injury. As determined by optical Laser-Doppler-flux and pO2-probes, the 20-HETE antagonist improved recovery of medullary blood flow as well as of medullary and cortical reoxygenation during early reperfusion phase. Compared to males, females featured significantly less functional and structural damage and postischemic inflammation. Pretreatment with the EET antagonist clearly reversed the protective phenotype in females and caused a similar extent of injury as in males. Immunostaining revealed upregulation of EET degrading enzyme soluble Epoxid hydroxylase in damaged tubules of renal cortex and outer medulla.
Our results suggest that sex-specific patterns in the production, action, and degradation of CYP-dependent AA metabolites determine differences in the severity of renal I/R-I in male and female rats and may offer novel sex-adapted therapeutic strategies for the prevention of acute kidney injury.

Pancreas

O-181 APLICATION OF THE CLAVIEN-DINDO CLASSIFICATION OF SURGICAL COMPLICATIONS AFTER PANCREAS TRANSPLANTATION: SINGLE CENTER EXPERIENCE

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Introduction: Commonly in pancreas transplantation, complications are expressed in terms of graft outcomes; factors implicated include surgical technique, donor and recipient characteristics.

Abstract O-181 – Table 1

	None (n=68)	Class I-II (n=68)	Class IIIa (n=43)	Class IIIb (n=64)	Class IV-V (n=6)	Total (n=249)	P value
Donor Age	27,26±10,43	29,34±10,16	33,12±10,34	31,19±10,78	26,17±10,02	29,82±10,86	0,037
Donor BMI	22,68±2,93	23,25±2,87	23,57±2,39	24±2.91	23,49±3.41	23,35±2.85	NS
Vasclar COD	26.5%	29,9%	32,6%	26,6%	33,3%	28,6%	NS
Recip Age	38,58±6.8	39,8±7.5	39,5±7.5	39,4±7.3	38±8.5	38.5±7.3	NS
Retx	10,3%	8,8%	27,9%	18,8%	0	14,9%	0,03
CIT (min)	690±216	646±231	694±232	692±222	580±114	676±223	NS
ICU (day)	2,3±0.8	3,3±1,8	3,5±3,2	5,8±8,1	10±7,3	3,8±4,9	0,001
HD (day)	12,2±3,3	18,6±10	19,9±9,1	33,7±31,6	23,5±20,6	21,1±19,2	0,001

BMI: body mass index; COD: cause of death; CIT: cold ischemia time; ICU: intensive care unit; HD: hospital days.

Aims: Evaluate the applicability of the Clavien-Dindo classification to pancreas transplantation (considered type C intervention) and assess the impact of such complications on graft outcomes.
Methods: Retrospective analysis of 249 pancreas transplants (214 SPK, 34 PAK, 1 PTA) over 10 year period (minimum follow up of 60 days). Data collection of the donor, recipient, transplant procedure and complications in the first month after transplantation are analysed. We excluded hemodialysis as treatment of delayed renal graft function and any complication derived from the immunosuppression therapy. For each graft, the higher graded complication was recorded.
Results: Cumulative Class I-II complications appeared in 26.9%, Class III 43% and 2.4% of Class IV-V. Blood transfusion accounted for 66.7% of the class II complications (15.5% of the total). Thrombosis was the most common among the Class IIIa (83.7%) the remaining were drainage of percutaneous collections (16.3%). In type IIIb, surgical exploration for bleeding occurred in 48.4%, thrombosis in 20.3% and anastomotic leak in 21.6%. There were no significant differences in donor age, BMI, COD, recipient age and CIT among the groups; understandably, the ICU and hospital stay was increased.
The 1 and 5yr graft survival for all causes of graft loss are shown.

Table 2

	1 yr (%)	5 yr (%)
None	98.3	90
I–II	95.2	95.2
IIIa	94.7	94.7
IIIb	95.2	74.4
IV–V	93.2	67.7

Failure to treat was considered pancreas removal (n=27), in 7 cases was during the same transplant procedure due to poor organ perfusion, the remaining as a consequence of a complication (graft thrombosis or enteric leak).
Conclusion: The classification of surgical complications after pancreas transplantation does not reflect on graft outcomes. Some complications are derived from the treatment required and are expected as normal postoperative course.

O-182 TREATMENT OPTIONS FOR PANCREAS ALLOGRAFT THROMBOSIS: 10 YEAR EXPERIENCE

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Aims: Graft thrombosis is the main cause of non-immunological failure in the first year. Doppler-Ultrasound (dUS) surveillance can identify thrombosis before it becomes clinically significant and improve the possibility of graft survival. We analyze the treatment options for graft thrombosis in our cohort of patients.
Methods: Retrospective evaluation of 249 pancreas transplants over ten-year yield 58 graft thrombosis (24.2%), either venous (40), arterial (4) or both (14). All patients received prophylaxis with low-molecular weight heparin within 24hrs and surveillance dUS within 48 hours after transplantation, or whenever clinically indicated. Therapeutic approach depended on clinical manifestation and extent of thrombosis. We analysed the treatment options, patient and graft outcomes.
Results: Diagnosis made by dUS in 95% of the cases but only 15 patients (26%) were symptomatic at that time. Complete thrombosis of the splenic or portal vein was detected in 19 grafts, half were symptomatic. Median time to diagnosis was 4 days (range 1-56 days). The treatment options were: percutaneous thrombectomy/lysis (32.7%), surgical thrombectomy (8.6%), full anticoagulation (18.9%) and no treatment (25.8%). Transplantectomy was performed in 8 patients as the first line of treatment, all were symptomatic with complete venous thrombosis and had also an arterial component. Resolution of the thrombus was complete in 40% cases, partial in 21% and 39% did not resolve. Despite this, only 12 grafts were ultimately removed. Graft loss due to vascular complications represents 4.4% of our series. With a median follow-up of 2.4 years, the 1-yr patient and graft survival were 99.2% and 95.6%.
Conclusions: Two-thirds of the patients were asymptomatic at time graft thrombosis diagnosis, making the surveillance techniques key for early diagnosis. Most patients can be treated medically or percutaneously with a salvage rate of 80%, even without thrombus resolution. Symptomatic or arterial thrombosis are poor prognostic factor.

Table 1. Treatment aproach

	No treatment (n=15)	Anticoag (n=11)	Perc. treat (n=19)	Surgical (n=5)	Tmy (n=8)	Total (%) (n=58)
Asymptomatic	14	8	16	4	0	42 (72%)
Throbosis Complete	5	0	4	3	8	20 (34%)
Arterial Thrombosis also	4	1	3	2	8	18
Thrombus Resolution*	5+2p	9+2p	5+8p	3	0	23+12p
Graft Loss (thrombosis)	0	1	3	0	8	12 (20%)

Anticoag: anticoagulation; Perc. treat: percutaneous thrombectomy or thrombolysis; Tmy: transplantectomy. *p: partial resoluton of the thrombus with treatment.

O-183 PRE-TRANSPLANT THROMBOELASTOGRAPHY AND THROMBOPHILIA SCREENING DO NOT PREDICT THROMBOSIS FOLLOWING PANCREAS TRANSPLANTATION

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Introduction: Pancreatic graft thrombosis is the largest non-immunological cause of graft failure following combined kidney and pancreas (SPK) transplantation. In SPK transplant recipients, thromboelastrographs (TEG) taken intra-operatively indicate a patient's current coagulation status, thus assisting in real-time management decisions about anticoagulation.
Patient factors are relevant in thrombus development but are difficult to define and quantify, resulting in a paucity of information about their influence on thrombus formation. In diabetic patients, there is a complex balance between pro- and anti-thrombotic factors.
We postulated that screening SPK recipients in a steady state may help to predict the likelihood of developing a graft thrombosis.
Methods: Between January 2001 to November 2010, 115 SPK transplants were carried out at our centre. At assessment, 105 had a TEG and a thrombophilia screen.
Patients who experienced a pancreatic graft thrombosis were identified and proportions of abnormal coagulation profiles were compared between those who had developed a thrombus and those who had not. Comparison was made between the 2 groups to look for any association between an abnormal coagulation profile and pancreatic graft thrombosis.
Results: Overall, 45% of patients had a normal TEG and thrombophilia screen, with the remaining 55% having some degree of abnormality in the coagulation profile (Table 1).
Twelve patients developed a pancreatic graft thrombosis, of which 8 patients lost the graft and 4 were managed with anticoagulation.
Of the patients developing a thrombus, 42% had a normal TEG and thrombophilia screen (Table 2).

Results of TEG and thrombophilia screens from SPK patients

Result	Number (N=105)
Normal TEG and normal thrombophilia screen	48 (45%)
TEG abnormal and thrombophilia screen abnormal	15 (14%)
Abnormal TEG and normal thrombophilia screen	37 (36%)
TEG normal and thrombophilia screen abnormal	5 (5%)

Table 2. TEG and thrombophilia screens of SPK recipients who had a thrombus

Results	Number (N=12)
Normal TEG and normal thrombophilia screens	5
Abnormal TEG and abnormal thrombophilia screen	4
Abnormal TEG and normal thrombophilia screen	2
Normal TEG and abnormal thrombophilia screen	1

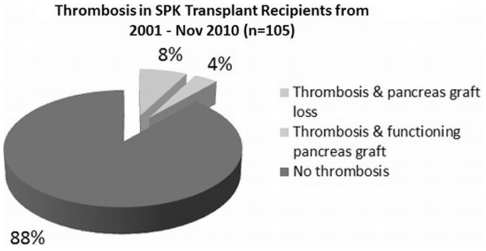

Figure 1

Conclusions:
- Graft thrombosis in SPK recipients often results in loss of the graft.
- Patients with diabetes being assessed for SPK commonly have an abnormal thromboelastogram.
- There is no association between an abnormal pre-transplant TEG taken at assessment and graft thrombosis in SPK recipients.
- An abnormal pre-transplant thrombophilia screen is not associated with graft thrombosis.

O-184 PANCREAS TRANSPLANTATION IN RECIPIENTS AGED ≥50 YEARS

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Improving outcomes resulted in pancreas transplantation being offered to older recipients. We investigated if this expansion was associated with adverse outcome.
Methods: Between March 2004 & February 2011, 407 pancreas transplants (353 DBD, 54 DCD) were performed. All grafts were implanted intraperitoneally with enteric - caval drainage. 95% received Alemtuzumab induction. Morbidity, graft and patient and outcomes were compared between older (OR) and younger recipients (YR).
Results: Within 407 transplants, 86 recipients (21%) were ≥50 years old at transplant; 321 recipients were <50 yrs. Of the 86 recipients in the OR group, 50 (58%) were aged 50-54 yrs, 27 (31%) were aged 55-59 and 9 (11%) were ≥60 yrs (max 67 yrs). OR had 16% grafts from DCD; YR had 12% (p=0.3). OR had 64 SPK, 12 PTA and 10 PAK, whereas YR had 236 SPK, 38 PAK and 47 PTA. OR donors' median age was higher (43, range 5-63) than YR median donor age (36, range 15-63) (p=0.0002) reflecting attempts to match donor-recipient age. Both groups had 35-month median follow up (range 1-84). Actuarial patient (91% YR vs. 93% OR), pancreas (83% vs. 83%), and kidney graft survival (89% YR vs. 88% OR) was similar. Both groups had similar DGF of the kidney (14% YR vs. 23% OR, p=0.09) and of the pancreas (4% YR vs. 3% OR). Rejection occurred in similar frequency – 11% YR vs. 9% OR, p=NS. Readmission (27% YR vs. 24% OR), reoperation (23% YR vs. 27% OR, p=0.3), and peri-operative cardiac events (5% YR vs. 2% OR, p=0.3) were similar. Bacterial (5.3 vs. 5.8%), viral (7% vs. 8%) and fungal (1% vs. 3%) infections' incidence was similar.
Conclusion: Offering pancreas transplantation to older recipients appears to be safe with similar graft & patient survival and comparable morbidity.

O-185 ANALYSIS OF RENAL FUNCTION AFTER SOLITARY PANCREAS TRANSPLANTATION: IS CNIs THE ONLY CULPRIT?

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Background: The level of renal function is an important parameter determining the type of pancreas transplant, Pancreas After Kidney (PAK) or Pancreas Transplant Alone (PTA), offered to patients with type 1 diabetes, as this may indicate the risk of a clinically important post-transplant deterioration. This retrospective, single-centre analysis has been performed to assess possible factors affecting renal function.
Methods: 57 patients, with a mean age of 43.2±8.8 years at the time of transplant, who received PAK (n=33) or PTA (n=24) over a 4-year period, were follow-up for a mean period of 25.8±14.9 months (range: 2-62). eGFR levels were used to evaluate renal function pre- and post-operatively. Various parameters and characteristics were included in the risk and time-to-event analysis separately for the two subgroups.
Results: In the PAK subgroup, a trend for substantial deterioration in renal function was identified among patients who suffered from CMV disease post-transplant (p=0.090) and those with rejection before the pancreas transplant (0.078). Renal function was significantly affected in patients suffering from gastropathy (p=0.040) and in those with high tacrolimus levels (>12 mg/dl) at 12 months post-PAK (p=0.040).
In the PTA subgroup, the only pre-transplant characteristic that significantly affected renal function was severe proteinuria (OR=14.000, CI=1.057-185.492, p=0.045), while high tacrolimus levels (>12 mg/dl) at 6 months after-transplantation was identified as an independent risk factor (HR=14.300, CI=1.271-160.907, p=0.031).
Conclusions: Our results suggest that:
– Substantial deterioration of renal allograft function after PAK is more likely in patients suffered from prior renal allograft rejection, symptomatic gastropathy, CMV disease and high tacrolimus levels 1 year post-transplantation.
– PTA may be justifiable even in patients with borderline renal function, provided they do not suffer from severe proteinuria and careful monitoring and tailoring of immunosuppression is ensured.

O-186 ALEMTUZUMAB INDUCTION IN PANCREAS TRANSPLANTATION

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Alemtuzumab is a humanized anti-CD52 depleting antibody with effects on T & B lymphocytes. The efficacy and safety of alemtuzumab-based immunosuppression and its ability to achieve steroid-free maintenance was analyzed retrospectively.
Methods: 407 consecutive pancreas transplants performed with systemic-enteric drainage between June 2004 and February 2011 were analyzed. Patients received alemtuzumab 30mg on day 0 & 1 with methyl prednisolone, and tacrolimus (target trough 8-12 ng/ml) & Mycophenolate mofetil (1-1.5g/day) as maintenance immunosuppression. Patient, graft survival and complications were analyzed.
Results: 390 of the 407 pancreases (96%) received alemtuzumab induction resulting in 288 SPK, 55 PTA, and 47 PAK including 9 re-transplants. 50 grafts (13%) originated from DCD. Median (range) recipient age and BMI were 43 years (23-67) & 23 (16.5-37). Median cold ischemia was 11hrs 30 min and hospital stay, 14 days. With 32-month median follow-up (range 1-80), overall patient, pancreas and kidney graft survival were 93%, 82% and 88%. DGF occurred in 15% of kidneys & 4% pancreases. Pancreas grafts were lost to chronic rejection (5%), DWFG (5%), pancreatitis (3%), thrombosis (2%, mostly DCD), bleeding (0.5%), leak (0.5%), and staple failure (0.5%). Renal grafts were lost to DWFG (5%), thrombosis (2%), rejection (2%), non-function (0.7%), BKV (0.2%), and PTLD (0.2%). Rejection episodes (11%) were rescued with corticosteroids; 2% developed vascular rejection. 25% recipients had reoperations. Other complications include neutropenia (2.6%), viral (CMV 3.6%, varicella zoster 1.3%, herpes simplex 0.5%), bacterial (6.4%), and fungal (4%) infections. PTLD occurred in 0.7%. 40 patients (10%) received single dose only (bleeding- 4%, low platelets - 1.5%, rash – 1.5%, age >60 – 1.5%, others – 1.5%). 89% recipients never received any maintenance steroids.
Conclusion: Our results suggest that alemtuzumab is safe and efficacious, enabling steroid free maintenance in 89% of pancreas transplant recipients.

Liver transplantation (longer-term outcome)

O-187 THE JOINT IMPACT OF DONOR AND RECIPIENT CHARACTERISTICS ON THE OUTCOME OF LIVER TRANSPLANTION IN GERMANY

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Introduction: The shortage of donor organs in Germany has led to the use of organs from donors with extended donor criteria (EDC). EDC have been defined in national guidlines on the basis of expert opinions, but their clinical relevance is controversial. This situation may lead to the loss of organs otherwise available for transplantation. We evaluated the impact of donor and

recipient factors in liver transplants with EDC on patient and graft survival in a nationwide German multicenter analysis.
Material and Methods: An anonymzed database was created from data on livers donated and transplanted in Germany between 2006 and 2008 as provided by Deutsche Stiftung Organtransplantation and BQS Institute for Quality and Patient Safety. Cox regression (significance level 5%, risk ratio [95%-CI]) was used including only recipients with first liver transplants (n=2095) for calculating impact on patient survival and all transplants (n=2175) for impact on graft survival.
Results: Patient and graft survival were significantly affected only by donor age (1.012 [1.006-1.019] and 1.011 [1.005-1.017]) per year), recipient age (1.019 [1.010-1.029] and 1.014 [1.006-1.022] per year), creatinine (1.248 [1.174-1.327] and 1.205 [1.135-1.278) per mg/dl), bilirubin (1.022 [1.014-1.030] and 1.023 [1.016-1.030] per mg/dl), and high urgency status (1.783 [1.312-2.423) and 1.809 [1.398-2.342]). Inferior organ quality resulted in lower graft survival (1.243 [1.001-1.545]).
Conclusion: Multiple Cox regression revealed no significant impact of EDC on patient and graft survival except for donor age, which is probably attributable to donor selection. Among recipient variables, only age, creatinine and bilirubin, and high urgency status were associated with poorer outcome.

O-188 TRENDS IN LIVER RETRANSPLANTATION IN EUROPE: EVALUATION OF THE LAST 28 YEARS

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Introduction: In patients with unsalvageable graft failure, liver retransplantation (ReLT) remains the only effective. Aim of this study was to evaluate trends in adult ReLT in Europe between 1980 and 2008.
Methods: Retrospective analysis was based on the prospective European Liver Transplant Registry (ELTR), including patients aged $\geq$16 years at primary liver transplantation (LT). Patients were grouped and analyized according to three decades of retransplantation.
Results: Of 69010 adult LTs performed, 6397 (9%) patients required 7114 ReLT. Median recipient age at ReLT was 47.4 (range 16-74) years, 60% were male and 54% were carried out three months after LT; 719 (11%) patients required multiple ReLT. Despite increasingly older donors and recipients ($p<0.001$), overall 1-, 5- and 10-year actuarial survival increased from 36%, 28% and 26% in the 1980s to 56%, 45% and 37% in the 1990s, to 67% and 56% (10-year data not available yet) in the 2000s ($p< 0.001$). ReLT for rejection accounted for 36% of ReLTs in the 1980's, decreasing to 11% in the 2000's ($p< 0.001$). Conversely, hepatic artery thrombosis (23%) and HCV recurrence (8%) - one of the main ReLT indications in the 2000's –accounted for 4% and <1% in the 1980's ($p<0.001$). Main causes of death were sepsis, disease recurrence and cardiovascular complications.
Donors <40 years, recipients <50 years, cold ischaemia of <8.5h, timing of ReLT >3months and era of ReLT were among those factors predictive of improved survival.
Conclusion: ReLT still provides the only treatment for irreversible graft loss. Although the indications for ReLT have changed significantly from the 1980's to the 2000's - reflecting a general change for all transplants - advances in perioperative care and immunosuppressive regimes have significantly improved survival in the last three decades.

O-189 SECOND AND SUBSEQUENT LIVER RETRANSPLANTS IN EUROPE: ARE WE JUSTIFIED TO CONTINUE?

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Introduction: Liver retransplantation (ReLT) remains challenging, with multiple ReLT often associated with worse outcomes. Aim of this study was to evaluate trends in adults necessitating two or more ReLT in Europe between 1980 and 2008.
Methods: Retrospective analysis was based on the prospective European Liver Transplant Registry (ELTR), including patients aged $\geq$16 years at primary liver transplantation (LT).
Results: 6397 patients required 7114 ReLT- 653 (10%) received a second, 62 (1%) a third ReLT. Second ReLT declined from 12% to 6% over the 28 years. While median recipient and donor age for first and subsequent ReLT increased between 1980 and 2000 ($p<0.001$), there was a parallel improvement in graft survival with 1-, 5- and 10-year rates for second ReLT increasing from 33%, 31% and 25% to 48% and 35% (10-year data not available yet) in the 2000s ($p=0.05$) and similar trends for third ReLT.
Although overall actuarial survival following ReLT, especially multiple ReLT remain worse compared to primary LT, this trend is less obvious in the last two decades.
Similar to first ReLT, rejection accounted for 40% of second ReLT in the 1980's, decreasing to 11% in the 2000's ($p< 0.001$), while hepatic artery thrombosis (5%) and HCV recurrence (2%) increased substantially (31%, respectively 5%) from the 190's to 2000's. In the 1980's more patients received a first ReLT within 3months (71%, versus 51% in 2000's), with an opposite trend for second ReLT ($p<0.001$).
Main causes of death were sepsis, vascular complications and primary non-function.
Conclusion: Multiple ReLT remain technically challenging. Fewer patients received $\geq$2 ReLT over the last three decades, owing to optimized patient selection, improved patient management and increased organ shortage. Despite improvements, outcome after multiple ReLT remains poor.

O-190 LONG-TERM SURVIVAL AFTER HEPATIC ARTERY THROMBOSIS

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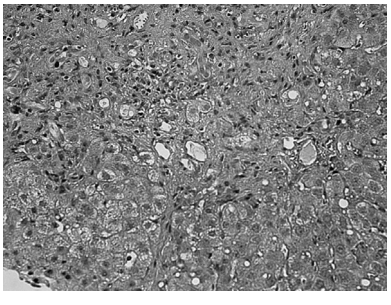
Background: After liver transplantation (LT), the only arterial pathway is the hepatic artery. However, in cases of hepatic artery thrombosis (HAT) the long term arterial supply can only be the result of neovascularization (NV). We present a series of patients with HAT who have evidence of NV at long term follow-up. We named this entity "venous liver" (VL).
Methods: HAT was established by CT-scan, angiography or at operation in all cases. The patients with VL and a normal liver function were strictly followed.
Results: HAT was noticed in 30/407 cases (7.37%). Of these, 14 (46.7%) patients had a vascular reconstruction during the back-table and 8 (26.7%) had a transarterial chemioembolization. HAT occurred early in 12 patients (40%)



and late in 18 (60%) (> 30 days). At the time of the study 11 (35.4%) patients had a VL, of these 10 (91%) had evidence of NV. An echo-Doppler arterial signal was recorded in all patients. The mean interval time between HAT and NV was 4.1 months (3-5.5). Liver histology showed an arterial structure in 4 (36.4%) of these patients.
Four factors were associated with the development of NV (late HAT, early HA stenosis, site of thrombosis, Roux-en-Y anastomosis). The overall survival rate at 54 months was 90.9%.
Conclusion: Considering the high survival rate of the VL patients, in case of late HAT the wait and see policy seems to be the better option, postponing the re-transplantation.

O-191 LONG TERM FOLLOW-UP (15–20 YEARS) AFTER LIVER TRANSPLANTATION IN ITALY: AN OBSERVATIONAL MULTICENTER STUDY

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Background: There are scarce data on the long term follow-up (15–20 years) of patients having undergone liver transplantation (LT). We have investigated this issue through a nationwide observational study conducted in 12 different LT Centers.
Methods: A specific electronic Case Report Form (eCRF, OpenClinica) was created to capture clinical data of patients having undergone LT in Italy before January 1, 2000. The eCRF included the following main sections: baseline (main recipient and donor characteristics at the moment of LT), patient/graft failure (date and cause), long term survivor details (data on *de novo* and/or recurrent diseases). Data harvest began on July 2010. We present here the first planned interim analysis performed on November 2010.
Results: On November 17th 2010, data on 3008 LTs performed in Italy covering the period 1983–1999 having been entered in the eCRF were subject to preliminary analysis. We considered 2846 patients with sufficient follow-up data to calculate long-term patient survival. There were 869 females (31%), median recipient age was 55 years, 38% of patients had hepatitis C, 19% had hepatocellular carcinoma, median donor age was 28 years. Median follow-up was 141.6 months (0.7 – 211.6). One, 10 and 20 years patient survival was 76%, 61% and 51% respectively. A preliminary univariate survival analysis was performed. Hepatitis C cirrhosis, Child C class, and LT before 1990 were all significant predictors of survival. Interestingly, recipient age had no impact on long term survival. In particular, 194 patients (7%) older than 60 years had an excellent long term survival profile (10 and 20 year survival = 63% and 52% respectively).
Conclusion: LT provides excellent survival profiles also in the very long term perspective (15–20 years). Effective pre-LT selection has the potential to minimize the intrinsic prognostic impact of recipient age.

O-192 A RECORD OF DE NOVO NON-SKIN AND MELANOMA MALIGNANCIES POST LIVER TRANSPLANTATION IN AUSTRALIA AND NEW ZEALAND

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Background: Various studies report that the development of denovo nonskin and melanoma malignancies is a serious long-term complication after liver transplantation and that the risk is greater than in the general population.
Aim: This study compares the incidence of denovo nonskin and melanoma malignancies in patients who received a liver transplant in Australia and New Zealand and compares the risk with the Australian general population.
Methods: The Australian general population data, reported by the Australian Institute of Health and Welfare (AIHW), was used as a comparison. Age groups identified and compared. All 3510 patients who received a liver transplant in Australia and New Zealand since 1985 were included in the study. Post transplantation malignancies were recorded in the ANZLTR Cancer Registry and the standardised incidence ratios (SIRs) for different types of cancers were calculated as compared to the Australian general population.
Results: A total of 260 post-transplant malignancies were found in 244 patients. Cumulative incidence at 1, 5, 10, 15 and 20 years were 1.4%, 5.2%, 9.1%, 13.6% and 18.3%, respectively. Median age at diagnosis was 55.6, range 1.2-76. Median time to diagnosis was 4.8, range 0.8-20 years. The overall SIR was 2.27, 95% confidence interval 2-2.56. The most common malignancies were of the alimentary tract (82, SIR 3.0, 95% confidence interval, 2.38-3.72). Other malignancies were lymphoma (62, SIR 8.98, 95% confidence interval, 6.95-11.4), and melanoma (22, SIR 1.77, 95% confidence interval, 1.11-2.68). Malignancies of genitourinary system (31) had a reduced SIR 0.54, 95% confidence interval 0.37-0.77.
Conclusion: Overall, Australia and New Zealand liver transplant recipients may be 2.27 times more likely to develop non-skin or melanoma malignancy than the Australian general population. Malignancies of the genitourinary system may be an exception.

Kidney (comorbidity)

O-193 RECIPIENT AND DONOR BODY MASS INDEX CORRELATE WITH INITIAL KIDNEY GRAFT FUNCTION

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Introduction: Recipient obesity is associated with worse outcome after kidney transplantation, whilst the number of overweight patients on the waiting list is increasing. To date, no data is available on cadaveric organ donor body mass index (BMI) and it's impact on the outcome after kidney transplantation. We investigated whether donor and/or recipient BMI correlate with the occurrence of delayed graft function (DGF) after kidney transplantation.
Patients and Methods: Single center retrospective analysis of 1132 consecutive cadaveric kidney transplants between January 2000 and December 2009. Recipients/donors were devided into four groups according to their BMI (<20, 20-25, >25-30, >30). Delayed graft function was defined as the requirement for more than one dialysis within the first post-transplant week. Impact of recipient and/or donor BMI, gender, age, re-transplant, cold ischemia and anastomosis time on the occurrence of delayed graft function were analyzed using uni- and multivariate analyses.
Results: Overall DGF rate was 32.1%, mean BMI was 23.75 (SD ±3.8) for all recipients and 24.68 (SD ±3.6) for all donors (median age 44.0; 40.3% female). In univariate analyses DGF rate was 25.2%, 29.8%, 40.9% and 52.6% in recipients with a BMI <20, 20-25, >25-30 and >30 respectively ($p<0.0001$). Donor BMI <20, 20-25, >25-30 and >30 resulted in a DGF rate of 22.5%, 31.0%, 37.3% and 51.2% ($p<0.0001$) in univariate analyses. Multivariate analyses revealed overweight in the recipient, besides cold ischemia and anastomosis time but not age or gender, as an independent risk factor for DGF ($p=0.002$).
Conclusion: Not only recipient but donor BMI as well closely correlates with the incidence of DGF after cadaveric kidney transplantation. Awareness thereof should have an impact on peri- and post transplant measures in order to avoid DGF and complications thereof in cadaveric renal transplant recipients.

O-194 TRANSPLANTATION WITH KIDNEYS REMOVED ELECTIVELY FOR RENAL CELL CARCINOMAS

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Introduction: In view of the ongoing shortage of organs for transplantation new sources have been explored with variable success. Each year in the UK 7000 new cases of renal cell carcinomas (RCC) are diagnosed with around 50% of these being T1a tumours (<4cm). These patients could safely undergo partial nephrectomy. However due to technical challenges only 20% undergo this procedure, the rest have radical nephrectomy (RN). Evidence also exists to suggest that these radically removed kidneys can be transplanted after resection of small tumours (D. Nicol et al BJU International, vol. 102, no. 2, pp. 188–193, 2008). However there is a need to audit current practice in UK in order to assess the real potential for using such restored organs.
Methods: This is a retrospective analysis comparing partial or radical nephrectomy for all the patients undergoing surgery for a T1a tumour in Freeman hospital, Newcastle, between 2004 and 2008. The National database from the British Association of Urological Surgeons (BAUS) was also analysed from 2004 to 2010 for the mode of treatment.
Results: Percentages of partial nephrectomy have gradually increased.
Data collection for BAUS was started recently with only 15 recorded cases between 2004 to 2007. With improved reporting there were 256 cases between 2008 and 2010. Although incomplete but still 61% of patients underwent radical nephrectomy.

Table 1. Trends of mode of treatment for small RCCs

Year	Total	Partial Nephrectomy	Radical Nephrectomy
2004	21	3 (14%)	18 (86%)
2005	18	6 (33%)	12 (67%)
2006	49	12 (24%)	37 (76%)
2007	42	19 (45%)	23 (55%)
2008	31	12 (38%)	19 (62%)

Conclusions: A significant proportion of patients with small RCC still undergo RN and potentially these kidneys could be used for transplantation after resection of tumour *ex vivo*. A more robust national database would help in the estimating the real pool of kidneys from this source. The issues of patient safety and ethics would have to be overcome before this approach is widely adopted

O-195 ANEMIA AS A RISK FACTOR FOR GRAFT LOSS AND MORTALITY IN KIDNEY TRANSPLANT

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Anemia is associated with increased cardiovascular events and mortality in dialysis patients. However, in kidney transplant this relationship has not been so clearly established, as only limited evidence is available.
Aim: In this study we have analysed the prevalence of anemia and its association with long-term patient and graft outcomes, in a prospective cohort of kidney transplant from the Andalusian Kidney Transplant Registry (Spain).
Methods: From January 2000 to December 2007, 2584 incident kidney transplant patients were studied. Anemia was defined as WHO criteria (Hb$<$13g/dL in men, $<$12g/dL in women). Hemoglobin (Hb) level at first post-transplant year was used as primary variable.
Results: At first year after transplantation, 29% of patients presented anemia. Hb level was 13.6$\pm$1.7 g/dL (range 6-19.5). 16.5% of patients were receiving erythropoiesis stimulating agents; of them, 67% were in CKD stage 4T or 5T. Both CKD stage and GFR correlated with Hb (r=0,34, p$<$0.001). Among anemic patients, diabetes, acute rejection episodes and delayed graft function were more prevalent. Also, lower FGR, elder age and proteinuria was more frequent in patients with anemia compared to non-anemic patients. Both graft and patient survival at 5 and 10 yr were decreased in the anemic cohort (univariant analysis, log-rank p=0.001). Moreover, reduced survival was observed in severe (Hb$<$11g/dL in men, 10g/dl in women) or moderate (Hb11-12g/dL in men, 10-11g/dL in women) anemic patients but not in those with mild anemia (Hb 12-$<$13 in men, 11-$<$12 in women). After adjusting for age, gender, FGR, cold isquemia, diabetes and rejection rates, anemia was an independent predictor of patient survival (relative risk 2,31, p=0.03) and graft loss (relative risk 2,01, p=0.001; multivariate Cox regression analysis).
Conclusions: anemia is an independent risk factor for both graft loss and mortality in kidney transplant patients.

O-196 UNDIAGNOSED GLUCOSE METABOLISM DISORDERS IN GERMAN HEMODIALYSIS PATIENTS

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Background: Post-transplant diabetes mellitus (PTDM) or new-onset diabetes mellitus (NODM) after renal transplantation is considered a major health threat for renal transplant recipients, that goes along with decreased patient and graft survival.
Methods: Screening for undiagnosed diabetes mellitus was done with the use of oral glucose tolerance test (oGTT) in 4 dialysis centers in Germany according to ADA criteria. Impaired glucose metabolism disorders were defined as a fasting glucose level $\geq$ 100 – 125 mg/dL (impaired fasting glucose IFG) and/or a 2 h glucose level 140-199 mg/dL (impaired glucose tolerance IGT). Overt diabetes mellitus was defined as a fasting glucose level $\geq$ 126 mg/dL and/or a 2 h glucose level $\geq$ 200 mg/dL.
Results: 237 adult hemodialysis patients were considered for inclusion in this trial. 91 patients (=38.4%), that were known to be diabetic were excluded from the trial leaving 146 non-diabetics. Since oGTT was not performed in 40 of these nondiabetics due to refusal to participate or inability to give informed consent or to participate or nonadherence, 106 patients underwent oGTT. From these 106 patients, 12.3% had an abnormal fasting glucose ($\geq$ 100 and $<$ 126 mg/dL), 18.9% had an impaired glucose tolerance (2 h glucose level $\geq$ 140 and $<$ 200 mg/dL), and 9.4% had overt diabetes mellitus.
Conclusion: There is a considerably high number of undiagnosed glucose metabolism disorders in German hemodialysis patients. These patients may eronneously be classified as PTDM or NODM after renal transplantation.

O-197 THE EFFECT OF RITONAVIR ON PHARMACOKINETICS OF TACROLIMUS IN PRETRANSPLANT KIDNEY FAILURE PATIENTS WITH HIV

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Background: Life expectancy of HIV patients has improved and therefore kidney failure is seen more frequently. An increasing number of HIV patients is accepted for kidney transplantation. Ritonavir-boosted protease inhibitors are frequently used in HAART. Ritonavir inhibits the cytochrome P450 CYP 3A enzyme and transporting protein P-glycoprotein (Pgp). Tacrolimus is a substrate for both CYP 3A and Pgp. Here we present data on the influence of ritonavir on tacrolimus pharmacokinetics in pretransplant kidney failure patients with HIV.
Methods: Six HIV patients accepted for kidney transplantation and treated with a ritonavir-containing regimen were included. At least 12 blood samples were drawn in each patient after an oral test dose of tacrolimus (Prograf®). Pharmacokinetic curves of tacrolimus in all patients were analyzed. A population model was created.
Results: In ritonavir users the area under the curve (AUC) after a 5 mg dose of tacrolimus was 50-fold higher than in non-ritonavir users (8680 vs 174 ng•h/mL). The mean oral clearance in ritonavir users was 25-fold lower than in non-ritonavir users (0.98 vs 24.2 L/h). The pharmacokinetic curve of tacrolimus in ritonavir users did not show the usual peak and trough pattern, but rather resembled a flat line with a half life up to 20 days. This resulted in a 40% lower estimated AUC when conventional target trough levels were applied.
Conclusion: Ritonavir changes pharmacokinetics of tacrolimus in pretransplant kidney failure patients with HIV dramatically, requiring lower dosing and close pharmacokinetic monitoring in these patients. As a result of "flat line" pharmacokinetic curves due to extremely prolonged half lives, target tacrolimus trough levels should be higher (17.5 ng/mL one month and 10 ng/mL one year after transplantation) in these patients compared to non-ritonavir users in order to achieve an equal exposure in terms of AUC.

O-198 THE DEVELOPMENT OF BK VIREMIA AFTER RENAL TRANSPLANTATION IS ASSOCIATED WITH A REDUCED CD8 CELL IL-2 RESPONSE

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Polyomavirus-associated graft nephropathy (PAN) has emerged as a significant risk factor for kidney graft loss. Risk factors for the development of PAN are ill defined. We performed a randomized prospective single-center study in 105 renal transplant recipients who were randomized to receive cyclosporin A/mycophenolate mofetil (CsA/MMF; n=31), tacrolimus/MMF (Tac/MMF; n=32) and Tac/MMF with conversion to everolimus (T/Erl; n=32), respectively. 10 patients were not randomized (contraindications against MMF). The impact of immunosuppressive therapy and pre- and posttransplant (4 months, 1 and 2 years) immune responses (intracellular cytokine responses and cytokine receptor expression using triple fluorescence flow cytometry) on the incidence of BK viremia and PAN was analyzed. BK virus screening was performed by rt-PCR testing in serum and urine specimens obtained on days 0, 14, 30, 60, 90, 120, 180, 270, 360 and 720.
7/105 (6.7%) patients developed biopsy-proven PAN (CsA/MMF: n=1, Tac/MMF: n=3, T/Erl: n=2, not randomized: n=1), and 4 of these lost their grafts (Tac/MMF: n=1, T/Erl: n=2, not randomized: n=1). 21/105 (20.0%) patients had documented BK viremia. BK viremia preceded PAN in all cases and was significantly associated with Tac/MMF immunosuppression (4/31 (12.9%) CsA/MMF, 11/32 (34.4%) Tac/MMF, and 5/32 (15.6%) T/Erl patients, p=0.034; 1/10 (10.0%) not randomized patients). Patients with BK viremia showed significantly diminished Il-2 producing CD8 cells 4 months (14.2% vs 20.4%, p=0.011) and 1 year posttransplant (14.1% vs 20.5%, p=0.014) compared with patients without BK viremia.
Patients on a Tac- and MMF-based immunosuppression were at higher risk of developing BK viremia. Patients with BK viremia differed from those without in a more profoundly reduced Il-2 response of CD8 cells. Our data suggest that low CD8 cell Il-2 responses may predict the risk of developing BK viremia.

mTor inhibition

O-199 IMPROVED RENAL FUNCTION OF AN EVEROLIMUS/ ENTERIC-COATED MYCOPHENOLATE SODIUM REGIMEN AFTER CALCINEURIN INHIBITOR WITHDRAWAL IN DE NOVO RENAL TRANSPLANT PATIENTS: 3 YEAR FOLLOW-UP OF THE ZEUS TRIAL

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To follow-up on renal function, efficacy and safety after a conversion to an Everolimus/Enteric-Coated Mycophenolate Sodium (EC-MPS) regimen after Cyclosporine (CsA) withdrawal in *de novo* kidney allograft recipients at Month 36 post-transplantation.
In this prospective, open-label, controlled, multi-center study patients were randomized 4.5 month after transplantation to either Everolimus/EC-MPS or CsA/EC-MPS. After completion of the core study (Month 12), patients were included in an observational 24 month follow-up.
300 pts were randomized to either Everolimus/EC-MPS (n=155) or CsA/EC-MPS (n=145), 242 (80.7%) pts completed the 36 month visit. BPAR was reported in 20 (13.0%) Everolimus/EC-MPS-treated vs. 7 (4.8%) CsA/EC-MPS-treated patients between randomization and Month 36. After 12 months two additional BPAR occurred in the Everolimus/EC-MPS vs. 5 BPAR in the CsA/EC-MPS group. Three death and 2 graft loss were observed in CsA/EC-MPS-treated, one death and one graft loss in Everolimus/EC-MPS-treated pts. The number of patients with infections [31 (20.0%) vs. 29 (20.0%); Everolimus vs. CsA)] and hospitalizations [48 (31.0%) vs. 41 (28.3%); Everolimus vs.CsA)] in the follow-up period (month 24-36) was comparable. Renal function expressed as calculated GFR (Nankivell) was similar in both groups at randomization with an improvement by 7.27 mL/min/1.73m^2 in favor of the Everolimus/EC-MPS regimen (p=0.0098) at Month 36 (60.6±16.4 vs. 67.9±21.6 mL/min/1.73m^2) compared to 10.6 mL/min/1.73m^2 at Month 12. The observed GFR slope from randomization to month 24 was +4.8 [+0.4,+9.3] for Everolimus and -2.7 [-7.3,+2.0] mL/min/1.73m^2 for CsA/EC-MPS pts. Fewer patients in the everolimus group had a decline of GFR (26.6% vs. 41.8%; p=0.0034) compared to cyclosporine.
The conversion to Everolimus/EC-MPS in *de novo* renal transplant patients reflects a novel therapeutic approach which significantly maintains renal function over a period of 36 months without compromising efficacy and safety.

O-200 RENAL FUNCTION IN RENAL TRANSPLANT RECIPIENTS AFTER 24 MONTHS OF IMMUNOSUPPRESSION WITH CONCENTRATION-CONTROLLED EVEROLIMUS PLUS REDUCED CYCLOSPORINE EXPOSURE: UPDATE FROM THE A2309 STUDY

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Introduction: Everolimus (EVR), an mTOR inhibitor exhibits synergistic effects with cyclosporine (CsA) in renal transplant recipients (RTxR).
Methods: A2309 is a 24-month (24M) randomized, multicenter, open-label study in 833 *de novo* RTxR comparing two targets of EVR exposure (C0 3-8 or C0 6-12 ng/mL) with reduced CsA exposure vs. a control group receiving enteric-coated mycophenolate sodium (MPA) 1.44 g/day with standard CsA exposure. At 24M, study endpoints were composite efficacy failure (incidence of BPAR, graft loss, death or loss to follow-up), renal function (calculated GFR [MDRD]) at Months 12, 18 and 24, and safety comparisons between the EVR groups and the MPA group.
Results: Donor and recipient characteristics were comparable between treatment groups. Composite efficacy and RF at 12 M were non-inferior across groups as previously described. Mean CsA exposure at 24M was 52, 50 and 135 ng/mL for EVR 3-8ng/mL, 6-12ng/mL and MPA groups, respectively. A 60% reduction in CsA exposure was observed in the EVR groups vs MPA group at 12M and was maintained at 24M. Mean GFR for all groups remained stable throughout the 24M study period. Mean GFR in both EVR groups was non-inferior to MPA at both M12 and 24. At 24M, more patients in the EVR groups vs. MPA were in the GFR≥60 range (Table).
Conclusion: This analysis confirms that EVR with reduced CsA exposure as compared to MPA results in comparable and stable renal function from 12M to 24M. A higher number of patients with a GFR≥60 were observed at 24M in the EVR treatment groups versus MPA.

GFR data (MDRD, mL/min/1.73m^2) in the ITT population

	EVR 3–8 ng/mL (N=277)	EVR 6–12 ng/mL (N=279)	MPA 1.44 g (N=277)
Month 12			
Mean calculated GFR	54.66	51.41	52.24
Month 24			
Mean calculated GFR	52.20	49.44	50.51
Patient with GFR ≥60 (%)	37.1	36.4	30.3
Patients with GFR 30–<60 (%)	53.7	53.0	59.2
Patients with GFR <30 (%)	9.3	10.6	10.6
Efficacy parameters at Month 24			
Graft loss, n	16	17	11
Graft loss or death, n	23	26	18

O-201 EFFICACY AND SAFETY OF EARLY CYCLOSPORINE (CsA) CONVERSION TO SIROLIMUS (SRL) WITH MYCOPHENOLATE MOFETIL (MMF): 5-YEAR RESULTS OF THE POST-CONCEPT STUDY

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CNI induces long-term nephrotoxicity and is associated with moderate renal dysfunction.
In the CONCEPT study, the early conversion to SRL demonstrated an improved renal function at one year. The 60 months follow up study investigates the long term impact of this strategy. In this prospective randomized trial, renal function by MDRD formula (eGFR) and safety were assessed from 12 to 60 months in group A: SRL+MMF and in group B: CsA+MMF. The changes of immunosuppressive treatment were left to the discretion of the centers.
At M60, 135 patients were evaluated; 65 in group A, 70 in group B. eGRF was significantly higher in group A, 58.31 [54.4; 62.23] *versus* 49.89 [46.03; 53.75] ml/mn/1.73m^2 (adjusted-mean [95%CI]) (p=0.0012). This difference was observed particularly in population "on treatment" (group A, n=36 and group B, n=55), 65.05 [60.27; 69.83] *versus* 50.31 [46.30; 54.32] ml/mn/1.73m^2 (p<.0001). eGFR increased between randomization and M60 in both ITT and OT: +12.5% *versus* -1.5% (p=0.01) and +27.1% *versus* -0.8% (p<0.0001) respectively.
On ITT analysis, 7 deaths (3 in group A and 4 in group B), 2 graft losses in group A and 4 BPAR (2 in each group) were observed between M12 and M60. Anemia, lipid profile and proteinuria were similar in both groups but the number of NODAT was increased in group A (8 vs 2).
The occurrence of cancers was lower in patients treated by MMF+SRL (n= 4) than MMF+CNI (n=12) (immunosuppressive treatment when the cancer diagnosed).
The significant improvement in renal function observed at 12 months in patients receiving MMF + SRL was maintained and even more pronounced at 60 months.

O-202 EFFICACY AND SAFETY OF CALCINEURIN-INHIBITOR-FREE DE-NOVO IMMUNOSUPPRESSION WITH EVEROLIMUS AFTER LIVER TRANSPLANTATION

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A prospective randomized study was designed to assess the efficacy and safety of a calcineurin-inhibitor (CNI)-free de-novo immunosuppression with everolimus, a mammalian target of rapamycin (mTOR) inhibitor after liver transplantation (LT) for HCV-related cirrhosis. Sixty-four LT recipients were enrolled at a single Center: 28 received an immunosuppressive schedule including everolimus + basiliximab + steroids and 36 received tacrolimus + basiliximab + steroids starting from the day of transplant. The rate of rejection and of HCV infection recurrence were the primary endpoints. Mortality rate at one year after LT, immunosuppression conversion rate, kidney function, and overall incidence of adverse events (infections, neurotoxicity, nephrotoxicity, incidence of de-novo-diabetes, hyperlipidemia and edema) were secondary end-

points. The 2 groups were comparable for main donor and recipient characteristics. Rejection rate (everolimus=11% vs. tacrolimus=3%, p=NS), HCV recurrence rate (everolimus=54% vs. tacrolimus=33%, p=NS), and one-year mortality (everolimus=18% vs. tacrolimus=19%, p=NS) were comparable between groups. Immunosuppression conversion rate was higher in the everolimus group (57% vs. 23%, p=0.01). One-year post-LT serum creatinine levels were comparable between groups. The most frequent side effect in the everolimus group was lower limb edema (everolimus=54% vs. tacrolimus=11%, p=0.001). The prevalence of other complications was similar between groups. A de-novo CNI-free immunosuppressive regimen seems feasible, but without significant overall improvement compared to a CNI-based immunosuppression.

O-203 INCIDENCE OF INTERSTITIAL LUNG DISEASE WITH EVEROLIMUS IN TWO LARGE, RANDOMIZED, MULTICENTER STUDIES IN DE NOVO HEART OR KIDNEY TRANSPLANT RECIPIENTS

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Background: mTOR-inhibitors have been associated with interstitial lung disease (ILD), a serious non-infectious lung disorder. Rare cases have been reported for everolimus (EVR) in kidney and heart transplantation (KTx, HTx), but no systematic evaluation of study databases has been published to date.
Methods: A2310 and A2309 are two 24-month (M), multi-center, randomized, open-label studies comparing efficacy and safety in de novo HTx- (A2310) or KTx (A2309) recipients receiving EVR dosed at 1.5 or 3mg/day (C0: 3-8 or 6-12ng/mL) with reduced-dose cyclosporine versus MMF/MPA with standard-cyclosporine. The study adverse event (AE) databases were searched for terms covering a wide range of pulmonary events. Identified cases were reviewed to assess likelihood of drug-induced ILD based on symptoms, history, concurrent conditions, diagnostic test results and possibility of treatment-relation. Concomitant infectious lung disease was ruled out.
Results: Safety populations for A2310 and A2309 consisted of 714 HTx- (EVR 1.5mg=279, EVR 3mg=167, MMF=268) and 825 KTx-recipients (EVR 1.5mg=274, EVR 3mg=278, MPA=273). Three cases were identified as possible ILD in A2310: one event of pulmonary toxicity (EVR 1.5mg) prompted EVR discontinuation. The event continued but the patient could be discharged. One ILD event (EVR 3mg) resolved 21 days after EVR termination. One mild AE reported by the investigator as ILD (EVR 3mg), did not lead to treatment adjustment but was reported as continuing at M12. One patient (EVR 1.5mg) in study A2309 experienced pulmonary alveolar proteinosis resulting in EVR dose reduction. This patient subsequently died from pneumonia and sepsis (Table 1).
Conclusion: Four cases (0.4%) of interstitial lung disease/pulmonary alveolar proteinosis were identified in this series of 998 HTx- and KTx-recipients receiving everolimus. ILD is a potentially serious event and rare occurrence needs to be considered with EVR-based immunosuppression.

O-204 ADVERSE EVENTS ASSOCIATED WITH mTOR INHIBITION: RESULTS FROM THE A2310 STUDY COMPARING EVEROLIMUS AND MMF IN DE NOVO HEART TRANSPLANT RECIPIENTS

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Background: Everolimus can reduce the development of cardiac allograft vasculopathy, a major contributor to long-term mortality after heart transplantation (HTx). However, mTOR-inhibitors have been associated with class-related complications possibly limiting treatment adherence.
Methods: A2310, a 24-month (M), multi-center, randomized, open-label study compared efficacy and renal function at M12 in de novo HTx recipients receiving everolimus (EVR) dosed at 1.5 or 3mg/day (C0: 3-8 or 6-12ng/mL) with reduced dose cyclosporine (CsA) or MMF 3g/day with standard dose CsA. All patients received steroids. Center-specific induction therapy (basiliximab/thymoglobulin/no induction) was allowed. Most relevant adverse events (AEs) were compared between groups.
Results: 714 HTx recipients constituted the safety population (EVR 1.5mg N=279, EVR 3mg N=167, MMF N=268). Enrollment in the EVR 3mg arm was prematurely terminated due to higher mortality. This analysis only compares low-dose EVR (1.5mg) and MMF patients. The EVR1.5mg group showed an increased incidence of new onset diabetes, pericardial effusions, proteinuria, stomatitis/mouth ulcerations, and thrombotic/thromboembolic events. Incidence of neutropenia and leukopenia was more frequent in the MMF group (Table 1). Management of these AEs rarely required the discontinuation of treatment (<3%). Incidence of serious infections (any infection) was 28.3% (64.9%) for EVR 1.5mg and 25.0% (63.1%) for MMF with serious bacterial infections being more frequent with EVR 1.5mg (14% vs. 8.6%) and serious viral infections more frequent with MMF (2.5% vs. 10.8%). Rates of serious fungal infections were 3.6% with EVR 1.5mg vs. 3.4% with MMF.

Table 1. Incidence of relevant adverse events, n (%)

	EVR 1.5 mg (N=279)	MMF (N=268)
Thrombocytopenia	33 (11.8)	29 (9.3)
Leukopenia	34 (12.2)	62 (23.1)
Neutropenia	44 (15.8)	94 (35.1)
Hyperlipidemia	83 (29.7)	60 (22.4)
New onset diabetes	27 (9.7)	16 (6.0)
Sternal wound complication	68 (24.4)	52 (19.4)
Pericardial effusion*	121 (43.4)	76 (28.4)
Pericardial effusion with hemodynamic compromise*	20 (7.2)	4 (1.5)
Pleural effusion	78 (28.0)	62 (23.1)
Peripheral edema	151 (54.1)	133 (49.6)
Proteinuria	9 (3.2)	5 (1.9)
Stomatitis, mouth ulceration	23 (8.2)	13 (4.9)
Thrombotic and thromboembolic events	43 (15.4)	25 (9.3)

*P<0.01.

Conclusion: The occurrence of mTOR-inhibitor associated AEs should be expected during treatment of HTx recipients with everolimus, although management of the event will rarely require discontinuation of everolimus. The use of systematic postoperative pericardial drainage may reduce the incidence of pericardial effusions.

Experimental immunosuppression

O-205 PHOSPHOSPECIFIC FLOW CYTOMETRY TO MONITOR P38 MAP KINASE ACTIVITY IN T LYMPHOCYTES OF RENAL TRANSPLANT PATIENTS

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Introduction: Transplant patients frequently suffer from lack of efficacy by immunosuppressive medication or from toxicity, which can be attributed to inter-individual variation in drug sensitivity. This problem can be overcome by pharmacodynamic monitoring, which focuses on measuring the biological effects of drugs. We used phosphospecific flow cytometry to study the effect of tacrolimus (TAC) on P38 MAP kinase (MAPK), the activator of NFAT (nuclear factor of activated T cells), in T cells.
Materials & Methods: Freshly obtained whole blood samples were stimulated with PMA/Ionomycin, and P38 MAPK phosphorylation was measured by flow cytometry.
Results: In vitro TAC inhibited P38 MAPK in a dose dependent manner: the IC50 in T cells was 37 ng/mL (95% CI 32 to 44 ng/ml).
In healthy volunteers (N=4), 2 hours after an oral dose of 10 mg TAC, the P38 MAPK activation was inhibited by 35.3% (median, range 16.4–60.3%) and

Abstract O-203 – Table 1. Identified cases of ILD in studies A2310 and A2309

HTx/KTx (treatment)	Event name (preferred term) / causality with treatment	Event start date / stop date or continuing	Severity / AE or SAE	Comments
HTx (EVR 1.5 mg)	Pulmonary toxicity / suspected	Day 110 / cont.	Severe / SAE	Ground glass opacities in CT scan. EVR discontinued Day 323
HTx (EVR 3 mg)	ILD / suspected	Day 94 / Day 127	Severe / SAE	Diagnosis by CT scan. Resolved after EVR discontinuation Day 106
HTx (EVR 3 mg)	ILD / suspected	Day 31 / cont.	Mild / AE	No diagnostic evidence provided. No action taken
KTx (EVR 1.5 mg)	Pulmonary alveolar proteinosis / not suspected	Day 316 / cont.	Moderate / SAE	Diagnosis by CT scan and bronchoscopy. EVR reduced. Death from pneumonitis/sepsis

cont., continuing; SAE, serious AE.

gradually recovered to baseline after 24 hours. In patients, one week and one month after renal transplantation, P38 MAPK activation did not decrease 2 hours after intake of TAC. Nevertheless, one week after Tx the P38 MAPK activation in T lymphocytes had significantly decreased in 22 out of 25 patients compared to pre-transplantation ($p<0.001$). Three patients had elevated P38 MAPK activity and at the same time, a biopsy proven cellular rejection.
Conclusion: In vitro and in healthy subjects TAC inhibits P38 MAPK. After transplantation P38 MAPK reflects an overall immunosuppressed state, which is not only the result of TAC alone. Elevated P38 MAPK activation might be a useful marker for cellular rejection.

O-206 *IN VIVO* IMMUNOSUPPRESSIVE EFFECTS OF INTRAVENOUS IMMUNOGLOBULIN ON INNATE AND ADAPTIVE IMMUNE CELLS INVOLVED IN ALLOGRAFT REJECTION

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Background: Intravenous Immunoglobulin (IVIg) is a safe and effective immunosuppressive therapy in autoimmune diseases. Surprisingly, we observed that anti-HBsAg IVIg reduces the acute rejection incidence after liver transplantation[1]. To understand these clinical benefits, we studied the immunomodulating effects of IVIg treatment in patients on IVIg-monotherapy.
Methods: Blood was collected before IVIg infusion, and 1 and 7 days thereafter from sixteen hypogammaglobulinemia patients. Changes in leukocyte markers were measured by flowcytometry, and serum cytokine and IgG levels by ELISA. The suppressive capacity of $CD4^+CD25^+CD127^-FoxP3^+$ regulatory T-cells (Treg) was studied *ex vivo* by co-culturing purified Treg with conventional $CD4^+CD25^-CD127^+$ T-helper cells (Tconv) from the patients stimulated with phytohemagglutinin (PHA).
Results: Serum IgG levels increased 2.4-fold directly post-IVIg treatment ($p<0.001$). IVIg selectively activated Treg as revealed by enhanced expression of the activation marker HLA-DR (T=0→T=7: +19%, $p<0.01$), while not affecting Tconv. In *ex vivo* suppression assays, Treg suppressive capacity increased 2.2-fold after IVIg treatment (p=0.02). On dendritic cells (DC), IVIg reduced the activating Fcγ receptor (FcγR) IIa expression (T=0→T=1: -28%, p=0.02), while the inhibitory FcγRIIb expression remained unchanged. Hence, IVIg balances DC towards a refractory status towards pathogenic immune-complexes. Additionally, IVIg induced a 4.8-fold decline of IFNGR2 expression, which is part of the signalling receptor for the potent DC activator, IFN-γ (T=0→T=7: -79%, p=0.01). Serum concentration of the anti-inflammatory cytokine IL-10 increased 2.5-fold one day after treatment (p=0.02).
Conclusion: We show that IVIg has selective immunomodulating effects on innate and adaptive immune cells involved in autoimmune diseases and allograft rejection *in vivo*. By both inhibiting the DC function and activating the Treg function, IVIg may be a promising safe and effective candidate for immunosuppression after organ transplantation.
Reference:
1. Kwekkeboom, J. *et al.* (2005). *Am J Transplant* 5, 2393-2402.

O-207 PHARMACOLOGICAL APOPTOSIS MODULATION IN ACTIVATED T CELLS PREVENTS ALLOGRAFT REJECTION

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Background: Apoptosis controls the adaptive immune response by regulating lymphocyte survival and therefore represents a potential target for immunosuppression. The small-molecule ABT-737 binds the anti-apoptotic proteins Bcl-2, Bcl-XL and Bcl-w (but not A1 and Mcl-1), induces lymphocyte apoptosis and inhibits autoimmune and allogeneic T cell responses. However, ABT-737 had only limited efficacy to prevent murine skin allograft rejection. We hypothesized that pharmacological combination therapy might prevent allograft rejection targeting the apoptosis pathway.
Methods: The transgenic mouse BM3.3 bearing a transgenic TCR specific for H-2Kb was used to analyze the pro-apoptotic potency of ABT-737 alone or in combination with classical immunosuppressive drugs on a homogeneous population of allo-reactive CD8 T cells in either naive or activated status in vitro. These data were correlated with the mRNA expression of anti-apoptotic Bcl-2 family members. Mechanistic experiments in vivo were performed generating syn-chimeric mice expressing the transgenic BM3.3-TCR only on a fraction of the CD8 T cell population. Finally, the most appropriate pharmacological combination was tested in a skin transplantation model.
Results: Activated CD8 T cells – in contrast to naive cells – were completely resistant to ABT-737. This resistance correlated with the up-regulation of anti-apoptotic A1 after TCR engagement. Calcineurin inhibitors, but not signal 2 or signal 3 blockers, prevented up-regulation of A1 and therefore broke resistance to ABT-737 after allo-stimulation in vitro and in vivo. As a consequence, in a MHC-single antigen mismatched skin graft model ABT-737 in combination with low dose cyclosporine A (CsA) potently inhibited rejection (mean graft survival, CsA vs. ABT-737+CsA, 16 days vs. undefined, p=0.008) and induced long-term graft survival without significant inflammatory infiltrates in 50% of the recipients.
Conclusion: Targeting the apoptosis pathway in lymphocytes represents a promising approach to prevent allograft rejection and to decrease calcineurin inhibitor exposure.

O-208 A NOVEL COMPOUND, DTCM-GLUTARIMIDE MARKEDLY PROLONGS MURINE CARDIAC ALLOGRAFT SURVIVAL IN COMBINATION WITH DONOR SPECIFIC TRANSFUSION

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Background: Donor specific transfusion (DST) has been shown as an attractive approach for the tolerance induction. DTCM-Glutarimide (DTCM-G) is a novel compound, which has been shown to inhibit LPS-induced c-Jun phosphorylation in murine macrophages. We examined the immunomodulatory effects of DST+DTCM-G on mouse cardiac allo-transplantation.
Methods: C57BL/6 (B6: H-2^b) mouse was given BALB/c (H-2^d) splenocytes (2×10^7, i.v.) as for DST on day -7, and administered DTCM-G (40 mg/kg/day, i.p.) thereafter for 2 weeks,. On day 0, BALB/c heart was transplanted and graft survival was assessed (n=6). Allo-immune responses and regulatory T cells (Tregs) population were assessed on day 0 and 7 using recipient splenocytes. The underlying mechanisms of DTCM-G were exploited *in vitro* using purified B6 mouse T cells.
Results: Control mice rejected grafts within 8 days. DTCM-G+DST treatment markedly prolonged graft MST to 70.5 days and allowed long-term graft acceptance in 2 of 6, whereas DTCM-G and DST monotherapy modestly prolonged graft MST to 19 and 24 days, respectively.

Figure 1A

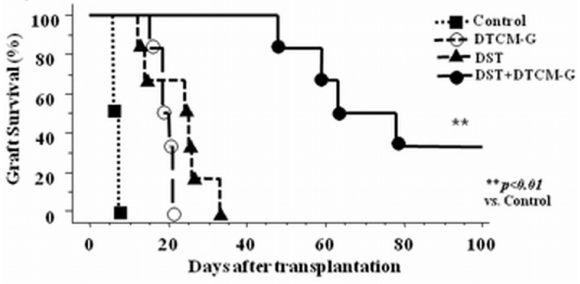
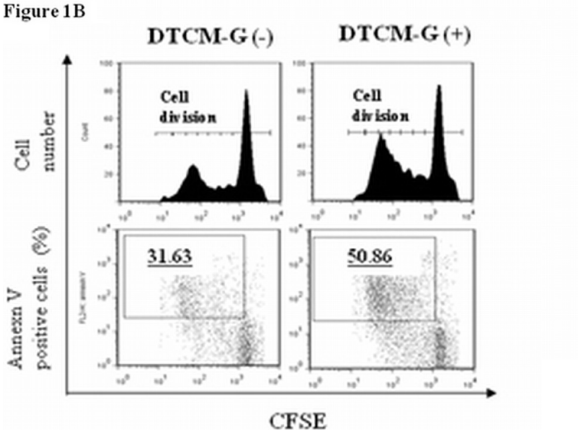


Figure 1B



Following DTCM-G+DST treatment, allo-reactive CD4+CD154+ T cells and IFN-γ producing cells significantly declined (n=4, $p<0.05$) on day 0, while CD4+CD25+Foxp3+ Treg population increased on day 7 (n=4, $p<0.05$). Addition of DTCM-G induced apoptosis on αCD3+αCD28mAbs triggered T cells *in vitro*, as assessed by Annexin V/PI. This was true in a GVHD model, in which DTCM-G promoted apoptosis on proliferating CFSE-labeled allo-

geneic T cells. Under stimulation with αCD3+αCD28mAbs or irradiated BALB/c splenocytes, treatment with DTCM-G (5 μg/ml) increased CD25+Foxp3+ population in cultured B6 CD4+T cells as compared to vehicle control (48.9±8.1% vs. 18.2±6.9%).
Conclusion: DTCM-G promotes apoptosis on activated T cells and induces Tregs. Such immunomodulatory properties of DTCM-G seem to synergize with DST that permits marked prolongation of allograft survival.

O-209 RAPAMYCIN INHIBITS INNATE AND ADAPTIVE FUNCTIONS OF PLASMACYTOID DENDRITIC CELLS

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Introduction: Plasmacytoid dendritric cells (PDC) are critically involved in innate immunity as the principal producers of IFN-α and, upon maturation, in adaptive immunity as Antigen-Presenting Cells (APC). PDC are activated by viral TLR-ligands, or by T cells via CD154-CD40 interaction. Here we report that the mode of PDC-activation dictates the strength of their capacity to stimulate allogeneic T cell responses, and show that rapamycin, which is an immunosuppressive drug that inhibits mTOR, reduces both innate and adaptive immune functions of PDC.
Methods and results: Human PDC stimulated by TLR-7 or TLR-9 ligands produced high amounts of IFN-α, but exhibited a weak capacity to stimulate allogeneic T cells. Conversely, PDC stimulated by CD154-expressing cells failed to produce IFN-α but exhibited a robust capacity to stimulate allogeneic T cell proliferation and cytokine production. The weaker allo-stimulatory capacity of TLR-stimulated PDC was associated by selective induction of programmed-death ligand-1 on their surface upon TLR-ligation, but not upon CD40-engagement. CD154-driven induction of allo-stimulatory capacity in PDC was strongly inhibited by rapamycin. This loss of allo-stimulatory capacity was caused by suppression of CD40-expression on PDC, which reduced their sensitivity to CD154-stimulation. Accordingly, the inhibitory effects of rapamycin on TLR-mediated induction of allo-stimulatory capacity in PDC were present but more limited. In addition, rapamycin potently inhibited TLR-7 induced IFN-α production (-66%±16%), while TLR-9-driven IFN-α production was modestly reduced (-19%±17%).
Conclusion: mTOR regulates TLR-7-stimulated IFN-α production by PDC, and is involved in T-cell-dependent induction of allo-stimulatory capacity in PDC. Rapamycin inhibits both innate and adaptive immune functions of human PDC.

O-211 DANAZOL INDUCED PROLONGED SURVIVAL OF FULLY ALLOGENEIC CARDIAC GRAFTS AND GENERATED REGULATORY CD4$^+$ CELLS

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Background: Danazol, a modified testosterone, which has been used as medicine for endometriosis, has a valid effect in pretreatment of patients receiving in vitro fertilization and embryo transfer, although its reproductive mechanism remains unclear. We investigated the effect of Danazol on alloimmune responses in a murine model of cardiac allograft transplantation.
Methods: CBA male mice ($H2^k$) underwent transplantation of C57BL/6 (B6, $H2^b$) hearts and received a single dose of Danazol by intraperitoneal injection. An Adoptive transfer study was performed to determine whether regulatory cells were generated. Immunohistochemical studies and cell proliferation assessments were performed.
Results: Untreated CBA mice rejected B6 cardiac grafts acutely (median survival time [MST], 7 days). When CBA mice were treated with Danazol from the day of transplantation to 6 days afterward, allograft survival was prolonged to MST, 63 days. Moreover, secondary CBA recipients given whole splenocytes from primary Danazol-treated CBA recipients 30 days after B6 grafting had prolonged survival of B6 hearts (MST, 29 days), compared to that in the secondary recipients with adoptive transfer of naïve splenocytes (MST, 10 days). When CD4$^+$ cells were purified from the spleen of the Danazol-treated recipients and were adoptively transferred into naïve secondary CBA recipients, the secondary recipients enjoyed prolonged survival of cardiac allografts (MST, 38.5 days), compared to that in the recipients with adoptive transfer of naïve CD4$^+$ cells (MST, 8 days). Proliferation of splenocytes was suppressed in Danazol-treated mice. Moreover, Danazol in MLRs showed the suppression of alloproliferation with dose dependent manner when added in culture medium. Flow cytometry studies showed an increased CD4$^+$CD25$^+$Foxp3$^+$ cell population in splenocytes from those mice.
Conclusion: Danazol could induce unresponsiveness of fully allogeneic cardiac allografts and generation of regulatory CD4$^+$ cells.

O-211A HISTONE DEACETYLASE-6 (HDAC6) PLAYS A CRITICAL ROLE IN DENDRITIC CELL MATURATION AND REVEALS A NEW MECHANISM FOR HDAC-TARGETED IMMUNOSUPPRESSION

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Background: Histone deacetylase inhibitors (HDACi) are clinically useful antineoplastic agents, which are emerging as a new class of immunosuppressant agents. Recent studies have demonstrated a critical role for HDAC in promoting T cell effector responses both *in vitro* and *in vivo*. However, the role of HDAC proteins in antigen presenting cell (APC) biology remains unknown. This study seeks to determine whether HDACs promote APC function *in vitro*.
Methods: Dendritic cells (DCs) were derived from peripheral blood monocytes from healthy donors using standard techniques. They were treated to promote differentiation into immature DCs with GM-CSF and IL-4 and further matured with the addition of IL-6, TNF-α, IL-Iβ, Poly I-C and LPS. DCs were matured in the presence or absence of a pan-HDACi (SAHA), a novel HDAC6-specific inhibitor (KAR3000) or Vitamin D. DC differentiation was assessed by CD14, CD11c and HLA-DR expression and maturation evaluated by the expression of CD80, CD83 and CD86 using flow cytometry.
Results: Differentiation of DCs, measured by a reduction in CD11c and HLA DR expression, was markedly suppressed by the HDAC-6 specific inhibitor KAR3000 ($p<0.0001$) in a dose dependent fashion. Subsequent DC maturation was also significantly impaired by KAR3000, with suppression of CD80 and CD83 expression ($p<0.02$). There was no difference in cell viability between the agents used.
Discussion: Histone deacetylases represent a novel class of targets for mediating immunosuppression. These data support a critical role for HDAC6 in both the differentiation and maturation of DCs. Such effects may have the potential to alter the antigen presenting capacity of DCs and subsequently have a role for HDACi-mediated immunosuppression within the field of transplantation.

Kidney (long-term outcomes II)

O-212 DE NOVO KIDNEY GRAFT TUMORS: RESULTS FROM A MULTICENTRIC RETROSPECTIVE NATIONAL STUDY

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Background: De novo tumors in renal allografts are rare and their incidence is probably underestimated. Knowing the prognosis of such tumors is important for deciding upon safe oncological treatments and preventing return to dialysis. We therefore analyzed epidemiology, diagnosis, treatment and outcomes of renal cell carcinomas (RCCs) arising in renal allografts through a large retrospective renal transplant cohort in France.
Methods: We performed a retrospective and multicentric study. All kidney graft tumors diagnosed after transplantation were considered as de novo tumors.
Results: Seventy one de novo kidney graft tumors were identified among 40407 patients who underwent a renal transplantation during the same period (0.17%). Patients were 48 men (67.6%) and 23 (32.4%) women with a mean age of 49 years (20-82) at the time of diagnosis. Mean tumor size was 3.2 cm (1.2-10). Tumor related symptoms were noted in only 11 patients (15.5%). Sixty one (85.9%), 44 (62.7%) and 30 tumors (42.2%) were organ confined (T1-2), low grade (G1-2), and papillary carcinomas, respectively. Only 3 patients had synchronous distant metastases (4.2%). Mean time between transplantation and RCC diagnosis was 122.6 months (0.9-300). Thirty five (49.3%) and 5 (7%) patients underwent a conservative treatment by partial nephrectomy and radiofrequency ablation, respectively. Estimated 5 years cancer specific survival rate was 96%.
Conclusions: This is the largest series of RCCs arising in renal allografts reported so far. Even though occurring in the context of immune suppression, most of these tumors are small, incidental, low stage and low grade carcinomas. Papillary histology is more frequent than in the general RCC population. Oncological outcome is excellent and conservative treatment should be preferred at each time it is feasible for preventing return to dialysis.

O-213 THE CRITICAL ROLE OF RENAL FUNCTION, PROTEINURIA AND CARDIOVASCULAR DISEASE ON THE RESULTS AFTER RENAL TRANSPLANTATION.A PROSPECTIVE MULTICENTRE STUDY AT FIVE YEARS ACCORDING RECIPIENT AGE

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Aim: To evaluate the impact of cardiovascular disease in kidney transplant patients in Spain according to the recipient's age.
Methods: From a database focus on posttransplant cardiovascular disease (CDV) we prospectively analyzed the results at five years of 2600 patients transplanted during 2000-2002 in 14 Spanish units. We established 3 groups according recipient age: <40, 40-60 and > 60 years. The more frecuent immunossuppression was Tac+MMF+St.
Results: Patients were (670)25.85% <40years, (1321) 50.9%, 40-60 years and (601) 23.19% > 60. 5-Year patient survival were 97.4, 90.8 and 77.7% respectively and graft survival (censored death) was 88, 84.2 and 79.1% respectively and 82.1, 80.3 and 64.7% without censured death. The main cause of death was CVD 33.9%: according recipient aged: (7/16) 43.7% <40yr; (36/101) 35.6% 40-60 yr and (34/110) 30.9% >60yrs. The main causes of graft loss were chronic allograft nephropathy in patients <40 years and death with function in the two remaining groups. In the multivariate analysis graft survival, just elevated creatinine and proteinuria > 1g at 6 months posTx were risk factors for graft loss in all groups. In the multivariate analysis for death we did not find a common risk factor for the three groups.
Conclusion: Five-year results showed an excellent patient and graft survival especially in the younger age group. CVD have a great impact in all patients and notably is the main cause of mortality in recipients under forty. Renal function and proteinuria early after transplantation are risk factors for graft loss in all patients. Early improvement of renal function and proteinuria togheter with strict control of cardiovascular risk factors, especially in young patients are mandatory.

O-214 POSTTRANSPLANT sCD30 AS A PREDICTOR OF KIDNEY GRAFT OUTCOME

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Background: Reliable markers for assessing the biological effect of immunosuppressive drugs and identification of transplant recipients at risk of developing rejection are not available.
Methods: In a prospective multicenter study we investigated whether posttransplant measurement of the T cell activation marker soluble CD30 (sCD30) can be used for estimating the risk of graft loss in kidney transplant recipients. Pre- and posttransplant sera of 2,322 adult deceased-donor kidney recipients were tested for serum sCD30 content using a commercial ELISA.
Results: sCD30 decreased posttransplant and reached a nadir on day 30. Patients with a high sCD30 of ≥40 U/mL on day 30 showed a subsequent graft survival rate at 3 years of 78.3±4.1%, significantly lower than the 90.3±1.0% rate in recipients with a low sCD30 of <40 U/mL (log-rank $P<0.001$; hazard ratio 2.02, $P<0.001$). While an association was found between pre- and posttransplant sCD30 levels, patients with high sCD30 on posttransplant day 30 demonstrated significantly lower 3 year graft survival irrespective of the pretransplant level. When sCD30 was analyzed in combination with ELISA-reactive HLA antibodies, 19 patients with high sCD30 of ≥40 U/mL and high HLA class I or II antibody reactivity on posttransplant day 30 showed a 3-year graft survival rate of only 65.2±13.5%, in contrast to a 90.9±1.1% rate in 835 patients with low sCD30 and low HLA antibody reactivity (log rank $P<0.001$). Even in 87 patients with low HLA antibody reactivity, high sCD30 on day 30 was associated with an impaired 3-year graft survival rate of 82.2±4.2% ($P=0.005$ as compared to low sCD30 and low antibody).
Conclusions: Our data suggest that posttransplant measurement of sCD30 on day 30 is a predictor of subsequent graft loss in kidney transplant recipients and that sCD30 may potentially serve as an indicator for adjustment of immunosuppressive medication.

O-215 PROSTAGLANDIN E2 RECEPTOR IDENTIFIES PATIENTS AT RISK OF KIDNEY ALLOGRAFT FUNCTION DETERIORATION

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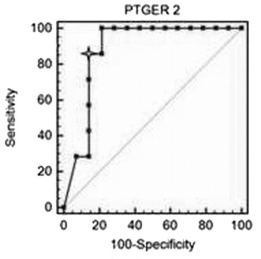
Background: Subclinical acute T-cell mediated rejection (SAR) diagnosed from protocol biopsies is thought to be a risk factor of long- term allograft dysfunction. It is unclear whether the degree of immune activation may explain the absence of graft dysfunction at the time biopsy. The aim of this study was to evaluate the transcriptome patterns of SAR and acute T cell mediated rejection (AR) from early case biopsies.
Methods: The intragraft expression of 375 target genes involved in chemokine defense, apoptosis, inflammation, tolerance and TGF-β signaling pathways was measured using quantitative real-time RT-PCR (2^{-DDCt}) method in SAR (n=10) and AR (n=10) and the results were correlated with the clinical outcome.
Results: The gene expression patterns in SAR were different from AR and included the decreased expression of cytokines mediating chemotaxis (CCL1, CC17, CCL24, CCL25, CCL26), cytokine receptors (CCR1, CCRL2, IL1RAPL2), proinflammatory cytokines (IL12A, LTA), complement protein C3, executioner protein of apoptosis (CASP7), growth factor (TGFA), costimulation (CD274), colony stimulating factor (GM-CSF), proteins involved in dendritic cells differentiation and interaction (CD209, LAMP3) and in regulation of immune response (LILRB2, LILBRB4). The quantity of FoxP3 gene expression in SAR was lower than in AR. Differences in transcripts signature between SAR and AR are consistent with stronger proinflammatory setting of AR. There was no difference in incidence of renal function impairment between SCR and ACR group (4/10 vs. 3/10) at 2 years. Logistic regression analysis showed that lower prostaglandin E2 receptor (PTGER2) gene expression levels in studied biopsies predicted graft function deterioration (Nagelkerke $R^2=0,74.3$, $p<0.0001$).



RQ < 1.46

85% senizitivita
83% specificita

Area under the ROC curve (AUC)	0,878
Significance level P (Area=0.5)	<0,0001

Conclusion: Subclinical acute kidney allograft rejection has transcriptional profile of acute injury of lower extend than clinical acute rejection. The lower intragraft PTGER2 gene expression may predict the progression of kidney graft dysfunction.
This work was supported by IGA grant (NS10516-3/2009).

O-216 SUB-CLINICAL ACUTE REJECTION (SAR) IN PROTOCOL BIOPSIES PERFORMED 3 MONTHS AFTER RENAL TRANSPLANTATION

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Background: We instituted protocol biopsies 3 months after transplantation with the aim of optimizing choice of long-term immunosuppression. The reported incidence of SAR of at least Banff grade 1 is 4.6-8.7% (Rush, et al. AJT 2007; 7: 2538., Naesens, et al. AJT; 2007: 2114). Naesens identified number of HLA-DR mismatches as a predictor of SAR at 3 months. We aimed to identify predictors that may allow stratification of risk of SAR, to target biopsies, and reduce exposure of patients to an invasive procedure.
Materials and Methods: We performed a retrospective analysis of the records of 210 kidney transplant patients, comparing timing of biopsy, and key patient characteristics, with histology findings. 85 patients (40.5%) had an indicated biopsy within 3 months and were excluded. Protocol biopsy was omitted in 4 patients for other reasons (1.9%) leaving 121 protocol biopsies for analysis.
Results: Protocol biopsy findings are shown in table 1. There was a significantly higher incidence of SAR in CMV negative recipients from CMV seropositive donors (17.9%) than those with other CMV combinations (4.4%) p=0.02. HLA-DR mismatch may be associated with SAR with 3.8% incidence with no DR mismatch, and 12.1% with at least 1 DR mismatch. This was not statistically significant, perhaps due to insufficient power in the study. Patients on my-

cophenolate mofetil (MMF) displayed similar levels of SAR (7.7%) compared to patients on tacrolimus and prednisolone (7.1%).

Table 1. Findings at 3 Month Protocol Biopsy

≥Banff 1a	9 (7.4%)
Borderline	20 (16.5%)
Normal	73 (60.3%)
Other	6 (5.0%)
Inadequate sample	13 (10.7%)
Total	121

Conclusions: The presence of SAR in 7.4% of patients at 3 months merits caution in reducing immunosuppression at this point without biopsy. CMV mismatch and possibly HLA-DR mismatch predict the occurrence of SAR but are not 100% predictive. We would advocate continued use of 3 month protocol biopsies.

O-217 ARE RENAL ALLOGRAFT POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLD) DIFFERENT FROM EXTRA-GRAFT PTLD?

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Renal allograft post-transplant lymphoproliferative disorders (R-PTLD) represent a singular PTLD subclass, but large series are scarce in the literature. We described a series of 62 R-PTLD, recorded in the French Registry, diagnosed between 1998 and 2008. We compared their characteristics with PTLD occurring outside the graft (n=427).

Time to R-PTLD was 33±46 months, and 53% occurred in the first post-transplant year. A single localization of PTLD in renal allograft was reported in 71%. Sixty three percent of R-PTLD were of donor origin (12 of 19 studied cases). Compared to the extra-graft PTLD, R-PTLD occurred earlier ($p<0.001$), more often in the first post-transplant year ($p<0.001$), more often as a single localization (p=0.05), and with a higher creatinine level (p=0.03) at time of diagnosis. R-PTLD were more often polymorphic (p=0.07), CD20+ (p=0.04) and associated with EBV (p=0.03). Patients with R-PTLD have more HLA-B mismatches (p=0.024) than patients with other localizations. Rejection after diagnosis were more frequent ($p<0.001$) in case of R-PTLD. Donor origin was more common in R-PTLD (p=0.001) than in extra graft PTLD. Restart of dialysis ($p<0.001$) and retransplantation ($p<0.001$) were more frequent in R-PTLD. In patients with R-PTLD, age>45 years at transplantation (p=0.003) or age>53 years at diagnosis (p=0.004), time to PTLD < 12 months, (p=0.01), single localization (p=0.001), polymorphic PTLD (p=0.05), and LDH level <480 UI/L (p=0.007) were associated with a better survival. Lymph nodes (p=0.018), splenic or bone marrow ($p<0.001$) infiltration were associated with a poorer survival. Survival was better in R-PTLD patients than in patients with extra-graft PTLD (p=0.002).

In conclusion, R-PTLD are different than lymphoma developing outside the allograft, suggesting a specific patho-physiology, highlighting by the frequency of donor origin of the tumoral cells.

O-218 INCREASE OF T CELL SUBSETS EXPRESSING MARKERS OF ACTIVATION AND CYTOXICITY IN PATIENTS WITH POST-TRANSPLANT MALIGNANCY

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Introduction: Markers of T-cell activation and cytotoxicity have been widely investigated as cancer indicators in the general population. Expanded cytotoxic T-lymphocytes (CD8+CD57+) as tumor infiltrating cells as well as circulating cells has been proven. Poor data are currently available for post-transplant malignancy. This is as a comprehensive analysis of lymphocyte subsets, activated (CD3+HLA-DR+) and cytotoxic (CD8+CD57+) T-cells in renal transplant recipients with neoplasm.

Methods: From a population of 904 kidney allografts transplanted between 1995 and 2006, we selected 89 patients (9.8%) with post-transplant malignancy: 26 (29.2%) were classified as non-melanoma skin cancer, 13 (14.6%) as Kaposi sarcoma, 13 (14.6%) as PTLD, 2 (2.2%) as melanoma and 35 (39.3%) as solid organ cancer. The follow-up was 8.9±3.5 years. Percentages of lymphocytes subsets (CD3+ T-cells, CD3+CD4+ T-helper, CD3+CD8+ T-suppressor/cytotoxic, CD3-CD19+ B-cells and CD3-CD16+ or CD3-CD56+ NK), activated T-lymphocytes (CD3+HLA-DR+) and cytotoxic T-lymphocytes (CD8+CD57+) were assayed using FACS analysis. In our centre, these quantitations are executed twice a week during transplant hospitalization and in case of hospitalization during the follow-up period, and every six months after discharge.

We collected all the data available and compared the assessments closer to cancer diagnosis (±6 months) with those performed at least one year before.

Results: The assays at time of cancer diagnosis (n=159) compared to those performed previously (n=659) revealed higher percentages of T-suppressor/cytotoxic cells (CD3+CD8+: 30.3±13.7% vs 26.5±11.0%, $p<0.001$), activated T-cells (CD3+HLA-DR+: 17.6±14.2% vs 14.3±9.8%, $p<0.001$), and CD57-positive cytotoxic lymphocytes (CD8+CD57+: 14.6±6.6% vs 14.35.8%, p=0.007; $CD8+CD57^{dim}$+: 6.7±4.9% vs 5.9±4.1%, p=0.039; $CD8+CD57^{bright}$: 7.1±5.8% vs 6.0±4.3%, p=0.009).

Conclusions: Our results show an increase in T-cell subsets expressing markers of activation (HLA-DR) and cytoxicity (CD57) in patients with post-transplant malignancy, suggesting a potential role of these expanded T-cell clones in cancer after renal transplant.

O-219 COST-EFFECTIVENESS MODEL TO EVALUATE 200-DAYS VS 100-DAYS TREATMENT WITH VALGANCICLOVIR PROPHYLAXIS TO REDUCE CYTOMEGALOVIRUS DISEASE IN HIGH-RISK (D+/R-) KIDNEY TRANSPLANT RECIPIENT IN SPAIN

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Background: IMPACT trial showed that prolonged prophylaxis of 200 days with valganciclovir (VGC 200) compared with 100 days (VGC 100) significantly decreases the incidence of cytomegalovirus (CMV) disease. Therefore, a cost-effectiveness model was developed to evaluate prolonged prophylaxis of 200 days with valganciclovir and its long term economic impact.

Methods: A Markov model with 9 health states was designed to simulate the CMV disease progression; costs and outcomes associated with the use of VGC 200 vs VGC 100 in a cohort of 10,000 patients over 10 years was examined. Data of the disease evolution were obtained from the IMPACT (Humar, Am J Transplant 2010) for year 1 and the available scientific evidence for years 2-10. The analysis was conducted from the perspective of the Spanish National Healthcare System (SNHS), considering direct medical costs. Unit costs (€2010) were obtained from a Spanish database. Utility values were obtained from literature. The annual discount rate was 3% for costs and outcomes.

Results: Treatment with VGC 200 provides better results in health than VGC 100 (50,020 vs. 47,640 QALY/patient). The average overall cost per patient is €1,121,327 with VGC 200 and €1,131,187 with VGC 100. The savings per patient treated with VGC 200 in 10 years is €986. Sensitivity analysis confirm the stability of the results.

Conclusion: Treatment of patients with prolonged prophylaxis valganciclovir reduces the incidence in high risk kidney transplant recipients and is a cost-saving strategy in CMV disease management from the perspective of the SNHS.

O-220 POOR OUTCOME OF SECOND KIDNEY TRANSPLANTATION: A DELAYED EVENT

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Background: Whilst graft and patient survival rates following second kidney transplantation have improved substantially in recent years, their poor prognosis compared to primary transplantations is still the subject of discussion.

Patients and Methods: To assess long-term survival of second transplantations compared to first transplantations and to identify graft outcome risk factors, we performed a prospective cohort-based study from the validated French DIVAT databank between 1996 and 2010 (n=3112 with 642 second transplantations). All patients were treated with at least steroids and mycophenolate mofetil for maintenance therapy. Patient-graft survival and acute rejection episode (ARE) free time were analyzed using multivariate Cox models adjusted on all potential confounding factors. A piece-wise Cox model allowed the non-proportionality of the graft rank to be taken into account.

Results: Even with careful adjustment, particularly for pre-transplant immunization, retransplanted patients appear to have a significantly higher risk of graft failure than primary transplanted patients (RR = 2.27, P = 0.0007) but only after four years. This time-dependent effect of the graft rank is not correlated with the ARE frequency. Indeed, there is no difference between second and primary transplantations in occurrence of overall ARE (RR = 0.99, P = 0.9108) nor cortico-resistant ones (RR = 1.22, P = 0.4766).
Conclusion: The risk of return to dialysis or death following second transplantations remains consistently higher than that observed in primary transplantations when adjusting for confounding factors, mainly immunization, but is delayed after four years. This time-dependent effect of the graft rank, which, to our knowledge, has not been reported previously, is not related to the frequency of ARE (even cortico-resistant ARE) but could be explained by a higher severity of ARE once they have occurred, or by a shorter occurrence of post graft donor-specific immunization under current investigations.

O-221 ARTERIAL AND ARTERIOLAR LESIONS ASSOCIATED WITH CHRONIC TRANSPLANT GLOMERULOPATHY

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Introduction: Up till now the only morphological correlates of chronic transplant glomerulopathy (TG) that were discussed in the literature encompass the acute and chronic lesions in glomeruli and PTC.
The aim of the study was to characterize the arterial and arteriolar lesions that are present in renal transplant biopsies with TG, as well as their impact on the graft survival.
Material and Methods: We retrospectively analyzed in the biggest so far group of 159 chronic TG cases, recognized in Transplantation Institute since 1996, and compare it with 85 non-TG biopsies with other types of chronic vascular and/or interstitial lesions.
Results: There were four types of vascular lesions that were significantly more common in TG group than in non-TG biopsies: endarteritis (10% vs 0 respectively, p=0.003), transplant vasculopathy, defined by the intimal sclerotization without the multiplication of lamina elastica (19% vs 4%, p=0.001), sclerotization of subendothelium in arterioles (47% vs 19%, $p<0.0001$), as well as hypertrophy/hyperplasia of arteriolar smooth muscle cells layer (40% vs 12%, $p<0.0001$). Among these lesions transplant vasculopathy was found to be an independent risk factor for the graft loss in the multivariate analysis (HR 1.5, p=0.002).
Conclusions: TG is associated with an increased risk of several types of vascular lesions, including acute vascular rejection. In almost half of the cases, TG is accompanied by arteriolar lesions, that are suggestive of chronic thrombotic microangiopathy. Transplant vasculopathy, a lesion that may be a complication of both: endarteritis and thrombotic microangiopathy is present in about 20% of TG cases and constitutes an independent risk factor for the graft loss. Presented results not only help to better characterize the morphology of TG cases, but also put a new light on the complexed pathogenesis of this lesion.

Applied biology II

O-222 THE ROLE OF HEPATIC HEMATOPOIETIC PROGENITOR CELLS IN INTRAGRAFT LEUKOCYTE CHIMERISM AFTER LIVER TRANSPLANTATION

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Leukocyte chimerism within the liver graft after LTx may have regulatory effects on recipient immune responses to the allograft. In this study we analyzed the duration of leukocyte chimerism within human liver grafts, and studied the role of hepatic hematopoietic stem cells (HSC) in this phenomenon.
Using explants of non-functioning liver grafts obtained during re-LTx, we observed that donor leukocytes (among which $CD14^+$ monocytes/Kupffer cells, NK, NKT and T cells) remained detectable in liver grafts up to 2 years after LTx. Leukocytes isolated from liver grafts before transplantation contained $CD34^+$ HSC, among which we detected NK-cell precursors and cells giving rise to granulocyte/monocyte colonies (CFU-GM). Surprisingly, one week after LTx donor HSC within explanted grafts were fully replaced by recipient HSC, indicating that persistence of donor leukocytes within liver grafts after LTx is not caused by sustained presence of donor-derived hepatic HSC. Interestingly, early after LTx donor HSC migrated from the graft and were detected in the recipient circulation. To study whether hepatic HSC can give rise to mature progeny, human liver $CD34^+$ cells transduced with a luciferase reporter gene were transferred into irradiated NOD/SCID mice. After 7 weeks CFU-GM were detected in the mouse bone marrow, but a robust luciferase signal indicating presence of progeny was only observed in the liver.
Conclusion: Donor-derived leukocytes remain present in human liver grafts for at least 2 years after LTx, while hepatic HSC are rapidly replaced by recipient HSC. Sustained leukocyte chimerism within liver grafts after LTx may be caused by long-living intrahepatic leukocytes, or may be derived from donor HSC that early after transplantation engraft into the recipient bone marrow and give rise to progeny leukocytes that home to the liver graft.

O-223 PRIMARY HUMAN HEPATOCYTES FROM METABOLIC DISORDERED CHILDREN RECREATE HIGHLY DIFFERENTIATED LIVER TISSUE-LIKE SPHEROIDS ON ALGINATE SCAFFOLDS

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Background: Human hepatocyte transplantation has not been routinely established as an alternative to liver transplantation. In vitro engineering of liver tissue using primary human hepatocytes on alginate scaffolds could be an alternative model to create functional liver neo tissue for transplantation.
Methods/Materials: Alginate scaffolds were seeded with 1×10^6 freshly isolated human hepatocytes. The scaffolds were incubated in modified Williams' medium under a humidified atmosphere of 5% CO2 and 95% air at 37°C for 14 days. Cell loading efficiency was quantified via DNA measurement. Cell viability and glycogen storage capacity were evaluated by HE and PAS staining. Biochemical assays for albumin, alpha1-antitrypsin, urea and LDH were established. The activities of liver-specific factors (ZO-1, HNF-4, CK18) as well as cytochrome P450 enzyme activity were analyzed by immunofluorescent staining. Actin filaments in bile canaliculi were labeled with Phalloidin. Furthermore, quantitative RT-PCR for liver specific factors was created.
Results: Formation of hepatocyte spheroids was detected from day 3 onwards. Only a marginal loss of hepatocytes was observed during the culture period of 14 days. Biochemical assays for albumin, alpha1-antitrypsin and urea revealed excellent metabolic function with its maximum at day 7. Low LDH enzyme release demonstrated minor cellular membrane damage. HE and PAS staining displayed high cell viability and well preserved glycogen storage until day 7. Immunofluorescent staining of liver-specific factors revealed highly differentiated hepatocytes in spheroids with a tissue-like structure on scaffolds. Fluorescent labeling of cytochrome P450 and bile canaliculi demonstrated detoxification ability as well as a well-shaped bile canaliculi network. Almost constant expression levels in most target genes were detected by quantitative realtime-PCR.
Conclusion: Alginate scaffolds provide a favourable microenvironment for viability and neo tissue regeneration of primary human hepatocytes in vitro.

O-224 MESENCHYMAL STEM CELLS REDUCE HEPATIC ISCHEMIA REPERFUSION INJURY

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Introduction: Mesenchymal stem cells (MSC) are one of the most promising cell populations for cell based immune therapy in solid organ transplantation. A transplanted organ is up against immune response of the recipient but also stress and tissue damage caused by ischemia and reperfusion. Besides their immunosuppressive function, MSC can have beneficial influence on tissue regeneration as well.
Methods: Influence of MSC were investigated in a murine ischemia/reperfusion injury (IRI) liver damage model. We used two different types of MSC: Exogenous MSC, isolated and cultivated from murine bone marrow and endogenous MSC, mobilised from recipients bone marrow into peripheral blood (done by daily application of VEGF (100 μg/kg/d KG) and Plerixafor® (5 mg/kg KG)). IRI was performed by clamping of the hepatic artery for 45min. Blood and tissue analysis was done at 6, 24 and 72 hours after reperfusion.

Results: Therapy by exogenous MSC reduced early liver damage significantly as assessed by ALT measurement in blood serum samples (5 times lower ALT levels in MSC treated animals). Expression of pro-inflammatory cytokines within the liver was also reduced after MSC administration (8 times lower TNFα expression, 2 times lower IL-6 expression). Mobilizing endogenous MSC resulted in significant increase of MSC frequency in peripheral blood (8 times more). When applied in the IRI setting, liver damage was also reduced, but to a lesser extent compared to exogenous MSC (3 times lower ALT levels). Mobilized MSC were absent in the peripheral blood of IRI animals, indicating that MSC migrate to damaged liver.
Discussion: In this murine model MSC are a potent inhibitors for transplantation related hepatic ischemia reperfusion injury. Since MSC are currently discussed as an alternative immunosuppressive strategy, the presented data might strengthen their beneficial use as novel cell therapeutics for solid organ transplantation.

O-225 TRANSFORMING GROWTH FACTOR-β1 POLYMORPHISM AND KIDNEY FUNCTION AFTER LIVER TRANSPLANTATION

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Background: The development of kidney dysfunction is one of the most important after liver transplantation (LT). Genetic variants of pathogenetically relevant cytokines may influence the development and course of the disease. The aim of our study was to evaluate the role of TGF-β1-polymorphism in this context.
Methods: 486 liver graft recipients were genotyped for TGF-β1 codon 25 (G->C) by polymerase chain reaction. Renal function before and after LT was characterized by estimation of glomerular filtration rate (GFR) using four-parameter-MDRD-formula on defined dates. GFR was compared among TGF-β1-genotype-groups of the entire cohort within 7.0 years (median) and among 10-year-survivors (n=213).
Results: Mean pre-transplant GFR differed significantly among TGF-β1-genotype groups (GG: 85.2ml/min vs. GC/CC: 75.3ml/min; p=0.014) regarding the entire cohort and 10-year-survivors (GG: 85.5ml/min vs. GC/CC: 67.4ml/min; p=0.006). Interestingly, lower mean GFR was observed among female compared to male recipients before (p=0.002), separately at all dates and cumulatively after LT ($p<0.001$). Moreover, mean post-transplant GFR among 10-year-survivors differed significantly during the whole period (p=0.033), at first day (p=0.026), after 3 months (p=0.004) and closely to significance after 3 years (p=0.051).
Conclusions: Genetic variants of TGF-b1 at codon 25 and recipient gender may influence the development of pre- and post-transplant kidney dysfunction suggesting an unfavorable effect of C-allele and female gender on renal function. Identification of further confounders seems to be promising.

O-226 TERMINALLY DIFFERENTIATED SUPPRESSOR CD8 T CELLS REDUCE THE RISK FOR ACUTE KIDNEY ALLOGRAFT REJECTION

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Introduction: End-stage renal disease (ESRD) is associated with premature immunological ageing of circulating T cells with a relative increase of terminally differentiated T cells.
We hypothesized that ESRD-related immunological ageing has a substantial effect on T cell immunity, thereby influencing the risk for acute rejection (AR) of kidney allografts.
Methods: In this prospective study, 185 ESRD patients receiving a kidney allograft were included and followed for 2 years. Prior to transplantation, circulating CD4 and CD8 T cells were quantified and T cell differentiation was established by determining the frequency of naïve T cells, central-memory and effector-memory T cells and the highly differentiated CD8 Temra ($CD8^+CD45RA^+CCR7^-$) cells. In addition, the frequency of T cells without expression of the co-stimulatory molecule CD28, which is associated with progressive T cell differentiation, was measured.
Results: In 47 patients a biopsy-proven AR occurred. Compared to healthy controls, ESRD patients were significantly immunologically aged. Patients with AR showed the least signs of immunological ageing with significantly more T cells, more naïve T cells and less terminal differentiation of memory T cells compared to non-rejecting patients. After multivariate analysis, only the frequency of terminally differentiated CD8 Temra cells (per percent;odds ratio 0.96, p=0.006) and the number of HLA mismatches (per mismatch;odds ratio 1.33, p=0.005) remained associated with AR. The cumulative incidence of AR at one year in patients within the lowest tertile of CD8 Temra cells was 3.5-fold increased compared to patients in the lowest tertile.
Conclusion: ESRD-related premature T cell ageing protects against AR after kidney transplantation, indicating a specific suppressor role for terminally differentiated CD8 T cells.

O-228 DIET-INDUCED INCREASE IN PLASMA OXIDISED LDL PROMOTES EARLY FIBROSIS IN A RENAL PORCINE AUTO-TRANSPLANTATION MODEL

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Background: Organ shortage drives renal transplantation centers to use marginal donors more prone to graft dysfunction. Pre-transplant hypercholesterolemia in the recipient increases the rate of acute rejections and the risk of late graft loss. This study aimed to characterize the effects of hypercholesterolemia on early renal graft outcome, investigating the role of oxidized low-density lipoprotein (oxLDL) and its pivotal lectin-like oxLDL receptor-1 (LOX-1).
Materials and Methods: We compared graft function and fibrosis 3 months after renal auto-transplantation in pigs fed either a normal diet (ND) or a hyperlipidemic diet (+20% lard, 2% cholesterol, HD) started 2 months before surgery and maintained until the sacrifice.
Results: HD induced a significant increase in plasma oxLDL levels at the time of surgery. Interestingly, oxLDL levels observed one day after transplant surgery were significantly correlated with the proteinuria measured 3 months later in the HD pigs, suggesting a detrimental role of oxLDL on graft outcome. Increased oxLDL levels, 3 months after surgery, were associated with renal activation of the LOX-1 signaling pathway, characterized by upregulation of LOX-1 and overexpressions of NFkB, Phospho-P38 and Gp91-phox (NAD(P)H oxidase subunit). Concomitantly, TGFb and CTGF protein levels were increased, whereas BMP-7 expression was decreased in HD pigs, giving rise to a profibrotic milieu. These results were supported by greater interstitial fibrosis revealed by Sirius red staining and increase of αSMA and vimentin protein expressions.
Conclusion: The significant correlation between plasma oxLDL and proteinuria, as well as the concomitant activation of both LOX-1 and TGFb signaling pathways, implicate oxLDL in the HD-induced fibrosis and tissue remodeling in renal transplantation, and reinforce the need to control cholesterol levels in both the donor and recipient of kidney transplants even in the early stage of transplantation.

O-229 INSULIN-PRODUCING CELLS DERIVED FROM ADULT HUMAN BONE MARROW MESENCHYMAL STEM CELLS CONTROL STREPTOZOTOCIN-INDUCED DIABETES IN NUDE MICE

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Background: Harvesting, expansion and directed differentiation of human bone marrow-derived mesenchymal stem cells (MSCs) would provide an autologous source of surrogate β-cells that alleviate the limitations of availability and/or allogenic rejection following pancreatic or islet transplantation.
Methods: Bone marrow cells were obtained from three adult type II diabetic volunteers and 3 non-diabetic donors. After 3 days in culture; adherent MSCs were expanded for 2 passages. At passage 3, differentiation was carried out in a 3-staged procedure. Cells were cultured in a glucose-rich medium containing activation and growth factors. These cells were evaluated in-vitro by immunofluoresce, electron microscopy and Rt-PCR. Insulin and c-peptide release in response to increasing glucose concentrations was determined.
Results: By immunofluorescence, some cells tested positively for insulin, c-peptide and glucagon with co-expression of insulin and c-peptide by the same cells. Nanogold immunostaining for electron microscopy demonstrated c-peptide at the rough endoplasmic reticulum. These insulin-producing cells (IPCs), expressed a variety of transcription factors and genes of pancreatic hormones closely similar to those of pancreatic islets. There was a stepwise increase in insulin and c-peptide release by these cells in response to increas-

ing glucose concentrations. One thousand clusters inserted under the renal capsule of diabetic nude mice resulted in control of their diabetic status for 3 months. The sera of treated mice contained human insulin, human c-peptide but negligible levels of mouse insulin. When the IPCs-bearing kidneys were removed, rapid return of diabetic state was noted. Histology of the removed kidneys showed insulin staining cells under the capsule.
Conclusion: MSCs could be differentiated to form IPCs. The implanted IPCs could control diabetic mice for 3 months. Optimization of culture conditions and/or differentiation techniques are needed to improve the functional performance of these cells.

Heart: factors affecting transplant outcome

O-230 GENDER MATTERS – GENDER-SPECIFIC OUTCOME ANALYSIS OF 67,000 HEARTS FROM THE ISHLT REGISTRY

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Background: Gender differences between donor and recipient might have an impact on outcome after heart transplantation (HTx). The literature review revealed controversial results. We reviewed the data of the ISHLT registry focusing on the influence of gender differences on short- and long-term outcome after HTx.
Methods: We performed a registry data analysis of all adult heart transplants performed between January 1, 1980 and June 30, 2009. In contrast to other studies the data for gender differences (donor and recipient gender) were calculated with respect to survival and conditional survival (conditional to 30-day mortality). Furthermore the patients were divided into 4 subgroups: male recipients/male donors (n=40,497), male recipients/female donors (n=13,480), female recipients/male donors (n=6,436) and female recipients/female donors (n=7,420).
Results: 67,833 hearts were analyzed including 53,977 male recipients (79.6%) and 13,856 female recipients (20.4%) who received hearts from 46,933 male (69.2%) and 20,900 (30.8%) female donors.
One-year survival stratified by recipient gender and donor gender was significantly inferior in male recipients of female donor hearts (m/f: 78.95%) compared to other combinations (m/m: 83.74%; f/m: 82.94%; f/f: 81.92%; log-rank p-value <0.0001). To exclude early mortality we performed a long-term-survival analysis stratified by recipient and donor gender, conditional on survival to 30 days. The analysis revealed superior outcomes for female recipients in general with a 15-year-survival of 40.95% in female recipients of female donor hearts (f/f) and 38.58% for f/m compared to 35.97% m/m and 33.41% m/f (log-rank p-value < 0.0001).
Conclusions: The combination male recipient/female donor carries a higher risk for early mortality whereas other gender constellations yield similar outcomes. In the long-term follow-up female recipients reveal superior results – especially in the combination female recipient/female donor.

O-231 HIGH INCIDENCE OF NON-HLA ANTIBODIES IN HEART TRANSPLANTED RECIPIENTS WITH CARDIAC ALLOGRAFT VASCULOPATHY

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Background: Cardiac allograft vasculopathy (CAV) after heart transplantation (HTx) is a major therapeutic challenge, occuring in more than 50% of HTx recipients in the first years after transplantation. Antibodies against human leukocyte antigens (HLA) or non-HLA antigens like major histocompatibility complex class I-related chain A (MICA), angiotensin type 1 receptor (AT1R) or endothelin receptor A (ETAR) increasingly gain in importance as modulators of allograft function and survival.
Methods: Sera of 114 HTx recipients were screened post-transplantation by Luminex-technology for HLA and MICA antibodies and for antibodies against AT1R and ETAR by ELISA. For statistical analysis the gender, age, status of CAV (IVUS detection) and the number of blood transfusions was documented.
Results: CAV was detected in n=43 recipients. There was no significant difference in gender and number of blood transfusions between recipients with or without antibodies. HTx recipients developed antibodies against HLA class I or class II to a lower extend than against non-HLA antigens (Tab.1), especially against AT1R and ETAR.
CAV appeared in averaged 27.1% of recipients with antibodies against non-HLA antigens, whereas averaged 5.8% of the recipients with HLA antibodies developed CAV. Recipients with non-HLA antibodies developed CAV earlier (69.1mo) than recipients without these antibodies (80.1mo).

Percentage of HTx recipients with positive antibody detection and CAV-positive recipients for HLA and non-HLA antigens

Antibody specifity	HTx recipients with positive antibody status [%] (n=114)	CAV-positive HTx recipients with positive antibody status [%] (n=43)
HLA class I	6.1 (n=7)	2.3 (n=1)
HLA class II	9.7 (n=11)	9.3 (n=4)
MICA	11.4 (n=13)	13.9 (n=6)
AT1R	33.3 (n=38)	30.2 (n=13)
ETAR	45.6 (n=52)	37.2 (n=16)

Conclusions: Non-HLA antibodies are connected to earlier and higher incidence of CAV after HTx. These results point out the necessity for monitoring HLA and non-HLA antibodies after HTx.

O-232 ALLOANTIGEN-PULSED HOST IMMATURE DENDRITIC CELLSINDUCE CD4+CD25+Treg IN RAT CARDIAC ALLOTRANSPLANTATION

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Background: To observe the effective amplification of recipient antigen-specific $CD4^+CD25^+$ regulatory T cells through Preoperative Infused alloantigen-pulsed host immature dendritic cells (imDC) via peripheral intravenous in the established abdominal heterotopic heart transplantation model.
Materials and methods: Established abdominal heterotopic heart transplantation model from SD rat to Wistar rat, Group A (control group): The heart from SD rat was transplanted into the abdomen of Wistar rat Group B (CsA group): The recipient was injected with CsA 10mg/kg/d via intraperitoneal after transplantation. Group C (imDC group): The recipient was injected with alloantigen-pulsed host imDC 2×10^6 via the tail vein before preoperative 7th day. Group D (imDC+CsA group): The recipient was injected with alloantigen-pulsed host imDC 2×10^6 via the tail vein before preoperative 7th day, the else as the Group B. The purity and quantity of $CD4^+CD25^+$ regulatory T cells in peripheral blood were detected by flow cytometry. The mean survival time (MST) of grafted heart, the proliferation of mixed lymphocyte reaction (MLR), the level of the expression of TGF-β1 and IL-10 were observed as well.
Results: The $CD4^+CD25^+$Treg cells of the peripheral blood in Group C and Group D were significantly higher compared with Group A and Group B ($P<0.05$). The MST of Group D: 49.2 ± 8.01d, was significantly prolonged than other groups ($P<0.05$).The expression lever of TGF-β1 and IL-10 of Group C and Group D was significantly higher compared with Group A and Group B ($P<0.05$).
Conclusion: 1.Infusion alloantigen-pulsed host imDC via peripheral intravenous can induce recipient antigen-specific CD4+CD25+Treg. 2. Cytokines were involved in $CD4^+CD25^+$Treg cells inducing immune tolerance process.

O-233 BOTH DOPAMINE AND ITS DERIVATIVE N-OCTANOYL-DOPAMINE PROTECT CARDIOMYOCYTES AGAINST COLD PRESERVATION INJURY

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Background: Treatment of deceased heart-beating donors with low-dose dopamine improves clinical outcome after heart transplantation. The present study was conducted to provide a biological plausibility of dopamine's ability to render cardiomyocytes less susceptible to cold preservation injury.
Methods: We treated neonatal rat cardiomyocytes (NRCM) for 1 hr with dopamine or a non-adrenoceptor activating dopamine derivative N-octanoyl dopamine (NOD). Thereafter, the cells were subjected to 8-12 hrs of hypothermic preservation. Lactate-dehydrogenase (LDH) release, intracellular ATP concentrations, contraction frequencies and β-adrenoceptor-induced cAMP formation were assessed immediately after preservation or 1 hr after rewarming.
Results: In untreated cardiomyocytes, cell damage was reflected by an increase in LDH in the preservation solution, a decrease in intracellular ATP and a loss of isoprenaline-stimulated cAMP-formation. In accordance, spontaneous beating of these cardiomyocytes was significantly decreased. Dopamine as well as NOD pre-treatment inhibited LDH release and preserved the cells from ATP depletion in a concentration dependent manner. The isoprenaline-induced cAMP formation as well as the spontaneous beating in dopamine and NOD treated cells was similar to NRCM not subjected to cold preservation injury. Interestingly, NOD exerted its protective effects at 10-fold lower concentrations than dopamine.
Conclusions: We conclude that NOD or dopamine treatment of cardiomy-

ocytes protects from cold preservation injury, as reflected by improved cell viability and cardiomyocyte function after re-warming. The beneficial effects of dopamine are apparently independent of its positive inotropic action, since NOD was superior in this regard.

O-234 SUCCESFULL TWELVE HOURS OF CARDIAC PRESERVATION WITH Custodiol-N, A NOVEL ORGAN PROTECTION SOLUTION IN AN ORTHOTOPIC HEART TRANSPLANTATION MODEL

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Objective: We currently developed a novel HTK based solution Custodiol-N which includes iron chelators to reduce oxidative injury and amino acids, in particular L-arginine, to improve endothelial function. We investigated the effects of Custodiol-N, on ischemia/reperfusion injury in a canine orthtopic heart transplant model.
Methods: After 4, 8, and 12 hours of ischemic preservation, 36 orthotopic heart transplantations were performed. The hearts were preserved with either HTK (Custudiol, control) or Custodiol-N solution. The slope of the left ventricular pressure-volume relationships (Ees) was calculated before explantation and after 120 minutes of reperfusion. Coronary blood flow (CBF), endothelium-dependent vasodilatation to acetylcholine (ACH) and endothelium-independent vasodilatation to sodium nitroprusside (SNP) were also determined.
Results: Custodiol-N led to significantly better recovery of Ees (91±5 vs. 49±8%, $p<0.05$). CBF was significantly higher in the Custodiol-N group (53±5 vs. 27±4, ml/min, $p<0.05$). While the vasodilatatory response to SNP was similar in both groups, ACH resulted in a significantly higher increase in CBF in the Custodiol-N group (72±8% vs. 29±7%, $p<0.05$). While none of the control animals could wean from cardiopulmonary bypass after 8 and 12 hours of ischemic preservation, all the animals could successfully weaned both after 8 and 12 hours in the Custodiol-N group. The recovery of Ees was 88±6% and 92±8%, respectively which did not differ significantly from baseline values.
Conclusions: The novel organ presrvation solution, Custodiol-N, reduces myocardial and endothelial reperfusion injury after orthotopic heart transplantation and successfully extends the safe hypothermic ischemic cardiac preservation times up to 12 hours.

O-235 DE NOVO EVEROLIMUS- VERSUS MMF-BASED IMMUNOSUPPRESSION IN HEART TRANSPLANT RECIPIENTS: EFFECT ON PROTEINURIA

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Background: High-grade proteinuria has been reported for maintenance heart transplant (HTx) recipients after switch to sirolimus-based immunosuppression. No data on proteinuria has been available to date for the mTOR inhibitor everolimus (EVR) in a de novo HTx setting.
Methods: A2310, a 24-month (M), multi-center, randomized, open-label study compared efficacy and renal function at M12 in de novo HTx recipients receiving EVR dosed at 1.5 or 3mg/day (C0: 3-8 or 6-12ng/mL) with reduced dose cyclosporine (CsA) or MMF 3g/day with standard CsA. All patients received steroids and induction therapy was center-specific (basiliximab/thymoglobulin/no induction).
Results: 721 HTx recipients were randomized (EVR 1.5mg N=282, EVR 3mg N=168, MMF N=271). Enrollment in the EVR 3mg arm was prematurely terminated due to higher mortality; only comparison of EVR 1.5mg vs. MMF is presented. At M12, everolimus was non-inferior to MMF for efficacy but not for renal function. In the subgroup of patients with CsA exposure within target (68.7%), renal function was non-inferior. For both treatment arms, protein:creatinine ratio values decreased from baseline to M12, however, to a significantly lower extent with EVR vs. MMF. Mean protein:creatinine ratio at M12 was significantly higher with EVR 1.5mg vs. MMF. The majority of patients in both groups showed mild proteinuria (30-<500mg/g) with values reaching nephrotic range in only two patients for both arms (Table). Proteinuria was rarely reported as adverse event (AE; EVR: 9 [3.2%] vs. MMF: 5 [1.9%]), with no cases of severe intensity. One serious AE of nephrotic syndrome was reported with MMF and one serious AE of proteinuria with EVR which required treatment discontinuation.

Protein:creatinine ratio (mg/g) at M12/treatment endpoint (TEP), ITT population

	EVR 1.5 mg	MMF	P-value
Month 12/TEP	319.35±618.43 (n=239)	224.63±920.03 (n=243)	0.001
Change from baseline	–55.39±879.59 (n=222)	–233.02±957.7 (n=227)	0.002
Categorical analysis for proteine:creatinine ratio (mg/g), n (%)			
30–<500	208 (87.0)	230 (94.7)	
500–<1000	14 (5.9)	6 (2.5)	
1000–<3000	15 (6.3)	3 (1.2)	
≥3000	2 (0.8)	2 (0.8)	

Conclusion: While everolimus was associated with higher levels of proteinuria compared to MMF, presentation was mostly mild and did not require adjustment of treatment.

O-236 DIFFERENTIAL PATTERNS OF RENAL FUNCTION WITH THE COMBINATION OF EVEROLIMUS PLUS REDUCED DOSE CYCLOSPORINE VERSUS MMF WITH STANDARD DOSE CYCLOSPORINE: INSIGHTS FROM THE A2310 STUDY

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Background: Worsening of renal function is an important long-term complication in heart transplant recipients (HTxR) and has been associated to cyclosporine (CsA) toxicity. However, other factors may contribute to renal function deterioration.
Methods: A2310, a 24-month (M), multi-center, randomized, open-label study compared efficacy and renal function at M12 in de novo HTxR receiving everolimus (EVR) dosed at 1.5 or 3mg/day (C0: 3-8 or 6-12ng/mL) with reduced-dose CsA or MMF 3g/day with standard-dose CsA. All patients received steroids and center-specific induction therapy (no induction/basiliximab/thymoglobulin).
Results: 721 HTxR were randomized (EVR 1.5mg N=282, EVR 3mg N=168, MMF N=271). Enrollment in the EVR 3mg arm was prematurely terminated for increased mortality; only comparison of EVR 1.5mg vs. MMF is presented. At M12, renal function (cGFR, MDRD [mL/min/1.73m^2]) was inferior for EVR 1.5mg vs. MMF (difference in mean: -5.6; 97.5%CI [-10.9,-0.2]; lower margin of 97.5%CI: -10). Patients with CsA exposure within protocol-targets (N=380) showed non-inferior renal function for EVR 1.5mg vs. MMF (difference in mean: -3.2; 97.5%CI: [-8.7, 2.2]). Renal function difference between treatments was established during the first month post-HTx, thereafter, renal function decreased less with EVR 1.5mg (Table 1).

Table 1. Renal function over time (cGFR, MDRD [mL/min/1.73m^2], ITT population – 12 month analysis)

By visit; mean±SD	EVR 1.5 mg	MMF	P-value
Baseline	66.45±36.31	67.10±30.30	0.793
Month 1	67.04±30.88	78.65±31.13	<0.001
Month 3	61.1±29.15	71.23±26.58	<0.001
Month 6	58.23±24.49	65.28±28.66	<0.001
Month 9	58.14±23.68	63.17±29.45	0.030
Month 12	59.21±23.11	64.37±28.37	0.011
Change in renal function; mean±SD			
Baseline to Month 12	–9.20±38.32	–4.11±31.08	0.223
Month 1 to Month 12	–8.83±25.37	–14.94±30.34	0.007

Analysis of covariance identified lower renal function at baseline, acute renal failure within 2 weeks post-HTx, and baseline diabetes as risk factors for worsening of renal function at M12 (Table 2).

Table 2. ANCOVA analysis for renal function at Month 12 (ITT population)

Risk factor	Parameter estimate	Standard error	t-value	P-value
Baseline cGFR	0.365	0.030	12.18	<0.001
Post-operative acute renal failure	–16.919	1.939	–8.72	<0.001
Diabetes at baseline	–4.527	1.716	–2.64	0.009

Conclusion: The renal function disadvantage in the EVR arm compared to MMF was largely due to worse renal function during the first month post-HTx, with less decrease in renal function with EVR past Month 1. Lower baseline cGFR, acute renal failure within 2 weeks post-HTx, and baseline diabetes were predictors of worse renal function at Month 12.

O-237 FACTORS AFFECTING SURVIVAL OF DE NOVO HEART TRANSPLANT RECIPIENTS RECEIVING EVEROLIMUS WITH THYMOGLOBULIN INDUCTION

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Background: Heart transplantation (HTx) is increasingly performed in higher risk patients. Little is known about the impact of different immunosuppressive protocols on more critical patients. Study A2310 enrolled 721 de novo HTx recipients (HTxR) to compare immunosuppression based on everolimus (EVR) and MMF.

Methods: This 24-month, randomized, open-label study, compared efficacy and renal function at Month 12 in HTxR receiving EVR 1.5 or 3mg/day (target C0 3-8 or 6-12ng/mL) and reduced-dose cyclosporine with MMF 3g/day and standard cyclosporine. All patients received steroids and centers could choose one of three induction strategies (basiliximab/thymoglobulin/no induction).

Results: Enrolment in the EVR 3mg arm was prematurely terminated due to increased mortality. Presented analysis focuses on the EVR 1.5mg and MMF groups. Increased mortality was seen in HTxR receiving EVR 1.5mg versus MMF (7.5% [21/279] versus 4.9% [13/268]). The higher mortality for EVR 1.5mg was driven by fatal infections (14/21), mainly during the first 3 months post-HTx, in patients receiving induction therapy, notably with thymoglobulin (Table 1).

Table 1. Incidence of death (n) by induction subgroup, time, and treatment group

	EVR 1.5 mg		MMF	
	by month 3	by month 12	by month 3	by month 12
No induction	N=91		N=88	
Deaths (thereof infections)	3* (2)	4 (3)	2 (0)	6† (2*)
Basiliximab	N=101		N=97	
Deaths (thereof infections)	3 (2*)	5* (2*)	1(0)	1 (0)
Thymoglobulin	N=86		N=83	
Deaths (thereof infections)	11 (8)	12 (9)	2 (0)	6 (2)

*One patient (†two patients) receiving thymoglobulin for acute rejection regardless of induction group.

Multivariate logistic regression analysis for the subgroup receiving thymoglobulin identified left ventricular assist device (LVAD; OR 4.591, 95%CI: [1.11, 18.96], P=0.035) and lower baseline renal function (eGFR$\times$10mL/min/1.73m^2; OR 0.659, 95%CI: [0.639, 0.679], P=0.008) as independent risk factors for mortality. The comparison of treatment effects in patients presenting these risk factors showed that for patients with LVAD prior to HTx, the combination of thymoglobulin with EVR 1.5mg but not with MMF increased the incidence of death. Baseline eGFR $<$40mL/min/1.73m^2 increased the risk of death for both treatment groups compared to eGFR $\geq$40mL/min/1.73m^2, but incidence was higher for EVR/thymoglobulin compared to MMF/thymoglobulin (Table 2).

Table 2. Subgroup analysis of incidence of death in patients receiving thymoglobulin (N=169)

Subgroup	EVR 1.5 mg, n/M (%)	MMF, n/M (%)	p-value
LVAD	5/9 (55.6)	1/14 (7.1)	0.091
no LVAD	7/77 (9.1)	5/69 (7.2)	
baseline GFR$<$40	6/21 (28.6)	2/20 (10.0)	0.041
baseline GFR$\geq$40	6/61 (9.8)	2/59 (3.4)	

n = patients with event in subgroup; M = total number of patients in subgroup.

Conclusion: In patients in a critical stage prior to HTx, the benefits and risks of the combination of thymoglobulin induction with EVR should be carefully evaluated.

O-238 EVEROLIMUS-RELATED INCREASE IN LIPID VALUES DOES NOT AFFECT THE ANTIPROLIFERATIVE EFFECT ON THE ARTERIAL INTIMA IN HEART TRANSPLANT RECIPIENTS

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Purpose: While mTOR inhibitors can reduce the development of cardiac allograft vasculopathy (CAV) after heart transplantation (HTx), they also induce hyperlipidemia. The interference between the two effects is not well characterized.

Methods: The A2310 intravascular ultrasound (IVUS) sub-study assessed change in average maximum intimal thickness (MIT) from baseline to Month (M) 12 in de novo HTx recipients. Lipid profiles (HDL-C, LDL-C, triglycerides [TG], total cholesterol [TC], TC/HDL-C) and their impact on MIT was analyzed at M12 in everolimus (EVR; C0 3-8ng/mL) plus reduced cyclosporine (CsA) group (N=88) vs. mycophenolate mofetil (MMF) plus standard CsA group (N=101). Statin use was protocol-required.

Results: M12 lipid parameters were comparable between the IVUS and the overall 2310 study population with significantly higher values for EVR vs. MMF in both populations (Table 1).

Table 1. Comparison of M12 lipid values between on-treatment groups for the overall study population and IVUS population

Lipid parameter at M12 (mg/dL)	Overall population*		IVUS population*	
Median value (min, max)	EVR 1.5 mg (N=183)	MMF (N=198)	EVR 1.5 mg (N=74)	MMF (N=86)
TC	205 (112, 503)	179 (96, 298)	205 (112, 394)	178 (117, 295)
LDL-C	116 (56, 363)	101 (35, 193)	114 (58, 298)	101 (51, 189)

*All treatment comparisons P$<$0.01.

In the IVUS subpopulation, the EVR 1.5mg group showed significantly higher increase from baseline to M12 for all lipid parameters vs. the MMF group (all P$<$0.05), except for TC/HDL-C ratio (P=0.827). Average daily dose of atorvastatin-equivalent statins was higher in the EVR 1.5mg group (17.2 mg/day) compared to MMF (13.4 mg/day) with 93.2% vs. 88.1% of patients having a statin exposure of $\geq$180 days. Change in average MIT (mm) from baseline to M12 was 0.03$\pm$0.05 with EVR vs. 0.07$\pm$0.11 with MMF (P$<$0.001) in the overall IVUS-group and 0.02$\pm$0.05 vs. 0.08$\pm$0.01 (P=0.002) in the subgroup with statin use $\geq$180 days (n=60 vs. 65). Patients on EVR 1.5mg showed consistently $\sim$50% to 70% less increase in MIT compared to MMF patients, irrespective of normal, high, or very high lipid values (Table 2).

Table 2. Change in average MIT by time-normalized lipid values in AHA categories

Lipid parameter	Category (mg/dL)	EVR 1.5 mg (N=88) mm	MMF (N=101) mm
TC	normal	0.020 (n=42)	0.062 (n=70)
	borderline high	0.025 (n=24)	0.070 (n=24)
	high	0.035 (n=22)	0.149 (n=7)
LDL-C	optimal	0.017 (n=35)	0.039 (n=42)
	near or above optimal	0.019 (n=28)	0.086 (n=47)
	borderline high	0.051 (n=16)	0.100 (n=8)
TG	normal	0.017 (n=22)	0.050 (n=48)
	borderline high	0.028 (n=29)	0.076 (n=23)
	high	0.027 (n=37)	0.098 (n=30)
HDL-C	low	0.016 (n=6)	0.101 (n=16)
	normal	0.035 (n=33)	0.064 (n=64)
	optimal	0.020 (n=49)	0.064 (n=21)

AHA, American Heart Association.

Conclusions: The antiproliferative effect of EVR on the arterial intima was maintained, irrespective of higher lipid values with EVR.

O-239 DE NOVO CALCINEURININHIBITOR-FREE IMMUNOSUPPRESSION WITH SIROLIMUS AND MYCOPHENOLATE MOFETIL AFTER HEART TRANSPLANTATION – 5-YEAR RESULTS

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Background: Despite improvements in immunosuppressive therapy chronic rejection, renal toxicity and malignancy are the major obstacles for long-term success after heart transplantation. Therefore we performed the worldwide first

pilot-trial to evaluate the efficacy and safety of a de novo CNI-free immunosuppressive protocol.
Methods: Between May 2003 and April 2005, 15 de novo cardiac transplant recipients (10 male, 5 female, mean age 55.1 years, diagnosis 8 DCM/7 ICM) were assigned to receive sirolimus, MMF and steroids. Antilymphocyte induction was given for 5 days; steroids were withdrawn after 6 months. 6/15 patients received CMV-prophylaxis for high risk CMV-constellation (R–/D+).
Results: Survival at 1 and 5 years was 87.5% (one death caused by pulmonary adenocarcinoma). Freedom from biopsy-proven rejection was 71.3% at 1 year; 59.4% at 5 years. Freedom from angiographically detectable vasculopathy was 100% after 5 years and only one CMV-infection occurred.
Mean serum-creatinine was 1.43±0.31 mg/dl prior to HTx, 1.29±0.56 mg/dl at 1 year and 1.23±0.53 mg/dl at 5 years. Cholesterol was 203±32 at 1 year and 199±40 at 5 years despite statins and hypertriglyceridemia (223±97 mg/dl) persisted after 5 years. No new onset diabetes occurred. Surgical interventions for pericardial effusions were necessary in 5 patients.
9 patients intermittently discontinued sirolimus treatment due to side effects or adverse events (4 acute rejections, 3 delayed wound healing, 2 GI-toxicity).
Conclusions: De novo CNI-free immunosuppression after heart transplantation is less efficacious in prevention of acute rejection and is associated with a variety of side effects. On the other hand de novo CNI-free immunosuppression is possible and long-term results are favourable for survival, malignancy, renal function, CMV-infections and vasculopathy.

Emerging immunosuppression

O-240 THREE-YEAR OUTCOMES FROM BENEFIT: A PHASE III STUDY OF BELATACEPT VS CYCLOSPORINE IN KIDNEY TRANSPLANT RECIPIENTS

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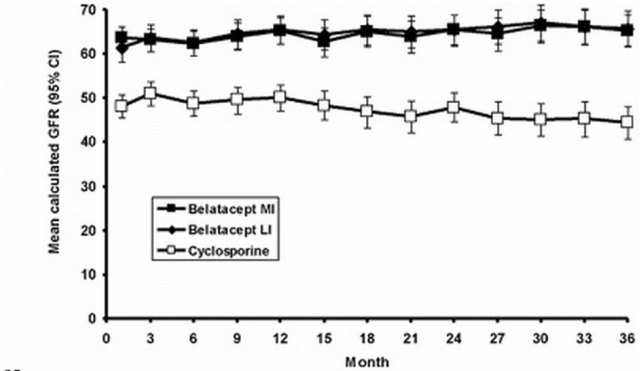
Background: Allograft function is associated with patient/graft survival in kidney transplant recipients. The efficacy and safety of belatacept was evaluated to determine if the results at 2 years in the BENEFIT study were sustained at 3 years.
Methods: BENEFIT is a randomized, phase III study in adults receiving a kidney transplant from a living or standard criteria deceased donor. Patients were randomized to a more (MI) or less intensive (LI) regimen of belatacept, or CsA.
Results: 471/666 intent-to-treat patients (n=158/219 MI; n=170/226 LI; n=143/221 CsA) completed at least 3 years of therapy. Patient/graft survival was 92% (MI), 92% (LI), and 89% (CsA) by Year 3. The mean calculated GFR (cGFR) was approximately 21 mL/min higher in the belatacept groups vs CsA by Year 3. The mean cGFR exhibited a positive slope over time in the belatacept groups (figure).



Despite an early increase in the rate/grade and impact of acute rejection (AR) in the belatacept groups, no new AR cases occurred in the belatacept groups from Years 2–3, with 1 AR case in the CsA group. There were no new safety signals and no new cases of PTLD after Month 18. A risk prediction model suggested that belatacept would extend the projected graft half-life by 23 months, from 11 to 13 yrs.
Conclusions: Belatacept-treated patients maintained a high rate of patient and graft survival, despite an early increased risk for acute rejection and PTLD. There were no new safety signals. Belatacept was associated with sustained improvements in renal function.

O-241 BELATACEPT COMPARED WITH CYCLOSPORINE IN RENAL ALLOGRAFT RECIPIENTS OF EXTENDED CRITERIA DONOR KIDNEYS: 3-YEAR OUTCOMES FROM THE PHASE III BENEFIT-EXT TRIAL

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Background: Recipients of extended criteria donor (ECD) kidneys have poor long-term outcomes compared to recipients of standard criteria donor kidneys. The efficacy and safety of belatacept in recipients of ECD kidneys were evaluated at 3 years to characterize longer-term outcomes and durability of treatment effect.
Methods: BENEFIT-EXT was a 3-year, phase III study in recipients of de novo ECD kidneys (n=543) who were randomized to a more intensive (MI) or less intensive (LI) belatacept regimen or cyclosporine (CsA).
Results: At 3 years, 323 patients remained on therapy (n=109 MI, n=114 LI, n=100 CsA). The proportion of patients surviving with a functioning graft was comparable between groups (80%-MI, 82%-LI, 80%-CsA). Mean calculated GFR at 3 years was 11 mL/min higher among belatacept-treated patients compared to CsA-treated patients (42.7-MI, 42.2-LI, vs. 31.5mL/min-CsA). Belatacept-treated patients showed less decline of renal function over time (mL/min/yr), with slopes of -0.9 (MI), -0.6 (LI), and -1.9 (CsA). More CsA-treated patients (44%) progressed to GFR<30mL/min (CKD stage 4/5) versus those receiving belatacept (27%-30%). Acute rejection (AR) occurred in 1 additional patient in each group after year 2; most AR occurred by month 6. PTLD risk was highest in the first 18 months (2-MI, 3-LI), with 2 additional cases (1-LI, 1-CsA) occurring after month 36. Tuberculosis was reported in 2 (MI), 4 (LI), and 0 (CsA) patients. A risk-prediction model suggested treatment with belatacept would extend graft half-life by 22 months, from 8 years to 10 years.
Conclusions: Among recipients of ECD kidneys, treatment with belatacept resulted in comparable patient and graft survival, similar rates of AR, with better renal function compared with CsA at 3 years after transplantation. No new safety issues were observed at 3 years.

O-242 3-YEAR SAFETY PROFILE OF BELATACEPT IN KIDNEY TRANSPLANT RECIPIENTS FROM THE BENEFIT AND BENEFIT-EXT STUDIES

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Background: Belatacept, a selective co-stimulation blocker, is associated with better renal function and similar patient/graft survival vs cyclosporine (CsA) in kidney transplant recipients in the BENEFIT and BENEFIT-EXT studies at 3 yrs. The current analysis focuses on pooled safety data for belatacept vs CsA through Year 3 of BENEFIT and BENEFIT-EXT.

Methods: Patients were randomized to a more intensive (MI) or less intensive (LI) regimen of belatacept, or CsA.
Results: The pooled analysis included 1209 intent-to-treat patients (MI = 403; LI = 401; CsA = 405). The incidence of deaths, serious adverse events, malignancies, and selected infections at Year 3 are listed in the table.

Table 1. Incidence of deaths, serious adverse events, malignancies, and selected infections at year 3

n (%)	Belatacept MI (n=403)	Belatacept LI (n=401)	CsA (n=405)
Deaths	31 (8)	25 (6)	32 (8)
Serious adverse events	282 (70)	270 (67)	296 (73)
Serious infections	151 (38)	144 (36)	157 (39)
All malignancies*	16 (4)	19 (5)	16 (4)
PTLD	5 (1)	6 (1)	2 (<1)
Infections			
CMV	54 (13)	53 (13)	56 (14)
BK polyoma virus	30 (7)†	17 (4)	27 (7)
Fungal	95 (24)	70 (18)	88 (22)
Herpes	61 (15)	55 (14)	46 (11)
Tuberculosis	6 (1)	6 (1)	1 (<1)

*Excluding non-melanoma skin cancer; post-transplant lymphoproliferative disorders (PTLD) includes all cases through Sept 2010 †Includes 1 case of progressive multifocal leukoencephalopathy (PML).

Infection was the most common cause of death in each study. More TB cases were observed in belatacept patients; most cases occurred in countries where TB is endemic. Although the frequency of PTLD, particularly CNS PTLD, was increased for belatacept patients regardless of EBV status at baseline, the highest risk remained in patients who were EBV(–). All but 1 case of PTLD in the belatacept patients occurred within the first 18 months post-transplant; 2 cases occurred after month 18 (n=1 LI; n=1 CsA). The incidence rate of all malignancies remained stable over time in each study, while the incidence rate of PTLD and most infections appeared to decrease over time.
Conclusions: Belatacept LI was associated with fewer deaths and serious infections vs MI or CsA. The primary risks with belatacept are PTLD, particularly CNS PTLD, and PML. The risk of PTLD is highest in EBV(–) patients, and appears to decrease after 18 months. There were no new safety signals through Year 3.

O-243 OUTCOMES AT 3 YEARS IN KIDNEY TRANSPLANT RECIPIENTS WITH PRE-TRANSPLANT DIABETES FROM TWO PHASE 3 BELATACEPT STUDIES

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Background: Pre-transplant diabetes mellitus is associated with poorer outcomes after kidney transplantation. We report outcomes at 3 years post-transplant in diabetic kidney transplant patients from 2 phase III studies (BENEFIT and BENEFIT-EXT) which assessed belatacept-based immunosuppressive regimens vs a cyclosporine-based (CsA) regimen.
Methods: Patients in each study were randomized to receive a more intensive (MI) or less intensive (LI) regimen of belatacept, or cyclosporine. Patients with a pre-transplant history of diabetes or who were taking anti-diabetic medication at the time of transplantation were included in this analysis.
Results: 180 patients in BENEFIT (n = 63 MI; n = 58 LI; n= 59 CsA) and 157 in BENEFIT-EXT (n = 52 MI; n = 39 LI; n= 66 CsA) were classified as having pre-transplant diabetes. Outcomes by year 3 are listed in the table.

Outcomes by year 3 in patients with pre-transplant diabetes

	BENEFIT			BENEFIT-EXT		
	Belatacept MI (n = 63)	Belatacept LI (n = 58)	CsA (n= 59)	Belatacept MI (n = 52)	Belatacept LI (n = 39)	CsA (n = 66)
Survived with functioning graft, %	94	90	86	75	77	67
cGFR, Ml/min/1.73 m2	61	65	42	41	39	23
Acute rejection, %	27	21	9	25	21	21
Serious adverse events, %	67	64	63	81	77	88
Serious infections, %	41	33	32	46	41	47
Serious neoplasms, %	8	7	5	12	3	8

The type and frequency of serious adverse events was generally reflective of the overall study populations.

Conclusions: In kidney transplant recipients who had diabetes mellitus at the time of transplantation, belatacept-based immunosuppression was associated with a numerically higher proportion of patients surviving with a functioning graft vs CsA and better renal function despite higher acute rejection rates in the BENEFIT trial. Acute rejection frequency and overall safety were comparable to the overall intent-to-treat population.

O-244 EVALUATION OF DONOR-SPECIFIC ANTIBODIES IN KIDNEY TRANSPLANT PATIENTS TREATED WITH BELATACEPT- OR CYCLOSPORINE-BASED IMMUNOSUPPRESSION IN BENEFIT AND BENEFIT-EXT

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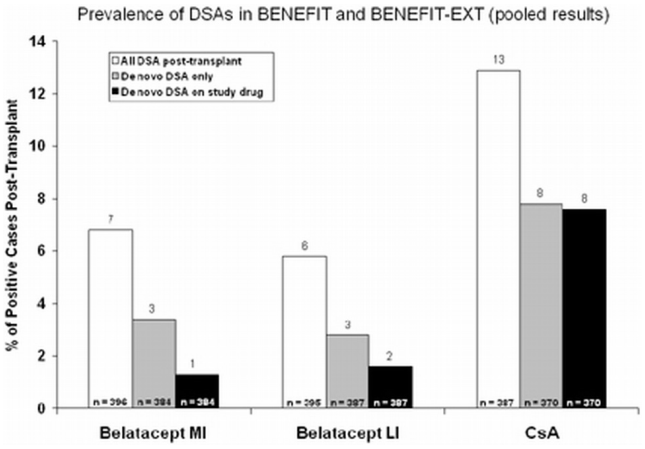
Background: In preclinical studies belatacept has been associated with suppression of donor-specific antibody (DSA) formation. This analysis was carried out to assess whether a similar trend was seen in Phase III studies in kidney transplant recipients comparing belatacept- and cyclosporine (CsA)-based immunosuppression.
Methods: Patients in 2 Phase III studies (BENEFIT and BENEFIT-EXT) were randomized to a more intensive (MI) or less intensive (LI) regimen of belatacept, or CsA. The presence of DSAs was centrally assessed (flow cytometry/Luminex) at baseline, at several points post-transplant over 3 years, and at the time of suspected rejection episodes. Gene profiling was conducted in a subset of patients using an Affymetrix whole genome array.
Results: By year 3, d*e novo* DSAs (DSAs in patients positive for a new specificity post-baseline) were detected in 3% (MI), 2% (LI), and 7% (CsA) of patients in BENEFIT, and in 4% (MI), 4% (LI), and 9% (CsA) of patients in BENEFIT-EXT. Interestingly, <1% (MI), 2% (LI) and 6% (CsA) of patients in BENEFIT had detectable *de novo* DSAs while on study therapy, as did 2% (MI), 1% (LI), and 9% (CsA) of patients in BENEFIT-EXT. In BENEFIT, 7% (MI), 5% (LI), and 11% (CsA) of patients had detectable DSAs at any point post-transplant, as did 7% (MI), 6% (LI), and 15% (CsA) of patients in BENEFIT-EXT. Several genes relating to immunoglobulin production (eg, IgG1, IgA1) had relatively lower expression in belatacept- compared to CsA-treated patients.



Conclusions: DSAs and *de novo* DSAs occurred less frequently among belatacept-treated patients. The results observed in these clinical studies were consistent with those previously reported in preclinical studies.

O-245 RENAL FUNCTION AT 2 YEARS IN KIDNEY TRANSPLANT RECIPIENTS SWITCHED FROM CYCLCOSPORINE OR TACROLIMUS TO BELATACEPT: RESULTS FROM THE LONG-TERM EXTENSION OF A PHASE II STUDY

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Background: At 1yr, patients who switched from a calcineurin inhibitor (CNI) to belatacept had significant improvements in renal function in a Phase II study. The current abstract addresses whether this clinical profile was sustained in the long-term extension (LTE) of this study.
Methods: This is a randomized, open-label, Phase II trial in renal transplant patients with stable graft function receiving either a cyclosporine (CsA)- or tacrolimus (TAC)-based regimen. Patients were randomized to either switch to belatacept or continue CNI treatment. After the first year, patients were eligible to enter the LTE.
Results: Of 173 randomized patients, 162 (n=81 belatacept; n=81 CNI) entered the LTE. 98% of patients in each group survived with a functioning graft. 2 patients (n=1 each group) had graft loss between Years 1 and 2. At Year 2, mean calculated GFR (cGFR) was 62.0 mL/min (belatacept) vs 55.4 mL/min (CNI). The mean change in cGFR from baseline was +8.8 mL/min (belatacept) and +0.3 mL/min (CNI). The relative renal benefit of belatacept was observed in patients switched from either CsA (+7.8 mL/min) or TAC (+8.9 mL/min). The frequency of AR was 4.9% (belatacept) and 3.7% (CNI) by Year 2. All AR occurred during the first year in the belatacept patients; all AR occurred in the CNI group in the second year. The overall safety profile remained similar between groups, except for more non-serious fungal infections (mostly skin) w/belatacept. No PTLD was reported.
Conclusions: Switching to a belatacept-based regimen from a CNI-based regimen resulted in further improvement in renal function over time, with no new cases of AR. Switching from a CNI to belatacept may be a viable approach, but merits confirmation in a larger controlled trial.

O-246 EFFICACY AND SAFETY OF ALEFACEPT IN COMBINATION WITH TACROLIMUS, MYCOPHENOLATE MOFETIL AND STEROIDS IN DE NOVO KIDNEY TRANSPLANTATION

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Primary objective of study was to evaluate efficacy/safety of alefacept (alef) (a CD2-LFA3 co-stimulation inhibitor) in conjunction with tacrolimus (Tac), mycophenolate mofetil (MMF) and steroids.
Method: Phase 2, multicenter, randomized (1:1), double-blind, placebo controlled, parallel arm study comparing investigational arm (alef) to control arm (placebo) in kidney transplant patients of 18 to 65 years of age. Exclusion criteria were HLA identical/UNOS ECD/DCD donors, donor organ cold ischemia time $\geq$ 30 hours, and recipients with PRA $>$ 20%. Experimental arm received 7.5 mg alef IV on Day 0 and 3, 15 mg SQ on Day 7 and then weekly for total of 12 weeks. All patients received maintenance therapy with Tac, MMF and steroids. Primary endpoint was incidence of biopsy proven acute rejection (BPAR) through 6 months.
Results: 212 adult patients received study drug. No statistical differences between treatment arms were observed for any baseline disease characteristic (age, gender, race). Mean 6 month results are shown below except T memory cell subsets (12 weeks). Adverse events are shown as% of patients with $\geq$ 1 event through 6 months. Four deaths occurred during study: 3 placebo (2 septic shock, 1 acute pulmonary edema) and 1 alefacept (pulmonary carcinoma). Two grafts were lost during study from acute rejection.
Conclusions: Alefacept was not statistically superior to placebo for the primary endpoint. Patient and graft survival as well as renal function was not statistically different between treatment arms. Alefacept was associated with statistically significant reduction in T memory lymphocyte subsets.

Table 1

Parameter	Alefacept (N=105)	Control (N=107)	P value
Completed 3 months on Assigned Drug (%)	79.0	83.2	
Delayed Graft Function (%)	7.6	12.1	0.270
BPAR cellular[1] (primary endpoint; %)	11.0	7.0	0.309
BPAR cellular or antibody[1](%)	12.0	10.0	0.485
BPAR $\geq$ Banff Grade 2a (%)	7.7	1.9	0.627
Clinically Treated Rejection* (%)	15.0	25.0	0.104
Tacrolimus level (ng/ml)	9.1	8.0	
MMF Daily Dose (mg/d)	1192.2	1179.1	
Pt. Survival[1,2] (%)	99.0	97.1	0.350
Graft Survival[1,2] (%)	95.2	90.6	0.233
All Infection (%)	61.0	57.9	
SAE (any; %)	53.3	57.9	
CMV (infection/viremia;%)	14.3	7.5	
BKV Infection (%)	2.9	9.4	
Malignancy (%)	6.7	0.9	
MDRD Creatinine clearance (ml/min/1.73 m2)	59.4	58.8	0.850
CD4+ T memory (cells/uL)	251.0	414.2	$<$0.001
CD8+ T memory (cells/uL)	94.9	152.5	0.004

[1]Kaplan Meier estimates, [2]Lost to follow-up is censored.

O-247 A PHASE 2 STUDY TO ASSESS THE SAFETY AND EFFICACY OF ALEFACEPT (ALEF) IN DE NOVO KIDNEY TRANSPALNT RECIPIENTS

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Primary objective of study was to evaluate efficacy/safety of alef in kidney transplant recipients in conjunction with tacrolimus (Tac) minimization or mycophenolate mofetil (MMF) replacement.
Methods: This was a randomized, open label, parallel group, multi-center study in adult recipients. *Control:* basiliximab, full Tac (levels 10-20 mg/mL Days 0-28), MMF and steroids. Experimental arms received 7.5 mg alef IV on Day 0 and 3. *Alef/Low Tac:* alef 15 mg SC Day 7 then weekly x 12 weeks, low dose Tac (levels 3-7 ng/mL Days 0-28), MMF and steroids; *Alef/Tac:* alef 15 mg SC Day 7 then weekly x 12 weeks, full dose Tac and steroids (MMF replacement). *QOW alef:* alef 30 mg Day 7 then 30 mg every other week x 12 weeks, low dose Tac, MMF and steroids. Alef groups received alef 15 mg SC at Months 4, 5 and 6. Primary endpoint was incidence of biopsy proven acute rejection (BPAR) at 6 months. Non-inferiority margin was 10%.
Results: 309 adult patients were randomized and received study drug. Mean 6 month results are shown except T cell subsets (12 weeks). Adverse events (AE) are% of patients with $\geq$ 1 event through 6 mos. No statistical differences between treatment arms were observed for any baseline disease characteristics. No differences were seen for patient/graft survival or renal function between study arms.

Parameter	Control (N=79)	Alef/Low Tac (N=77)	Alef/Tac (N=75)	QOW Alef (N=78)
BPAR (%)	12.7	26.3*	18.8	16.7
Difference in BPAR (%, 90%CI)		13.6 (3.2, 23.9)	6.1 (–3.6, 15.8)	4.0 (–5.3, 13.3)
Tac levels (ng/ml)	7.9	6.2	8.3	5.8
CMV Iinfection/viremia,%)	3.8	7.8	0.0	5.1
Malignancy (%)	2.5	2.6	1.3	1.3
BKV (infection/nephropathy,%)	17.7	15.6	16.0	21.8
MMF GI Specific AE (%)	93.7	79.2*	81.3*	87.2
Polys/neutrophil$<$1000 cell/mm3(%)	12.7	18.2	2.7	19.2
CD4+ T memory (cells/mm3)	538.6	335.2*	330.9*	268.8*
CD8+ T memory (cells/mm3)	146.3	84.8*	92.0*	56.2*

*P$<$0.05 compared to Control arm.

Conclusions: None of alef arms met non-inferiority criteria for primary endpoint. Patient/graft survival and renal function at 6 months were similar in all arms. MMF GI specific AEs were statistically lower in MMF replacement arm vs Control arm. T lymphocyte memory subsets were statistically reduced in alef treatment arms. There were fewer subjects with severe neutropenia in MMF replacement vs Control.

O-248 A PHASE 1 SINGLE ASCENDING DOSE STUDY OF ASKP1240 (ANTI-CD40 MAB) IN HEALTHY SUBJECTS

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Primary objective was to evaluate safety, tolerability, and pharmacokinetics (PK) of ASKP1240 after single intravenous (IV) dose in healthy subjects. Secondary objectives were evaluation of pharmacodynamics (PD) of ASKP1240 and determination of incidence of anti-ASKP1240 antibody formation.

Study Design: Study was double-blind, single dose, dose escalation. Doses were 0.00003, 0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 mg/kg. Follow-up was 60 days in all dose groups except 10 mg/kg group which was followed 90 days.
Results: 104 of 109 randomized subjects completed study. One subject was randomized but not treated, 3 subjects were lost to follow up between days 8 and 15, and 1 subject withdrew consent on day 8. Doses of 1, 3 and 10 mg/kg resulted in quantifiable serum ASKP1240 in all subjects through days 8, 29 and 43, respectively, with 5 of 6 subjects in 10mg/kg treatment group sustaining PK levels through day 60. In the 3 and 10 mg/kg ASKP1240 groups, maximal CD40 receptor occupancy was sustained through days 29 and 60, respectively. Dose proportionality was not met for either Cmax or AUC. No subjects experienced cytokine release syndrome (CRS) or thromboembolic event (TE). No subjects discontinued due to an adverse event (AE). AEs reported in the combined ASKP1240 treatment groups vs placebo included headache in 9.7% vs 11.1%, upper respiratory tract infection in 8.3%vs 2.8% and cough in 6.9% vs 2.8%. One mild rash occurred in subjects who received ASKP1240 10 mg/kg. Incidence of treatment emergent anti-ASKP1240 antibodies was 6.9%. Laboratory evaluations did not indicate platelet or endothelial cell activation nor activation of coagulation or fibrinolytic cascades.
Summary and Conclusions: ASKP1240 was well tolerated at all doses with no evidence of CRS or TE. ASKP1240 showed dose-dependent CD40 receptor occupancy.

Immune regulation and innate immunity

O-249 MOUSE MACROPHAGES DRIVEN TO A NOVEL STATE OF ACTIVATION PROLONG ALLOGRAFT SURVIVAL IN NON-IMMUNOSUPPRESSED RECIPIENTS

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Human regulatory macrophages (M reg) have been extensively investigated as a potential cell-based immunosuppressive treatment. Using culture conditions analogous to those under which human M regs arise, a mouse counterpart of the human cell has been generated. Mouse monocytes are driven to the M reg phenotype over a seven day culture period during which the cells are exposed to M-CSF and IFN-γ. The resulting M regs adopt a characteristic morphology and express markers distinguishing them from monocytes, monocyte-derived DCs and M0-, M2a-, and M2c-polarised macrophages. To assess the interrelation of M regs and these other cell types, whole genome expression profiling studies were undertaken: in clustering analyses, M regs were found to constitute a distinct subgroup of macrophages. At a 1:1 ratio, M regs completely suppressed polyclonally-stimulated T cell proliferation through a nitric oxide synthase-dependent mechanism which was blocked by the inhibitor L-NMMA. In a heterotopic heart transplant model, a single intravenous administration of 5×10^6 donor-strain M regs prior to transplantation significantly prolonged allograft survival in unconditioned, non-immunosuppressed recipients using both the stringent C3H-to-BALB/c (32.6$\pm$4.5 vs. 8.7$\pm$0.2 days; $p<0.001$) and B6-to-BALB/c (31.1$\pm$12 vs 9.7$\pm$0.4 days; p=0.002) strain combinations. The graft-protective effects of M regs were specific to donor cells as recipient (9.6$\pm$0.4 days; $p>001$) and third party-derived M regs (11.0$\pm$0.6 days; $p<0.001$) were not effective. Co-treatment with M regs and 1 mg/kg/day rapamycin for 10 days posttransplant further enhanced the effect of M regs (64.1$\pm$8.6 days) compared to M reg treatment alone (p=0.006) or rapamycin alone (p=0.022), and some recipients accepted grafts indefinitely. It is concluded that mouse M regs represent a novel, phenotypically distinct subset of macrophages which bear a resemblence to human M regs in their derivation, marker phenotype and *in vitro* functions.

O-250 THE KILLER-CELL IMMUNOGLOBULIN-LIKE RECEPTOR (KIR) GENOTYPE CORRELATES WITH ACUTE KIDNEY FAILURE IN THE EARLY POST- LIVER TRANSPLANTATION PERIOD

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Acute kidney injury (ARI) and acute renal failure (ARF) are major complications following liver transplantation (LT) leading up to chronic end-stage renal disease. We aimed to elucidate the role of NK cells and their receptors in the context of early post-liver transplant ARI and ARF more precisely. For instance, patients with impaired kidney function (serum creatinine levels $>$1.2 mg/dl, n=13) illustrated heightened peripheral NK cell frequencies prior LT compared with patients showing stable renal function (n=9) (17.22% $\pm$10.56 versus 12.98% $\pm$9.09). We further tested retrospectively 89 liver transplant recipients for their killer-cell immunoglobulin-like receptor (KIR) genotype and the risk of ARI and ARF. During the first week post-LT. ARI occurred in 12% and ARF in 22% of the patients, respectively. ARI was a significant risk factor for acute rejection (p=0.0009) and ARF led to elevated serum creatinine levels ($>$1.2 mg/dl) at the time of hospital discharge (p=0.008). Significantly less patients having a homozygous KIR haplotype A/A (characterized by the presence of only one activating KIR gene) displayed a stable early postoperative kidney function, compared to patients with a KIR haplotype B/x (more than one activating receptor) (p=0.025, odds ratio 2.3, CI=1.3-3.9). The absence of KIR2DL2/DS2 genes significantly influenced the risk of ARF (p=0.05). Multivariate regression of both clinical and genomic risk factors for acute kidney injury/failure confirmed a link between KIR haplotype A/A and post-LT acute renal failure (p=0.04). We observed a higher percentage of NK cells prior to LT in patients with impaired renal function and identified the KIR haplotype A/A as an independent genetic risk factor for ARF. Our data provide new aspects of an innate immune response within the setting of post-LT kidney injury and failure.

O-251 QUANTIFICATION OF DEMETHYLATED *FOXP3* DNA DEMONSTRATES A LOWER PROPORTION OF NATURAL REGULATORY T CELLS 1 YEAR AFTER KIDNEY TRANSPLANTATION

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Background: Circulating FOXP3+ regulatory T cells (Treg) can be subdivided into those developed in the thymus (natural (n)Treg) and those induced in the periphery (iTreg). Since specific markers for either Treg population were lacking, their relative importance in alloreactivity remains unknown. Based on the recent identification of nTreg-specific DNA demethylation in the *FOXP3* gene, we established a quantitative PCR assay to identify the DNA methylation status of *FOXP3*. Using demethylated *FOXP3* DNA as a marker for nTreg, the proportion of nTreg of total PBMC was studied in 15 patients before and 1 year after kidney transplantation.
Methods: Isolated DNA was treated with bisulfite to introduce methylation dependent changes in the DNA sequence. These changes were quantified with methylation- and demethylation-specific primers and probes in real-time PCR.
Results: FACS-sorted $CD4^+CD25^{bright}CD127^{low}$ cells (nTreg pool; demethylated at *FOXP3*) and $CD14^+$ cells (methylated at *FOXP3*) from a male donor demonstrated the expected discrimination between methylated and demethylated DNA with a proportion of demethylated *FOXP3* DNA of 96% and 0.03% respectively. In kidney transplantation patients, the proportion of nTreg of total PBMC before transplantation was similar to the proportion of nTreg in age and gender matched donors. One year after kidney transplantation, the proportion of nTreg (4.2%) significantly decreased (p=0.002) compared to the proportion of nTreg before transplantation (2.8%). This decrease was more prominent in tacrolimus/mycophenolate mofetil treated patients than tacrolimus/rapamycin treated patients (p=0.007 versus p=0.13).
Conclusion: Using *FOXP3* DNA demethylation as a marker of nTreg, a lower proportion of nTreg is present 1 year after kidney transplantation depending on their immunosuppressive regimen. Quantification of demethylated *FOXP3* DNA is the first specific marker for nTreg and will be used to study their relative contribution in alloreactivity.

O-252 IMMUNOSUPPRESSIVE ROLE OF FIBRINOGEN-LIKE PROTEIN 2 (FGL2) IN $CD8^+$ REGULATORY T CELLS-MEDIATED LONG-TERM GRAFT SURVIVAL

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Background: Despite of the importance of $CD8^+CD45RC^{low}$ regulatory T lymphocytes (Tregs) in allograft tolerance, mechanisms of tolerance-induction by these cells remain unclear (Guillonneau, *Transplantation*, 2005).
Materials/methods: A Lewis 1W rat heart, grafted in a heterotopic intra-abdominal and transversal position in a MHC non-compatible Lewis 1A rat, is infected with 2.10^{10} pi of adenovirus recombinant for CD40 molecule fused to the Fc part of an immunoglobulin (AdCD40Ig) the day of the graft. Spleen and graft are analyzed by quantitative PCR and immunohistology, and serum by Western Blot. Tregs, $CD4^+CD25^-$T lymphocytes (TL) and plasmacytoïde dendritic cells (pDCs) are purified from spleen by FACS Aria for *in vitro* cocultures. For *in vivo* FGL2 study, $4,5.10^{11}$ vg of FGL2-recombinant Adenovirus Associated Virus (FGL2-AAV) are injected in *i.m* in receivers one month before the graft.

Results: We have shown by micro-array the overexpression of FGL2 in splenic Tregs of AdCD40Ig-treated versus naïve rats. By quantitative RT-PCR and immunohistology, we have confirmed FGL2 overexpression in splenic Tregs and also in the graft of AdCD40Ig-treated versus non-treated and naïve rats. FGL2 involvement in Tregs immunosuppressive function has been shown by *in vitro* and *in vivo* experiments. Indeed, proliferation of TL cocultured with allogeneic pDCs is inhibited by adding of FGL2 protein. Moreover, Tregs from AdCD40Ig-treated rats inhibit TL proliferation in response to allogeneic pDCs, and this inhibition is annihilated by FGL2-blocking antibodies. Finally, we have shown that FGL2 overexpression prolongs graft survival. Moreover, the adoptive transfer of splenocytes from a FGL2-AAV-treated rat, whose graft is not rejected after 120 days, to two naïve rats, induces a prolonged graft survival for more than 80 days.
Conclusion: FGL2-treatment of transplanted patients could induce a long-term graft tolerance, reducing or dispensing of immunosupressors continuous taking.

O-253 ABROGATION OF GALECTIN-3 PROTECTS AGAINST CHRONIC RENAL ALLOGRAFT INJURY

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Background: Chronic allograft injury, characterised by interstitial fibrosis and tubular atrophy (IFTA), is a significant challenge in renal transplantation, leading to a progressive decline in function. It results in loss of 5% of renal transplants per annum and currently eludes specific therapies. Galectin-3 is a β-galactoside-binding lectin that is highly expressed in fibrotic tissue of diverse aetiologies, and mice deficient in galectin-3 have reduced fibrosis in kidney, liver and lung. The role of galectin-3 in chronic allograft injury is examined in this study.
Methods: We adopted a murine model of chronic allograft injury, characterised by a single class II mismatch between BM12 donor and C57B6 recipient strains. Syngeneic transplants served as controls (C57B6). Outcome measures included tubular count, fibrosis assessment following staining with picrosirius red (pan collagen), myofibroblast activation (α-smooth muscle actin) and collagen I. Leucocyte infiltration was studied: macrophages (F4/80), T cells (CD3) and B cells (B220). Galectin-3 (Mac2) and the marker of alternative macrophage activation (ym1) were assessed.
Results: Transplantation of BM12 kidneys into C57B6 mice was associated with the development of interstitial fibrosis ($p<0.0001$) and tubular atrophy ($p<0.0001$) at 4 weeks and marked upregulation in galectin-3 expression ($p=0.002$), compared with syngeneic (C57B6) controls (n=8). Transplantation of BM12 kidneys into galectin-3 null mice (C57B6) resulted in significant preservation of tubules ($p=0.008$) and reduced interstitial fibrosis ($p=0.01$), with decreased myofibroblast activation ($p=0.01$) and collagen I expression ($p=0.04$), compared with wild type controls. The number of infiltrating leucocytes was unaltered by abrogation of galectin-3, but reduced expression of ym1 ($p=0.0001$), a marker of alternative macrophage activation, in galectin-3 null mice suggests a possible mechanism by which galectin-3 promotes renal transplant fibrosis.
Conclusion: Our results suggest that galectin-3 plays a central role in IFTA in chronic allograft injury and provides a potentially exciting therapeutic target.

O-254 OVER-EXPRESSION OF HSP-27 IN DONOR HEARTS ALLOWS ENHANCED CARDIAC ALLOGRAFT SURVIVAL

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Hsp-27 is a constitutively expressed heat shock protein and its expression increases during stress. Previous studies have suggested that over-expression of hsp-27 protects against atherosclerosis in non-transplant patients and cardiac allograft vasculopathy in cardiac transplant patients. Murine hearts over-expressing human hsp-27 have been shown to be resistant to ex-vivo induced ischemic damage. The purpose of this study was to determine whether over expression of hsp-27 protects the heart from acute rejection and if so, to define the mechanisms of its protective effect. B10 A mice, over-expressing Ha-tagged human hsp-27 were used as donors (hsp-27 tg). Western blotting and immunocytochemistry revealed over-expression of hsp-27 in lung, liver and heart compared to wild-type litter mate controls. Immunocytochemistry demonstrated increased expression of hsp-27 in cardiomyocytes of transgenic mice. Hsp-27 tg hearts and wild-type hearts were exposed to 10 minutes of cold and 40 minutes of warm ischemia ex-vivo and the extent of apoptosis was determined using TUNEL assay and caspase-3 activity. Ischemia induced an increase in numbers of apoptotic cells in wild-type mice (250%increase) but hsp-27 tg hearts showed less apoptotic cells in response to ischemia (140%increase, $p<0.0001$). Similarly, the increase in caspase 3 activity was significantly reduced in transgenic hearts (150% increase) compared to wild-type (240% increase, $p<0.0001$) following ischemia. B10.A hearts from hsp-27 tg or wild-type litter mate controls were transplanted into the abdomen of C57BL/6 wild-type recipients, representing complete MHC mismatch. Daily palpation of the transplanted hearts revealed significantly prolonged cardiac allograft survival of hsp-27 tg hearts (time to heart beat cessation, 35 ± 10.37 days, n=10) compared to survival of hearts from litter mate controls (13.6 ± 3.06 days, n=10, $p=0.0004$). The data so far suggest that hsp-27 may delay acute allograft rejection by limiting ischemia-induced apoptosis.

O-255 EX-VIVO LUNG PERFUSION REMOVES DONOR DERIVED NON-CLASSICAL MONOCYTES THAT RAPIDLY DIFFERENTIATE INTO INFLAMMATORY DENDRITIC CELLS?

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Background: Ex-vivo lung perfusion (EVLP) is a novel procedure designed to rapidly assess and recondition unusable donor lungs for use in transplantation (LTx). EVLP may reduce graft immunogenicity and allorecognition via removal of passenger leukocytes. We aimed to explore this hypothesis using human EVLP and *in vitro* analysis.
Methods: Explanted human lungs (n=7) underwent standard EVLP. Perfusate samples (30 minute intervals) and leukocyte filter were collected, and cells characterised via flow cytometry. Isolated alveolar monocytes (from post-LTx BAL) were naturally differentiated (to DC or mΦ) and immunocharacterised. An *in vitro* (air/epithelial-liquid/endothelial) lung model was utilised to evaluate monocyte migration/differentiation within the lung.
Results: Non-classical monocytes (NCM, normally $<1\%$ of total WBC repertoire - range 0-8240 NCM/ml whole blood) mobilised within 30 minutes of EVLP and represented 80.04% of the passenger leukocyte population (mean 41,222 NCM/ml perfusate). *In vitro*, this subset readily differentiated to DC and secreted proinflammatory cytokines (IFN-γ and IL-2) following stimulation. NCM rapidly diapedesed from the vascular bed to the alveolus and when cultured on the alveolus, differentiated to DC with inflammatory phenotypes ($HLA\text{-}DR^{+}$, $CD11c^{+}$, $CD86^{++}$, $CD123^{++}$).
Discussion: For the first time, we have identified that the lungs possess a reservoir of NCM which can readily diapedese to the alveolus, or mobilise in the circulation. Following activation, NCM differentiate to an inflammatory DC with T cell costimulatory capacity. EVLP may impart additional benefits following LTx via the removal of these cells, which may be reflected in the low incidence of rejection observed in this cohort.

O-256 IL-2-INDEPENDENT INDUCTION OF NOVEL CD4+CD25+CD127-FOXP3+ T-CELLS BY MESENCHYMAL STEM CELLS AND NATURAL REGULATORY T-CELLS

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Background: Mesenchymal stem cells (MSC) and CD4+CD25+CD127-FOXP3+ natural regulatory T-cells (nTreg) represent candidates for cellular therapy in organ transplant patients due to their immunosuppressive functions. Hence, it is essential to investigate the interactions between these cell types and existing immunosuppressive medication to circumvent mutual impairment. An immunomodulatory mechanism employed by MSC is the induction of T-cells with a regulatory phenotype, associated with elevated levels of IL-2. This study aimed to elucidate whether a similar effect is evoked by nTreg and whether the effect is influenced by basiliximab, an anti-IL-2 receptor antibody.
Methods/Materials: MSC were isolated from perirenal fat tissue of kidney donors. PBMC and nTreg were obtained from healthy donors. The immunomodulatory effect of MSC (1:2.5) and nTreg (1:10) on PBMC subsets was studied without and in the presence of basiliximab (4μg/mL) by means of mixed lymphocyte reactions and subsequent flow cytometric analysis.
Results: MSC and nTreg dose-dependently decreased the proliferation of various allo-activated PBMC subsets. MSC and nTreg in combination resulted in a cumulative inhibition of proliferation. Further, it was observed that MSC and nTreg induced the generation of CD4+CD25+CD127-FOXP3+ T-cells which were not of nTreg origin. MSC and nTreg attained a 5.6-fold ($p=0.02$) and a 2.6-fold ($p=0.01$) increase, respectively. The addition of basiliximab maintained this phenomenon with MSC and nTreg achieving a respective induction of CD4+CD25+CD127-FOXP3+ T-cells by 6.2-fold ($p<0.001$) and 2.3-fold ($p=0.005$).
Conclusion: MSC and nTreg contribute to the generation of an immunosuppressive environment via two shared mechanisms: i. inhibition of allo-activated

effector T-cells and ii. induction of T-cells with a regulatory phenotype. The fact that basiliximab does not hamper the induction of novel CD4+CD25+CD127-FOXP3+ T-cells by MSC and nTreg indicates promising prospects for combined cellular- and drug-mediated immunotherapy in organ transplant patients.

O-258 ORGAN GRAFTS TOLERIZED THROUGH THIRD-PARTY BONE MARROW-DERIVED ADHERENT STEM CELLS CAN BE RE-TRANSPLANTED WITH NO IMMUNOSUPPRESSION

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Multipotent adult progenitor cells (MAPCs) are a unique population of bone-marrow derived, non-hematopoietic, adherent stem cells with a broad range of regenerative abilities. Both MAPCs and mesenchymal stem cells (MSCs) have immunological features that promise to be valuable for immunosuppressive therapy. Unlike MSCs, MAPCs are more pluripotent, can be cultured indefinitely, and have been shown to function across allogeneic barriers, making them ideal candidates for routine use. This is the first study to demonstrate that third-party MAPCs mediate long-term acceptance of allogeneic, vascularized heart grafts when administered concurrently with low-dose calcineurin-inhibitor (CNI)-free immunosuppression in rats. MAPC-induced allograft acceptance could further be transferred via organ upon re-transplantation, or via the recipient lymphocyte pool following adoptive splenocyte transfer into naïve recipients with no additional immunosuppression. We thus introduce a model for MAPC-mediated graft acceptance and transfer of such immune privilege to naïve recipients. Our findings add to previous studies in rodent transplant models that have either used (donor-type) MSCs or hematopoietic cell preparations, both of which would pose major obstacles for routine clinical application. Based on these findings, we are currently initiating a phase I clinical trial applying third-party MAPCs to liver allograft recipients.

Ethics, legal and psychosocial aspects of transplantation

O-259 HOME-BASED GROUP EDUCATION: THE SOLUTION FOR BREAKING BARRIERS IN COMMUNICATION AND KNOWLEDGE AMONG NON-EUROPEAN KIDNEY PATIENTS?

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Background: Non-Dutch patients are overrepresented on the deceased donor waiting list and underrepresented among recipients of a kidney from a living donor. We translated Rodrigue's home-based education programme to the Dutch situation, taking into consideration the cultural diversity of our patients. We present our first experiences with this programme.
Methods: During the first session, a genogram and sociogram of the extended family and social environment of the patient is constructed to determine who could be invited for the second educational session. All invitees receive written invitations with information about the session. During the second session, kidney disease, treatment options including living donor transplantation, and the impact on quality of life of these different treatment options are discussed. In order to create a safe environment for communication we use the tools provided by the therapeutic framework of Multi System Therapy (MST). Important components of our approach include: patient-centeredness and empowerment, respecting and tailoring to the different cultural and social norms, using independent interpreters and offering practical solutions.
Results: We piloted this new programme among patients from Moroccan, Turkish, Surinamese, Antillean, Cape Verdean and Dutch origin. 2-17 invitees attended each educational session. Patients and invitees welcomed this new way of education. However, patients find it daunting to invite their family and friends and therefore hesitate sometimes to participate. Clear explanation of the goal of the education helped remove these hesitations. The education generated increased understanding and compassion for the patient.
Conclusion: Misconceptions, anxiety and concerns about donation and transplantation are taken away during the sessions. The results of our pilot study encouraged us to embark on a randomised controlled study to ascertain the effectiveness of this approach.

O-260 INFORMED CONSENT AND EXTENDED CRITERIA DONORS (ECD) FOR LIVER TRANSPLANTATION

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ELPAT (Ethical, Legal and Psychological Aspects of Organ Transplantation), coordinated the distribution to European liver transplant centres of an electronic questionnaire concerning the definition of extended criteria (ECD) liver donation and the implication for informed consent of transplant receipients. Completed questionnaires were received from 30 centres in 13 countries. 28 centres accepted ECD liver donors. The criteria for defining a liver donor as ECD were: steatosis in 24 centres (85%), age up to 80 years in 23 centres (82%), serum sodium higher than 165 mmol/l in 17 centres (60%), ICU stay with ventilation longer than 7 days in 16 centres (57%), SGOT higher than 90U/l in 12 centres (42%), BMI more than 30 in 10 centres (35%), SGPT higher than 105U/l in 10 centres (35%), serum bilirubin higher than 3mg/dl in 10 centres (35%) and other criteria in 13 centres (46%). 23 centres informed the transplant candidate of the ECD status of the donor: 10 centres (43%) when the patient registered for transplantation, 3 centres (14%) when an ECD liver became available and 10 centres (43%) on both occasions above. 10 centres required the liver transplant candidate to sign a special consent form. 10 centres informed the potential recipient of the donor's serology. Only 3 centres informed the potential recipient of any high risk behaviour of the donor.

O-261 'TO GIFT OR NOT TO GIFT?' EXPLORING VIEWS ON DECEASED ORGAN DONATION FROM DIFFERENT CONGREGATIONS ACROSS THE UK

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Background: The UK transplant waiting list show 8,000 people waiting for an organ; 23% are people from south Asian and African-Caribbean minority ethnic communities who account for 8% of the total population. This group is three-to-four times more likely to develop end stage renal failure and need a kidney transplant, but are likely to wait twice as longer to receive one. Audit data shows that these families are 70% more likely to decline consent to deceased organ removal compared to 40% from the white population. The most common reason for declining deceased organ removal was the belief that desecration of the deceased body was not permissible within their faith. This study explores the views of different congregations to the concept of "giving a gift, and doing good deeds" from a theological perspective and then considers whether this concept could be stretched to cover giving the gift of life through deceased organ donation.
Methods/Materials: 13 focus groups across 10 faiths in three diverse locations of the UK were undertaken. The focus groups were tape recorded and the data was transcribed verbatim. A thematic approach to data analysis was used.
Results: Most participants agreed that the concept of giving/gifting from a theological perspective could be stretched to cover deceased organ donation. However, some were reluctant to make this link because they believed that desecration of the deceased body was not permissible within their faith.
Conclusions: Until more is done to raise awareness for the need of organs and of what is religiously permissible in terms of deceased organ donations, the congregation will be prevented from making an informed decision and the question of whether to "gift or not to gift" will remain a perplexing one.

O-262 GIVING THE GIFT OF LIFE THROUGH DECEASED ORGAN DONATION, EXPLORING THE VIEWS OF LOCAL FAITH LEADERS FROM ACROSS THE UK

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Background: The UK transplant waiting list show 8,000 people waiting for an organ; 23% are people from south Asian and African-Caribbean minority ethnic communities who account for 8% of the total population. This group is three-to-four times more likely to develop end stage renal failure and need a kidney transplant, but are likely to wait twice as longer to receive one. Audit data shows that these families are 70% more likely to decline consent to deceased organ removal compared to 40% from the white population. The most common reason for declining deceased organ removal was the belief that active desecration of the deceased body was not permissible within their faith. This study explores the views of local faiths leaders in the UK, to consider whether the concept of "giving a gift and doing good deeds" from a theological perspective could be stretched to cover the concept of giving the gift of life through deceased organ donation.
Methods/Materials: 23 semi-structured interviews across 10 faiths, in four diverse location of the UK were undertaken. The interviews were tape recorded and the data was transcribed verbatim. A thematic approach to data analysis was used.
Results: All participants agreed that the principle of giving/gifting from a the-

ological perspective could be stretched to cover deceased organ donation. However, the lack of awareness and thinking of giving/gifts in terms of time and money may prevent the link being made generally.
Conclusions: By working together, faith leaders and health professional are ideally placed to raise awareness, and highlight how giving the gift of life through deceased organ donation can change lives. It is quite plausible that people from different faiths would then actively register on the ODR.

O-263 REPORTING QUALITY OF RANDOMISED CONTROLLED TRIALS IN SOLID ORGAN TRANSPLANTATION

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Background: The reporting quality of randomised controlled trials (RCTs) is often inadequate. We reviewed the reporting quality of RCTs in solid organ transplantation.
Methods/Materials: All English reports of RCTs published in 2007 were identified and rated by two reviewers. Methodological quality was assessed using the 0-5 Jadad scale with trials scoring $\geq$3 considered as good quality trials. The two other quality items were allocation concealment and whether the analysis was by original randomisation assignment. Additionally, we assessed 25 selected items from the revised 2010 CONSORT statement. Whether a trial was single or multicentre and whether a flowchart was included in the report was also recorded.
Results: We identified 111 RCTs. Forty-four percent of trials were considered of good methodological quality according to the Jadad scale, 40% used concealed allocation and 43% analysed the data by original randomisation assignment. On average, reports described 12 out of 25 CONSORT items (range 3-21 items). A flowchart was included in 29% of reports. Fifty-three trials were single centre, 34 multicentre and for 24 trials it was unclear whether these were single or multicentre. The percentage of good quality trials according to the Jadad scale was higher among multicentre trials than single centre trials (56% vs 43%). Trial funding was declared in 66 trials with most trials receiving commercial funding (n=34) followed by non-commercial funding (n=22). Sixty-two percent of trials that received commercial funding and 80% of trials that received both commercial and non-commercial funding were considered of good quality according to the Jadad scale versus 43% of trials receiving non-commercial funding.
Conclusion: The CONSORT statement was first published in 1996 to improve the quality of reports of RCTs. However to date the reporting quality of most RCTs in organ transplantation is still unsatisfactory.

O-264 ELPAT'S NEW CLASSIFICATION FOR LIVING ORGAN DONATION

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Background: With an expansion in variety of live donor-recipient combinations, confusion currently exists regarding appropriate terminology for different types of donation. For example, some donations are termed "altruistic" yet all donation could be considered so. Alternatives, including "good samaritan" donation, and "non-directed" donation are similarly problematic. "Related" donations imply a genetic relationship but there may be no pre-existing emotional relationship, whilst in "unrelated" donation there may be a profound emotional but no genetic relationship. Further confusion arises when considering paired exchange programmes, in which the donation is directed to an unknown individual. This is an anonymous yet directed donation, but is performed in order to help a third individual with whom the donor has some relationship.
Methods: A working group of ELPAT, a section of ESOT, recently convened in Sofia (October 2010) and proposed a new classification. The aim was to propose a workable classification system for living organ donation that avoids morally or religiously loaded concepts and enables coherent discussion and comparisons.
Results: The proposed system is as follows:
Specified donation: a) Direct donation: when a person donates directly to his or her intended recipient: – donation to genetically and emotionally related recipient, – donation to genetically unrelated but emotionally related recipient, – donation to genetically related but emotionally unrelated recipient, – donation to genetically and emotionally unrelated recipient, but the recipient (or the group to which he/she should belong) is specified.
b) Indirect donation: when a person donates indirectly to his or her intended recipient: – donation to a specified recipient through an exchange programme. Unspecified donation: – donation to an anonymous and unspecified recipient.
Conclusions: The proposed new system of classification for living donor transplantation will ensure clarity and consistency as new approaches to such transplants are more widely adopted.

Hand and face transplantation

O-265 THE BONE COMPONENT OF CTA GIVES RISE TO DONOR HSC WHICH MIGRATE TO RECIPIENT THYMUS AND DIFFERENTIATE TOWARDS A MATURE T CELL PHENOTYPE

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Introduction: Composite Tissue Allotransplantation (CTA) is immunologically unique in that it represents the only type of graft to include a vascularized functional bone-marrow-component. Here we studied if the bone component of a composite tissue graft represents the source of HSCs that differentiate in the thymus and thereby reconstitute a functional immune system (CD3+T-cells in peripheral blood/lymphoid organs) in immunodeficient B6/SCID-recipients rather than originate from donor mature passenger T-cells that expand in the host.
Material and Methods: B6 (WT/nude) murine composite tissue grafts (osteomyocutaneous or myocutaneous) were transplanted heterotopically to B6 (WT/scid) recipients using a non-suture cuff technique for revascularization. Flow cytometry of peripheral blood (CD3, CD19) was performed at pod:7,14,21,28, and 56. In addition, histopathology (H&E) and immunohistochemistry of tissues was performed at indicated time points. To assess immunocompetence, allogeneic skin grafts (Balb/c) were transplanted to either naïve B6/nude, naïve B6/scid or B6/scid mice that prior received a B6/nude CTA.
Results: The surgical success rate was 85% in all groups. As expected no CD3+cells and no rejection of skin allografts were detected in B6/nude and B6/scid controls. B6/scid mice that received B6/nude osteomyocutaneous flaps demonstrated B and T cell immunity from pod 7 and 21 respectively. The percentage of CD3+ and CD19+ cells within peripheral blood mononuclear cells steadily increased to 57.7% and 17.1% respectively at pod 56. Allogeneic skin allografts were rejected 2 weeks after transplantation. However, no B and T cell reconstitution was observed in B6/scid mice receiving B6/nude myocutaneous flaps (without bone component).
Conclusion: The vascularized-bone-marrow-component of CTA provides an effective source of HSCs to restore immunocompetence in T-and B-cell-deficient mice. This might also contribute to chimerism induction and maintenance after CTA and facilitate the clinically observed immunoprivilege of CTAs.

O-266 OUTCOME 5 YEARS AFTER THE FIRST HUMAN PARTIAL FACE TRANSPLANTATION

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Background: The first human facial transplantation (Tx) was performed in November 27, 2005. Herein aesthetic aspect and functional recovery as well as the risk to benefit ratio are evaluated 5 years later.
Methods: The facial transplantation included nose, chip and lips The initial immunosuppressive protocol included tacrolimus, mycophenolate mofetil, prednisone and antithymocyte globulins. In addition, donor bone marrow (BM) cells were infused on days 4 and 11 after transplantation.
Results: Aesthetic and functional recovery was satisfying. She has normal pain and cold sensation. Discriminative recovery was normal 2 years after Tx. The analysis of motion recovery showed a rapid improvement of muscle func-

tion. After the first year she presented a normal mouth opening, and labial closure was complete despite a persisting light asymmetry. She can smile, chew, swallow and blow normally while pouting and kissing is still difficult. Phonation recovery was impressive and the patient can talk normally.
She presented only two episodes of acute rejection during the first year. Microchimerism was not detected in peripheral blood but was observed in CD34+ BM cells 2 months after Tx. Donor specific anti-HLA antibodies were never detected. Five-year protocol mucosal biopsy showed a slight perivascular infiltrate while skin biopsy was normal.
The main side effect of the immunosuppressive regimen was a progressive decrease in renal function 11 post-tx months that improved after switching from tacrolimus to sirolimus. In addition, she developed chronic hypertension and increase in lipid levels which were pharmacologically well controlled. Because of an increase in Gamma-GT values a hepatic biopsy was performed showing mild cholangitis.
Conclusion: Although the reported long-term complications, which are similar to those reported in solid organ transplantation, the patient is very satisfied of her new face and has normal social interaction.

O-271 RESULTS OF THE FIRST PROSPECTIVE PILOT STUDY ON BILATERAL HAND ALLOTRANSPLANTATION

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Background: This study includes five cases of bilateral hand allotransplantation performed in 2000, 2003, 2007, 2008 and 2009 with a period of follow-up ranging from 11 to 1 year.
Methods: The recipients were 33, 21, 27, 29 and 21 years old respectively at the time of transplantation; 4 men and 1 woman. The level of amputation was at wrist level bilaterally in 2 recipients, at wrist and distal forearm level in 1 recipient, at mid forearm and third distal forearm in other 2 cases.
All the recipients received the same immunosuppressive protocol which included tacrolimus, mycophenolate mofetil, prednisone and, for induction, antithymocyte globulins.
Results: All recipients showed a relevant sensorimotor recovery (protective and tactile sensitivity and a partial recovery of intrinsic muscles) and they were able to perform the majority of daily activities living a normal social life.
The first two recipients experienced two episodes of acute skin rejection which resolved by increasing oral steroid dose; the fourth and the fifth recipient experienced only one episode of acute rejection, resolved by increasing oral steroid dose and using intravenous steroids respectively; while the third recipient presented 4 episodes, which regressed using intravenous steroids and also antithymocyte globulins. Some complications occurred after the transplantation but they were successfully treated: the first and the fifth recipients presented transient hyperglycemia; the second recipient suffered from a thrombosis of the right ulnar artery and an osteomyelitis of left ulna; the third patient presented an Epstein Barr virus infection and the fifth patient developed several vascular complications (thrombosis of right radial artery and left ulnar artery) in the first post-operative period.
Conclusion: This study demonstrates that bilateral hand transplantation is an acceptable treatment for patients suffering from bilateral hand amputation with a remarkable benefice/risk ratio.

O-267 THE INNSBRUCK HAND TRANSPLANT PROGRAM: UPDATE AT 11 YEARS AFTER THE FIRST TRANSPLANT

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Introduction: We describe here the outcome after two bilateral hand, one bilateral forearm and one unilateral hand transplantation at 11/8/4.5 and 1.5 years after transplantation.
Methods: Four patients received a bilateral hand (n=2), a bilateral forearm (n=1) or a unilateral hand transplant between March 2000 and July 2009. Induction therapy with ATG (n=2) or alemtuzumab (n=2) was followed by tacrolimus, prednisolon ± MMF (n=3) or tacrolimus and MMF (n=1) maintenance IS. Later, sirolimus/everolimus was added under simultaneous withdrawal (n=2) or dose reduction (n=1) of tacrolimus (n=1) or MMF (n=1). Steroids were avoided in one and withdrawn in two patients.
Results: Range of motion reached up to 70% of normal with a grip strength of 2-10kg. Hand function correlated well with time after transplant and amputation level. Intrinsic hand muscle function recovery and discriminative sensation were observed in all patients. Complications included CMV infection, fungal infection, hypertension, hyperglycemia, transient creatinine increase and headache. Three, six, four, and one rejection episode were successfully treated with steroids, anti-CD25, anti-CD52 antibodies and/or intensified maintenance IS. Skin histology at current shows no or mild perivascular lymphocytic infiltrates without signs of progression. Vessels are patent without signs for luminal narrowing or intimal proliferation.
Conclusion: The overall functional outcome and patient satisfaction after bilateral hand, bilateral forearm and unilateral hand transplantation are highly encouraging. All patients are now free of rejection with moderate levels of IS.

O-268 FACE TRANSPLANTATION WITH COMBINED HEMATOPOIETIC STEM CELL INFUSION AND VASCULARIZED BONE MARROW: FIRST YEAR OF FOLLOW-UP

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The aim of the study was to report on the outcome of a partial face allotransplantation including vascularized bone marrow (VBM), combined with hematopoietic stem cell infusion.
Methods: The recipient was disfigured at the lower part of his face by a pyrotechnic explosion. Facial allograft included bilateral mandible (VBM), cheeks, lips and chin. Immunosuppression included Thymoglobulin, tacrolimus, prednisolone, mycophenolate mofetil (MMF). Donor bone marrow was infused at day 4 post-transplantation. EBV status was D+/R-. Follow-up included protocol mucosal and skin biopsies and search for chimerism using RQ-PCR (Taqman) in blood and bone marrow.
Results: The follow-up was marked by severe complications: i) HHV1 infection of lips and mouth that occurred at day 6, 86, 160 and 226 post transplant, treated by acyclovir (d6, d160) and foscavir (d86, d 226) ii) post-transplant monoclonal B lymphoma at 4 months after a primary EBV infection, treated with Rituximab and reduction of immunosuppression, with good initial outcome iii) 3 episodes of acute rejection (grade II-III) occurred at d41, d103 and month 6 involving simultaneously face, mucosa and sentinel skin flap that were successfully treated by high dose steroids.
Despite donor bone marrow infusion combined with the VBM transplantation, transient microchimerism was evidenced in the bone marrow at d7 (0.4% donor CD34 + cells), d14 (0.6% donor CD34 + cells), and d56 (0.4% donor CD34 + cells) and was detected once in peripheral blood (0.6% donor CD3+ lymphocytes at d28).
Conclusion: The one year-outcome of this partial face allograft transplantation was complicated by donor transmitted EBV-related post transplant lymphoma and the occurrence of 3 episodes of rejection. This suggests that D+/R- EBV status should be avoided in CTA. VBM transplantation combined with hematopoietic stem cell infusion induced only a transient microchimerism without inducing tolerance.

O-269 EXPRESSION OF PERIPHERAL NODE ADDRESSIN INDICATIVE OF TERTIARY LYMPHOID ORGANS IN SKIN AFTER HAND TRANSPLANTATION

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Background: Expression of peripheral-node-addressin (PNAd) on endothelial cells indicates presence of tertiary lymphoid organs (TLO) in chronic autoimmunity and allograft rejection. We herein investigated the expression of PNAd

in skin biopsies of human hand allografts for evidence of TLO after composite tissue allotransplantation.
Methods: 167 skin biopsies of 11 hand allografts were collected over 10 years and assessed by HE-histology and immunohistochemistry for PNAd, CD3, CD4, CD8, CD20, C4d, CD68, LFA-1, ICAM-1, E-selectin, P-selectin, VE-cadherin, HLA-DR, Psoriasin, IDO and Foxp3. Levels of PNAd expression was assessed semiquantitatively and correlated with rejection grade, characterization of the infiltrate, expression of adhesion molecules and time after transplantation.
Results: Rejection ranged from grade 0 to IV (mean score: 0.79±1.05). Upon rejection, expression of PNAd was increased in endothelial cells (grade 0:0.24±0.48 vs. all grades of rejection:0.44±0.62). Most often PNAd expression was only found in few vessels (1-10%). PNAd staining intensity was increased the higher the grade of rejection (grade 0: 0.38±0.76; grade I: 0.41±0.74; grade II: 0.67±0.80; grade III: 0.73±0.91; grade IV: 0.50±0.58). Intense PNAd-staining was associated with more CD4+ and CD8+ infiltrating T-cells, but less B-cells and macrophages, compared to mild PNAd staining intensity (CD4+cells 49.00%±29.89; CD8+cells 31.00%±22.34; CD20+B-cells 0.50%±1.54; CD68+macrophages 0.57±0.60 vs. CD4+cells 37.35%±40.82; CD8+cells 27.35%±35.93; CD20+B-cells 0.94%±2.02; CD68+macrophages 0.67±0.66). PNAd expression correlated well with CD3+cells and CD20+B-cells. Poor correlation was found for expression of adhesion molecules, IDO and Foxp3, except for LFA-1+ infiltrating cells. PNAd expression was observed at all time-points after transplantation; however, staining intensity was enhanced very early and late after transplantation.
Conclusion: PNAd expression in endothelial cells is increased in skin biopsies of human hand allografts indicating presence of TLO. Further invetigations are needed to enlighten the role of PNAd and TLOs in composite tissue allotransplantation.

O-270 EFFECT OF COLD ISCHEMIA AND PRESERVATION SOLUTIONS HTK AND UW ON COMPOSITE TISSUE ALLOGRAFTS

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Background: The effect of cold ischemia (CI) on tissue damage of a composite tissue allograft is unknown. We therfore investigated the impact of cold tissue storage and preservation with HTK and UW in a rat-hindlimb-transplant-model.
Methods: LEW-rat-limbs were flushed and stored for 0,2,10,30 and 40h in HTK or UW. Skin, muscle, bone and nerve biopsies were taken at any time point for H&E-histology. After transplantation (subsequent to 2,10 or 30h CI), limbs were analyzed for morphological alterations by histomorphology (0:no alterations; 1:mild alterations; 2:severe alterations; 3:necrosis) and confocal microscopy (percentage of vital cells) at 1+10 days.
Results: Appearance and histology of skin, bone and nerve remained unaltered at any time point during preservation. Histomorphologic changes of muscle was observed in some biopsies regardless of preservation-solutions and CI-time. 2,10 and 30h CI and subsequent reperfusion did not cause alterations in histomorphology of skin and muscle at 1 day. At 10 days, skin showed a mild lymphocytic infiltrate in all samples after 10 and 30h CI. In muscle a mild lymphocytic infiltrate was found in groups of 10h CI, after 30h CI highly affected and necrotic muscle fibers were obtained. Nerve showed myxoid degeneration, a perineural infiltrate and hyper-cellularity at 2,10 and 30h CI. In bone necrosis was observed in some samples, regardless of preservation-solution and CI-time. Skin and muscle were more affected of CI when flushed and stored in UW than HTK. At 10d and 10h CI HTK showed an advantage over UW in preservation of muscle (vital cells HTK: 48,59±23,41% vs UW: 42,97±19,68% vs NaCl: 31,44±21,85%).
Conclusions: CI causes mild histomorphologic alterations on muscle. In transplanted legs most severe histomorphological changes can be observed in nerve and muscle at 10 days with an advantage of HTK over UW for tissue preservation.

Lung: vaccins and alternative donors

O-272 THE IMPACT OF VACCINATION ON THE BREADTH AND STRENGTH OF ALLOSENSITIZATION IN LUNG TRANSPLANT CANDIDATES

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Background: Although guidelines advocate vaccinating organ transplant candidates, whether vaccination promotes sensitization remains unclear.
Methods: We studied 66 adult lung transplant candidates listed at our institution between 2005 and 2010 with documented history of vaccination. Inclusion required record of detailed antibody testing performed at least 3 times: 2 weeks to 3 months prior to vaccination, 2 weeks to 3 months following vaccination, and at least once in the subsequent pretransplant period.
The subjects' median age was 58 yrs (23–73 yrs), with 50 males, 57 Caucasians, 4 African-Americans, and 5 others/unknown race. We retrospectively studied changes in strength and breadth of allosensitization in proximity to time of vaccination. Strength of allosensitization was measured by fluorescent intensity on single-antigen flow beads (MESF) or Luminex assay (MFI), and breadth of allosensitization was measured by calculated PRA (cPRA).
Results: Response ranged from no change (53%), to increase in strength of allosensitization (37% of all subjects, 29% of these returning to baseline), to increase in breadth of allosensitization (3%, with all returning to baseline). See Figure 1 for further details.
The incidence of increased screening PRA following vaccination did not depend on pre-vaccine sensitization status: 8/28 sensitized (initial PRA $>$ 10%) and 6/38 unsensitized subjects showed an increase (p=0.17).

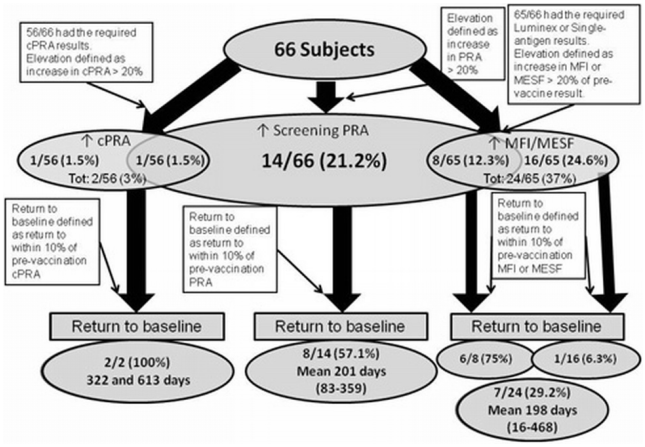

Figure 1

Figure 1 indicates the proportion of subjects that experienced an increase in allosensitization by the modalities indicated. The bottom row shows what proportion of subjects returned to baseline after experiencing an increase in allosensitization.
Conclusion: These observations suggest that patients may experience different patterns of change in allosensitization status, although these changes were independent of pre-vaccine sensitization status. Further study is needed to more precisely characterize these patterns and to investigate possible associations with number and/or type of vaccines.

O-273 CADAVERIC LOBAR LUNG TRANSPLANT: A VALID OPTION FOR SMALL-SIZED CF PATIENTS

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Background: Small-sized cystic fibrosis (CF) patients usually face long waiting times. Reduced-size lung transplantation (LTx) from a larger donor was promoted to shorten waiting times. We compared donor and recipient characteristics and outcome in lobar [L] versus full-size [FS] recipients.

Methods/Materials: Between 01/07/1991-28/02/2011, 535 isolated LTx were performed, including 74 (13.8%) patients with CF (8 L-66 FS). Median follow-up was 720 [44-2095] days vs 1906 [51-5894], respectively.
Results: Patients in [L] were younger (21 [13-25] vs 28 [14-57] years; $p<0.01$), smaller (152 [145-166] vs 168 [144-192] cm; $p<0.01$), lighter (42 [34-52] vs 51 [30-82] Kg; $p<0.01$). Gender (25%M vs 55%M) and waiting times (284 [29-921] vs 176 [3-1143] days) were comparable as well as donor data: age (30 [14-44] vs 34.5 [12-62] years), gender (75%M vs 63%M), height (178 [160-185] vs 171 [154-190] cm), weight (73 [55-85] vs 70 [35-100] Kg), PaO_2/FiO_2 (460 [261-601] vs 493 [258-669] mmHg), and ventilation time (50 [37-96] vs 44 [10-381] days). Cardiopulmonary bypass was used more often in [L] (75% vs 30%; $p<0.05$) but time was comparable (164 [115-344] vs 210 [97-465] minutes); cold ischemia was comparable for first lung (273 [240-471] vs 263 [310-521] minutes) but longer in [L] for second lung (473 [401-598] vs 410 [430-561] minutes; $p<0.01$). Implantation times were comparable (T1 60 [50-72] vs 60 [62-125], T2 60 [50-66] vs 59 [50-90] minutes). In-hospital mortality was 0% vs 3%. Both ICU (12 [4-49] vs 4 [1-60] days; $p<0.01$) and hospital stay (37 [25-67] vs 24 [1-91] days; $p<0.01$) were longer in [L]. Primary graft dysfunction (PGD) was more pronounced in [L] at T0 (75% vs 52% PGD3) and at T48 (75% vs 6% PGD1). FEV_1 increased significantly from preoperative value to latest follow up (25 [21-30]% to 67 [44-88]% in [L]; $p<0.05$ and 24 [12-52]% to 84 [16-126]% in [FS]; $p<0.01$). Bronchiolitis obliterans syndrome (BOS) was absent in [L] and diagnosed in 22 patients in [FS] accounting for 6/14 deaths. Actuarial 5-year survival was 100% in [L] vs 79.7% in [FS] (87.5% since the start of L-LTx).
Conclusion: Although hindered by a higher incidence of PGD, L-LTx is a viable option with excellent survival and pulmonary function comparable to FS-LTx.

O-274 IMPROVED CLINICAL OUTCOME OF PATIENTS TRANSPLANTED WITH RECONDITIONED DONOR LUNGS VIA EVLP COMPARED TO STANDARD LUNG TRANSPLANTATION

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Lung transplantation (LTx) offers improved survival & QoL in patients with end stage lung disease. However, LTx is severely limited by a scarcity of suitable donors, resulting in high waiting list mortality. Ex-vivo lung perfusion (EVLP) allows detailed evaluation and reconditioning of marginal donor lungs for use in LTx. This study aimed to comparitively assess clinical outcome of patients transplanted with reconditioned organs via EVLP with a standard LTx cohort.
This was a multicentre open technique trial. Group 1 included patients who were transplanted using EVLP reconditioned lungs. Group 2 consisted of date matched patients transplanted using standard LTx with acceptable lungs. The primary composite endpoint was incidence of acute rejction (AR) and infection between the groups at 3 and 12 months LTx.
20 patients were included in the study (n=8 in group 1, n=12 in group 2). There was no significant difference with immunosuppressive protocol, reperfusion injury or early/12month survival between the groups. There were significantly more AR episodes in group 2 at both 3 and 12 months compared to group 1 (p=0.002 and p=0.008 respectively). Interestingly there were also more treated incidences of infection in group 2 at 3 and 12 months compared to group 1 (p=0.002 and p=0.005). However, one patient died from sepsis (confirmed as Staphlococcus Aureus) within 3 months of transplantation in group 1.
Our data demonstrates that EVLP may be advantageous to standard transplantation in reducing incidence of AR and infection. The lack of AR in EVLP patients may be the result of reduced donor organ stress and the mechanical removal of passenger leukocytes which directly contribute to alloresponsiveness. Potentially, EVLP may be of benefit to all donor lungs prior to transplantation.

O-275 COMPLIANCE DURING IN SITU LUNG PERFUSION (ISLP) IS A PREDICTOR FOR LUNG INJURY IN NHBD CATEGORY I – II

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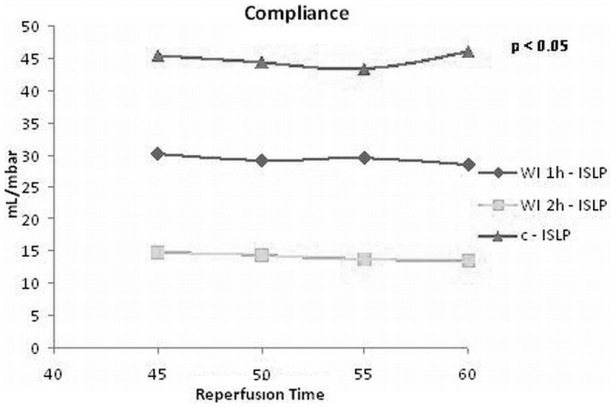
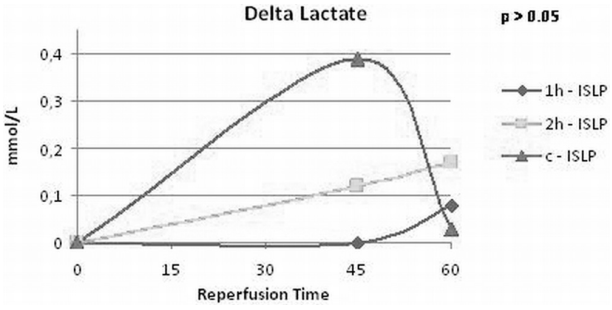
Introduction: NHBD are an important alternative to extend the donor pool in lung transplantation. In category I–II, lung function is often not available at the time of procurement.In this study we evaluate the use of a lung perfusion system in the donor for the assessment of these lungs.
Methods: Domestic pigs (n=12/n=4 per group) were sacrificed by ventricular fibrillation.This was followed by 20 minutes of cardiopulmonary resuscitation. Heparin was administered after a 5 minutes hands off period.In group I,this was followed by 1 hour of WI and 2 hours of topical cooling (TC) [1h-ISLP]. In group II,sacrification was followed by 2 hours of WI and 1 hour of TC [2h-ISLP].In group III, there was a minimal period of WI and no TC [C]. In all 3 groups the lungs were evaluated during 60 minutes in the pig with an in situ lung perfusion system.W/D weight ratio was calculated as an index of pulmonary edema.
Results: Compliance and $\Delta PO_2/FiO_2$ were significantly higher in [C] compared to [1h-ISLP] and [2h-ISLP] at 60 minutes of reperfusion (p=0,0052 and p=0,0077, respectively).



CO_2 retention (pCO_2) at end of the reperfusion was higher in [2h-ISLP] versus [C] (p=0.0463). Pulmonary vascular resistance was lower in [C] compared to [1h-SLP] and [2h-ISLP], ($p>0.05$). Δ lactate at 60 minutes was lower in [C] and [1h-ISLP] versus [2h-ISLP] ($p>0.05$).



W/D ratio was lower in [C] compared to [1h-ISLP] and [2h-ISLP] ($p<0.05$). The higher W/D in [2h-ISLP] was correlated with lower compliance and higher pCO_2 (r= –0.9489, p=0.026 and r=0,9581, p=0.021).
Conclusion: ISLP is a safe,fast and non-injurious way to assess lungs from NHBD category I–II in the donor.These data suggests that compliance and CO_2 retention are independent predictors for donor lung injury.

O-276 PROPHYLACTIC SURFACTANT INHALATION AMELIORATES ISCHEMIA-REPERFUSION INJURY IN RAT LUNGS FROM DONATION AFTER CARDIAC DEATH DONORS

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Background: To increase the number of organ donors, the use of donation after cardiac death (DCD) donors has come into practice in the last decades. Considering ischemia-reperfusion injury in lung transplantation from DCD donors, it is very important to alleviate lung injury during warm ischemia. It has been reported that after lung transplantation, the ischemia-reperfusion injury causes an impaired function of alveolar surfactant. Therefore, we investigated the effect of surfactant inhalation against pulmonary ischemia-reperfusion injury in rat lungs.
Methods/Materials: Male Lewis rats were used. One hundred and ten minutes after cardiac arrest induced by ventricular fibrillation, the lungs were initiated to ventilate and flushed by Low Potassium Dextran solution. Then heart-and-lung block was isolated, and ex vivo lung perfusion (EVLP) was conducted using diluted blood. Rats were randomly allocated into 3 groups: sham group (n=4, no ischemia before EVLP), control group (n=6, only ventilation before EVLP), and surfactant group (n=6, ventilation with surfactant inhalation before EVLP). We investigated the physiological lung functions during EVLP. In all groups,

lungs were perfused for 80 minutes in which lung functions were evaluated on time throughout the experiments.
Results: At 80 minutes of reperfusion, surfactant inhalation significantly increased pulmonary compliance ($p<0.0001$) and decreased airway resistance ($p=0.005$). In the surfactant group, shunt fraction and vascular resistance were lower than in the control group ($p<0.0001$ and $p=0.04$, respectively). Surfactant inhalation also inhibited pulmonary edema ($p=0.0002$).
Conclusion: Our results confirmed that surfactant inhalation at the last period of warm ischemia improved lung functions in various aspects. This method may contribute to improve the graft function from DCD donors.

O-277 beta2-ADRENORECEPTOR AGONIST INHALATION AMELIORATES ISCHEMIA REPERFUSION INJURY FOLLOWING LUNG TRANSPLANTATION FROM NON-HEART-BEATING DONORS IN A CANINE MODEL

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Background: It is a matter of great importance in a donation after cardiac death to attenuate ischemia reperfusion injury (IRI) related to the inevitable warm ischemic time (WIT). Our previous study in a canine transplant model demonstrated that the last 60-min ventilation in 240-min WIT ameliorated the IRI, but the protective effect of the last 60-min ventilation was insufficient for "300-min WIT". Then, we hypothesized that aerosolized beta2-adrenoreceptor agonist inhalation during the 60-min ventilation might ameliorate the IRI because our previous study proved the protective effect against IRI in a rat model (Chen, et al. Annals of Thoracic Surg 2006).
Methods/Materials: Donor dogs were rendered cardiac-dead and left at room temperature. The dogs were allocated into two groups: the inhalation group (n=5) received aerosolized beta2-adrenoreceptor agonist (Procaterol) and ventilation with 100% oxygen for 60 min starting at 240 min after cardiac arrest, and the control group (n=5) received only the ventilation. Lungs were harvested 300 min after cardiac arrest. The recipient dogs thereafter underwent left single lung transplantation. The right pulmonary artery was ligated at 60 min after reperfusion to evaluate the functions of the left transplanted lung for 240 min after the reperfusion.

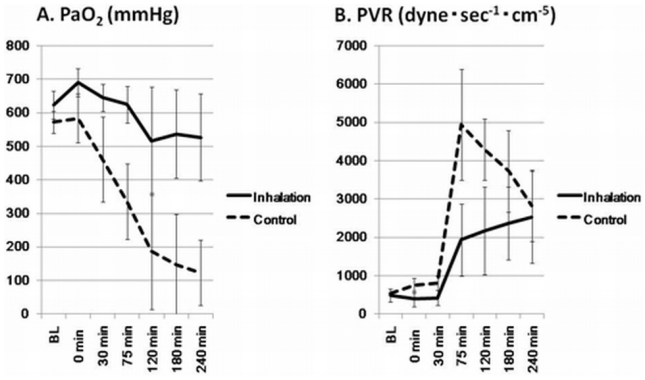

Figure 1

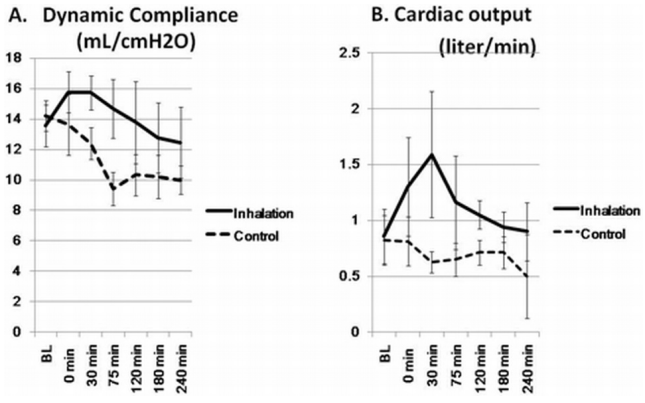

Figure 2

Results: In the inhalation group, all 5 animals survived for 240 min after reperfusion, while in the control group, one animal died of severe lung edema. Oxygenation (PaO_2, Figure 1A), dynamic compliance (Figure 2A) and cardiac output (Figure 2B) were significantly higher and pulmonary vascular resistance (Figure 1B) was significantly lower in the inhalation group (Repeated ANOVA, $P<0.05$).
Conclusion: The results suggest that aerosolized beta2-adrenorecpctor agonist inhalation can help to preserve pulmonary function and ameliorate IRI.

Deceased donation

O-278 THE LOST OPPORTUNITY IN END-OF-LIFE CARE? DONATION INFORMATION. A MULTI-CENTRIC STUDY

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Introduction: Despite all efforts, we still observe significant losses of donors because of poor hospital detection, due to both a lack of familiarity with the process and to the introduction of vital support limitation (VSL).
Objectives: 1) To analyse the evolution of patients with Critical Neurological Diseases (CND), defined by a Glasgow Coma Score <8.2) To know the number of CND cases receiving VSL and its effect on organ donation. 4)To detect losses of organ donors 5) To determine the degree of information provided to relatives of potential donors.
Methods: Prospective, multicentre and observational study of all CND patients up to death, (detailing the type of death, Cardiac-Arrest or Brain Death), release from hospital, or 30 days in-hospital stay. We also included type and place of VSL application. Statistical analysis.
Results: 10 hospitals with 607 patients followed over 9 months (2010). Mortality rate was 48.6%, and 40,6% of deaths were BD. Of BD patients, 56% became organ donors. VSL was applied in 28% of cases with 14 patients surviving. The average survivor age is similar to that of BD patients (52 years) and significantly lower than that for deaths due to VSL (70,7years). Non-admittance in ICU was the main VSL (40%), applied in emergency areas (46%). Relatives accept doctors' proposal of VSL in 79% of cases. Donation information was provided to 85,7% of BD families (organs) and only 11,8% in the LTSV group (tissues).
Conclusions: Mortality rates in CND are high, with 40% of our deaths patients evolving to brain death. Over half of them became organ donors. VSL was applied to 28% of the CND patients, mostly in the emergency areas. Donation information to VSL relatives is clearly insufficient.

O-279 REASONS FOR NOT FORMALLY DIAGNOSING POTENTIAL DONORS BRAIN DEAD: DONOR ACTION® MEDICAL RECORD REVIEW DATA FROM 7 COUNTRIES

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Formal brain death (BD) diagnosis is a crucial step in converting *potential* heart-beating (HB) donors into *actual* donors. This study aimed at investigating reasons for not declaring potential donors BD, whilst they met all criteria for a formal BD diagnosis according to national laws or guidelines.
Data from 59,064 Critical Care (CC) deaths between January 2007 and June 2010 in 7 countries, 228 hospitals and 620 CC units was entered to the Donor Action® System Database for further analysis. Potential HB donors were defined as ventilated patients, medically suitable on admission (V/MS), meeting all criteria for BD diagnosis and aged <76 years.
On a total of 19,683 V/MS patients, 53% met preconditions for BD diagnosis and 48.4% showed signs of severe brain damage. A total of 5,195 patients (mean: 39.2% of V/MS) met all criteria for formal BD diagnosis and therefore were considered *potential HB donors.* On average, BD was formally diagnosed in 65.9% of all potential donors (lowest in Poland (38.4%), highest

in Israel (90.7%) (P<.0001). Major reasons for not diagnosing BD were: *non-identification* as a potential donor: (25.8% on average, lowest in Switzerland (2.1%) and highest in Croatia (56.5%)), *cardiac arrest* and *failed resuscitation* (22.5% on average, lowest in Belgium (8.8%) and highest in Israel (47.6%)), *objections to donate* (15.6% on average, lowest in Croatia (0%) and highest in Switzerland (43.3%)), *treatment de-escalation* (11.8% on average, lowest in Israel (0%) and highest in France (17.6%)), patients becoming *medically unsuitable* (10.6% on average, lowest in Israel (0%), highest in Finland (28.6%)). Ultimate conversion rates averaged 41.7% (lowest in Poland (30.6%), highest in Finland (52.5%) (P<.0001).
Markedly different BD diagnosis practices demonstrate a significant room for improvement in donation processes of the 7 countries surveyed.

O-280 AGE AS A DETERMINANT OF POTENTIAL DONOR CONVERSION RATES: DONOR ACTION® DATABASE FINDINGS

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The conversion of *potential* heart-beating (HB) to *actual* organ donors is a stepwise process involving donor identification, referral, family approach and consent, clinical management and organ retrieval. The study presented aimed at assessing the impact of potential donor age on average conversion rates in 6 countries.
A total of 53,539 Donor Action® (DA) Medical Record Review (MRR) files were collected from Critical Care (CC) deaths between January 2007 and December 2009 in 6 countries (*Belgium, Finland, France, Israel, Poland and Switzerland*), 226 hospitals and 607 CC units that have implemented DA's methodology as a quality assurance tool to optimize their donation processes. Data was entered to the DA System Database for further analysis. Potential HB donors were defined as ventilated patients, medically suitable for donation on admission, and meeting all criteria for brain death diagnosis. Age groups were: <18 years (n=1014), 18-39 years (n=2809), 40-59 years (n=10,934), 60-79 years (n=24,599) and 80+ years (n=14,183).
On a total of 27,828 ventilated patients reported to be medically suitable for donation, 5,614 met all criteria for a formal brain death diagnosis (=potential HB donors). Areas for improvement, as illustrated in below table, are donor *identification* and *referral* in the 60+ age groups, *referral* in the <18 and 60+ age groups, family approach or registry checks in the 60+ age groups and *consent* to donate in the <18 age group.

Table 1

	<18 yrs	18–39 yrs	40–59 yrs	60–79 yrs	80+ yrs	P value
% donors identified*	75.6	78.0	79.0	62.7	38.3	<0.0001
% donors referred*	55.0	63.0	62.3	48.4	21.6	<0.0001
% family approaches*	69.4	70.4	70.3	53.5	27.0	<0.0001
% consents**	59.2	68.4	69.2	74.1	72.9	<0.05
% retrievals* (=conversion rate)	36.3	43.9	43.4	31.4	13.1	<0.0001

*As a % of potential donors; **As a % of family approaches.

Significant age-related differences could be observed with regard to donor identification, referral, family approach, consent to donate and organ retrieval. Properly addressing these bottlenecks in the donation process should result in optimal conversion rates in the 6 countries surveyed.

O-281 A DUTCH MULTI-CENTER STUDY ON THE DECISION-MAKING PROCESS OF FAMILIES REQUESTED FOR DONATION

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Backround: The consent process for donation is complex, and as a result family refusal is seen as a frequent outcome. In the Netherlands this is the main reason for the loss of potential donors. The Dutch Transplant Foundation therefore performed a multi-center study in an attempt to decrease the refusal rate.
Methods/Materials: Three hospitals participated. In the intervention hospital a special group of health-care professionals was trained according to the "Communication about Donation" programme ("*trained donation practioners*" (TDP)), their role was to guide the family throughout the time in the ICU until a decision regarding donation had been reached. The first control hospital had no special professionals for family care and donation was requested by the physician without any extra support. The second control hospital had "hostesses" for family support, but without any special training. The primary outcome measurement was the consent rate, and second the experiences of families measured by use of a questionnaire.
Results: The family consent rate was significantly higher in the intervention hospital with the TDP (57.6%) compared to the control hospital without hostesses (34.6%), and the control hospital with hostesses, but without special training (39.4%). No significant differences were seen between the participating hospitals based on the outcomes of the questionnaire, therefore no confounding variables could have influenced the consent rate.
Conclusion: It was possible to achieve higher consent rates with the implementation of the TDP, even though families in the intervention hospital were not more satisfied compared to the control group. The training and extensive contact between the TDP and the next of kin were the decisive factors in the statistically significant higher consent rate.

O-282 ACTIVATION OF HEMOSTASIS IN BRAIN-DEAD ORGAN DONORS

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Background: Brain death is associated with a systemic inflammatory response resulting in diminished organ function in individuals transplanted with these grafts. As inflammation is accompanied by activation of coagulation, we hypothesized that activation of hemostasis occurs in brain dead organ donors. Activation of hemostasis may result in formation of microthrombi in transplantable organs, which potentially contributes to deterioration of organ function.
Methods: In this study, we systematically assessed the hemostatic system in samples taken from thirty brain death organ donors. As controls, blood samples from 30 living kidney donors were included.
Results: Compared to the living donors, brain death donors showed significant platelet activation (assessed by glycocalicin plasma levels), and a profound dysbalance in the von Willebrand factor/ADAMTS13 axis, which is key in platelet attachment to damaged vasculature. Furthermore, compared to the living donors, brain death donors showed a significantly increased activation of secondary hemostasis with formation of fibrin (assessed by plasma levels of prothrombin fragment 1+2, fibrinopeptide A, and d-dimer). Finally, brain dead donors showed profound hypofibrinolysis as assessed by a global clot lysis assay, which could be attributed to a substantial elevation of plasma levels of plasminogen activator inhibitor type 1 (PAI-1).
Conclusion: Collectively, our results show activation of blood platelets, evidence for fibrin generation, and a decreased capacity to clear fibrin clots in brain dead organ donors. This prothrombotic state may contribute to formation of microthrombi in transplantable organs, which potentially contributes to deterioration of organ function.

O-283 THE MASTERPLAN FOR ORGAN DONATION IN THE NETHERLANDS: INTENSIVISTS IN THE LEAD

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Background: As a result of the growing demand for donor organs the organization of organ donation (OD) in the four Northern provinces of The Netherlands was changed. Key issues related to the 2009 "Masterplan for Organ Donation", were optimal detection of donors, improved donor management (DM) and reduction of refusal of consent.
Methods: All 21 hospitals of the Northern provinces were grouped in four clusters including a core centre and satellite hospitals. In the core centre a donation intensivist (DI) was appointed, who was given the task to introduce DM protocols in all intensive care units, promote a strategy for asking relatives for consent, provide a helpdesk function concerning OD, and evaluate all donation procedures with the physician in charge on all cases where a potential donor was not effectuated.
Results: The DM protocol was introduced in all hospitals. Misconceptions about OD and errors in checking the national donor registry were detected. The helpdesk was functional and resulted in two successful procedures that otherwise would have been abandoned. In 2009, 85% of the reported donation procedures were effectuated compared to 100% in 2010. The evaluation of procedures identified physicians not following the guidelines when checking the donor registry and asking relatives for consent. When not following the

guidelines the refusal rate of relatives was between 65 to 80%. The promotion of a strategy for asking relatives for consent resulted in a higher participation in courses in communication training on this subject.
Conclusion: The DI plays a crucial role in the OD organization by ensuring cooperation between cluster hospitals, stimulating focus on donation among intensivists. A structure has been devised, which allows immediate recognition and reporting of a potential donor. The responsibility to enhance OD has shifted from transplant to intensive care professionals.

Immune monitoring / pharmacokinetics

O-284 IMMUNE-MONITORING OF MEMORY/EFFECTOR T-CELL ALLORESPONSE FOR SELECTION OF CNI-FREE IMMUNOSUPPRESSION IN RENAL TRANPLANTATION

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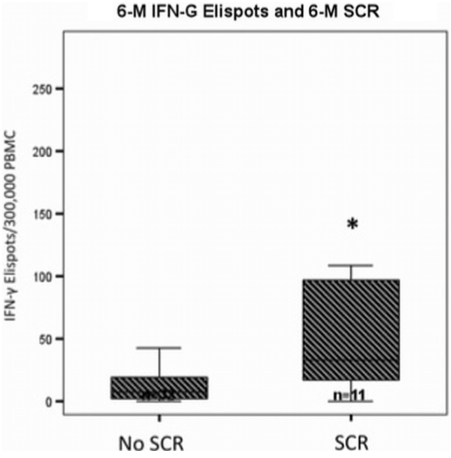
Introduction: Immune-monitoring of donor-specific memory/effector T cells using an IFN-γ Elispot before and after renal transplantation has been shown to discriminate patients at risk for immune-mediated graft dysfunction, suggesting to be a potential biomarker for immunosuppression individualization. We report the one-year immune-monitoring data of a prospective, non-randomized, multicenter, pilot study for selection of either CNI-based (TAC) or CNI-free (SRL) immunosuppressive regimen depending on donor-specific IFN-γ Elispot.
Methods: 61 renal transplant patients were enrolled in a 2-phase study. The first part lasted from pre-transplantation until month 6 and the second from month 6 until month 24. All patients received rATG, MMF and steroids. A donor-specific IFN-γ Elispot was assessed pre-transplantation and at month 6, where a protocol biopsy was also done. TAC or SRL was given if a positive or negative Elispot was obtained, respectively.
Results: Pre-transplantation, 61% of patients showed a positive ELispot and 34% were negative. 13% of Negative pre-transplant patients experienced BPAR (1 antibody-mediated and 2 T-cell-mediated) whereas none among the positive recipients. At month six, 74% of positive pre-transplant patients became negative and 64% of negative pre-transplant recipients remained negative. Thus, 76% of all patients were Elispot negative at 6 month and showed a significantly better 1-year graft function than those with a positive Elispot regardless the type of immunosuppression (POS/POS=49,8±13, POS/NEG=57±14, NEG/NEG=60,8±12, NEG/POS=48±24ml/min; $p<0,05$). Patients with subclinical rejection at 6-month had significantly higher Elispots than those without (107,7±11,7 vs 11,8±18, $p<0,05$).



Conclusion: Immune-monitoring of donor-specific T-cell alloreactivity using an IFN-γ Elispot may allow guided immunosuppression individualization in renal transplant recipients.

O-285 THE CD4⁺CD25⁺FoxP3⁺ AND CD4⁺CD25$^{low/-}$FoxP3⁺ CELLS ARE ASSOCIATED WITH LOW INCIDENCE OF KIDNEY ALLOGRAFT REJECTION

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Background: The aim of this prospective study was to evaluate the effects of different inductive agents on regulatory cells in the peripheral blood of kidney allografts recipients and to analyze their association with the short-term outcome.
Methods/Materials: Using flow cytometry, different regulatory and effector cell phenotypes were measured in the peripheral blood at days 0, 7, 14, 21, 28, 60 and 90 after kidney transplantation. Kidney transplant recipients were treated with CNI+MMF+steroid immunosuppression and induction with 1) rabbit antithymocyte globulin in a case of panel reactive antibodies (PRA) >49% (Thymoglobulin, rATG, n=28), 2) basiliximab (Simulect, n=18) in PRA 20-49% or received no induction in PRA <20% (controls, n=25). Phenotypes of regulatory and effector cells were correlated with clinical and laboratory data.
Results: Compared to controls, regulatory T cells (CD4⁺CD25⁺FoxP3⁺, Treg) frequencies were higher in rATG group in all post-transplant time-points ($P<0.001$) while lower in basiliximab group at days 7-60 ($P<0.001$). In basiliximab group, the transient decrease of Tregs was accompanied with the appearance of CD4⁺CD25$^{low/-}$FoxP3⁺ cells between day 7 and 60. Biopsy-proven acute rejection occurred in 16.7% of controls, 10.7% of rATG group and in 11.1% of basiliximab group. In rejectors in all groups, the Treg/Teff (CD8⁺CD45RA⁺CD62L⁻) ratio favors Teff in first 60 days ($P<0.05$). In controls with biopsy-proven borderline changes, Treg/Teff ratio was similar to patients without rejection. There was a significant positive correlation between Treg frequencies from first 21 days and GFR at day 60.
Conclusion: The reduction of acute rejection incidence is likely to be caused by elimination of Teff and expansion of Treg in patients who received rATG induction. Borderline changes are not associated with the expansion of Teff in the peripheral blood.

O-286 *CYP3A5* GENOTYPE IS NOT RELATED TO THE INTRA-PATIENT VARIABILITY OF TACROLIMUS CLEARANCE IN RENAL TRANSPLANT RECIPIENTS

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Introduction: The risk of long-term chronic allograft nephropathy and graft loss after kidney transplantation is increased in patients with a high intra-patient variability in the clearance of tacrolimus (Borra, L.C. et al. *Nephrol Dial Transplant* 2010;25(8):2757-2763). An individual's *cytochrome P450 3A* (*CYP3A*) genotype could be one explanation for this variability, as recently suggested by Korean investigators (Yong Chung, J. et al. *Ther Drug Monit* 2010;32(1):67-72).
In this study, intra-patient variability of tacrolimus clearance was correlated with *CYP3A5* genotype in a large cohort of renal transplant recipients from two renal transplant centres in the Netherlands and England.
Methods: The study population consists of 326 renal transplant recipients with stable renal function 12 months post transplant: 208 from Erasmus University, Rotterdam, and 118 from St. George's Hospital, London.

Table 1. Baseline Characteristics

	CYP3A5 expressers (n=96)	CYP3A5 non-expressers (n=230)	P-value
Male/female	61/35	144/86	0.9005
Mean age of recipient at transplantation (years)	47.2±13.0	44.5±13.9	0.1043
Mean transplant number	1.26±0.6	1.20±0.6	0.4111
Ethnicity			
Asian	22 (23%)	15 (6.5%)	<0.0001
Black	25 (26.1%)	5 (2.2%)	<0.0001
Caucasian	32 (33.3%)	182 (79.1%)	<0.0001
Other	6 (6.3%)	8 (3.5%)	0.3672
Unknown	11 (11.5%)	20 (8.7%)	0.4168
Mean tacrolimus concentration (ng/mL)	7.8±2.4	8.3±2.6	0.1066
Mean tacrolimus dose (mg)	9.3±4.2	5.2±2.7	<0.0001
Mean tacrolimus oral clearance (ng/ml per mg)	1.1±0.7	1.9±1.0	<0.0001

The intra-patient variability of tacrolimus concentration was calculated as described in Borra et al.
The patients were divided into low and high intra-individual variability groups using the median variability of tacrolimus clearance as the cut-off value.
Statistical analysis was performed using, as appropriate, the Chi square, Fisher's exact test or Student's T test. All values shown are means ± SD, unless otherwise stated.
Results: The baseline characteristics of the 326 patients are shown in Table 1. No differences were observed between the expressers (n=96) and non-expressers (n=230) except for ethnicity, which is in line with previous observations.
Tacrolimus dose requirement was significantly higher in patients expressing *CYP3A5*, confirming earlier observations ($p<0.0001$; Table 1). However, intra-individual variability of tacrolimus clearance was not related to *CYP3A5* genotype (p=0.3331; Table 2).

Table 2. Intra-patient variability of tacrolimus clearance and CYP3A5 genotype (n=326, median variability = 15)

	Low variability	High variability
CYP3A5 non-expressers (*3/*3)	117 (73.1%)	113 (68.1%)
CYP3A5 expressers (*1/*1 or *1/*3)	43 (26.9%)	53 (31.9%)
		p = 0.3331

Conclusion: The intra-patient variability of tacrolimus clearance is not associated with *CYP3A5* genotype in stable renal transplant recipients.

O-287 THE EFFECT OF CYP3A5 POLYMORPHISMS ON LONG TERM TACROLIMUS DISPOSITION IN RENAL RECIPIENTS

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Introduction: Tacrolimus is characterized by a narrow therapeutic index and a significant variability in dose requirements. The biological activity of CYP3A5 plays an important role in its metabolism.
The aim of this study was to measure the effects of the SNP CYP3A5 polymorphism on tacrolimus dose (mg/kg body weight) and Dose-adjusted trough blood levels (ng/ml per mg/kg body weight) on both early and late stages of Tacrolimus exposure in renal transplant patients.
Material and Methods: 34 renal allograft recipients were genotyped. (15 female,19 male,mean age 47.70).Polymorphism was correlated with the Tacrolimus dose, and dose-adjusted trough levels at week (w) 1,4,12,26 and 52 and 104 after transplantation.
Data are expressed as mean±DS. Genotype groups were compared by using the Kruskal–Wallis test. The p values <0.05 were considered statiscally significant.
Results: 23 recipients carrying CYP3A5 *3/*3 (67,64%), 8 CYP3A5 *3/*1 (23,52%) and 3 CYP3A5 *1/*1 (8.82%). There were no significant differences in Tacrolimus levels at any group except in the first month (w1:7.27 vs. 5.67 vs. 4.75ng/dl. p = 0.02; w4:8.61 vs 6.75 vs6.6ng/dl p=0.02).
Tacrolimus dose requirements was 3 fold higher in carriers of CYP3A5*1 alleles through the complete study period, whereas it decreased significantly over time in homozygous carriers of CYP3A5*3 alleles.
Dose-adjusted trough blood levels was 5-6 fold higher in carriers of CYP3A5*3 alleles and increased significantly over time specially after the sixth month post-transplantation, whereas it remained constant at recipient carriers of CYP3A5*1 alleles.
Conclusion: CYP3A genotype is a major factor in determining the dose requirement for tacrolimus. Patients with the CYP3A5*3/*3 genotype require less tacrolimus dose to reach target concentrations compared with CYP3A5*1.Tacrolimus dose-corrected exposure increases after renal transplantation through a period of 2 years in patients CYP3A5*3 genotype suggesting a continuous alteration in CYP3A activity.

O-288 COMPARATIVE BIOAVAILABILITY STUDY OF TWO MYCOPHENOLATE MOFETIL FORMULATIONS IN STABLE KIDNEY TRANSPLANT RECIPIENTS

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Background: The primary objective of this study was to compare steady state pharmacokinetics (PK) of mycophenolate mofetil (MMF) - Myfenax® (Teva) and CellCept® (Roche) - in stable kidney transplant recipients (KTR).
Methods: This was an international, multi-centre, randomized, open-label, two-treatment, two-sequence crossover study with a 3-months follow-up. We included adult male and female KTRs at least 12 months post-transplantation with stable renal graft function for at least 3 months. The maintenance treatment consisted of MMF in combination with tacrolimus. The dose of MMF (≥500 mg twice daily) had to be stable with no changes in the immunosuppressive regimen for at least 6 weeks prior to the start of the study. At the end of the two treatment periods 6-hour or 12-hour PK studies of mycophenolic acid (MPA, measured by HPLC) were performed. Tolerability was studied during the PK period and a 3-month follow-up period.
Results: A total of 43 patients (mean age: 50.7±13.5 years; 19 females, 24 males) was randomized. Exclusion of one patient before the first PK study and 1 protocol violation left 41 patients for pharmacokinetic analysis. The PK parameters (mean ± SD) as well as the log-transformed test to reference ratios (T/R) with 90% confidence intervals are shown in the table.

Pharmacokinetic parameters

	Myfenax	CellCept	T/R, % (90% CI)
$AUC_{(0-\tau)}$ (h*µg/mL)	48.3±21.2	49.9±20.8	95.9 (89.9–102,3)
$AUC_{(0-6h)}$ (h*µg/mL)	31.1±15.4	33.5±15.1	92.3 (86.5–98.4)
c_{max} (µg/mL)	14.3±8.3	16.2±10.0	87.3 (78.7–96.8)
c_{min} (µg/mL)	1.57±0.74	1.58±0.78	98.5 (87.7–110.6)
t_{max} (h)	1.34±1.14	1.12±0.75	

The numbers and types of adverse events were not different between the two treatments.
Summary: The steady state pharmacokinetics of MPA are comparable for Myfenax and CellCept in stable kidney transplant recipients, showing bioequivalence with regards to $AUC_{(0-\tau)}$, $AUC_{(0-6h)}$, and C_{min}. C_{max} showed greater variability confirming previous studies with different MPA brands. The safety profiles of the two formulations were comparable.

O-289 12 MONTH FOLLOW UP RESULTS OF AN INITIAL INTENSIFIED DOSING REGIMEN OF ENTERIC-COATED MYCOPHENOLATE SODIUM (EC-MPS) IN DE NOVO RENAL TRANSPLANT RECIPIENTS

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Background: A sufficient mycophenolic acid (MPA) exposure is required for

Abstract O-287 – Table 1. Tacrolimus dose(mg/kg body weight)

	Week 1	Week 4	Week 12	Week 26	Week 52	Week 104
CYP3A5 *3/*3	0.10±0.03	0.10±0.04	0.09±0.03	0.07±0.03	0.07±0.03	0.06±0.03
CYP3A5*1/*3	0.17±0.05	0.18±0.05	0.18±0.04	0.19±0.07	0.17±0.06	0.13±0.55
CYP3A5*1/*1	0.19±0.00	0.27±0.12	0.27±0.12	0.29±0.04	0.33±0.07	0.21±0.02
P	0.0006	0.0005	0.0003	0.0002	0.0003	0.01

Abstract O-287 – Table 2. Dose-adjusted trough blood levels (ng/ml per mg/kg body weight)

	Week 1	Week 4	Week 12	Week 26	Week 52	Week 104
CYP3A5*3/*3	80.54±34.45	98.23±45.63	109.84±45.94	118.98±57.57	138.12±69.64	136±95.86
CYP3A5*1/*3	32.90±12.38	38.47±16.10	38.47±16	47.98±28.86	50.01±21.93	59.6±26.95
CYP3A5*1/*1	20.24±1.41	25.50±7.77	25.50±7.77	18.50±2.12	18.0±8.4	26.94±1.33
P	0.001	0.000	0.000	0.000	0.000	0.005

effective rejection prophylaxis in renal transplant recipients (RTxR). However, approximately half of cyclosporine (CsA)-treated RTxR do not reach sufficient MPA exposure in the first weeks post-transplantation with standard MPA dosing regimens. In the present meta-analysis, data from two 6-month (M) parallel-run studies were pooled to explore the potential benefit of an initially intensified MPA regimen on rejections.

Methods: A total of 441 de novo RTxR were randomized (1:1) to either Intensified (2 weeks 2880mg/d; subsequently 4 weeks 2160mg/d; followed by 1440mg/day) or Standard (1440mg/day) enteric-coated mycophenolate sodium (EC-MPS), with CsA treatment and steroids with or without anti-IL2R induction. Primary endpoint was treatment failure (BPAR, graft loss, death) at M6 post-transplantation. Here we present the efficacy and safety data of 12-M follow-up.

Results: At M6, treatment failure was not significantly different between groups, but the incidence of BPAR was 13.8% vs. 19.3% (p=0.034; Intensified vs. Standard). The M12 follow-up population included 332 patients (160 Intensified and 172 Standard group; ITT population). At M6-12 the incidence of acute rejection (4.4% [intensified] vs. 4.1% [standard]) and the incidence of graft loss or death (0.6% [intensified] vs. 0.6% [standard]) were comparable between the two groups. No cases of death were reported in either of the groups. Renal function (mean GFR; MDRD, 49.1 mL/min/1.73m^2 [intensified] vs. 51.3 mL/min/1.73m^2 [standard]) and safety profile (patients with adverse events 98.6% [intensified] vs. 97.3% [standard]) were also comparable between the two treatment groups.

Conclusion: An initially intensified EC-MPS dosing regimen was associated with significantly lower rate of BPAR at Month 6 as compared to standard regimen, with a comparable efficacy and safety at 12 months.

Liver

O-290 LIVER TRANSPLANTATION FROM CARDIAC DEATH DONATION: CURRENT EXPERIENCE IN A SINGLE CENTRE

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Organ shortage has driven the search for expansion of the liver pool through donation after cardiac death (DCD). DCD is established practice, however, concerns remain regarding graft outcome and biliary complications, particularly cholangiopathy. We present a retrospective outcome analysis of our DCD liver transplant (LTx) program, started in 2001.

Our protocol was designed to clearly identify the period of warm ischaemia, obtain acceptable grafts and optimise post-transplant function. Donor selction criteria included ICU stay less than 5 days unless fed, warm ischemia time less than 30 min. Super rapid retrieval technique was adopted, with dual aorto-portal perfusion. In the first 20 cases only, upper donor age was 60 years with no specific recipient selection. Further experience excluded sclerosing cholangitis and previous abdominal surgery. Early arterial reperfusion was adopted as the preferred surgical technique.

From 2001 to 2010 191 livers were utilised. The number of transplants performed over that time remained the same, with an increasing DCD/DBD LTx ratio reaching 28%.

The tables show donor and recipient characteristics. 17 patients were lost in follow up, 25 died and 37 grafts failed. Median patient follow up was 27.6 months (0-119.6).

Table 1. Donor characteristics

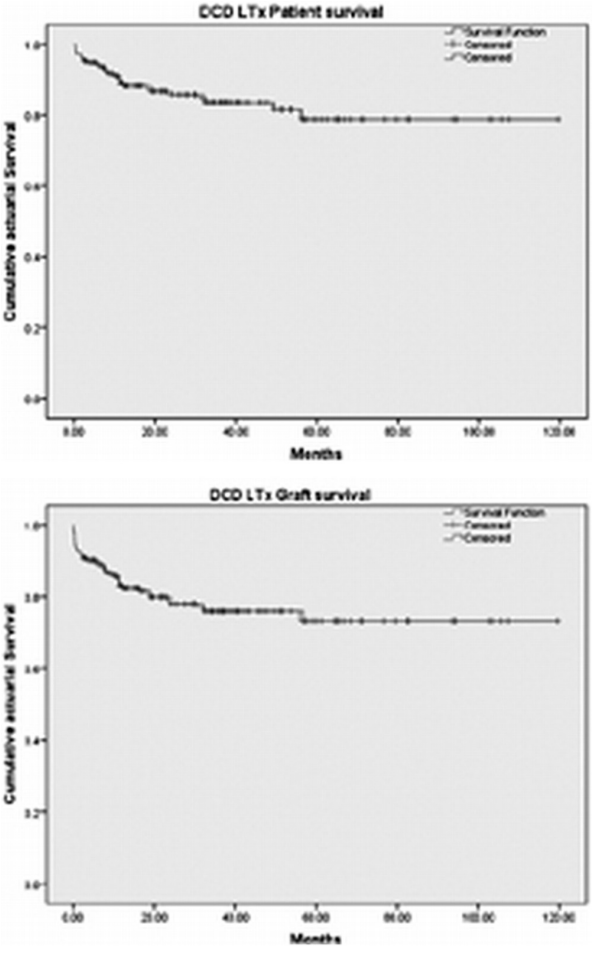
Gender	Female/Male	77 (40.3%) / 114 (59.7%)
Age (years)		42 (10–79)
Elderly donor	< / > 65 yrs	178 (93.2%) / 13 (6.8%)
ICU Days		2 (1–14)
Inotropes	Yes / No	96 (50.3%) / 94 (49.7%)
Cause of death	ICB	89 (46.6%)
	HBI	53 (27.7%)
	NT	43 (22.5%)
	Other	6 (3.1%)
BMI		24 (13–39)
Na+ (mmol/L)		146 (105-170)
AST (IU/L)		38 (8-834)
BIL (umol/L)		10 (3-86)
WIT (minutes)		19.5 (8-72)
Liver macroscopical assessment	Optimal	120 (62.8%)
	Non optimal	55 (28.8%)
	N/A	16 (8.4%)
Liver steatosis	Null/Mild	72 (37.7%)
	Moderate	0
	Severe	0
	N/A	112 (62.3%)

ICU: Intensive Care Unit; BMI: Body Mass Index; WIT: Warm Ischemia time; Liver steatosis: Null/Mild (<30%), Moderate (30–60%), Severe (>60%). Numerical values expressed in Median (range).

Table 2. Recipient pre- and post-transplant characteristics

Gender	Female / Male	54 (28.3%) / 137 (71.7%)
Age (years)		52 (1–73)
Age group	Adult / Paed	171 (89,5%) / 20 (10,5%)
Disease	Toxic	46 (24.1%)
	Viral	57 (29.8%)
	Autoimmune	26 (13.6%)
	Neoplasm	15 (7.9%)
	Congenital cholestatic	9 (4.7%)
	Other	39 (20.4%)
Presentation	ALF / CLD	7 (3.7%) / 184 (96.3%)
CP	A	25 (13.1%)
	B	68 (35.6%)
	C	97 (30.8%)
MELD Score		14 (2–43)
CIT (hours)		7 (2.9–14.3)
GRWR		2.1 (0.9-6.8)
Post AST Peak (IU/L)		1905 (211–21300)
Day 5 INR		1.1 (0.9–5.3)
Day 5 BIL (umol/L)		57 (10–719)
LOS (days)		18 (6-163)

ALF: Acute Liver Failure; CLD: Chronic Liver Disease; CP: Child Pugh; UNOS: United Network for Organ Sharing; MELD: Model for End stage Liver Disease; CIT: Cold Ischaemia Time; GRWR: Graft/Recipient Weight Ratio; AST: Aspartateaminotransferase; INR: International Normalised Ratio; BIL: Bilirubin; LOS: Length Of Stay. Numerical values expressed in Median (range).



Patient and graft actuarial survival at 3 months, 1 and 5 years were 95.4%, 88.3%, 78.3% and 90.8%, 82.4%, 73.3% respectively. Complications included 7 (3.6%) primary non functions, 6 (3.4%) arterial thromboses, 17 (9.7%) biliary complications, of which 5 (2.7%) were ischaemic cholangiopathies.

DCD LTx represent over one fourth of our current activity. DBD LTx has decreased by a similar amount. We utilise DCD as marginal organs, achieving acceptable outcome in selected patients. The DCD programme has maintained our transplant programme into acceptable results, while the incidence of cholangiopathy appears low despite donors up to 70 years of age.

O-291 A 10 MIN "NO TOUCH TIME" IN NON-HEART BEATING DONATION – IS IT ENOUGH?

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Among others, non heart beating donation (NHBD) is under investigation due to the lack of human donor organs to expand the organ donor pool. The amount of NHBD of the whole organ pool is rising steadily worldwide. The Maastricht criteria (I-V) are commonly used for the characterization of NHBDs and are clearly defined, but accomplishment is more difficult; the cardiac arrest varying from 75 seconds up to 27 min until declaration of death. EUROTRANSPLANT defined 10 min of cardiac arrest equal to brain death.
The aim of this study was to investigate brain activity after different times of cardiac arrest with successful CPR in order to simulate NHBD.
NHBD was simulated in 26 pigs; after opening the chest subcostally, cardiac fibrillation was induced using direct current (9V). The "no touch time" varied from 1 min up to 10 min; then, 30 min of CPR were performed in order to provide sufficient circulation. Brain activity was monitored continuously using EEG and brain stem reflexes were tested by a neurologist.
In all animals which suffered from cardiac fibrillation for at least 6 min, no EEG as well as brain stem activity was detectable. However, in animals which underwent 5 min of "no touch time", all animals showed an isoelectric EEG, but only 75% still showed brain stem activity. Reversibility of isoelectric EEGs was given until 4,5 min of cardiac fibrillation; recovery of brain stem activity was guaranteed until 5 min of cardiac fibrillation for 100%.
The definition of EUROTRANSPLANT (Newsletter 148) for the "no touch time" of 10 min of cardiac arrest seems to be sufficient to guarantee no brain as well as no brain stem activity. Shorter "no touch times" seem to be feasible but ethically not justifiable.

O-292 IMPACT OF ACUTE RENAL DISEASE DEFINED BY RIFLE CRITERIA IN LIVING DONOR LIVER TRANSPLANTATION

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Background: Acute renal injury (ARI) is serious complications after living donor liver transplantation (LDLT). The RIFLE criteria have been reported as useful prediction tools which provide a consensus definition for ARI in critically ill patients. The aim of this study was to prove the usefulness of RIFLE criteria in LDLT and to determine the risk factors for ARI after LDLT.
Methods: Using the RIFLE criteria, patients were categorized as risk (R), injury (I), failure (F), and loss (L) according to their renal function after transplantation. We retrospectively analyzed 200 consective adult LDLT patients. The risk factors for severe ARI were determined by multivariate analysis.
Results: According to the RIFLE criteria, ARI occurred in 60%: the R-class was 23.5%, the I-class was 21%, the F-class was 15%, and the L-class was 1%. Concerning the post-transplant prognosis, 1-year survival rates were 97.5% in non-ARI patients, 91.5% in the R-class, 90.5% in the I-class, 53.3% in the F-class, and 0% in the L-class. In multivariate analysis, the risk factors for the occurrence of severe ARI (F/L-classes) were determined as GW/RBW $<$ 0.8% (Odds 2.33, p=0.047), blood loss $>$ 50ml/kg (Odds 3.71, p=0.007), the use of mycophenolate mofetil (Odds 0.357, p=0.027), preoperative diabetes (Odds 5.01, p=0.005). Mild ARI (R/I-classes) didn't affect the post-transplant prognosis; the hospital mortality after LDLT in the R and I classes was 2.3% combined, compared with 37.1% in the F and L classes combined ($P<0.0001$). Seven patients (21%) in the F-class developed chronic kidney disease (Stage-3/4 under the K/DIGO guideline).
Conclusion: We concluded that the RIFLE criteria are useful predictive tools after LDLT. Mild ARI, defined as R/I-classes, was not linked a poor outcome. Also, the RIFLE criteria can stratify severe ARI which has a risk of early post-transplant death and CKD occurrence.

O-293 MONITORING LIVER-GRAFT DRUG METABOLZING CAPACITY USING PERIPHERAL BLOOD SAMPLE. CYP-PHENOTYPE AND GENOTYPE FREQUENCIES OF CYP2C9, CYP2C19, CYP3A4 AND CYP3A5

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Drug-metabolizing capacity of the liver depends on levels and activities of cytochrome P450 enzymes (CYP). Liver injury increase the morbidity and mortality rate after liver surgery or transplantation. Inter-individual variations in CYP genes result in differences in drug metabolism.
Metabolomic and transcriptomic tools were used for CYP-phenotyping, based on the fact that strong correlation exists between CYP enzyme activities in the liver and expression at mRNA level in leucocytes. For CYP-genotyping genomic DNA was extracted from the leukocytes or from the liver tissues of the donors and genotype analysis for single nucleotide polymorphism (SNPs) was performed by PCR.
Phenotyping 105 liver donors, the incidence of transplanted poor metabolizer liver grafts was up to 37%. CYP gene expression in the donor leucocytes presented poor metabolism in 37% for CYP3A4 and CYP2C9, in 13% for CYP2C19. Intermediate and extensive metabolizers were documented too. Biopsy showed drug toxicity in only 48%. Permanent "poor metabolism" for CYP2C9, CYP2C19 and CYP3A5 was attempted to be estimated by CYP-genotyping of 102 liver donors. In this donor group, 13.7% carried one, and 0.98% carried two CYP2C9*2 mutated alleles, while 12.7% carried one, no donors carried two CYP2C9*3 mutated alleles. Furthermore, 33.3% of the donors were found to be heterozygous and 2% homozygous for the CYP2C19*2, while no CYP2C19*3 was detected, 10.78% carried one and all the others 89.22% carried two CYP3A5*3 mutated alleles. No homozygous wild types were detected for CYP3A5 gene. Frequencies were similar to those of other Caucasian populations.
In conclusion, prospective non-invasive investigation of CYP status of the liver together with individual medication can be beneficial for liver and kidney function or encephalopathy.

O-294 BEYOND THE MILAN CRITERIA: WHAT RISKS FOR PATIENTS WITH HEPATOCELLULAR CARCINOMA PROGRESSION BEFORE LIVER TRANSPLANTATION?

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Background: To date the selection of the best candidates for Liver Transplantation (LT) due to Hepatocellular Carcinoma (HCC) has been mainly based on tumor morphological characteristics (nodule diameter and number), which have resulted to be independent risk-factors for short long-term survival and a high rate of tumor recurrence.
Methods: The study cohort included 118 patients among the 166 with HCC transplanted at our unit from January 2000 to December 2007. Patients were classified according to response to Loco-Regional Treatments (LRT) before LT: progressive Group-A; complete Group-B; partial Group-C; stable Group-D.
Results: The 3- and 5-year overall survival rates were 65.5% and 48.9% for group-A vs 84.8% and 74.6% for Group-BCD (p= 0.01). The 3- and 5-year disease-free survival rates were 74% and 74% for Group-A and 95.7% and 93% for groups BCD (p= 0.007). HCC progression was the only independent risk factor according to Cox-regression p= 0.014 - OR 4.4 (1.35-14.3).
Conclusion: Following aggressive HCC treatment before LT, imaging progression while on the waiting list was a strong predictor of high HCC recurrence rate also in patients who met the Milan criteria. Lack of imaging progression can contribute towards the selection of good transplant candidates for HCC together with the Milan criteria.

O-295 HISTOLOGICAL EXAMINATION OF THE DONOR LARGE BILE DUCTS ISOLATED AFTER REPERFUSION DURING LIVER TRANSPLANTATION

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Background: Biliary complications such as anastomotic and non-anastomotic strictures (ITBL) are of major concern after liver transplantation (LT) leading to long-standing morbidity and graft loss. Regarding pathogenesis, ischemia, epithelial alteration by inadequate bile duct flushing and inadequate preservation of the bile ducts has been discussed among other factors.

Methods: We have analyzed donor bile ducts taken 1 hour after portal and arterial graft recirculation in order to assess bile duct damage caused by preservation and recirculation. Bile duct tissue specimens of 36 donors were fixed in PBS-buffered formalin and processed according to standard protocols.
Results: Loss of epithelium of the bile duct being complete in 17% was a common feature in all specimens. The majority of cases (87%) showed diffuse transmural bleeding of the bile. Inflammation was generally only sparsely detected. The most remarkable alterations were observed in the arterioles: In 36% of cases, we found damage of the endothelial lining characterized by loss of endothelial cells and sub-endothelial edema. Additionally, 47% of the specimens revealed variable numbers of necrotic arterioles. In these patients, necrotic walls of the bile ducts occurred (with a total number of 20 donors). Vessels with thrombi could be detected in 42% of the specimens.
Conclusions: To our knowledge, this is the first study analyzing the histology of donor bile ducts immediately after recirculation during LT. The most prominent finding was a remarkable vascular damage leading to arteriolonecrosis. Further studies should elucidate if these lesions are caused by inadequate preservation or by natural variations of the blood supply to the bile ducts.

Wednesday, 7 September 2011

Non-renal DCD / ECD

O-296 SEPTUAGENARIAN AND OCTAGENARIAN DONORS PROVIDE EXCELLENT LIVER GRAFTS FOR TRANSPLANTATION

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Background: Wider utilization of liver grafts from donors ≥70 yo could substantially expand the donor pool, but their use remains limited by fear of their poor outcome. We examined the results of liver transplantation (LTx) using livers from donors ≥70 yo at our center.
Methods: From February 2003 to August 2010, 450 LTx were performed. Of those 58 (13%) were performed using donors ≥70 yo. Their outcome was compared to that of LTx using donors <70 yo.
Results: Cerebrovascular causes of death predominated in donors ≥70 yo (85% versus 47% in donors <70 yo) (p<0,001) whereas traumatic causes of death predominated in donors <70 yo (36% versus 14% in donors ≥70 yo) (p=0,002). Unlike grafts/donors <70 yo, older grafts/donors had no additional risk factor (steatosis, high sodium, hemodynamic instability). Both groups were comparable for cold and warm ischemia times. No difference was noted in posttransplant peak transaminase, incidence of primary non-function, hepatic artery thrombosis, biliary strictures, and retransplantation between both groups. The 1 and 5-year patient survival were 90% and 80% in recipients of livers <70 yo versus 88% and 80% in recipients of livers ≥70 yo (p=0,74). Recipients of older grafts were 9 years older than recipients of younger grafts (p<0,001) and tended to have a lower laboratory MELD score (p=0,074).
Conclusion: Short and middle-term survival following LTx with donors ≥70 yo can be excellent providing that donors and recipients are adequately selected. Septuagenarian and octogenarian victims of cerebrovascular ischemia and bleeding represent a large pool of potential donors whose wider use could substantially reduce the mortality on the waiting list.

O-297 APPLICABILITY OF DONATION AFTER CARDIAC DEATH LIVER TRANSPLANT USING MAASTRICHT TYPE 2 DONORS

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Unlike Maastricht type 3 donation after cardiac death (DCD) donors, in whom cardiac arrest (CA) is induced by the removal of life support, type 2 donors arrest unexpectedly, typically outside the hospital. These donors have significant potential to expand the donor pool (US Institute of Medicine, 2006).
Aim: Analyze the results of type 2 DCD livers used for transplant as well as all potential liver donors treated under our center's type 2 DCD protocol.
Methods: Cardiac arrest was extrahospitalary. Potential donors arrived with cardiorespiratory support (CRS). Death was declared and femoral vessels canulated to establish normothermic extracorporeal membrane oxygenation (NECMO), which was maintained until organ recovery.
Results: From 4/02 to 12/10, there were 400 activations of our type 2 DCD protocol; 34 liver transplants were performed (9%). Causes for rejecting a type 2 DCD liver were classified as absolute or relative, the latter including a prolonged phase (CA >15 min, CRS >150 min, NECMO > 4 hours), high AST/ALT during NECMO, and poor macroscopic liver aspect at recovery. Overall, 130 livers (33%) were turned down due to relative contraindications.
Among transplanted livers, median follow-up was 24 months (range 0-111). One-year graft and patient survival rates were 71% and 82%, respectively. Ischemic cholangiopathy developed in three patients (8%), who were retransplanted at 5, 8, and 13 months.
Comment: This is largest series of type 2 DCD liver transplants to date. Based on protocol activations, the applicability of type 2 DCD liver transplant was less than 10%. Thirty three percent of livers were turned down based on relative contraindications. It is possible that with better means of preservation *ex vivo*, such as normothermic machine perfusion, we may be able to improve the viability of these grafts and improve the applicability of this procedure.

O-298 LIVER TRANSPLANTATION FROM DONATION AFTER CARDIOCIRCULATORY DEATH (DCD) DONORS: BELGIAN EXPERIENCE 2003-2009

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Introduction: The Belgian experience with DCD liver transplantation (LT) was retrospectively updated with the aim to evaluate patient and graft survivals, and biliary complications.
Patients and methods: From 2003 to 2009, 111 DCD-LTs have been performed in Belgium. The characteristics of donors, recipients, transplantation procedure and outcomes were retrospectively reviewed.
Results: Mean donor age was 47.6±15.5 years (range: 13-79). Donor causes of death were anoxia (26.1%), head trauma (31.5%), stroke (36%) and euthanasia (5.4%). Mean duration of treatment withdrawal to aortic cold perfusion was 24.4±13 min, mean cold ischemia time (CIT) was 367.3±128.9 min. Mean recipient age was 55.9±11.2 years (range: 10-73). The most frequent indications for LT were cirrhosis (49.5%) and hepatocellular carcinoma (39.6%). The rate of primary non function was 4.5%. Overall patient and graft survival was 88% and 80% at 1 year, 75% and 65% at 3 years, respectively. Thirty-seven patients (33.3%) developed biliary complications with the need for endoscopic or surgical management in twenty-eight and retransplantation in seven. In univariate analysis, HU indication, younger donor age, elevated donor bilirubin level, absence of heparin administration to the donor, CIT, secondary WIT (liver implantation), were significantly (p<0.05) associated with transplant failure. Risk factors for biliary complications were short donor ICU stay, elevated donor bilirubin level, long duration between switch off to cold perfusion, long CIT, while local allocation decreased the risk. In multivariate analysis, elevated donor bilirubin level and duration of donor hepatectomy were associated with transplant failure while higher donor bilirubin level, CIT and Meld score were associated with occurrence of biliary complications.
Conclusions: In an era of organ shortage, DCD transplantation is a valuable treatment option with an adequate selection of donors and recipients.

O-299 ISCHEMIC CHOLANGIOPATHY IN LIVER TRANSPLANTATION USING DONATION AFTER CARDIAC DEATH DONORS: ANALYSIS OF A MATCHED CONTROL STUDY IN A SINGLE LARGE VOLUME CENTER

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Background: Shortage of available organs is a limiting factor in liver transplantation (LT). The use of donors after cardiac death (DCD) offers potentials to increase the organ pool. The early results with DCD liver grafts were associated with a greater incidence of non-anastomotic biliary complications, leading to several programs to abandoning this source of organs. The UNOS data

also raise concerns regarding DCD-LT results and the incidence of cholangiopathy.
Aim: To assess the impact of cholangiopathy and biliary complications in DCD recipients at a single institution.
Methods: A retrospective analysis from April 2001 to 2010 was undertaken focusing on 167 consecutive DCD-LT. Each DCD transplant was matched with two DBD (brain death donors) grafts (n= 333) according to the period of transplantation. Primary outcome measures were biliary complications and ischemic cholangiopathy including the severity of complications, graft survival and patient survival.
Results: The most common type among biliary complication was anastomotic stricture (DCD= 30, 19% vs. DBD= 41, 13%). Most of them were treated endocoscopically (grade IIIa = 72%), while hepatico-jejunostomy (grade IIIb) was performed in 22%. Primary ischemic cholangiopathy occurred in 4 (2.5%) recipients from the DCD group, while such complication were absent in the DBD group (p=0.005). However, none of these patients required re-transplantation. Patient and graft survival at 1-, 3- and 5- years were similar between DCD and DBD groups (p=0.106, p=0.138, p=0.113 respectively).
Conclusions: In contrast to previous reports, the incidence of ischemic cholangiopathy in DCD recipients was low, and has had no impact on graft or patient survival to-date. These encouraging results of DCD-LT are likely due to a stringent selection of DCD grafts and clear definition of warm ischemia.

O-300 AGGRESSIVE LUNG DONOR MANAGEMENT INCREASES GRAFT PROCUREMENT WITHOUT INCREASING THE RISK OF RENAL GRAFT LOSS AFTER KIDNEY TRANSPLANTATION

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Background: To determine the impact of an aggressive protocol on the rate of lung grafts available for transplant. We also analyzed the impact of this management on kidney graft survival and on the development of delayed graft function (DGF) after kidney transplantation.
Methods: A cohort study. Lung donors and kidney recipients from January 2009 to December 2010 were considered the prospective cohort with 2003-2008 as the historical control period. The number of lungs available for transplantation and kidney graft survival and the appearance of DGF (in recipients) were the main outcome measures.
Results: We quadrupled the number of lung donors in the period 2009-2010 compared with the historical control. In this period lungs were procured from 53.2% of all organ donors. Lung donors in the prospective cohort were older than those in the historic control (p=0.001). Management in the prospective cohort included higher use of positive end-expiratory pressure (PEEP) ($p<0.0001$), increased use of hormonal resuscitation therapy (HRT) ($p<0.0001$), and lower level of central venous pressure (p=0.05) than historical control.
Kidneys transplanted in 2009-2010 were obtained from older donors ($p< 0.0001$), with a greater rate of diabetes (p=0.024) and hypertension (p=0.045) than in the historical control.
The probability of renal graft survival at 1 year after transplant was 88.6% (CI 95%: 74.8-95.1) in historical control and 94.7% (CI 95%: 81-98.7%) in the prospective cohort (p=0.226). There were no significant differences in the rate of DGF in both groups (p=0.108)
Conclusions: Aggressive management strategy in potential lung donors, which includes ventilator recruitment maneuvers, PEEP $\geq$8 cm H_2O, the use of HRT and restrictive fluid balance increases the rate of lung grafts available for transplant negatively impacting on neither kidney graft survival nor DGF development.
This work was supported, in part, by the Fundacion Marques de Valdecilla-IFIMAV.

O-301 EXTRA CORPOREAL MEMBERANE OXYGENATION (ECMO) VS COLD PRESERVATION (CP) IN PORCINE DONATION AFTER CARDIAC DEATH (DCD) CATEGORY-2 DONOR LIVERS

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Aims: We aimed to compare the effects of ECMO on liver viability, with those from CP method of intra-vascular and -peritoneal cooling.
Methods: 11 landrace pigs were studied as 2 groups; ECMO (n=5) and CP (n=6). Under general anaesthesia, all animals underwent laparotomy, cannulation of great vessels, placement of microdialysis catheters, followed by abdominal closure, euthanasia and thrombolysis after 30mins warm ischaemia. In CP group, a peritoneal cooling circuit was established for continuous aortic infusion of cold HTK solution (2 hours). In ECMO circuit, normothermic, oxygenated, autologous blood was perfused within the aorta (2 hours). Liver was retrieved, cold stored and re-perfused (2 hours) on another ex-vivo oxygenation circuit using mixture of autologous blood and RS-I solution. Multiple readings and samples were taken to assess liver viability. Tissue biopsies were analysed using a semi-quantitative. ANOVA, Mann-Whitney U and Paired t-tests were used to analyse the results, as appropriate.
Results: Preservation phase: tissue lactate was significantly higher at 2hrs in the ECMO group (p=0.034). Lactate pyruvate ratio was significantly lower in ECMO at 1hr (p=.014); trends continuing to be better at 2 hrs.
Histologically, no significant damage was noted in either of the groups (p=ns).
Re-perfusion phase: bile production increased in the ECMO group (p=0.0240). Trends in AST levels were higher in the CP group (p=ns). No differences were found in the oxygen consumption, weight gain, lactate, Albumin, ALT and Factor VII levels.
Histologically, Liver parenchyma in ECMO group was significantly better preserved (p=0.016) with extensive damage consistently noted in CP group such as shrunken nuclei andsinusoidal dilatation.
Conclusion: In our experiments, ECMO preserved DCD livers suffered much lesser histological damage and were biochemically superior. Therefore ECMO is probably better in preserving the tissue viability, in comparison to the CP method.

Clinical immunosuppression

O-302 MECANO: MYCOPHENOLATE SODIUM VS EVEROLIMUS OR CICLOSPORIN WITH ALLOGRAFT NEPHROPATHY AS OUTCOME STUDY: CLINICAL RESULTS

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In a prospective, open, randomized, multicenter study comparing the effects of everolimus versus mycophenolate sodium or ciclosporin as maintenance therapy in renal allograft recipients, we investigated the effects of early withdrawal from a triple maintenance immunosuppressive regimen consisting of prednisolone (P), cyclosporine (CY) and mycophenolate sodium (MPS) following an IL2R blocker. 367 consecutive renal transplant recipients were included. Exposure to immunosuppressive drugs was monitored. At 6 months patients were randomized in the absence of subclinical rejection (SCR) in a protocol biopsy into one of three study groups. Group 1: CY and P, MPS was withdrawn. Group 2: MPS and P, CY was withdrawn. In group 3 both CY and MPS were withdrawn and substituted for everolimus (EVL). At 6 months 273 patients underwent biopsy of which 49 had SCR. The remaining 224 patients were randomized to one of the 3 study arms. In group 1: 8/89 = 9% had a rejection episode. In group 2: 8 rejection episodes occurred when only 39 patients were included (22%). This arm was stopped. In Group 3: only 3/96 patients suffered from a rejection episode (2,8%). There was a significant higher number of adverse events in the patients treated with EVL necessitating cessation of EVL and return to other maintenance immunosuppressive therapy. Renal function in group 3 was superior (serum creat. group 3: 121 versus 139 μmol/L in group 1: $P < 0.02$ Wilcoxon test) compared to group 1.
Conclusion: Early overnight conversion to EVL and steroid duo therapy resulted in a low late rejection rate, no graft loss and better renal function at 2 years, however this comes at the expense of a higher amount of adverse events.

O-303 A HIGHER DECREASE IN TACROLIMUS TROUGH BLOOD LEVELS FOLLOWING TWICE-DAILY TO ONCE-DAILY TACROLIMUS CONVERSION OCCURS IN STEROID FREE RENAL ALLOGRAFT RECIPIENTS

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Background: Conversion from the classical twice-daily tacrolimus (Prograf®) to the new modified-release once-daily formulation (Advagraf®) produces a decrease in tacrolimus trough blood levels. Steroid withdrawal in kidney transplantation (KT) is also associated to a lower tacrolimus exposition for a decrease in hepatic and bowel CYP3AP glycoprotein induction. The possible distinct impact of the absence of steroids in the treatment of KT recipients converted from Prograf to Advagraf has not previously been explored.
Methods: We have assessed safety of this 1:1 conversion in 133 KT recipients with eGFR >20 ml/min converted to the once daily formulation >6 mo post-KT. 37 were on steroids (36 on stable prednisone over 2.5 mg/day, 1 withdrawing) and 96 were not.
Results: We did not find statistically significant differences in demographics between these two subgroups, but there were more retransplant patients (27.8 vs 11.4%) and worse renal function in the group on prednisone (SCr 1,92±0.50 vs 1.58±0.36 mg/dl, eGFR-MDRD 39,7±20.1 vs 48±13.3 ml/min and proteinuria 460±604 vs 258±409 mg/day). During follow-up after conversion, we did not find significant changes in renal function or in tacrolimus relative dose (tacrolimus mg per kg of body weight). But we detected a clinically significant reduction in tacrolimus blood through level (11.5% at 6 months and 13% at 12 months in the whole population). This reduction was more important in patients on steroids (17-19%) than in those without steroids (8-9%), as shown in Table 1.

Tacrolimus dose and through levels in renal allograf recipients with and without steroid treatment

All mean (SD)	With Steroids (n=36)	Without Steroids (n=96)
Baseline Tacrolimus dose (mg/kg/day)	0,061 (0,041)	0,054 (0,04)
Tac dose at 2 weeks	0,061 (0,042)	0,055 (0,04)
Tac dose at 3 months	0,065 (0,044)	0,057 (0,04)
Tac dose at 6 months	0,064 (0,042)	0,057 (0,04)
Tac dose at 12 months	0,070 (0,054)	0,055 (0,04)
Baseline Tacrolimus through level (ng/ml)	10,07 (4,11)	9,62 (3,10)
Tac through level at 2 weeks	8,10 (4,39)	8,10 (2,50)
Tac through level at 3 months	7,82 (2,33)	7,93 (2,59)
Tac through level at 6 months	8,38 (4,02)	8,83 (2,36)
Tac through level at 12 months	8,13 (2,79)	8,77 (2,47)

Tac: tacrolimus.

Conclusions: Conversion from Prograf to Advagraf is followed by a decrease in tacrolimus through blood levels, more important (up to 20%), in patients receiving steroids than in those without steroids. It is important to consider this information when changing between the two tacrolimus formulations in KT.

O-304 STEROID FREE DESENSITIZATION IN LIVING DONOR KIDNEY TRANSPLANTATION: A CASE CONTROL STUDY

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Introduction: The presence of anti-donor antibodies has been a contraindication to rapid steroid withdrawal (5 days) in renal transplantation. Steroids cannot prevent/reverse antibody mediated rejection, yet they are included in immunosuppressant strategies for desensitized renal transplant recipients. This is a case control study comparing post-transplant outcomes of steroid-free desensitized living donor kidney transplant recipients to a matched group of standard steroid free living donor recipients.
Methods: A 1:2 case:control match was completed from recipient records from 2005-2008: recipient age ±5 years, donor age ±5 years, race, primary diagnosis, and previous transplant. Rapid steroid withdrawal with Thymoglobulin (6mg/kg) induction was used for all recipients. Anti-donor antibodies were defined pretransplant using luminex testing. Patients with complement-dependent cytotoxicity (CDC) positive crossmatches were desensitized with plasmapheresis (PP) and low-dose (10g) IVIG. Flow cytometric crossmatch (FCXM) positive recipients with a negative CDC crossmatch received high-dose (2g/kg) IVIG prior to transplant. Rituximab (150 mg/m^2) was given POD 1.
Results: Follow up was 31 months (range 12-84). Demographics were similar. See table for outcomes.
Conclusions: Rapid steroid withdrawal is safe in living donor renal transplant recipients undergoing desensitization. Steroid free desensitized recipients have similar 1 year graft/patient survival and renal function compared to non-desensitized living donor controls. The higher rate of rejection in the desensitized group was able to be overcome without the need for chronic corticosteroids. Literature controls for desensitized living donor recipients *receiving steroids* have 1-year graft survival of 94% and rejection rate of 50%.

	Living donor steroid-free Desensitization	Living donor non-sensitized Control	p-value
NUMBER (1:2 match)	37	74	
Initial immunology			
Panel reactive antibody I (PRA I)	34	0	<0.001
Panel reactive antibody II (PRA II)	22	0	<0.001
Human leukocyte antigen (HLA) mismatch	3	3	0.16
Median fluorescence intensity (MFI)	9750	N/A	
Outcomes			
Any rejection, 1-year (patients)	49%	11%	<0.001
Antibody mediated (episodes)	10 /22 (45%)	8 / 10 (80%)	
Cell-mediated (episodes)	7 / 22 (32%)	0 / 10 (0%)	
Both (episodes)	5 / 22 (23%)	2 / 10 (20%)	
Survival, 1-year			
Graft	95%	99%	0.21
Patient	97%	100%	0.15
Glomerular filtration rate, 1-year	76	78	0.64

O-305 LOBSTER – LIVER OBSERVATIONAL STUDY TO ASSESS THE EFFECT OF MYCOPHENOLATE MOFETIL ON RENAL FUNCTION IN LIVER TRANSPLANT PATIENTS

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Introduction: The benefit of a MMF (CellCept®)-based regimen with respect to renal function has been shown by several clinical trials following liver transplantation (LT). However, little is known about the evolution of renal function under every-day conditions where recipients may start MMF at various time points after LT.
Methods: Non-interventional study of patients who start MMF de novo or at any time point after LT. Reflecting the time after LT, four strata were analyzed: start of MMF up to day 6 (group A), between day 7 and day 30 (group B), from day 31 up to 1 year (group C) and more than 1 year after LT (group D). Calculated GFR (abbreviated MDRD) was measured at start of MMF and at 3, 6, and 12 months thereafter.
Results: 543 patients (pats) were enrolled in 32 centers. At start of MMF median GFR was 73, 82, 63, 54 ml/min/1,73m^2 in groups A, B, C and D. In group A and B median GFR decreased to 66 ml/min/1,73m^2 at 12 months. Of note, GFR remained stable in group C and increased in group D by 11 ml/min/1,73m^2 12 months after start of MMF. In group C and D CNI exposure was lowered by up to 30%. In contrast, in groups A and B only a slight reduction of CNI exposure was observed. At 12 months MMF was administered at a mean daily dose of 1204, 1363, 1504 and 1578 mg in groups A-D respectivley and was well tolerated throughout the observation period.
Conclusion: These findings demonstrate that the decline of renal function after LT can be reversed by initiating a MMF-based regimen while reducing CNI between one and beyond twelve months post LT.

O-306 INTERLEUKIN 2 RECEPTOR ANTAGONISTS FOR LIVER TRANSPLANT RECIPIENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS OF CONTROLLED STUDIES

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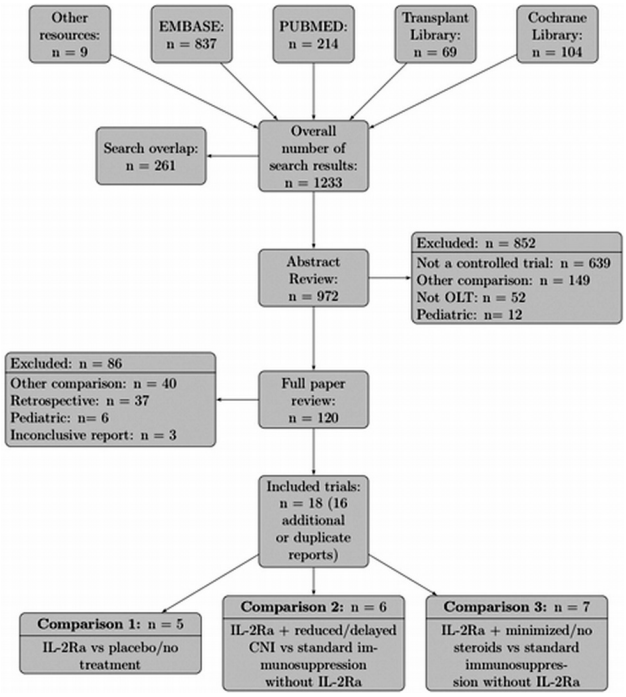
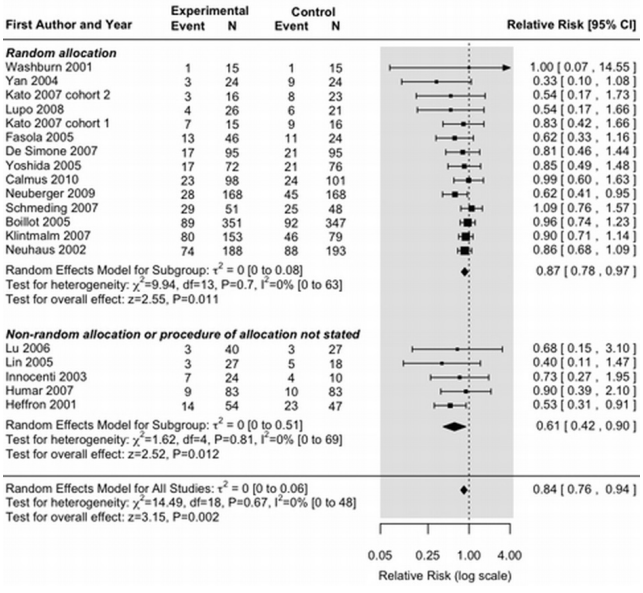
Introduction: Interleukin 2 receptor antagonists (IL-2Ra) are frequently used as induction therapy in liver transplant recipients to decrease the risk of acute rejection while allowing the reduction of concomitant immunosuppression. We conducted a systematic review of prospective, controlled studies to test the hypothesis that the use of IL-2Ra is associated with a decrease in acute rejection and/or a decrease in the side-effects of concomitant medication.

Methods: We performed a search of all major databases and secondary sources from inception to December 2010. Random effects models were used to assess the incidence of acute rejection, graft loss, patient death and adverse side-effects, with or without IL-2Ra. Subgroup analysis and meta-regression were used to explore differences in effect and sources of heterogeneity.
Results: Eighteen studies (13 randomized and 5 non-randomized) met the inclusion and exclusion criteria.



Acute rejection at 12 months or later favored the use of IL-2Ra (relative risk (RR) 0.83; 95%-confidence interval (CI) 0.76 to 0.94) and steroid-resistant rejection was also less frequent in patients receiving IL-2Ra (RR 0.66; CI 0.48 to 0.91). Graft loss and patient death did not differ significantly between treatments. Patients who received IL-2Ra in addition to reduced or delayed calcineurin inhibitors (CNI) had better renal function (mean difference of estimated glomerular filtration rate: 6.29 mL/min; CI 1.66 to 10.91) and a lower incidence of renal dysfunction (RR 0.46; CI 0.27 to 0.78). The use of IL-2Ra was also associated with a lower incidence of post-transplant diabetes mellitus, whereas the incidence of other adverse events was similar.



Conclusion: The use of IL-2Ra is associated with a lower incidence of acute rejection after transplantation. Concomitant immunosuppression can be reduced, avoiding long-term side-effects of immunosuppression.

O-307 LONG-TERM EFFECTS OF ATG INDUCTION THERAPY ON THE HUMORAL IMMUNE RESPONSE

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Background and methods: We showed previously that rabbit ATG induction therapy induces a strong decrease of CD4+ T cells together with impaired in-vitro IL-2 secretion up to 1 year post-transplant. To further characterize long-term immunological effects of ATG induction 2 and 5 years post-transplant, we used sensitive intracellular cytokine analysis in the same prospective study of 84 renal transplant recipients (with ATG, n=44).
Results: 5-year kidney graft outcome was not significantly different between the low-risk non-ATG group and the immunological risk ATG group. This favorable clinical result of ATG-treated patients coincided with comparable levels of the immune parameters sCD30 and neopterin (Neo/Cr) at 1 year, which were associated with immune-mediated graft deterioration or loss within 5 years (logistic regression: p=0.006, sCD30; p=0.064, Neo/Cr). Long-term humoral effects of ATG induction included a profoundly downregulated production of the B-cell growth and differentiation factor IL-10 by CD4 cells at 2 years post-transplant (p=0.004; logistic regression: p=0.054) and a persistent decrease of CD4+ T helper cell counts in peripheral blood even at 5 years post-transplant ($p<0.0005$ versus non-ATG treatment) which was associated with suppressed T-cell dependent B cell responses (p=0.026) but not with suppressed CD4 cell functions. In contrast to non-ATG patients, ATG patients showed no rise in CD19+ B cell counts between 2 and 5 years post-transplant (non-ATG: 192±35 versus 96±13/μl, p=0.001; ATG: 119±25 versus 84±13/μl, p=0.101).
Conclusion: Long-term suppression of humoral responses by ATG induction (profoundly diminished CD4 cell IL-10 production at 2 years, persisting low CD4 cell counts associated with suppressed T-dependent B cell responses even at 5 years, and the absence of a rise in B cells between 2 and 5 years) may provide long-term graft-protective effects which may affect HLA antibody formation.

Histocompatibility

O-308 ALLOSENSITIZATION IN SIMULTANEOUS LIVER-KIDNEY TRANSPLANTS: DO LIVERS EVEN PROTECT THEMSELVES?

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Background: The immunoprotection of liver allografts to simultaneously transplanted organs from the same donor has been investigated in numerous studies and remains controversial. Recent studies suggest that a positive T cell crossmatch (TXM) may have a deleterious effect on liver retransplants and transplants from living donors. Recipients of simultaneous liver/kidney transplants (SLK) often have more detailed PRA and TXM records compared to other liver transplant recipients in the SRTR Registry of the US. In this study we investigate the association between liver allograft survival and the presence of HLA antibodies (PRA%) and TXM results in SLK performed in the US.
Methods: Using data from SRTR pertaining to SLK transplants (1995 and

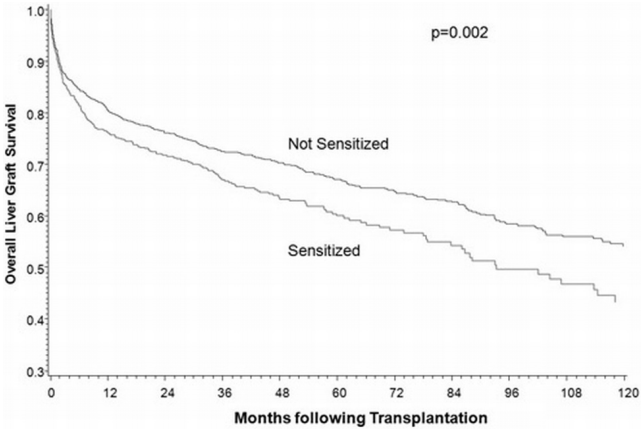

Figure 1. Time to liver graft loss by Sensitization

2008), we examined liver graft and patient survival by indication of sensitization (PRA>10% or positive TXM). We evaluated incidence and outcomes for patients utilizing uni- and multivariable models after adjusting for common risk factors.
Results: Among 2,484 SLK recipients with available PRA or TXM information, 30% had positive TXM or PRA>10%. Among those with TXM information, 12% had a positive crossmatch (n= 234). The time to liver graft loss was statistically significantly associated with sensitization (Figure 1, p=0.002). This association remained consistent after adjusting for potential confounding factors included in the Cox model (AHR=1.21, 95% C.I. 1.04-1.42). Similarly, time to patient death was also independently associated with sensitization (AHR=1.22, 95% C.I. 1.04-1.43)
Conclusion: These results suggest that allosensitization is associated with statistically significant lower liver allograft survival in recipients of simultaneous liver kidney transplants. Although the observed increased risk does not appear to be large enough to discourage the decision to pursue a life saving SLK, it may be particularly relevant in patients with multiple additional risk factors and warrants further investigation.

O-309 IMPACTS OF DONOR-RECIPIENT HLA MATCHING ON OPERATIONAL TOLERANCE FOLLOWING PEDIATRIC LIVING-DONOR LIVER TRANSPLANTATION

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Backgrounds: Although clinical operational tolerance (COT) has been documented after orthotopic liver transplantation (OLT), it remains elusive whether and how donor-recipient HLA matching would impact COT.
Methods: We conducted a prospective cohort study of 134 pediatric primary living-donor OLT that were performed between 1990 and 2008. Haploidentical grafts were implanted in all cases. COT was defined as stable normal graft function for more than 1 year with no use of maintenance immunesuppression (IS). Logistic regression was used to determine independent predictors of COT.
Results: There were 84 COT patients (Gr-Tol) and 50 non COT patients (Gr-Intol) who were unable to stop IS due to rejection. Multivariate logistic regression analysis showed that absence of early rejection within one month after OLT (odds ratio (OR) 2.83, 95% confidence interval (CI) 1.13-7.09, p=0.03), higher average trough level of tacrolimus in the first week after OLT (TAC) (OR 1.10, 95%CI 1.02-1.20, p=0.02), and HLA-B mismatches (OR 8.51, 95%CI 1.05-69.2, p=0.045) were positive independent predictors of COT. HLA-A mismatches (OR 0.18, 95%CI 0.04-0.91, p=0.04) was negative predictors of COT. HLA-DR mismatches or ABO compatibility did not affect COT. In addition, frequency of $CD4^+CD25^{++}IL\text{-}7R^{low+}$ Tregs in the peripheral $CD4^+$ cells at > 10 years post-OLT was significantly lower in HLA-B matched cohort than that in HLA-B mismatch cohort (7.3 vs. 4.7%, p< 0.05) (Figure 1). Such a correlation was not observed between Tregs and HLA-A (Figure 2).
Conclusions: HLA matching was one of determinants for COT in semi-allogeneic pediatric OLT. HLA-B but not HLA-A match was accompanied by down-regulation of the circulating Tregs frequency. The classical concept that HLA matching does not affect outcomes of OLT must be revisited.

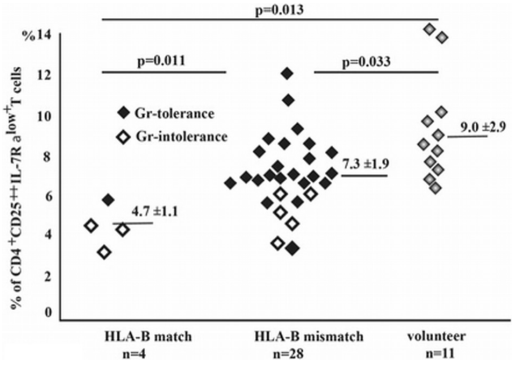


Figure 1

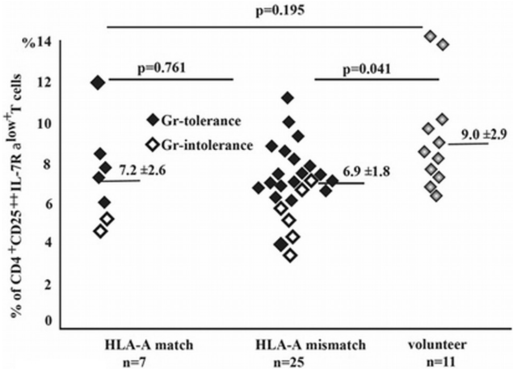
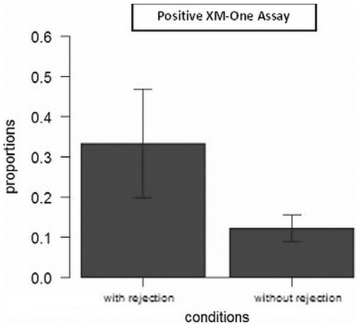


Figure 2

O-310 PRE-TRANSPLANT ENDOTHELIAL CROSSMATCH CORRELATES WITH ACUTE REJECTION EPISODES IN LIVING DONOR KIDNEY TRANSPLANT RECIPIENTS

John Friedewald, Shivani Shah, Charles Jie, Jennifer Wehner, Joseph Leventhal, Anat Tambur. *Comprehensive Transplant Center, Northwestern University, Chicago, USA*

With the goal of more personalized immunosuppression, accurate immunologic risk stratification is important in the care of kidney transplant recipients. We studied 113 consecutive living donor kidney transplant recipients done at our transplant center in 2009 and 2010. Pre-transplant sera were tested using the XM-One (Absorber AB, Stockholm) crossmatch test. The XM-One assay isolates donor endothelial cells and precursors and tests for recipient sera reactivity *in vitro* using a flow cytometry based assay.
Methods: 113 consecutive living donor transplant recipients at our center consented and were tested with the XM-One assay against their donor. Subjects were then followed per our local standard of care. Protocol kidney biopsies are done routinely at 12 months post transplant, and for cause. Our primary outcome was episodes of acute rejection. Secondary endpoints include chronic vascular changes seen on biopsy, proteinuria, and renal allograft function (GFR) which are part of our ongoing analysis of this cohort.
Results: 15 of 113 patients had an episode of acute cellular rejection during the study period (none had humoral rejection). Of those, 5/15 (33%) had a positive pre-transplant XM-One crossmatch, while 12/98 (12.2%) of patients without rejection had a positive XM One assay (difference, p= 0.0252). Only 2 of the 15 patients with rejection were sensitized (by PRA) pre-transplant.



Discussion: Our initial results suggest that kidney transplant recipients with a positive pre-transplant XM-One crossmatch assay have significantly higher rates of acute cellular rejection. These outcomes did not correlate with HLA sensitization pre-transplant. Ongoing analysis is needed to determine the effects on markers of chronic transplant outcomes, including graft and vascular fibrosis, as well as proteinuria and GFR. Further analysis of these chronic endpoints is planned as our cohort matures.

O-311 THE IMPACT OF A VIRTUAL CROSSMATCHING POLICY ON COLD ISCHAEMIC TIMES AND OUTCOME FOLLOWING DECEASED DONOR TRANSPLANTATION: A SINGLE CENTRE EXPERIENCE

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Background: It is possible to proceed with transplantation without awaiting the results of a pre-transplant cross-match (XM) in patients where a negative XM can be predicted from sensitization history and antibody screening, and this is known as a virtual XM (vXM). We assessed the effect of the introduction of a

vXM policy on cold ischaemia times (CIT), delayed graft function (DGF), and transplant survival in a single transplant centre.
Methods: Data from deceased donor kidney transplants between January 2009 and February 2011 were analyzed (n=157). Transplants went ahead with a vXM in 65 (41%) transplants. 126 (80%) of kidneys were donated after brain death (DBD) and 31 were donated after circulatory death (DCD) (20%).
Results: All patients who underwent the transplant with a vXM had a negative cytotoxic crossmatch confirmed the following day. The median CIT was 13.8 hrs with a prospective XM test and 10.5 hrs with a vXM ($p< 0.001$). The DGF rate was significantly lower in those with a vXM in both DBD and DCD recipients (18.5% vs 39.1%, p=0.006). Logistic regression analysis of combined DBD and DCD data, after adjustment for variables that influenced DGF, showed that the vXM was not a predictor of DGF independent of CIT, but DGF was significantly lowered by reduced CIT ($p<0.001$). Omission of the prospective XM test influenced neither acute rejection nor graft survival.
Conclusion: Within a short time period, the introduction of a vXM policy has a significant effect on CIT. This analysis also showed an influence of CIT on DGF in both DBD and DCD recipients.

O-312 RESULTS OF COMPLETELY HLA MISMATCHED LIVING DONOR KIDNEYS ARE AS GOOD AS THOSE OF HLA IDENTICAL DECEASED DONOR KIDNEYS

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Background: HLA mismatch as a categorical variable is known to influence graft survival in deceased donor kidney transplantation. We wondered whether the effect of HLA mismatch would be different in deceased and living donor transplantation.
Methods: All 1821 transplantations performed in our center between 1990 and 2009 were included in the analysis.
Univariate and multivariate Cox proportional hazard analyses were performed. HLA matching was studied in different ways: continuous and categorical total number of mismatches, and zero versus non-zero mismatches. Other variables included were year of transplantation, number of previous transplantations, pre-treatment, maximum PRA, current PRA, recipient gender and age, and donor type, gender and age.
Results: 941 patients received a deceased donor kidney and 880 a living donor kidney. There were 465 failures, 324 in recipients of deceased donor kidneys and 141 in recipients of living donor kidneys.
In multivariate Cox analysis, donor type and age, current PRA, and recipient age were found to have a significant influence on graft failure, censored for death. HLA mismatch had a significant influence in all studied ways. There was no interaction between donor type and HLA mismatch.
Conclusion: HLA mismatch as a continuous variable turns out to have a significant influence on graft survival. This holds true for both deceased and living donor transplantation. However, a 2-2-2 mismatch living donor kidney has a risk for graft failure comparable to a 0-0-0 mismatch deceased donor kidney (Figure 1).

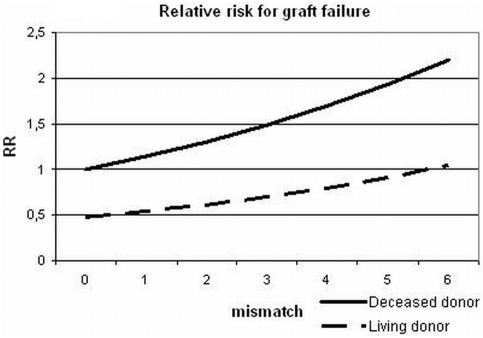
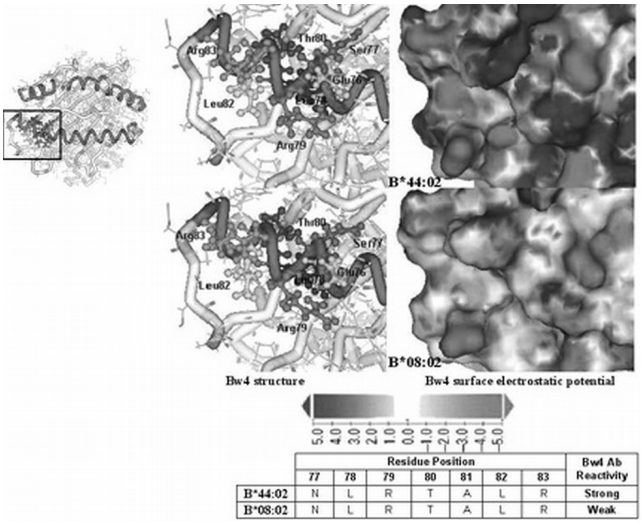


Figure 1

O-313 HIGH RESOLUTION THREE-DIMENSIONAL MODELLING OF HLA CLASS I STRUCTURE AND SURFACE ELECTROSTATIC POTENTIAL REVEALS THE MOLECULAR BASIS FOR ALLOANTIBODY BINDING EPITOPES

Vasilis Kosmoliaptsis[1,2], Timothy R. Dafforn[3], Afzal N. Chaudhry[4], David J. Halsall[5], J. Andrew Bradley[2], Craig J. Taylor[1]. *[1]Tissue Typing Laboratory, University of Cambridge, Addenbrooke's Hospital, Cambridge, [2]Department of Surgery, University of Cambridge, Addenbrooke's Hospital, Cambridge, [3]School of Biosciences, University of Birmingham, Birmingham, [4]Department of Renal Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, [5]Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Cambridge, United Kingdom*

Background: The potential of HLA to stimulate humoral alloimmunity depends on the orientation, accessibility and physiochemical properties of polymorphic amino acids (aa). We have generated high-resolution structural and physiochemical models of all common HLA-class I alleles and analysed the impact of aa polymorphisms on surface electrostatic potential.
Methods: Atomic resolution three-dimensional structural models of HLA-class I molecules were generated using comparative structure prediction (Modeller) based on known crystallographic structures. The electrostatic potential at the surface of the HLA structures was computed by numerically solving the Poisson-Boltzmann equation for macromolecules in a simulated aqueous solution (pH: 7.4, ionic strength: 0.145). To confirm that electrostatic motifs (distinct topographic patterns of electrostatic potential in three dimensional space) reflect known HLA B-cell epitopes, we examined Bw4 and Bw6 and ascertained the impact of aa polymorphisms on their tertiary and physiochemical composition.
Results: The HLA protein structures generated performed well when subjected to stereochemical and energy-based testing for structural integrity. The conformation and electrostatic patterns of Bw4 and Bw6 epitopes are commonly maintained among HLA molecules even when expressed in a different structural context. Importantly, variation in epitope aa composition does not always translate into a different electrostatic motif, providing an explanation for serological cross-reactivity. Conversely, heterogeneous antibody binding to Bw4 epitopes, despite aa sequence identity, can be explained by observed differences at the structural and physiochemical level (figure1). Mutations of critical amino acids that led to abrogation of Bw6-specific antibody binding also induced distinct changes in the Bw6 epitope electrostatic properties.



Conclusion: Serological patterns of HLA-specific antibody binding can be explained by high-resolution structural modelling and electrostatic potential analyses allowing novel insights into HLA immunogenicity.

Paediatric transplantation

O-314 FAMILY STRAIN AFTER PEDIATRIC LIVER TRANSPLANTATION

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Background: Only little is known about the psychosocial adjustment of fami-

lies with a liver transplanted child. In this study we focused on family strain and the association with several transplant-related variables.
Methods/Materials: Parents of 170 liver transplanted children (50.6% girls, aged 8.4±4.6 years) were examined late postoperatively (i.e., 5.9±3.9 years after liver transplantation (Ltx)). The mean age at Ltx was 2.5±3.1 years. 39,4% received an organ from a living-related donor. Assessment included a semi-structured interview and the German version of the Impact on Family Scale.
Results: Regarding the family strain, the results of our sample were comparable with a sample of 271 parents with a chronically ill or disabled child. In the subscale "Burden of the siblings" the present sample showed significantly more strain (t=3.01, p=0.003). Parents who reported financial problems because of the Ltx (35%), and family problems after Ltx (42%) had a significantly higher "Total Score" (r=0.42, p<0.001; resp. r=0.26, p=0.001). A median split regarding time since transplantation (at 5 years postoperatively) showed lower strain in families, which were examined 5 or more years after Ltx in all subscales except in "Problems with Coping" (t=0.45, p=0.66).
Conclusion: The results corroborate our hypotheses that parents of liver transplanted children have to sustain a high family strain. In the subscale "Problems with Coping" parents scored high even 5 or more years after Ltx. However, for long term medical success coping is vitally important, and problems with coping may lead to non-adherence. Our results underline the importance of psychosocial diagnostics and support pre and post Ltx to ensure the best possible adjustment to the chronic condition after pediatric Ltx.

O-315 COGNITIVE FUNCTIONING IN CHILDREN WITH BILIARY ATRESIA AFTER LIVER TRANSPLANTATION

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Purpose: Because different primary diseases might bias cognitive functioning outcomes of liver transplanted children, we exclusively examined children with biliary atresia after transplantation (Ltx). We hypothesized that children are below the population mean.
Method: The sample consisted of 70 liver transplanted children (age at assessment: 8;4±3;5 years, range: 5;0-15;2; age at Ltx: 1;5±2;3 years, range: 0;1-14;8 years). 49% had received a living related donation (LRD). Assessment of cognitive functioning included: K-ABC (5-7 years, n=27) and WISC-III (8-17 years, n=43).
Results: Children scored within the normal range (100±15) but significantly below the population mean in several subscales: K-ABC-Achievement-Scale (AS): 90.5±16.2, t(25)= -3.0, p=0.006; WISC-III-Performance-IQ (PIQ): 86.2±16.0, t(42)=-5.7, p=.000; WISC-Full-Scale-IQ (FIQ): 91.5±17.4; t(42)=-3.2, p=0.003. Between 18.5% (SES) and 51.2% (PIQ) of the children scored below the normal range. Regarding all WISC-III-subscales, children with a LRD performed significantly better than children with a postmortem donation (PMD): LRD-VIQ: 102.4±18.4 vs. PMD-VIQ: 89.8±13.3, t(41)= -2.5, p=0.014; LRD-PIQ: 92.2±17.6 vs. PMD-PIQ: 80.0±11.4, t(41)= -2.7, p=0.010; LRD-FIQ: 97.4±18.6 vs. PMD-FIQ: 85.3±14.0, t(41)= -2.4, p=0.021). LRD was associated with higher maternal educational level (r=0.28, p=0.019). High correlations between the height percentile pre-Ltx and intelligence subscales (r=0.44, p=0.023 to r=0.57, p=0.003) were obtained.
Conclusion: By assessing exclusively children with biliary atresia, we eliminated confounding due to various primary diseases. We confirmed our main hypothesis: Liver transplanted children with biliary atresia revealed more cognitive restraints compared to the norm. Children's preoperative decelerated body height could be regarded as an index of children's decelerated brain growth, which might be relevant to explain the interrelation between height percentile (pre-Ltx) and cognitive functioning after transplantation. However, family socioeconomic status seems to have a mediating role. Our results emphasize the urgent need of routinely performed psychological diagnostics and support to liver transplanted children.

O-316 BEHAVIOUR AND SOCIAL FUNCTIONING AFTER PAEDIATRIC LIVER TRANSPLANTATION

Tanja Kaller[1], Nadine Langguth[1], Rainer Ganschow[2], Björn Nashan[3], Karl-Heinz Schulz[1,3]. *[1]Medical Psychology, [2]Children's Hospital, [3]Transplantation Center, University Hospital Eppendorf, Hamburg, Germany*

Purpose: Liver transplanted children have an increased risk to develop serious developmental problems. Based on previous research, we hypothesized that liver transplanted children show more behavioural problems and poorer social functioning compared to the norm.
Method: The sample consists of 117 children (53% girls, aged 10.3 3.7 years) that completed a behavioural questionnaire late postoperatively (i.e., 8.8 4.3 years after transplantation). The mean age at transplantation (Ltx) was 41.0 46.1 months. Assessment included: behaviour (SDQ, self- and proxy-report) and intelligence (WISC, K-ABC).
Results: Regarding behaviour 77% to 91% of the assessed children scored within the normal range. In the subscales (proxy-report) hyperactivity (t=5.0, p<.001), problems with peers (t=8.7, p<.001), and total sum score (t=3.0, p=.004) results were significantly below the population mean. In the self-report only problems with peers (t=10.4, p<.001) was significantly below population mean. Here 22.7% of the children scored within the borderline or abnormal range compared to 13.3% in the norm population. Moreover, problems with peers was highly correlated with all subscales of the K-ABC (r=-.37, p=.04 to r=-.54, p=.002) and total IQ-score of the WISC (r=-24, p=.04). In semi-structured interviews, parents with children that experience more problems with peers reported more social problems at school (r=.49, p<.001), more problems with teachers (r=.38, p=.004) and more problems with subject materials (r=.38, p=.001).
Conclusion: The results corroborate our hypotheses in parts. Regarding social adjustment, our results provide evidence suggesting that liver transplanted children might be at risk of interpersonal difficulties, i.e. peer problems. Further research is needed to understand the origin of these problems. Interrelations between behaviour and intelligence support the previous findings that comprehensive psychological diagnostics and psychosocial support are necessary to ensure children's social integration.

O-317 LIVER Re-TRANSPLANTATION (LReT) IN CHILDREN – ANALYSIS OF EUROPEAN LIVER TRANSPLANT REGISTRY OVER THREE DECADES

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Background: Improved techniques, perioperative care and immunosuppression have influenced indications and outcome of LReT in children. This ELTR study looked at the LReT data in children over 3 decades.
Methods: P: aediatric (<16.0 years) LReT across the ELTR between 1/1980 to 12/2008 was studied, divided into three eras - Group 1 (1980-1989), Group 2 (1990-1999) and Group 3 (2000-2008); and analyzed for indication for primary graft and LReT, timing of LReT, type of graft (whole/reduced/split) and survival.
Results: LReT constituted 15.8% of total pediatric transplantation. Number of retransplants in Group1 were 119 (16%), 565 in Group 2 (16.8%), and 517 in Group 3 (14.8%). The indication for LReT was rejection-28.6%, 25.3%, and 14.9%; primary non-function-16.8%, 16.8%, and 10.6%; hepatic artery thrombosis, 9.2%, 12.4%, and 18.9%; and biliary complication, 2.5%, 5.1%, and 6.6%, respectively. The median age at LReT was 5years in all three groups. The proportion of whole: partial: reduced graft was 23:1:14.6 (Group 1), 2.3:1:2.2 (Group 2) and 2.2:1.9:1 respectively. Patient survival at 1, 5, and 10-years following regraft was 45%/39%/38% (Group1), 66%/53%/49%(Group 2) and 71%/64% (Group 3) p<0.001. There was no difference in patient survival following LReT against the different graft types used. One year, 5- and 10-years patient survival for those re-transplanted early (<3 months) versus late (>3 months) following primary transplant was 43,39,and 38% versus 54, 41, and 38% (Group 1, p=n.s), 58, 46, and 42% versus 79, 63, and 58% (Group 2, p<0.001), and 66, 61% versus 75, 68% (Group 3, p=0.001).
Conclusion: LReT constitutes about 16% of total paediatric transplantation. Rejection as a cause for retransplantation has reduced over the years, while arterial thrombosis continues to be a common indication. Patient survival has improved over the three eras, p<0.001 independent of the type of graft (whole, partial, or reduced). However late retransplants had better survival compared to those having early regrafts.

O-318 OUTCOME OF EARLY AND LATE LIVER RETRANSPLANTATION IN CHILDREN IN COMPARISON TO PRIMARY TRANSPLANTATION

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Introduction: In case of acute or chronic liver graft failure retransplantation (re-LTX) remains the only option. However, due to the increasing organ shortage and the postulated inferior outcome following re-LTX this procedure is controversial. To date only few data are available regarding re-LTX in children. In this study we analyze outcome following pediatric re-LTX.
Methods: Retrospective review of all pediatric LTX at our institution between 2000-2010.
Results: Overall, 371 children (median age 1.8yrs, range 0-16yrs) underwent

LTX, thereof 294 patients primary LTX (79%) and 77 patients re-LTX (21%; first re-LTX n=56, second re-LTX n=17, third re-LTX n=4). Children were divided into early (≤30d; median 8d, range 1-28d; n=32) and late (>30d; median 2.4yrs, range 0.3-15yrs; n=45) re-LTX. Causes for early re-LTX were primary non function (n=18) and vascular complications (n=16), for late re-LTX chronic rejection (n=34), vascular complications (n=5), and other (n=6). Patient survival was significantly worse for children undergoing early re-LTX compared to primary LTX and late re-LTX (Log Rank test p=0.014 and p=0.02, respectively). In contrast, patient survival for children with primary LTX and late re-LTX was comparable. 1- and 5-year patient survival rates were 96%/94% for primary LTX, 100%/98% for late re-LTX and 87%/81% for early re-LTX. Also, graft survival was significantly worse in children with early re-LTX compared to primary LTX and late re-LTX (Log Rank test p=0.001 and p=0.002, respectively), again comparable in primary LTX and late re-LTX. 1- and 5-year graft survival rates were 86%/77% (primary LTX), 91%/74% (late re-LTX), and 67%/51% (early re-LTX).

Conclusion: We found an excellent graft and patient outcome that was comparable for pediatric primary LTX and late re-LTX, supporting liver retransplantation in case of liver graft failure. Early re-LTX had an increased risk of early graft loss and patient death.

O-319 CAMPATH FOR INDUCTION OF IMMUNOSUPRESSION IN PEDIATRIC KIDNEY TRANSPLANTATION

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Costimulatory pathway (CD28-B7) is considered to play a key role in rejection and is realized mostly through the mesenhimal cells. We supposed that Campath, being infused several weeks pretransplant can affect mesenhimal cells and promote donor-specific tolerance.

Ninety-nine children, aged 10,9±5,2), were transplanted from 2006 to 2009. Patients were followed 841±385 days post transplant. Immunosupression (IS) protocol: First dose of Campath, 30 mg, was infused to live donor kidney recipients 14-27 days prior to transplantation (18.2±2.8). Next dose of Campath was given on Day 0. Maintenance IS was based on CNIs and mycophenolates. Steroids were discontinued after achievement of target CNI level. Protocol biopsies were taken 1 month, 1 and 3 year post transplant.

Graft and patient survival was 95% and 97% for one year and 91% and 94% for two years. Delayed graft function occurred in 12 patients. Biopsy proven acute rejection (BPAR) developed in 18% patients at one year and in 24% at two years, BANFF score: borderline in 59%, 1a in 34%, 1b in 0.5%, 2a in 5% and 2b in 1% of all rejections.

Among survived patients, the change of graft function by comparison of CFR and proteinuria at discharge and at the last control was not significant: 82±23 and 82±32 ml per min; 221±241 and 161±139 mg per day. IS at the last control is follows: CNI-based 61 pts, PSI based 14 pts, MMF based 8 pts. Free of steroids remain 84% of patients.

Conclusion: Preconditioning of children with Campath 1-H 14-27 days before kidney transplantation allows to reach satisfactory short-term results with little maintenance IS and significant proportion of steroid freedom important issue in pediatric population.

O-319A THE ASSOCIATION BETWEEN PRETRANSPLANT SOLUBLE CD40 LIGAND PLASMA LEVELS, THE PRESENCE OF ANTI-HLA ANTIBODIES AND GRAFT DYSFUNCTION IN PEDIATRIC LIVING DONOR LIVER TRANSPLANT RECIPIENTS

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Living-donor liver transplantation (LDLT) is the accepted treatment for pediatric patients with end-stage liver disease (ESLD). Transplant patients who present anti- human leukocyte antigen antibodies (anti-HLA antibodies) in the pre- or posttransplantation period have shown higher incidences of acute and chronic allograft rejection. It was found that soluble CD40 ligand (sCD40L) is a predictor of adverse outcome in heart transplant recipients. The objective of the study was to evaluate the presence of pretransplant anti-HLA antibodies and plasma levels of sCD40L and their association with the occurrence of graft dysfunction in children after LDLT.

Methods: 67 pediatric patients with ESLD, 32 boys and 35 girls, aged 14±6.0 (4-36) months were followed for 2 months after the LDLT. The procedures in recipients included hepatectomy, orthotopic implantation of left lateral sector, biliary reconstruction by hepaticojejunostomy. Plasma concentrations of sCD40L and the presence of anti-HLA antibodies before LDLT were measured by ELISA.

Results: Graft dysfunction at day 26-32 posttransplant, diagnosed by clinical and laboratory criteria, was observed in 15 pediatric patients: in 13 (40.6%) patients with pretransplant sCD40L levels above median (≥3.3 ng/ml) and only in 2 (5.7%) patients with low sCD40L (<3.3 ng/ml). Pretransplant plasma levels of sCD40L were significantly higher in patients with graft dysfunction than in those without graft dysfunction (5.5±1.7 ng/ml vs 3.1±2.8 ng/ml, resp., p<0.01). The presence of anti-HLA antibodies before LDLT was not associated with graft dysfunction in children after LDLT.

There was no correlation for sCD40L with age, gender, liver enzymes, plasma levels of C-reactive protein, interleukin-6, neopterin, and homocysteine.

Conclusion: Elevated pretransplant sCD40L plasma levels, but not the presence of anti-HLA antibodies, are associated with graft dysfunction after living-donor liver transplantation.

Kidney

O-320 POSTTRANSPLANT HLA ANTIBODY OCCURRENCE CORRELATES WITH HUMORAL REJECTION IN PEDIATRIC KIDNEY RECIPIENTS

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The detrimental role of donor-specific HLA antibodies (DSA) on kidney allograft outcome has been extensively evaluated. In recent times, the emerging role of humoral immunity in the pathogenesis of chronic allograft damage, together with the evolution of alloantibody detection assays from cell-based to solid-phase methods, has rekindled the interest in post-transplant alloantibody monitoring, and has allowed identification of patients with low-level *de novo* antibodies. However, the clinical significance of these findings is still unclear. Utilizing a unique source of sera collected pre-transplant, three-monthly in the first year, and annually thereafter, we evaluated 70 consecutive pediatric recipients of first allograft transplanted between 03-2003 and 01-2010 and with a minimum follow-up of 12 months, for *de novo* occurrence of HLA-antibodies by Luminex screening kit and single-antigen analysis. The patients were followed for a median time of 38 months (range 12-94 months). Sixteen patients (23%) developed *de novo* DSA (3 class I only, 10 class II only, and 3 class I + class II; in 13 of 13 cases, class II DSA were directed to DQ) at a median time of 30 months. Six patients (9%) developed *de novo* non-DSA (4 class I only, 2 class II only) at a median time of 12 months. Among the 70 patients, 10 developed acute cellular rejection (ACR) and 7 symptomatic C4d+ humoral rejection (CHR). CHR correlated with *de novo* DSA (44% vs 0% in patients with non-DSA or without de novo HLA Ab). No such correlation was found for ACR. DSA appearance preceded CHR by a median of 2 years. In our cohort of pediatric kidney recipients, DSA monitoring identifies a cohort at risk of CHR; treatment started at the time of antibody emergence might prevent CHR occurrence and/or progression to graft failure.

O-321 EFFICACY OF DESENSITIZATION THERAPY IN LIVING DONOR KIDNEY TRANSPLANT RECIPIENTS WITH LOW-LEVEL DONOR-SPECIFIC SENSITIZATION

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Introduction: Patients with high-level donor-specific HLA-antibodies (HDSA), defined by positive cytotoxicity crossmatch (XM), who were previously denied transplantation, can be successfully transplanted after achieving negative XM with Plasmapheresis/IVIG (PP/IVIG) desensitization regimens. In contrast, both live and deceased transplantation with low-level DSA (LDSA), defined arbitrary by negative XM with high PRA or re-transplantation, is allowed without pre-emptive desensitization.

Aim: To determine efficacy of desensitization in patients with LDSA by comparison with control group not having pre-emptive desensitization. The endpoints were graft and patient survival, renal function and complications.

Patients and methods: Study group - 34 XM negative living kidney transplant recipients, who underwent pre-transplant desensitization, because of the presence of DSA, PRA >50% and/or positive Flow-XM. Control group - 40 XM negative patients with 2nd and 3rd retransplantation from deceased donor, without preemptive desensitization. Patients in both study and control group had similar demographics and same Tacrolimus-based maintenance immuno-

suppression. The desensitization was achieved by PP/IVIG at 0.5 gr/kg/bw ± Rituximab/ATG.
Results: There was no difference in type or rate of complications between the two groups. One, 3 and 5 yr patient survival in study group was 100% versus 90%, 87% and 87% in control group, respectively (p 0.058). One, 3 and 5 yr graft survival in study group was 93%, 93% and 69% versus 79%, 73% and 62% in control group, respectively (p 0.029). Mean serum creatinine in study group was 1.24±0.72 mg% versus 1.99±1.4 mg% in controls (p 0.13).
Conclusions: In this study, the desensitization in low immunological risk living kidney transplantation have resulted in superior patient and graft survival than those achieved in similar risk cadaveric transplants.

O-322 COMBINED INTRODUCTION OF ANTI-IL2 RECEPTOR ANTIBODIES, MYCOPHENOLIC ACID AND TACROLIMUS: EFFECT ON MALIGNANCIES IN A SINGLE CENTRE RETROSPECTIVE CO-HORT STUDY OF 929 RENAL TRANSPLANT RECIPIENTS

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Aim: The aim of the present study was to investigate the effect of the combined introduction of the potent immunosuppressive drugs tacrolimus, mycophenolic acid (MPA) and anti-IL2 receptor monoclonal antibodies (aIL2R) more than a decade ago on the incidence rate of post-transplant malignancies.
Methods: A retrospective single-centre cohort study on 929 adult renal transplant recipients. Investigation of the effect of two consecutive immunosuppressive regimens (1993-1998; N=405: anti-lymphocyte antibodies, cyclosporine and azathioprine; 1999-2007; N=524: aIL2R, tacrolimus and MPA) on the incidence rate of skin cancer, solid tumours and post-transplant lymphoproliferative disease (PTLD).
Results: In total, 365 malignancies developed among 113 patients. As compared to the previous cyclosporine and azathioprine-based immunosuppression the introduction of the new immunosuppressive regimen was not associated with an increase in the incidence rate ratio (IRR) for skin cancer 0.84 (95%CI 0.48 to 1.46), solid tumours 0.89 (0.46 to 1.67) and PTLD 0.82 (0.28 to 2.21). Patients treated with the more recent regimen less frequently developed multiple skin cancers and invasive squamous cell cancer. The development of skin cancer was strongly associated with the development of solid tumours (odds ratio 5.2; $P<0.0001$). The introduction of the new immunosuppressive drugs reduced the incidence of first year acute rejection from 34.8% to 13.2% ($P<0.0001$).
Conclusion: Although significantly more efficient in the prevention of acute rejection, the introduction of tacrolimus, MPA and aIL2R-based immunosuppression after kidney transplantation was not associated with an increased incidence of skin cancer, solid tumours or PTLD. This suggests that immunosuppressive regimens can differentially affect mechanisms involved in graft rejection and tumour surveillance.

O-323 LYMPHOPROLIFERATIVE DISORDERS IN RENAL TRANSPLANTATION: ANY CHANGE IN TWO DECADES?

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We studied the incidence of lymphoproliferative disorders (PTLD) and its relationship with Epstein-Barr Virus (EBV) in 1176 renal recipients transplanted in our hospital from 1988 to 2009. We evaluated the variation of PTLD incidence, risk factors and outcome of these patients.
All patients received calcineurin inhibitors, prednisone and azathioprine, switched to MMF since 1998. Immunological risk recipients had antilymphocyte antibodies. PTLD was diagnosed histologically and EBV determined by hibridation in situ. Two periods were analized:1988-1998 and 1999-2009 with 472 and 704 recipients. The follow-up was between 1 and 255 months.
Twenty-eight recipients, 22 males and 6 females, mean age 46.5±15.3 years, with a time after grafting of 72.9±56.3 months developed PTLD (2.3%). Ten patients CD20 positive were treated with rituximab.
Thirteen of 28 recipients (46.4%) had no classical risk factor.
EBV was confirmed in 18 of 26 studied recipients (69.2%). Twenty-five out 28 proliferations were B lymphocytes (89.2%).
The density incidence PTLD/year/patient was similar in both periods,0.003922 and 0,003995.
The patient survival after transplantation in recipients with PTLD was 73,6% at 5 years and 36.9% at 10 years against 87,8% and 73,9% in patients without PTLD $p<0.0001$. The graft survival was 62.6% at 5 years and 27.3% at 10 years while was 72.4% and 53.9% patients without PTLD. $P<0.0001$.
The patient and graft survivals were 30.9% and 15.5% at 1 year; 23.2% and 7.7% at 2 years after diagnosis.
There was no significant difference in the survival of patients treated with rituximab.
In conclusion, PTLD is a disease with a poor prognosis in renal transplant recipents. Most of the proliferations are B lymphocytes. It seems to exist a close relationship between EBV and PTLD, which can develop without classical risk factors. The incidence has not changed in the last two decades.

O-324 LONG-TERM IMMUNOLOGICAL EFFECTS OF ATG INDUCTION ASSOCIATED WITH AN INCREASED RISK OF INFECTION

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Background and methods: We showed previously that rabbit ATG induction therapy induces a strong decrease of CD4+ T cells together with impaired in-vitro IL-2 secretion up to 1 year post-transplant. To analyze long-term immunological effects of ATG induction at the 2- and 5-year post-transplant time points, we used sensitive intracellular cytokine analysis in the same prospective study of 84 renal transplant recipients (wit ATG, n=44).
Results: 5-year kidney graft outcome was not significantly different between the low-risk non-ATG group and the immunological risk ATG group. However, ATG induction was associated with an increased frequency of severe infectious disease within 2 years (20/44 (46%) versus 9/40 (23%) patients, p=0.027) but not beyond (2-5 years post-transplant: 11/40 (28%) versus 11/38 (29%); p=0.887). This increased risk of infection coincided with suppressed T cell functions (T cell proliferation (CD69 expression), p=0.011; intracellular CD4 cell IL-2 and IL-10 responses, p=0.036 and p=0.004, respectively) at 2 years which were no longer detected at 5 years post-transplant. A persistent decrease of CD4 cell counts was evident even 5 years post-transplant in ATG compared with non-ATG patients ($p<0.0005$), however, this was not associated with impaired CD4 cell functions (CD4 helper activity and cytokine production) nor with an increased risk of severe infectious disease or malignancy.
Conclusion: A strongly increased risk of severe infectious disease within 2 years after ATG induction coincided with an impairment of T cell functions within this timeframe. Profoundly decreased CD4 cell counts persisted even 5 years after ATG treatment but were not associated with suppression of CD4 cell functions nor with an increased risk of severe infectious disease following year 2.

O-325 FUNCTIONAL PARALYSIS OF CD8 MEMORY T CELLS AFTER RABBIT ANTITHYMOCYTE GLOBULIN INDUCTION THERAPY IN KIDNEY TRANSPLANT PATIENTS

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Introduction: Rabbit ATG induction therapy depletes lymphocytes, followed by repopulation skewed to regulatory and memory T cells. While this effect has been studied extensively by FACS analysis, functional studies on repopulating T cells are lacking. Here we report on the functional capacities of repopulated T cells after rATG induction therapy.
Materials and Methods: Phospho-specific flow cytometry on whole blood was used to study IL2 mediated phosphorylation of signal transducers of activated T cells (P-STAT5), which function as nuclear transcription factors in T cells, before and 3 months after transplantation. Patients were treated with rATG (3 x 2mg/kg/day, n=14) or the non-depleting anti-CD25 antibody basiliximab (day 0, 4; 20 mg, n=23) induction therapy in combination with tacrolimus, MMF and steroids.
Results: At 3 months after rATG therapy an incomplete recovery of absolute numbers of CD4 naive (5% of pre-transplant levels), regulatory (13%) and memory (11%) T cells and CD8 naive T cells (39%) were measured (all $p<0.001$), while the CD8 memory T cells almost fully recovered (71%). No statistically significant effect was seen on the absolute T cell numbers of basiliximab treated patients. At the functional level, CD4 naive, regulatory, effector and central memory populations of rATG and basiliximab treated patients, responded to IL2 by phosphorylating STAT5. In contrast, after 3 months, recovered CD8 memory T cells of rATG but not of basiliximab treated patients, showed impaired responses to IL2.This functional paralysis of the CD8 memory cells was found in all memory subsets; effector memory, central memory and EMRA (p=0.02; p=0.02; p=0.03).

Conclusion: After rATG induction therapy CD8 T cell subsets repopulate more rapidly than CD4 T cells. However, while rATG recovered CD4 subsets show cytokine driven reactivity, CD8 subsets remained paralyzed at the functional level.

Late Breaking

LB-O-001 EVALUATING SAFETY AND EFFICACY OF TOL101 INDUCTION TO PREVENT KIDNEY TRANSPLANT REJECTION, PART A INTERIM ANALYSIS

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Background: TOL101 is currently in First in Man Phase 2a/b testing. Induction therapy using biological agents remains a highly potent tool for the prevention of early rejection episodes in clinical transplantation. However, induction therapies are associated with significant adverse events and safety issues, which are often related to prolonged depletion of T cell function. TOL101, a IgM monoclonal antibody targeting the αβTCR, is non-mitogenic, has the potential to be safer than currently utilized antibodies.
Methods: The study was designed in two parts, with Part A, the dose-finding portion of the study, to enroll up to 42 subjects. TOL101 dosing began with 1/10 the Minimum Anticipated Biologic Effect Level. Eligible subjects were low-risk (recipients of first transplant, living and standard criteria deceased donor, crossmatch negative, ABO compatible patients with <20% PRA, EBV seropositive) and ranged in age from 18-60. Maintenance therapy included tacrolimus, mycophenolate mofetil, and steroids. The following safety parameters were evaluated: infusion reactions, adverse events, standard laboratory evaluations, cytokine release syndrome, malignancies, and infections. Preliminary efficacy will be assessed based on recovery of renal function, biopsy-proven acute rejection, graft survival and subject survival.
Results: Several doses of TOL101 were tested, with escalating doses demonstrating more substantial effects on the αβ-TCR+ T cell population. Despite significant T cell-modulating effects, no serious adverse events were reported. Infusion reactions were infrequent, mild, and have thus far not resulted in drug discontinuations. No cytokine release syndrome was observed, and no malignancies or serious infections developed during the 6 month observation period.
Conclusions: TOL101 appears to be a safe and well tolerated induction agent at doses having substantial T cell modulating effect.

LB-O-002 THE VASCULARIZED BONE MARROW COMPONENT OF COMPOSITE TISSUE ALLOGRAFTS (CTA) COMBINED WITH COSTIMULATORY BLOCKADE PROMOTES DONOR-DERIVED HEMATOPOIETIC STEM CELL ENGRAFTMENT, CHIMERISM AND TOLERANCE

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Background: The creation of hematopoietic chimerism through BM transplantation remains the most reliable method for inducing transplantation tolerance. However, the contribution of a vascularized bone graft in CTA, such as knee and hand/arm transplants, towards such effects is largely unknown. Therefore, this study investigates the long-term effects of vascularized BM within CTA under costimulation blockade-based immunosuppression.
Materials and Methods: Balb/c mice were utilized as osteomyocutaneous (OMC) or myocutaneous (MC, without bone components) CTA donors and C57BL/6 mice as recipients. Immunosuppression consisted of combined costimulation blockade (anti-CD154, 1 mg IP at day 0 and CTLA4Ig, 0.5 mg IP at day 2) and rapamycin (3 mg/kg/day IP x 7 days, then QOD for 3 weeks) treatment. Viability of BM and efficacy of donor-specific hematopoietic cells engraftment was determined in recipient blood and tissue samples at POD 30, 60, and 120.
Results: Combined costimulation blockade and rapamycin treatment significantly prolonged OMC CTA survival (4/6, >120 days) in comparison with untreated group (MST 10 days) and CTLA4Ig (x 4 doses) treated group (MST 36 days). Moreover, OMC CTA showed significantly prolonged survival in comparison with MC CTA in either combined costimulation blockade and rapamycin treated group (MST 87 days) or CTLA4Ig treated group (MST 22 days). Macrochimerism could be detected at POD 30 in 4 animals which showed long-term survival, but it tended to diminish and disappeared at POD 60. However, viability of vascularized BM was confirmed by histopathological sections at POD 120.
Conclusion: Vascularized BM transplantation is effective in inducing chimerism and CTA tolerance in mice that had undergone combined costimulation blockade (anti-CD154 and CTLA4Ig) and rapamycin treatment. These findings suggest that vascularized BM plays a critical role in the immunoprevileged features of CTA.

LB-O-003 BETTER GRAFT SURVIVAL WITH MACHINE PERFUSION THAN COLD STORAGE AFTER THREE YEARS: FOLLOW-UP ANALYSIS OF THE EUROPEAN MULTICENTRE RCT IN DECEASED-DONOR KIDNEY TRANSPLANTATION

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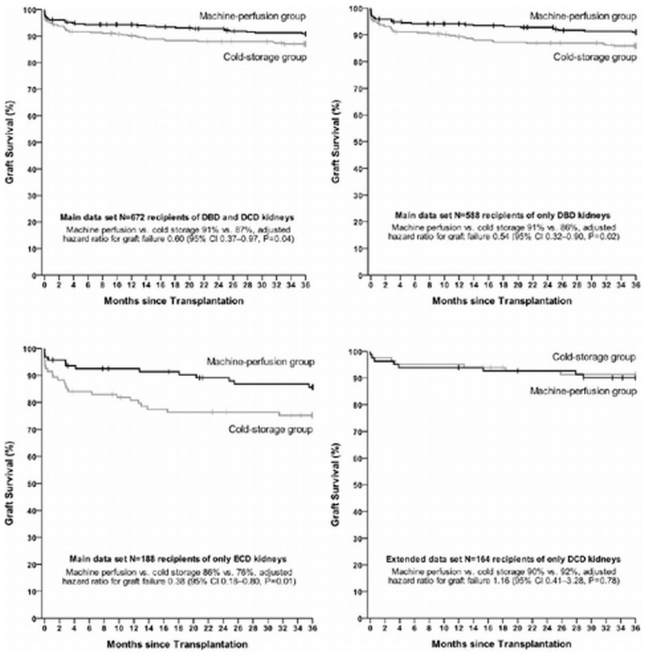
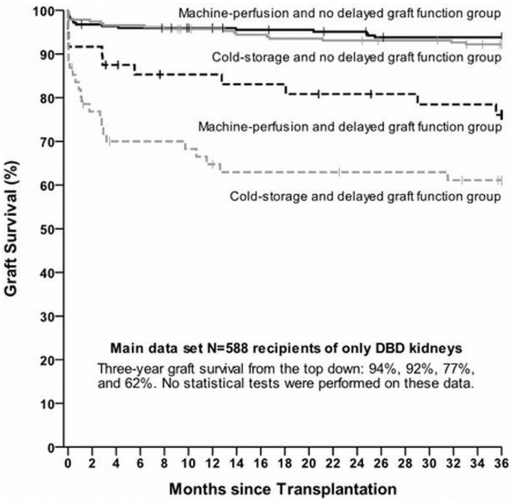
Background: In 2009, we showed in an international RCT that machine perfusion (MP) of deceased-donor kidneys significantly reduced the risk of delayed graft function (DGF) compared to cold storage (CS) preservation. We also found that one-year graft survival was significantly better after MP. We decided to extend the follow-up period of our study and investigate whether this important graft survival advantage would persist three years after transplantation.
Methods: In our RCT, one kidney of each included donor was randomly assigned to MP, and the contralateral organ to CS. For the present analysis, the 60 collaborating transplant centres were contacted and three-year follow-up data were collected of all 672 recipients of consecutive DBD and DCD kidneys in the main dataset, as well as 164 recipients of DCD kidneys is the study's extended dataset. End points were three-year death censored graft survival, patient survival, and serum creatinine.
Results: In the main dataset, three-year graft survival was significantly better



for MP kidneys (91% vs. 87%, adjusted HR for graft failure 0.60, p=0.04). When differentiated to donor type, MP was superior to CS for DBD kidneys (91% vs. 86%, adjusted HR 0.54, p=0.02), but not for DCD kidneys. For ECD kidneys the graft survival advantage after MP was most pronounced (86% vs. 76%, adjusted HR 0.38, p=0.01).
DGF had a profound impact on graft survival. Three-year patient survival and serum creatinine were equal in the two study arms.



Conclusion: Three years posttransplant, graft survival of DBD kidneys remains significantly better after MP compared to CS, especially in the ECD subgroup. Despite the large reduction in DGF by MP we found no effect on graft survival of DCD kidneys, suggesting a different type of DGF.

LB-O-004 IMPROVED RENAL FUNCTION BY OVERNIGHT SWITCH FROM CYCLOSPORINE TO EVEROLIMUS AT WEEK 7 AFTER RENAL TRANSPLANTATION. ONE YEAR RESULTS FROM A RANDOMIZED, CONTROLLED TRIAL

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Background: The benefits of early conversion from calcineurin inhibitor (CNI) to mTOR inhibitor-based immunosuppression in long-term kidney transplant patients remain uncertain.
Methods: Rejection free kidney transplant recipients, receiving cyclosporine-based immunosuppression, were randomized 7 weeks after transplantation to either overnight switch to everolimus (EVE) or to continued CNI treatment (controls). The difference in measured GFR between the two groups was analyzed at month 12 after transplantation (primary endpoint).
Results: 341 patients were included at time of transplantation. Of these, 137 were not randomized due to withdrawal of consent, acute rejection or adverse event. At week 7, 100 patients were randomized to continue CNI unchanged and 102 to switch to EVE. At 12 months there was a difference in measured GFR of 5.5 mL/min in favor of EVE treatment (ITT analysis, p=0.008). This difference was 9.2 mL/min ($p<0.001$) in the per protocol population. Acute rejection episodes following randomization were 32% and 14% in the EVE and CNI arms respectively (p=0.003). The majority of biopsies were Banff grade I in both groups. There were 5 grade IIA and 2 grade IIB rejections in the CNI arm and 3 grade IIA and 2 grade IIB in the EVE arm. All rejections were resolved and no grafts were lost at month 12. Albumin/creatinine ratio was only slightly and insignificantly increased in the EVE arm (p=0.13). Three deaths occurred, one in the control arm and 2 in the EVE arm. The profile of adverse events in the EVE group was consistent with those reported previously for mTOR inhibitors.
Conclusion: A rapid switch from cyclosporine to everolimus at week 7 after transplantation resulted in a significant improvement in renal function at one year.

LB-O-005 RENAL TRANSPLANTATION AFTER EX-VIVO NORMOTHERMIC PERFUSION: THE FIRST CLINICAL SERIES

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Introduction: Cold storage techniques have served organ transplantation well over many decades but are limited by the process of refrigeration, which primes the mechanisms responsible for ischaemia-reperfusion injury. Experimental studies have suggested that normothermic preservation techniques may have advantages over hypothermic methods. We report the first seven cases of *ex-vivo* normothermic perfusion in human renal transplantation.
Methods: The *ex-vivo* normothermic perfusion (NP) circuit was designed using paediatric cardio-pulmonary bypass technology consisting of a centrifugal pump, heat exchanger and membrane oxygenator. NP was performed after a period of static cold storage and immediately prior to transplantation. Kidneys underwent a period of ideal perfusion with cross-matched packed red cells suspended in Ringer's solution supplemented with anti-oxidants, steroids and a vasodilator; mean±SD perfusion temperature was 34.3±0.8 °C.
Results: 1 donor after circulatory death and 6 extended criteria donor kidneys were perfused *ex-vivo* for 35-90 minutes. Donor age was 62±7 yr and the cold storage time was 11±5 hr. During NP all kidneys functioned with a mean±SD urine output of 168±66 ml/hr. 6 of the 7 transplant recipients had initial graft function with no requirement for dialysis in the first 7 days. In 2 cases the paired kidney from the same donor was transplanted after static cold storage; both had delayed graft function. There were 3 rejection episodes (Banff 1a, 1a, 1b) in the first 6 weeks post-transplant. Lowest serum creatinine at 6 weeks post-transplant was 138±28 μmol/l.
Conclusions: These early results demonstrate that *ex-vivo* normothermic kidney perfusion with a blood-based solution is feasible and can be performed without compromising the transplant kidney. The preliminary findings suggest that NP may improve early graft function in kidneys from marginal donors.

LB-O-006 Reduced rate of cutaneous squamous cell carcinoma in a randomised, prospective, multi-centre controlled trial of conversion to sirolimus-based immunosuppression: The RESCUE Trial

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Background: Squamous cell skin cancer (SCC) causes significant morbidity and mortality in renal transplant recipients (RTR). The mTOR-inhibitor sirolimus has anti-proliferative effects resulting in the suggestion of a reduced incidence of skin SCC in transplant registry data. This multi-centre, prospec-

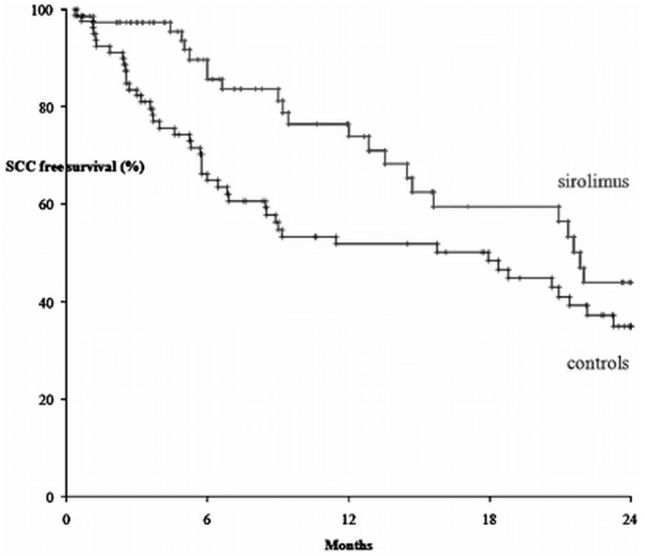

Figure 1. Squamous cell carcinoma free survival in patients who were switched to sirolimus and control patients who remained on their original immunosuppressive regimen (P=0.039).

tive, open-label randomised controlled trial was designed to investigate if switching maintenance immunosuppression to sirolimus could diminish the recurrence rate of skin SCC in RTRs on maintenance immunosuppression.
Methods: One hundred and fifty four RTR in the Netherlands and United Kingdom with at least one biopsy-proven skin SCC and stable transplant function were randomised to sirolimus (n=74) or continuation of their original maintenance immunosuppression (n=80). Each patient was screened for the development of skin SCC every 3 months by a dermatologist for 2 years.
Results: Survival analysis revealed that the time to recurrent skin SCC was delayed in the sirolimus group (p=0.039)[Figure 1]. Risk of SCC recurrence in the sirolimus arm was significantly reduced (HR=0.59: CI 0.36-0.98; p=0.04). There was no difference in renal function or proteinuria between treatment arms (control arm: serum creatinine 127 SD 48 umol/L, sirolimus arm 113 SD 38 umol/L; control arm: 0.36g/24h proteinuria and sirolimus arm 0.43 g/24h). In the sirolimus arm 39% who had switched to sirolimus discontinued treatment due to side-effects.
Conclusion: Conversion to sirolimus-based immunosuppression in RTR delays the development of subsequent skin SCC and total number of skin SCC.