Parallel Sessions 19–36

Session 19. Experimental & clinical islet transplantation

O-170 IN VITRO XENOGENEIC IBMIR IS MORE AGGRESSIVE THAN ALLOGENEIC AND IS MEDIATED BY CLASSICAL AND ALTERNATIVE COMPLEMENT PATHWAYS

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Purpose: Intraportally transplanted islets provoke an instant blood-mediated inflammatory reaction (IBMIR), resulting in acute islet destruction. In a simplified in vitro model, we compared the mechanisms of IBMIR induced by human (allogeneic, A) and pig (xenogeneic, X) islets.
Methods/Materials: 2,500IEQ were incubated with fresh non-anticoagulated human blood in petri dishes in an incubator-shaker (37°C, 100rpm). Clotting times were recorded. Levels of complement activation products (iC3b for common pathway and Bb for alternative pathway) and C-peptide were compared to dishes with blood alone at 5, 30 and 60min. At 60min, clots were fixed for immunofluorescent staining. The effect of 1.6mg/mL low molecular weight dextran sulfate was investigated.
Results: Exposure of human and pig islets to human blood induced equally rapid clotting (A: 3:13+1:31min vs. X: 3:54+1:42, P=0.35; compared to blood alone: 46:25+16:23min, $P<0.0001$). In X, average porcine C-peptide levels increased from 118 to 627ng/mL between 5 and 60min, indicating islet cell lysis. In A, human C-peptide only increased from 41 to 64ng/mL ($P<0.05$). IgM and IgG binding were observed on pig but not on human islets. In X, supernatants released by clots at 30min contained 50% more iC3b, and 77% more Bb than controls. These increases were only 7% and 38% in A ($P<0.05$). Dextran sulfate completely prevented clotting and reduced iC3b and Bb to control levels. However, in X, it did not prevent antibody binding and porcine C-peptide release.
Conclusion: Xenogeneic IBMIR was more aggressive than allogeneic IBMIR in an in vitro model of islets exposed to human blood. Dextran sulfate prevented complement activation through the alternative pathway, but did not prevent antibody binding to pig islets and C-peptide release, suggesting an important role for the classical pathway of complement activation.

O-171 PRO-INFLAMMATORY CYTOKINE MEDIATED DYSFUNTION IN CULTURED RAT ISLETS IS PREVENTED BY IL-1ra TREATMENT

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Aims/Hypothesis: Pro-inflammatory cytokines (PIC) impair islet viability and function by activating inflammatory pathways that induce both necrosis and apoptosis. The aim of this study was to utilize an in vitro rat islet model with exogenous PIC treatment to evaluate the efficacy of a clinically approved IL-1b receptor antagonist (Anakinra) in blocking PIC induced islet impairment.
Methods: Isolated rat islets were cultured ± IL-1b, IFNg, and TNFa and ±IL-1ra and assayed for cellular integrity by flow cytometry and gene expression by RT-PCR. Nitric oxide (NO) release into the culture media was measured by Griess reaction. Islet functional potency was tested by glucose stimulated insulin secretion (GSIS) and by transplantation into streptozotocin-induced diabetic NOD.scid mice.
Results: IL-1ra completely abrogated the effects of PIC with respect to NO production, necrosis, apoptosis, mitochondrial dysfunction, GSIS, and in vivo potency. Rat islets cultured alone, with IL-1ra alone, or the combination of PIC and IL-1ra were indistinguishable and showed high viability, low apoptosis and equivalent glucose-induced insulin secretion. PIC treated rat islets showed strong induction of iNOS and NO release into the culture media. IL-1ra treatment abrogated PIC induced iNOS gene expression and NO production. Indicators of mitochondrial integrity (JC-1 staining and glucose induced metabolic flux) were diminished by PIC treatment, but prevented by co-culture with IL-1ra. The recipients of untreated, IL-1ra alone, and PIC + IL-1ra showed rapid restoration of normoglycemia and stable blood glucose control over the duration of the experiment (28 days).
Conclusion: These data demonstrate that Anakinra is an effective agent to inhibit the activation of IL-1b dependent inflammatory pathways in cultured rat islets and support the extension of its application to human islets in vitro and potentially as a therapy for islet transplant recipients.

O-172 QUANTIFICATION OF TRANSPLANTED IRON OXIDE-LABELLED ISLET CELLS BY 3-DIMENSIONAL 3T MAGNETIC RESONANCE IMAGING (MRI)

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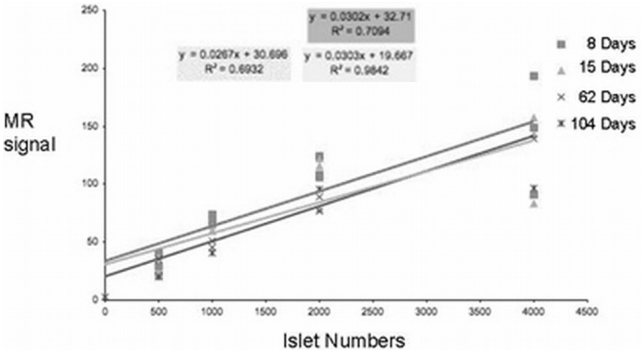
Background: Monitoring mass and function of islet grafts is vital for the improvement of results of islet transplantation in type I diabetes. MRI provides non-invasive imaging for iron-labelled islets. Quantification of the engrafted islet mass has not yet been reported.
Methods: Syngeneic Resovist-labelled islets were transplanted into the portal vein of SD rats. Increasing islet numbers were transplanted (0, 500, 1000, 2000, 4000). Imaging was carried out on a clinical 3T MRI scanner. Scanning was performed 1 day, and 1, 2 and 8 weeks after surgery. Respiratory triggering was performed with a trigger delay of 150ms. Images obtained with a novel 3-dimensional Ultrashort-Echo-Time (UTE) technique were compared with conventional 2-dimensional acquisition sequences. Quantitative assessment included measurement of the number of iron-related pixels (over all liver slices) and correlation with number of transplanted islets.
Results: The isotropic 3-dimensional images can be viewed with the same resolution in all three orientations. When imaging at day 1, surgical disturbance makes visualization of clusters difficult. At 1, 2 and 8 weeks, cell visualisation and quantification is more defined with isolated enhanced spots within an uniform background. UTE images show a good correlation between the number of counted pixels and the number of transplanted islets, and this is reproducible over time.



A rapid decrease of the signal was observed in rats transplanted with xenogeneic human islets. The novel technique also offers an improved signal-to-noise ratio, due to lower background and better signal detection due to control of motion artifacts.
Conclusion: This novel MR imaging technique offers reproducible quantification of transplanted islet grafts. Development of the technique on a clinical MRI scanner makes its application in a human clinical study promising.

O-173 2009 UPDATE FROM THE COLLABORATIVE ISLET TRANSPLANT REGISTRY

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Background: This report summarizes primary efficacy and safety outcomes of islet transplantation reported to the NIDDK and JDRF funded Collaborative

Islet Transplant Registry (CITR), currently the most comprehensive collection of human-to-human islet transplant data.
Methods: CITR collects and monitors comprehensive data on allogeneic islet transplantation in North America, Europe and Australia since 1999.
Results: As of February 2009, the CITR registry comprised 396 adult recipients of 796 islet infusions derived from 937 donors. At three years post first infusion, 27% of islet-alone recipients were insulin independent (II, >2 weeks), 30% were insulin using with detectable C-peptide, 26% had lost function, and 18% had missing data. 70% of IA recipients achieved sustainted II at least once, of whom 70% were still II 1 year later and 50% at 2 years. Higher number of infusions, greater number of total IEQs infused, lower pre-transplant HbA1c levels, processing centers related to the transplant center; and larger islet size are factors that favor the primary outcomes. Protocols with daclizumab or etanercept during induction had higher rates of II and lower rates of function loss, respectively, which endorse the current approaches. Infusion-related AE incidence was 0.7 events/person-year (EPY) in year 1, while immunosuppression-related AE incidence was 0.9 EPY, both declining to less than 0.07 EPY thereafter.
Conclusions: Clinical islet transplantation needs to be evaluated using the most clinically relevant endpoints such as glucose stabilization and severe hypoglycemia prevention. The cumulative results of the Registry confirm the inarguably positive impact of islet transplantation on metabolic control in T1D.

O-174 DIFFERENCES IN BASELINE LYMPHOCYTE COUNTS AND AUTOREACTIVITY ARE ASSOCIATED WITH DIFFERENCES IN OUTCOME OF ISLET CELL TRANSPLANTATION IN TYPE 1 DIABETIC PATIENTS

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Purpose: The metabolic outcome of islet cell transplants in type 1 diabetic patients is variable. This retrospective analysis examines whether differences in recipient characteristics at the time of transplantation are correlated with inadequate graft function.
Methods: Thirty non-uremic C-peptide negative type 1 diabetic patients had received an intraportal islet cell graft of comparable size under an ATG-tacrolimus-mycophenolate mofetil regimen. Baseline patient characteristics were compared with outcome parameters during the first 6 posttransplant (PT) months, ie plasma C-peptide, glycemic variability and gain of insulin-independence. Correlations in univariate analysis were further examined in a multivariate model.
Results: Patients that did not become insulin-independent exhibited significantly higher counts of B-lymphocytes, as well as a T-cell autoreactivity against IA2 and/or GAD. In one of them a liver biopt during PT year 2 showed B-lymphocyte accumulations near insulin-positive beta cell aggregates. Higher baseline total lymphocytes and T-cel autoreactivity were also correlated with lower plasma C-peptide levels and higher glycemic variability.
Conclusion: Higher total and B-lymphocyte counts and presence of T-cell autoreactivity at baseline are independently associated with lower graft function in type 1 diabetic patients receiving intraportal islet cells under ATG-Tacrolimus-MMF therapy. Prospective studies are needed to assess whether control of these characteristics can help increase the function of islet cell grafts during the first year posttransplantation.

Session 20. Ischemia/reperfusion injury & intervention

O-175 THE ROLE OF COMPLEMENT REGULATORY PROTEINS IN CARDIOPROTECTION OF ISCHEMIC POSTCONDITIONING IN HEART ISCHEMIC-REPERFUSION INJURY

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Complement activation during ischemic-reperfusion injury (IRI) is an important reason that impacts cardiovascular and myocardium after heart transplantation. Recent evidence showed that ischemic postconditioning (Pos-con) may obviously lessen heart injury on the heart IRI. We explored the hypothesis that the Pos-con protects heart, at least in part, through up-regulation of decay-accelerating factor (DAF), CD59, or/and membrane cofactor protein (MCP). Isolated working rat hearts were subjected to a global total ischemia, followed by 36 min of reperfusion. Post-con were performed in the first minutes of reperfusion as 3 cycles of 10-sec sequence of I/R. The blood and the heart samples were taken. The mRNA and protein expression of DAF, MCP and CD59 were examined by real-time PCR, western blot and immunohistochemistry, and C3d deposition in tissue will be detected by immunofluorescence, further heart injury were also observed by histologic examination. In addition, serum creatine phosphokinase-MB (CPK-MB) and lactate dehydrogenase1 (LDH1) values were measured. Compared with Pos-con and control, the mRNA/protein expression of DAF and CD59 were significantly decreased, while C3 deposition and CPK-MB/LDH1 values were markedly increased in the IRI group. However, no statistical difference of DAF/CD59 expression, C3d deposition and CPK-MB/LDH1 values between Pos-Con and control has been found. Moreover, the injury of heart was attenuated and the pathological scale was reduced significantly in the Pos-Con group compared with IRI group. Of interest, the mRNA and protein expression of MCP among all groups have not statistical difference. These observations provide evidence that down-regulation of DAF and CD59, not MCP, plays a key role in complement-mediated heart dysfunction resulted by IRI, and this cytolysic effect would be inhibited, at least in part, through Pos-Con.

O-176 TETRAHYDROBIOPTERIN OFFSETS MICROVASCULAR RENAL ISCHEMIA REPERFUSION INJURY VIA SUSTAINMENT OF NO HOMEOSTASIS

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Purpose: Tetrahydrobiopterin (BH4) is an essential cofactor for nitric oxide synthases (NOS) and thus a critical determinant of NO production. BH4 depletion during cold ischemia leads to uncoupling of NOS and contributes to reperfusion injury (IRI) due to increased superoxide formation. The role of BH4 during warm ischemia, however, is still largely unknown.
Material and methods: Ischemic renal injury was induced by clamping the left renal artery for 45 min in male Lewis rats immediately after right-side nephrectomy. Reperfusion was studied at R0 (no reperfusion), 15min (R1), 2hours (R2) and 7days (R3). Animals received either BH4 (20mg/kg/BW) prior to reperfusion (GroupI) or saline (GroupII). Sham operated animals served as controls (GroupIII). Renal function was determined by plasma creatinine/urea. BH4 tissue levels were assessed by HPLC. Morphologic changes were quantified by H&E histology. Peroxynitrite formation was assessed by nitrotyrosine-immunostaining and kidney microcirculation was analyzed by means of functional capillary density and capillary diameters using intravital microscopy.
Results: BH4 tissue levels significantly decreased after 45min of warm ischemia ($p<0.05$) up to two days (R1,R2) when compared to non-ischemic controls. Additional BH4 treatment prior to ischemia significantly improved renal function at all time points studied following reperfusion (all $p<0.001$). Furthermore, BH4 reduced ischemia induced histologic damage (increased inflammation, interstitial edema, hemorrhage, tubular atrophy and focal areas of necrosis) and diminished peroxynitrite formation and hence nitrotyrosine staining (R1-R3). Subsequently, microcirculatory changes correlated with kidney peroxynitrite generation, and improved considerably through BH4 treatment.
Conclusion: BH4 treatment significantly improves post-ischemic renal function as well as histologic and microcirculatory function and might be a promising novel therapeutic strategy in attenuating IRI via maintenance of NO homeostasis.

O-177 A NEW P38MAPKINASE-INHIBITOR IN ISCHEMIA AND REPERFUSION INJURY IN PIGS

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Introduction: We investigated the effect of the new MAPKinase-Inhibitor CBS-3830, (c-a-i-r biosciences, Germany) in an isolated ex-vivo kidney hemoperfusion system on ischemia and reperfusion injury (IRI) and the activated intracellular signaling pathways. CBS-3830 is a highly potent inhibitor of p38 MAP-kinase, JNK-2 and -3 and showed no further interactions with any of the 380 tested protein kinases.
Material and methods: Kidneys from 9 donor animals (n=18; german landrace pigs, mean body weight 41,5 kg) were perfused with cold solution (HTK, 72 ml/kg BW): Group V (verum, n=10 kidneys) included 10 μM CBS-3830; group P (placebo, n=8 kidneys) only vehicle. Cold storage time was mean 26.1 hrs.for V and 25.8 hrs. for P (n.s.) Organs were reperfused with preserved and heparinized pig blood from the same donor including CBS-3830. The test compound (dose: 1 mg/kg) was administered i.v. starting 70 minutes prior organ removal. Simultaneously we determinated CBS-3830 plasma concentrations and an ex vivo pharmacodynamic analysis (TNF-release after LPS-stimulation). Probes were taken at 1, 5, 10, 15, 30, 45, 60, 90, 120 minutes after reperfusion.
Serum concentrations for Indoleamine 2,3 dioxygenase (IDO), IL-6, TNF-a, LDH,MDA as well as HMGB-1 (high motiliy group box 1) and NKG2D were performed. Urine analysis included electrolytes, a1-microglobulin and heparansulphate (HPSG).
Results: HMGB-1, LDH were high in both groups (n.s.). TNF-α was nearly 0 in V and highly elevated in P. Significance was found for IDO, urine flow at 30 min beginning, HPSG and oxygen consumption in favor for the verum group. Ex vivo pharmacodynamic analysis demonstrated a $>$90% inhibition of TNF-α-release after LPS stimulation.
Conclusion: The activation of p38 MAPKinase through IRI can be treated with CBS-3830. There is evidence that the damage and the activation of innate response is decreased. This experiment is limited by time.

O-178 PROTECTION OF AGED DONOR LIVER FROM ISCHEMIA REPERFUSION INJURY BY RNAi

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The aged donor liver is more sensitive or susceptive to ischemia reperfusion (I/R) injury, which results in more severe tissue damage, earlier graft dysfunction and poorer outcome, therefore the utilization of aged liver is limited in clinic. Complement activation is a critical factor in liver I/R injury. We hypothesized that knock down of complement pathway may prevent I/R injury.
Methods: shRNA expression vectors were constructed for specifically targeting C3 and C5aR genes. 12-month old BALB/c mice were treated with shRNA by hydrodynamic injection. Liver I/R injury was induced by interrupting blood supply to the left lateral and median lobes of the liver for 60 min followed by reperfusion 6 hrs. I/R injury was evaluated using liver histopathology, as well as levels of serum alanine transferase (ALT) and aspartate transaminase (AST). Neutrophil accumulation was determined by a myeloperoxidase (MPO) assay and immunohiscochemical staining. Lipid peroxidation was assessed by malondialdehyde (MDA) levels. Quantitative PCR was used to test gene silencing efficacy in vitro and in vivo.
Results: We demonstrated that an significant increase of C3 and C5aR in the aged livers in I/R injury. shRNA-treatment effectively knocked down the expression of C3 and C5aR in I/R livers. In comparison with control mice, the serum levels of ALT and AST were significantly reduced in mice treated with C3 and C5aR shRNA. Additionally, the neutrophil accumulation and lipid peroxidase-mediated tissue injury, detected by MPO and MDA respectively, were improved after shRNA treatment. Tissue histopathology showed an overall reduction of injury area in shRNA-treated mice.
Conclusions: This is the first demonstration that I/R injury in aged livers can be effectively prevented through gene silencing of complement genes, highlighting the potential of shRNA-based therapy in clinical liver transplantation when the aged organs are used.

O-179 DONOR TETRAHYDROBIOPTERIN PRE-TREATMENT PROLONGS RECIPIENT SURVIVAL IN A MURINE GRAFT PANCREATITIS MODEL

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Ischemia-reperfusion injury (IRI) is a major cause for the occurrence of graft pancreatitis following pancreas transplantation. Recent findings showed significantly reduced early parenchymal damages following murine pancreas transplantation if donors were pre-treated with tetrahydrobiopterin (H4B), an essential cofactor of nitric oxide synthases and strong antioxidant. In this study we analyzed if H4B supplementation was also able to prolong recipient survival, since occurrence of graft pancreatitis in this model showed to be lethal.
Male syngenic C57BL6 (H-2b) mice were used as size-matched donor and recipient pairs. Murine cervical pancreas transplantation was performed with a modified no-touch technique. To induce graft pancreatitis grafts were subjected to 16h prolonged cold ischemia time (CIT) as well as to 45min warm ischemia time. Different treatment regiments were applied: untreated animals (I), H4B 50mg/kg i.m. (II) and VitC 350mg/kg i.m. (III), both prior to organ retrieval. Median survival of the different groups was analyzed. Intravital fluorescence microscopy was used for graft microcirculation analysis (functional capillary density – FCD). Parenchymal damage was analyzed by histology (H&E) and by determination of peroxynitrite formation.
Following prolonged CIT only pancreatic grafts treated with H4B displayed markedly higher values of FCD compared to non-treated animals ($p<0.01$). In contrast, the strong antioxidant VitC did not improve microcirculation. Compared to non-treated animals application of both agents significantly attenuated peroxynitrite formation ($p<0.05$). However, reduction of early parenchymal damage in pancreatic grafts was more pronounced following H4B pretreatment ($p<0.05$). Finally, pancreatic grafts treated with H4B prior to organ explantation showed markedly longer survival rate compared to non-treated animals ($p<0.0001$). In contrast, pre-treatment of donor animals with VitC did not improve recipient survival.
H4B attenuates IRI related graft pancreatitis and significantly improves recipient survival rate and might therefore be a novel promising agent preventing graft pancreatitis.

O-180 THE RENAL COMPARTMENT SYNDROME: NEW PERSPECTIVES IN KIDNEY TRANSPLANTATION

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Purpose: Following kidney transplantation, chronic allograft nephropathy represents a major cause of allograft dysfunction and loss. Efficient therapeutic strategies to counter disease progression are missing. As ischemia-reperfusion is associated with edema formation, we investigated the extent and role of edema-mediated pressure elevation within the renal compartment for the long-term functional outcome of kidney transplants.
Methods: The increase in intracapsular pressure at different time points following ischemia was determined in a murine model of renal ischemia-reperfusion injury. Renal dysfunction was evaluated using 99mTc-MAG3 scintigraphy, laser Doppler perfusion measurement, and histological analysis.
Results: Prolonged ischemia was associated with a significant rise in pressure. Compared to baseline, a 7-fold increase was found 6 h after reperfusion. Pressure values continued to be unphysiologically high until returning to baseline by 48 h post ischemia. Strikingly, we found a de novo increase in pressure in long-term follow-up examination.
In reference to the healthy kidney, scintigraphy revealed early marked functional impairment by almost 70% with no evidence for spontaneous restoration in the long term. Blood flow was significantly decreased by 30%. Kidneys exposed to prolonged ischemia exhibited severe tissue damage including edema, excessive tubular atrophy, necrosis, and fibrosis. In contrast, shorter ischemia time did not lead to significantly increased pressure. Consistently, renal function and blood flow were completely restored with only mild changes in histological appearance.
Conclusion: We show that increased pressure within the renal compartment may play a major role in the development of chronic allograft dysfunction via microcirculatory impairment, increased inflammatory response, and direct cell damage. Our data support the notion of a renal compartment syndrome as a significant trigger to a pathophysiological cascade of immunological and

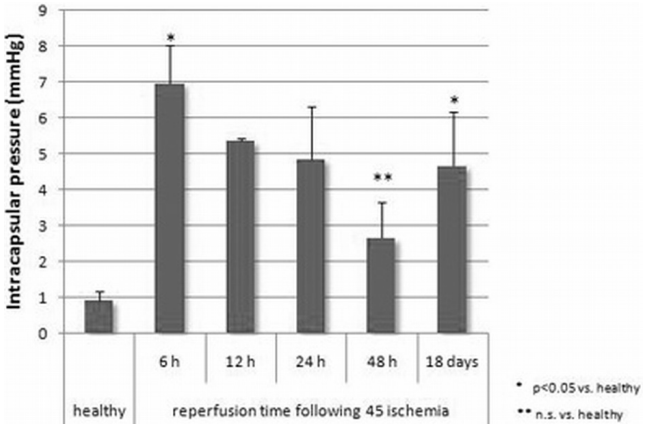

Figure 1. Increase in intracapsular pressure following prolonged ischemia.

non-immunological events. Peri-operative pressure relief by pharmacological or surgical treatment modalities represents a promising strategy to improve function and survival of the renal allograft.

Session 21. Long term complications in kidney transplantation

O-181 AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE: RISK FACTOR FOR NON-MELANOMA SKIN CANCER FOLLOWING KIDNEY TRANSPLANTATION

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Introduction: Non-melanoma skin cancers (NMSC) are the most common malignant tumors following solid organ transplantation. Risk factors for NMSC mainly include immunosuppression, age, sun exposure and patient phototype. Recent findings have suggested that autosomal dominant polycystic kidney disease (ADPKD) may increase the risk of developing NMSC.
Patients and methods: We performed a monocenter retrospective study including all kidney recipients between 1985 and 2006 (n=1019). We studied the incidence of NMSC, solid cancers and post transplantation lymphoproliferative disease (PTLD) and analyzed the following parameters: age, gender, phototype, time on dialysis, graft rank, immunosuppressive regimen at 3 months post-transplantation, history of cancer and kidney disease (ADPKD vs others).
Results: ADPKD was the cause of renal failure in 156 patients (15.3%). A second kidney graft was performed in 10.5% of all patients and a third graft in 0.9%. Median follow-up was 5.5 years (range:0.02-20.6; 79,838 patient-years). The cumulated incidence of NMSC ten years after transplantation was 12.7% (9.3% for solid cancers and 3.5% for PTLD). ADPKD and age were risk factors for NMSC (HR 2.63; $p<0.0001$ and HR 2.21; $p<0.001$, respectively) using univariate analysis. The association between ADPKD and NMSC remained significant after adjustments for age, gender and phototype using multivariate analysis (HR 1.71; p=0.0145) and for immunosuppressive regimens ($p<0.0001$). Patients suffering from ADPKD were not at increased risk to develop a solid cancer (HR 0.96; p=0.89) or PTLD (HR 0.98; p= 0.96) after transplantation.
Conclusion: Our findings confirm that ADPKD is an independent risk factor for developing NMSC after kidney transplantation. Further studies should include genetic analysis to evaluate the mechanisms of this association.

O-182 MEASURING TOTAL BLOOD CALCIUM DISPLAYS A LOW SENSITIVITY FOR THE DIAGNOSIS OF HYPERCALCEMIA IN RENAL TRANSPLANT RECIPIENTS

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Background: Hypercalcemia is a common complication in renal transplant recipients and has been associated with nephrocalcinosis and poor graft outcome. The Kidney Disease: Improving Global Outcomes (KDIGO) position states that the measurement of ionized Ca (iCa) is preferred and that if total Ca (tCa) concentration is used instead, then it should be adjusted in the setting of hypoalbuminemia.
Methods: We compared the ability of noncorrected and albumin-corrected tCa concentration to identify low, normal, or high iCa concentration in an unselected cohort of 268 renal transplant recipients (RTRs). Patients were studied 3 and 12 months after successful engraftment (male 61%, age 53.3±13.4 yrs).
Results: Hypercalcemia, defined as a iCa >1.29 mmol/L was present in 58.6 and 44.8% of the patients at month 3 and 12, respectively.

Parameters of mineral metabolism 3 and 12 months after successful renal transplantation

Parameter	Month 3	Month 12	p-value
Creatinine (mg/dL)	1.52±0.40	1.36±0.37	<0.0001
tCa (mg/dL)	9.7±0.6	9.7±0.6	NS
hypercalcemia (tca, %)	13.1	13.1	NS
iCa (mmol/L)	1.32±0.09	1.29±0.08	<0.0001
hypercalcemia (iCa,%)	58.6	44.8	0.0002
Bicarbonate (mmol/L)	22.2±2.6	22.9±2.6	0.0002
Bicarbonate < 22 mmol/L	48.1	37.3	0.01
Albumin (g/L)	43.5±3.4	44.1±2.8	0.3
Albumin < 35 g/L	3.0	0	NS
Phosphorus (mg/dL)	2.6±0.6	3.0±0.6	<0.0001
hypophosphatemia (%)	31.4	11.8	<0.0001
whole PTH (ng/L)	55.0±48.4	48.2±42.1	0.03

NS: not significant (Month 12 vs Month 3)

Noncorrected and albumin-corrected tCa concentrations > 10.3 mg/dL, conversely, were observed in 13.1% of the patients only. Measuring tCa had a low sensitivity (20.3 and 23.5% at month 3 and 12, respectively) for the diagnosis of hypercalcemia. The agreement (k coefficient [95%CI]) between noncorrected tCa concentrations and iCa was poor (Month 3: 0.11 [0.05-0.17]; Month 12: 0.20 [0.11-0.30]). Albumin-corrected tCa does not predict iCa better than noncorrected tCa. The risk for underestimating iCa was increased by a low total bicarbonate concentration. In
Conclusion: Hypercalcemia is common after successful engraftment. The high prevalence of metabolic acidosis requires the measurement of iCa for the accurately assessment of blood calcium levels in renal transplant recipients, at least in the early posttransplant period.

O-183 LOW SERUM MANNOSE BINDING LECTIN AS A RISK FACTOR FOR NEW ONSET DIABETES MELLITUS AFTER RENAL TRANSPLANTATION

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Introduction: In renal transplant recipients, infections and new onset diabetes mellitus after transplantation (NODAT) are frequent complications. Alterations in innate immune function may contribute to both infection and type 2 diabetes susceptibility. We aimed to evaluate whether the immune, liver-synthesized protein mannose binding lectin (MBL), which circulates at high concentrations in plasma, was associated with NODAT and infection.
Patients and methods: Between March 2005 and October 2006 consecutive non-diabetic renal transplant recipients were recruited. MBL, soluble tumor necrosis factor receptor 2 (sTNFR2) and neutrophil gelatinase associated lipocalin (NGAL) (as markers of chronic inflammation) were determined before transplant, and at 1 and 3 months following transplantation. An oral glucose tolerance test was also performed at 3 months.
Results: A total of 125 patients were recruited and 111 patients had a functioning graft at 3 months. MBL levels remained unchanged following transplantation. Subjects with low MBL (lower tertile) had higher pretransplant sTNFR2 (40±13 vs. 35±11 ng/ml, p=0.05), higher pretransplant NGAL (638±114 vs. 553±185 ng/ml, p=0.03), an increased incidence of bacterial/fungal infection (p=0.021) and an increased prevalence of NODAT at 3 months (44.4 vs 22.6%, p=0.01). Multivariate analysis confirmed that MBL was a risk factor for NODAT (relative risk 3.3, 95% confidence interval 1.3-8.3; p=0.013) after adjusting for recipient age and BMI.
Conclusion: Pretransplant MBL constitutes a new risk factor for NODAT development in addition to infection susceptibility.

O-184 THE USE OF ORAL GLUCOSE TOLERANCE TEST TO STRATIFY THE RISK OF DIABETES MELLITUS AFTER KIDNEY TRANSPLANTATION

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Background: Impairment in glucose metabolism after kidney transplantation

confer risk of various complications with negative effect upon patient and graft survival. The aim of this study was to stratify the risk of impaired glucose tolerance (IGT) and diabetes mellitus (DM) by means of serial oral glucose tolerance tests (OGTT).
Methods: A total of 62 recipients who underwent living-related kidney transplantation were included in this retrospective study. The recipients who were diabetic pretransplantly were excluded. All the recipients received tacrolimus, mycophenolate mofetil and basiliximab, and 58% of the recipients received chronic steroid. Fasting plasma glucose (FPG), hemoglobin A1c (HbA1c) and OGTT were tested before transplantation and 1 year after transplantation. A diagnosis of DM, IGT, and impaired fasting glucose (IFG) were made based on the guidelines of World Health Organization.
Results: Although all the recipients showed normal pretransplant FPG and HbA1c, OGTT identified 31 (50%) recipients to have IGT pattern pretransplant. IGT pattern improved to normal in 19 (61%) of 31 recipients at 1 year after transplantation but 8 (26%) remained in IGT category. Deterioration to DM pattern was seen in 4 (12%). On the other hand, 19 (61%) of 31 recipients with normal pretransplant glucose tolerance, 19 (61%) recipients also remained normal at one year after transplantation, but 11 (39%) recipients were found to have abnormal glucose tolerance: DM, 2 (6%), IGT, 8 (26%) and IFG, 2 (6%). Five of 6 recipients with posttransplant DM received diet therapy alone and tacrolimus was converted to cyclosporine in one recipient.
Conclusions: Our results confirm that FPG and HbA1c underestimate the prevalence of impaired glucose metabolism. These findings suggest serial OGTT after renal transplantion is useful to stratify the risk of developing post-transplant DM.

O-185 POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS IN RENAL ALLOGRAFT RECIPIENTS: FRENCH REGISTRY UPDATE AT 10 YEARS

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PTLD are a well-recognized complication occurring in transplant patients. In order to evaluate the incidence, identify clinical and histopathological features, and assess patients outcome, a French Registry of PTLD occurring after renal transplantation was set up.
Methods: We prospectively identified 378 new cases of PTLD between 1/1/1998 and 31/12/2007.
Results: Patients (256 M, 122 F) ranged from 18 to 75 years (mean: 46±13 years). The median time between grafting and PTLD was 83 months (1 m to 28 y) with 21% of early-onset PTLD. 12% of recipients were EBV negative. Lymphoma involved a single site in 69% of cases and multiple sites in 31% of cases. Locations were grafted kidney in 61 cases, brain in 48 cases, gastro-intestinal tract in 92 cases and lymph nodes in 80 cases. Only 20 PTLD expressed markers of T lineage, others were of B lineage. 2/3 of tumors were EBV positive, 50% of tumors were polymorphic and 70% monoclonal. Most patients were treated with immunosuppression reduction. Rituximab was used alone in 73 patients and associated with chemotherapy in 96 patients. Chemotherapy alone was chosen in 99 patients. 88 patients were treated with surgery and 24 with radiotherapy. 155 patients died, mostly in the first year, half of them from PTLD progression. The median survival was 92 months. Patient survival is 73% at 1 year and and 56% at 10 years. Factors positively influencing survival were: recipient's age above 50 years (p=0.001), early-onset occurrence (p=0.006); single vs multiple location (p=0.013); creatininemia $<$ 150 μmol/l (p=0.04) and PTLD localized in the graft (p=0.02).
Conclusion: This ongoing registry is designed to better understanding epidemiology, risk and prognostic factors of PTLD occurring after renal transplantation in order to further establish consensus on treatment modalities.

O-186 PREVALENCE OF VITAMIN D DEFICIENCY IN RENAL TRANSPLANT RECIPIENTS. EFFECTS OF 25OHD SUPPLEMENTS

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Background: The K/DOQI clinical practice guidelines in chronic kidney disease (CKD) give some recommendations about diagnosis and treatment of vitamin D deficiency. These guidelines may also be applied to renal transplant patients. However, there are few studies about 25-hydroxyvitamin D (25OHD) deficiency in renal transplant recipients. The aim of the present study was to assess the vitamin D status and the effects of vitamin D3 supplements in a cohort of kidney graft recipients.
Patients and methods: 320 renal transplant recipients non treated with vitamin D supplements and with a follow-up of over 12 months were included in a retrospective cross-sectional study.
Results: Serum creatinine was 1.7±0.7 mg/dl and estimated GFR 46.8±17.7 ml/min/1.73m^2. The iPTH levels were 143±118 pg/ml, 25OHD and 1,25OHD concentrations 19.9±11.3 ng/ml and 36.1±21.3 pg/ml respectively. When stratified according to 25OHD levels: 39.1% had 25OHD deficiency ($<$ 16 ng/ml), 47,5% had 25OHD insufficiency ($>$16 and $<$ 30 ng/ml) and 13.4% had normal 25OHD levels ($>$30 ng/ml). Moreover, 25OHD concentrations correlated with several other variables such as age, gender, time of follow-up, graft function, total CO2 concentrations, iPTH, 1,25OHD concentrations, treatment with ACEI/ARB and season of 25OHD determination. On multivariate analysis: gender, length of follow-up, iPTH and 1,25OHD concentrations, season of 25OHD determination and treatment with ACEI/ARB were the variables that remained in the model. Twenty patients were treated with 25OHD2 supplements (400 IU/day). At 6 months iPTH and 1,25OHD levels showed no change and 25OHD levels increased (14.2±6.7 vs 21.6±10.2 ng/ml; p=0.045).
Conclusions: 25OHD deficiency or insufficiency is frequent after renal transplantation even in sunny regions. Low 25OHD concentrations may be, at least in part the cause of persistent hyperparathyroidism. Treatment with 25OHD supplements improved vitamin D status without any effect on iPTH levels.

Session 22. Pediatric liver transplantation

O-187 BENEFICIAL USAGE OF ANATOMICAL FULL-LEFT SPLIT LIVER GRAFT FOR MIDDLEWEIGHT PEDIATRIC RECIPIENT UNDER HIGH URGENCY STATUS

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Background: Commonly used left lateral segmental split grafts (LLGs) are normally not suitable for middleweight pediatric recipients of liver transplantation (LTx) especially under high urgent (HU) status. Therefore anatomical full-left segmental split grafts (FLGs) are sometimes selected for such recipients. Objective: To assess the impact of LTx using FLGs on middleweight recipients under HU status
Patients: We analyzed the data of 73 middleweight (15-35kg) pediatric recipients who received diseased organ graft among 1205 LTx between 1998 and 2007. All split grafts were made in ex situ splitting.
Results: Sixteen cases (21.3%) were under HU status and 57 (78.7%) cases were not. Fulminant hepatitis and Re-transplantation were major indications in the HU group. Biliary disease and metabolic disease were common in non-HU group. FLGs were used more frequently in HU group (37.5% in HU group vs. 8.3% in non-HU group, p$<$0.05). Fewer LLGs were adopted in HU group (31.3% in HU group vs. 48.3% in non-HU group, p$<$0.05). One and 5 year patient survival was 80.0% and 80.0% in HU group, and 94.2% and 92.0% in non-HU group (P=0.09), respectively. One and 5 year graft survival was 73.3% and 73.3% in HU group and 87.0% and 83.0% in non-HU group (P=0.16), respectively. In HU group, 2 of 5 LLGs and 1 of 6 FLGs were lost but no graft loss was occurred in whole liver grafts (WLGs). Eight remnant full-right grafts were used in our institute to non-HU recipient and only one graft was lost
Conclusion: With the view of severe shortage of organ graft, FLGs are a comparable option to WLGs even for the pediatric recipients under HU status.

O-188 EFFICACY OF VALGANCICLOVIR AS PREEMPTIVE THERAPY OF INFECTION DUE TO EPSTEIN BARR VIRUS IN PEDIATRIC LIVER TRANSPLANTATION

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Aim: To analyze the response to preemptive therapy with valganciclovir (VGC) in children with liver transplantation and high quantitative EBV-PCR
From June 2005- Dec. 2007, we have tested 979 EBV-PCR in 80 children. 21/80 PCR were tested from the date of transplant, 59/80 belonged to the historical cohort. Patients were divided in 2 groups depending if they received VGC treatment (n=22) or not (n=16). The response to VGC was considered complete if PCR was negative at 30 and 60 days of treatment, partial if PCR decreased at least 50%. Ganciclovir (GAN) blood levels were tested in 109 instances and were correlated with the EBV-PCR.
Results: A total of 369 (33%) + PCR were detected in 36/80 patients (m: 75.000 copies). Of the 22 episodes treated for 30 days, 34% had a complete response, 41% partial, 23% no response. In non-treated group it was: 6%, 25% and 68% respectively (p=0.01). We did not find differences in episodes treated during 60 days. No patients reached recommended GAN therapeutic levels at

2 hour (6 mg/L).However, mean PCR was lower when the GAN levels were higher than 4 mg/L. The PCR in blood was negative in 3/8 biopsied patients with presence of EBV (EBER). Four patients were long-term treated because of persistent high viral load, 1/4 developed PTLD 2 months after stopping VGC.
Conclusion: There is a response in the EBV viral load to VGC after 30 days of treatment. There is a high interpatient variability of GAN serum concentrations suggesting the need of pharmacokinetic monitoring to optimize treatment. There was a relationship between GAN levels and the EBV viral load. Presence of EBV in tissue can occur with negative EBV-PCR.

O-189 LONG-TERM EVOLUTION OF DE NOVO HEPATITIS C INFECTION (HCV) AFTER PEDIATRIC LIVER TRANSPLANTATION

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To establish HCV characteristics in long term follow-up of de novo HCV infection in children with LTX.Between 1985-2008, 175 p. underwent 218 LTX. 10 (5.7%) developed de novo HCV,1 pt also was coinfected with HBV. 8 pts were transplanted before HCV screening. Mean age at LTX: 8,4 yrs (r: 1.8-16 years). 1 pt. was liver-kidney graft.
Diagnosis: HCV antibodies (Ab), HCV-RNA by PCR. Mean age at time of tx: 8.2 y (r: 1.9-16 y). Patients were divided in 2 groups depending if they received antiviral treatment with Peg IFN and ribavirin.
Results: All patients are alive. Mean time of HCV diagnosis after LTX: 10.8 y r: 2 m-19 y. 6/10, HCV diagnosis was by screening, 4/10 because of liver dysfunction. 9/10 had HCV+ Ab. HCV genotypes (G): G 1a-1b:1; G 1b:7; G 3:1. 1 pt had spontaneous virus clearance. Liver biopsy was performed in 7/10 pts: 3/7: Active chronic hepatitis with F2, 4/7: Minimal changes, 1 of those with concomitant chronic rejection. Treatment group n=4: (1 G1a-1b; 3 G1b). In all of them, virus clearance occurred with sustained virological response and normal ALT. 2/4 pts developed chronic rejection solved on TAC and MMF. No treatment group n=6: (4 G1b, 1 G3 and 1 VHC Ab + RNA- 2 pts (1 G1b, 1 HCV Ab+) had spontaneous viral clearance, 2 pts (G1b) are RNA + normal liver function, and the remaining 2 pts (1 G3/VHB, 1 G1b hepatorenal tx) are RNA + with liver dysfunction.
The behaviour of de novo HCV after pediatric liver tx is better than in adults. After treatment, the sustained viral response of HCV G1b is good; There is a risk for chronic rejection development.Spontaneous viral clearance may occur.

O-190 CENTRAL SINUSOIDAL FIBROSIS (CSF) AS AN INDICATOR OF INADEQUATE IMMUNOSUPPRESSION IN CHILDREN WITH TACROLIMUS WITHDRAWAL AFTER LDLT

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We have withdrawn tacrolimus (Tac) in 191 of 675 children undergoing LDLT. Initially, Tac was decreased slowly and finally discontinued as far as liver function tests (LFT) were normal without liver biopsy, and was discontinued in 103. We began follow-up biopsy for all children in 2004 and found CSF in considerable number of children with normal LFT after discontinuing Tac. Hence, we hypothesized that CSF is an indicator of inadequate immunosuppression and began a prospective study of Tac resumption
Methods: Patients with CSF and normal LFT after Tac withdrawal were enrolled and patients with ongoing rejection, biliary or vascular complications were excluded
Results: Tac was resumed in 25 children (age: 0.7-9 years at LDLT, 6-24 years at resumption) because of CSF. Tac had been discontinued in 12, administered every month in 4, every 2 weeks in 2, every 1 week in 3, every 3 days in 2 and every 2 days in 2 and resumed finally to daily in all. Duration of minimum immunosuppression ranged from 6 to 98 months (median: 23). CSF was graded from stage 0 to IV according to Dixon criteria. The CSF was graded in IV in 1, III in 9, II in 7, and I in 8, and C4d deposition was endothelial&stromal in 10, endothelial in 6, and no in 7, and not available in 2 at resumption. CSF was subsequently improved in 5 (follow-up: 12-48 months), not progressed in 12 (6-16 months), progressed in 2, and not assessed because of short follow-up in 6. C4d deposition turned to negative in 4 and decreased in 1 among the 5 CSF-improved children.
Conclusion: CSF might be an indicator for inadequate immunosuppression and the mechanism was possibly humoral immunity.

O-191 PREDICTORS OF OPERATIONAL TOLERANCE AFTER PEDIATRIC LIVING DONOR LIVER TRANSPLANTATION; THE ABSENCE OF EARLY REJECTION, THE PRESENCE OF DONOR-RECIPIENT HLA-B MISMATCH AND FEMALE DONOR

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Background: After our pediatric living-donor liver transplantation (Tx), a substantial number of the patients achieved complete cessation of IS (operational tolerance), which had been long exceptional in clinical Tx. On the other hand, some patients encountered rejection while they were undergoing weaning from IS. Nonetheless, reliable predictors of operational tolerance are not available yet.
Method: The study group consisted of group-tolerance (Gr-Tol) in which 50 patients were successfully weaned off from IS 10 years post-Tx and group-intolerance (Gr-Intol) in which13 patients were still on maintenance IS due to the experience of rejection during or after weaning IS at identical time point. Two groups were compared with respect to following clinical parameters to identify predictors of operational tolerance;donor/recipient age and gender, ABO compatibility, HLA mismatch, graft-size, early ($<$ 1 month) rejection episode, initial tacrolimus trough level ($<$1week).
Results: Among clinical parameters tested, multiple logistic regression identified the presence of HLA-B mismatch (Gr-Tol and Gr-Intol; 93% and 80%, OR [odds ratio] =33.36, p=0.05) and the absence of early rejection episode (Gr-Tol and Gr-Intol; 90% and 31%, OR=34.48 p=0.002) as independent predictors of successful IS withdrawal. Univariate analysis revealed that female donor was associated with successful IS cessation (Gr-Tol and Gr-Intol;77% and 38%, $p<0.01$), By multiple logistic regression, OR of female donor reached as high as 5.32, although it was not a statistically significant predictor of successful IS cessation (p=0.16).The other parameters were not associated with operational tolerance.
Conclusion: This is the first report showing the possibility that the presence of donor-recipient HLA-B mismatch, the absence of early rejection and female donor could be useful as predictors of operational tolerance after pediatric living-donor liver transplantation.

O-192 THREE HUNDRED LIVING DONOR LIVER TRANSPLANTATIONS IN ADULTS AND CHILDREN: TECHNICAL EVOLUTIONS AND LESSENS LEARNED

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Purpose: Living donor liver transplantation (LDLT) has become a legitimate and accepted alternative in such countries where cadaveric organs are scarce. In this study, we present our technical evolutions and critically evaluate the result of our consecutive 300 LDLTs to identify risk factors for poor outcome.
Methods: The selection criteria for grafts are based on graft-to-standard liver volume ratio (GV/SLV). Left lobe (LL) grafts are the primary choice for all patients, however, right lobe (RL) grafts are considered if calculated GV/SLV$<$35%. Graft types included left lobe (LL) grafts (n=187), right lobe (RL) grafts (n=93), left lateral segment (LLS) grafts (n=19) and dual grafts (n=1). Grafts were defined as extra-small (ES, GV/SLV$<$30%, n=18), small (S, 30-40%, n=94), medium (M, 40-50%, n=114) and large (L, $>$50%, n=74) grafts according to the size.
Results: The mean GV/SLV of the LL, RL and LLS grafts were 40.0%, 48.6% and 81.3%, respectively. The mean MELD score in adult patients was 14.5 (range 1-54). Several technical evolutions were phased in including introduction of RL grafts (n=93), duct-to-duct biliary reconstruction (n=205), planned simultaneous splenectomy (n=71), auxiliary partial orthotopic liver transplantation (n=2), temporal hemiportocaval shunt (n=2), and dual-graft LDLT (n=1). Overall 1-, 5 and 10-year patient survival rates were 85.1%, 75.6% and 69.9%, respectively. On multivariate analysis, high MELD score$>$20 ($p<0.05$) was the only significant factor for poor prognosis while other factors graft type and size were not.
Conclusion: Our left lobe-centered policy can offer acceptable results and should be a viable option in LDLT. However, indications for LDLT in patients with a high MELD score should be limited due to poor prognosis.

Session 23. Mechanisms & evolution of chronic allograft nephropathy

O-193 THE AUTOANTIBODY LEVEL OF CITRATE SYNTHASE IS CORRELATED WITH C4D DEPOSITION IN RATS WITH CHRONIC ALLOGRAFT NEPHROPATHY

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Purpose: This study is to investigate the relationship between C4d deposition and autoantibodies of citrate synthase (CS) in rats with chronic allograft nephropathy (CAN).
Methods: Fisher344 rat renal grafts were orthotopically transplanted into Lewis rats following the procedure of Kamada with our modification. All the recipients were given CsA 10mg/kg^{-1} d^{-1} $\times$10d and then divided into three groups: (1) Vehicle; (2) CsA: 6mg/kg^{-1} d^{-1}; (3) MMF: 20mg/kg^{-1} d^{-1}. At 4w, 8w, 12w after transplant, the renal allografts were harvested and the sera was collected. The SCr was measured and the pathological changes were assessed according to the Banff 97 criteria. The IgM and IgG isotype of CS antibodies in all the recipients were detected by binding indirect enzyme-linked immunosorbent assay (ELISA)The deposition of C4d was detected by immunofluorescence and analyzed by Integrated Optical Density (IOD).
Results: The level of IgM of CS autoantibodies was more obviously stable and higher than IgG isotype in all blood samples. With the progression of CAN from 4w,there was a strong positive correlation between the level of IgG isotype of CS and C4d deposition (r=0.973, p=.000) in vehicle group. CsA didn't prevent the formation of IgG isotype of CS ($\triangle$OD values) ($p>0.5$) and the deposition of C4d (IOD)(p=0.016) at 8w and 12w.The differences of IgG and deposition of C4d between MMF and other two groups were all statistically significant (p=0.000) at 12w.
Conclusions: This study shows that there was a strong positive correlation between the level of IgG isotype of CS and C4d deposition. The CS autoantibodies may contribute to progression of CAN. MMF may inhibit the progression of CAN by decreasing the formation of IgG autoantibodies of CS and the deposition of C4d. CsA has no these effects.

O-194 INTERSTITIAL FIBROSIS AND TUBULAR ATROPHY (IFTA) AFTER RENAL TRANSPLANTATION: TIME COURSE AND FACTORS INFLUENCING SCARRING

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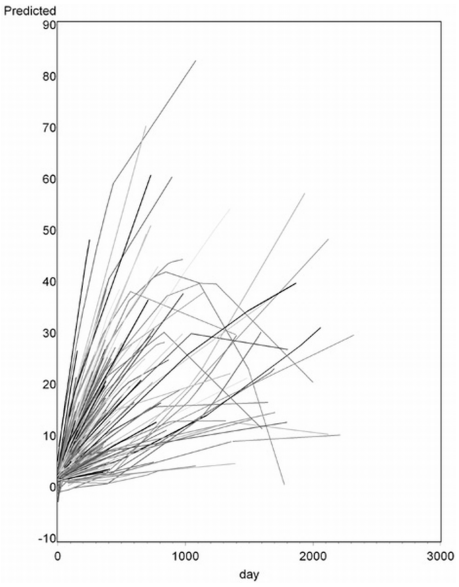
subgroup of 86 patients had a 12 months protocol biopsy. Ordinal logistic regression was used to look at possible factors influencing the degree of scarring at that time. Variables included *patient:* age, gender, BMI, time on dialysis, diabetes, smoking, HLA mismatches, peak PRA, *donor:* gender, status, age, serum creatinine, *and:* cold ischaemia time, delayed graft function, GFR at 3 months, ACE inhibitor or statin use, compliance, immunosuppressant (tacrolimus, cyclosporine, everolimus), OKT3 use, IL2 antagonist induction, ATN on biopsy during 1st week, number of borderline or acute rejections.
Results: 1. There was strong evidence of an increase in scarring over time ($p<0.001$), with a significant negative quadratic effect showing a tendency for the increase to reduce over time ($p<0.001$). The mean curve's turning point, indicating a slow down in scarring, was 1238 days (see figure).
2. The odds ratios for the variables included in the final model are given below, where it is modelling the probability of the degree of scarring being low. No compliance problems, shorter cold ischaemia time, use of ACE inhibitors or IL2 antagonist induction were associated with less scarring at 12 months.

Odds Ratio estimates

Effect	Point Estimate	95% Wald Confidence Limit		Significance
Donor age	0.977	0.940	1.016	p=0.25
Donor status (deceased vs living)	8.256	0.833	81.809	p=0.07
Compliance problems (no vs yes)	33.391	2.306	483.585	p=0.01
Cold ischaemia time (longer vs shorter)	0.869	0.760	0.993	p=0.04
Time on dialysis	1.015	0.998	1.032	p=0.09
GFR at 3 months	1.042	1.006	1.079	p=0.02
ACE inhibitor therapy (no vs yes)	0.086	0.019	0.377	p=0.001
Statin therapy (no vs yes)	2.552	0.771	8.448	p=0.13
IL2 antagonist induction (no vs yes)	0.148	0.033	0.657	p=0.01
ATN on biopsy	0.400	0.150	1.068	p=0.07

Conclusion: Interventions to prevent the development of IFTA need to be instigated early. The use of IL2 antagonist induction, ACE inhibitors, and early recognition of compliance problems might prove beneficial.



Abstract O-194 – Figure 1.

O-195 INDIRECT ALLORECOGNITION OF HLA 'PUBLIC' T-CELL EPITOPES IS ASSOCIATED WITH CHRONIC ALLOGRAFT DYSFUNCTION

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Introduction: We previously reported on human CD4$^+$ T lymphocyte responses to peptide epitopes derived from HLA class I that exhibit little polymorphism, some that are identical to self and many of which bind promiscuously to MHC class II ('public T cell epitopes' PTE). This contrasts with other studies in which there is an assumption that epitopes arise from highly polymorphic sequences. If responses to PTE's are representative of indirect alloimmunity in general, then they may allow the development of a standardised assay of cellular immunity for chronic allograft dysfunction (CAD).
Methods & results: Immune response to a restricted set of HLA PTE's was assessed by PBMC γ-interferon production using ELISPOT in 110 kidney transplant recipients under long-term follow-up at our centre. The relationship between these responses and two indicators of (CAD): late transplant biopsy (LTB) for clinical indication after the 1st post-transplant year and deteriorating renal function in the previous 3 years (DRF) was assessed. 30 patients underwent LTB and an intersecting group of 30 defined as having DRF. In both groups, 22 patients made a significant response in the ELISPOT (ER). This was significantly higher than in patients in whom there was no LTB or DRF (22/30 vs 32/80, 73.3% vs 40%; p=0.002 in both). In multivariate analysis ER was the variable most strongly associated with LTB and the only variable associated with DRF. Of 54/110 transplant recipients with an ER, 22/54 had undergone LTB compared to 8/56 non-responders.
Conclusion: These data suggest that responses to PTE's are significantly associated with a diagnosis of CAD in a population unselected for HLA type. Although limited by the retrospective nature of the analysis, the strength of these associations suggest that this is a viable biomarker of 'chronic rejection' meriting further investigation.

O-196 DIFFERENTIAL EVOLUTION OF STRUCTURAL AND FUNCTIONAL CHANGES DURING THE FIRST YEAR OF KIDNEY TRANSPLANTATION (KT)

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The usefulness of measured GFR during the first year post KT is not well established. In this study, we determined if there was a correlation between the mGFR impairment and BANFF lesions worsening during the first year post KT

Between January 1998 and March 2007, a 51Cr-EDTA mGFR and a screening biopsy were performed in 565 and 376 KTR at 3-months and 1-year post KT, respectively.
The mean mGFR was 55.4±17.5 mL/min/1.73m^2 and 52.8±17.0 mL/min/1.73m^2 at 3 months and 1 year, respectively ($p<0.0001$). At 3 months and 1 year, none of the Banff acute scores were correlated with the concomitant mGFR, but interstitial fibrosis/tubular atrophy (IFTA), transplant glomerulopathy (cg), and arteriosclerosis (cv) scores were strongly associated with concomitant mGFR ($p<0.0001$ in all cases). The prevalence of IFTA and of cg lesions increased from 53% to 72%, and from 1.8% to 5.5% between 3 months and 1 year, respectively ($p<0.001$).
Among the 298 patients having both mGFR and adequate biopsy at 3 months and 1 year, 173 (58%) worsened their GFR while 125 (42%) patients had stable or improved GFR from 3-months to 1-year. These 2 groups were similar in term of IFTA progression (42% *vs* 38% in groups 1 and 2, respectively), arteriosclerosis lesions worsening (37% *vs* 41%, respectively), and cg lesions progression (7.9% vs 4.1%, respectively). Both 3-months and 1-year mGFR were strongly predictive of allograft survival.
This study suggests the absence of parralelism between a marginal decrease of mGFR but a much higher histological worsening during the first year post KT. Consequently, the GFR variation between 3 months and 1 year cannot be used as a decision criterion in performing a screening biopsy at 1 year, but might be useful to predict long-term graft outcome.

O-197 INTERSTITIAL FIBROSIS AND TUBULAR ATROPHY IN KIDNEY ALLOGRAFTS ARE ASSOCIATED WITH TUBULAR ENDOPLASMIC RETICULUM STRESS DETECTION

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Although Endoplasmic Reticulum (ER) stress has been implicated during various nephropathies and kidney insults, its exact pathological and prognostic role during these nephropathies remains to be elucidated. Since ER stress ultimately leads to tubular apoptosis and cell death, ER stress markers such as GRP78 could be evaluated as biomarkers for early kidney injury with the goal of detecting the pathological process that precedes morphological changes or cell death.
The aim of our study is to demonstrate whether the detection of GRP78 on tubular cells is significantly associated with chronic tubulo-interstitial changes in kidney allograft biopsies performed at 3 months post-transplantation.
Immunohistochemical staining for GRP78, used as surrogate marker of ER stress, was analyzed in 56 consecutive kidney transplant protocol biopsies performed at 3 months post-transplantation.
A positive GRP78 staining was observed in 25 biopsies (44%). Whereas donor and recipient demographic characteristics, cold ischemia time, preimplantation histology, acute rejection episodes, and immunosuppressive regimens were all similar between the two groups, GRP78 staining was associated with significantly higher interstitial fibrosis score (ci score, 0.5±0.1 vs 0.2±0.1, p=0.001), tubular atrophy score (ct score, 0.5±0.1 vs 0.2±0.1, p=0.017), and chronic allograft nephropathy score (IF/TA score, 0.4±0.1 vs 0.2±0.1, p=0.05).
In conclusion, using GRP78 as a surrogate marker of ER stress, immunohistochemical analyses performed on kidney allograft protocol biopsies suggested that ER stress occurs *in vivo* and is associated with tubular atrophy and interstitial fibrosis. The detection of ER stress may reflect ongoing injuries on tubular cells, which adapt with the unfolded protein response. Further studies are needed to validate ER stress detection as an early marker of tubular injury in renal allografts.

O-198 LONG-TERM RENAL FUNCTION AFTER ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ADULTS PATIENTS: A SINGLE-CENTRE STUDY

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Purpose: Long-term organ damage is an increasingly identified cause of morbidity and mortality in patients who have undergone hematopoietic stem cell transplantation HSCT. Reported data regarding chronic kidney disease (CKD) in HSCT recipients have yielded contrasted results.
Methods/Methods: We undertook a retrospective single-centre study in order to assess the rate, risk factors and outcome of HSCT-associated CKD in 123 patients who had undergone allogeneic HSCT in the hematology department at Hopital Necker (Paris, France) betwween January 1995 and December 2005. All patients medical files were reviewed and relevant data collected
Results: Twenty-four months after HSCT, CKD, defined as glomerular filtration rate (GFR) <60ml/min/1.73m^2, was noted in 49 patients (40%). Age ≥ 45 years, early acute renal failure and a baseline GFR <80ml/min/1.73m^2 predicted the occurrence of CKD. The drop in GFR occurred mainly during the three months after HSCT. One hundred and six patients were followed more than 36 months (range 36-142). At last follow-up, among 45 patients with CKD at 24 months after HSCT, 30 (67%) had persistent CKD, 10 (22%) showed a slight and transient improvement in GFR but retained CKD and ten patients (22%) had a sustained improvement of GFR. Among 62 patients without CKD at 24 months after HSCT, 3 (5%) developed CKD during follow-up.
Conclusion: HSCT-related CKD probably includes two subsets: a frequent early-onset CKD occurring during the first two years after HSCT, mainly as a consequence of ARF in older patients with pre-existent renal impairment and a rare late-onset CKD occurring after 2 years following HSCT and probably related to radiation nephropathy and calcineurin inhibitors toxicity.

Session 24. Clinical immunosuppression: news

O-199 SOTRASTAURIN: PHARMACOKINETICS AND EXPOSURE-EFFICACY RELATIONSHIP IN RENAL TRANSPLANT RECIPIENTS

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Pharmacokinetics of sotrastaurin, a novel, selective inhibitor of protein kinase C, and relationship between normalized average trough blood levels and biopsy-proven acute rejection (BPAR) were assessed in 216 *de novo* renal transplant recipients.
Methods: Recipients were randomized to a control regimen of mycophenolic acid (MPA) with standard-exposure tacrolimus (n=74), or sotrastaurin 200mg bid with either standard (n=76) or reduced-exposure tacrolimus (n=66). At month 3, tacrolimus was replaced with MPA in the sotrastaurin arms. All study arms used basiliximab and corticosteroids.
Results: By week 2 sotrastaurin trough levels remained stable at 615±419 ng/ml till month 6. Sotrastaurin levels were neither affected by combination with standard- vs reduced-exposure tacrolimus (p=0.99) nor altered when tacrolimus was replaced by MPA (p=0.11). Sotrastaurin C_{max} and AUC, pooled over treatments and across visits till month 6, was 1603±636 ng/ml and 12222±4179 ng.h/ml, respectively. Pooled AUC intersubject variability was moderate (27%) and not influenced by age, weight, or sex (p=0.86, p=0.37, p=0.12, respectively). Sotrastaurin levels significantly correlated with AUC (r^2=0.62, $p<0.001$). From month 1 to month 3, four BPARs occurred in both sotrastaurin arms, whereas post-conversion from tacrolimus to MPA (months 3 to 6) 15 BPARs occurred. Recipients were classified based on their average sotrastaurin levels (<400, 400-600, >600ng/ml) and the freedom (%) from BPAR was determined in each group (Table): fixed-dose sotrastaurin+tacrolimus showed good efficacy in contrast to sotrastaurin+MPA, especially for the lower sotrastaurin exposure.

Freedom (%) from BPAR by sotrastaurin exposure group

Time	Regimen	Low Sotrastaurin	Medium Sotrastaurin	High Sotrastaurin
Months 1-3	sotrastaurin + tacrolimus	93%	100%	98%
Months 3-6	sotrastaurin + MPA	82%	92%	87%

Conclusion: Sotrastaurin exposure was stable from week 1 onwards regardless of the combined immunosuppressant. Sotrastaurin intersubject pharmacokinetic variability was similar to that of other immunosuppressants. In combination with MPA, higher sotrastaurin exposure may be needed for equivalent efficacy to sotrastaurin+tacrolimus. Future studies are necessary to ascertain the best regimen for sotrastaurin.

O-200 COMPARISON OF COMBINATION PLASMAPHERESIS/ IVIG/ANTI-CD20 VERSUS HIGH-DOSE IVIG IN THE TREATMENT OF ANTIBODY-MEDIATED REJECTION

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Different strategies appear to improve the success in treatment of antibody-mediated rejection (AMR), although no one best method has yet emerged. The objective of this study was to compare the efficacy of the combination of

Plasmapheresis/IVIg/anti-CD20 based regimes versus high-dose IVIg alone in the treatment of AMR. Group A (12 patients) was treated with high-dose IVIg between 01/2000 and 12/2003; Group B (12 patients) was treated by Plasmapheresis/IVIg/anti-CD20 between 01/2004 and 12/2005. The evaluation of response was based on graft survival at 36 months and histologic and serologic data (detection of DSA by ELISA and Luminex SA) gathered at the time of AMR diagnosis and 3-months post-rejection. Graft survival at 36 months was 91.7% in Group B versus 50% in Group A (p=.02). DSA levels detected 3 months post-rejection are significantly lower in Group B than in Group A: DSA ELISA score 6-8 (p=0.02), DSA MFImax 2671 633 vs 9010 1851 (p=0.05) and DSA mean MFI 1030 489 vs 4437 1534 (p=0.004). The persistence of elevated DSA levels post-treatment is more frequent in patients with graft loss as compared to those with preserved renal function: score 6-8 on ELISA (p=0.04); mean MFI (p=0.0001); and MFImax (p=0.006). The presence of a MFImax post-AMR $>$ 5000 is associated with graft loss with a sensitivity of 100% and a specificity of 77.8% (ROC curve with AUC of 0.88, p=0.004). DSA levels at the time of rejection were weakly predictive for graft loss (ROC curve with AUC of 0.74, p=0.08). We conclude that i) high dose IVIg alone is inferior to Plasmapheresis/IVIg/anti-CD20 as therapy for AMR and ii) DSA post-rejection can be quantified using solid phase assays, showing that 3 months after AMR DSA levels are higher in patients with graft loss.

O-201 EVEROLIMUS WITH LOW OR VERY LOW EXPOSURE OF TACROLIMUS IN *DE NOVO* RENAL TRANSPLANT RECIPIENTS: THE ASSET STUDY

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Everolimus, a potent immunosuppressant and proliferation signal inhibitor (PSI) has been developed for the prevention of renal and heart allograft rejections and is used in combination with calcineurin inhibitors (CNIs). Renal function is impacted negatively by CNIs and early reduction may allow for better outcome. The ASSET study investigates whether everolimus with either low dose or very low dose tacrolimus can preserve renal function whilst maintaining efficacy in *de novo* renal transplant recipients (RTxR).

Methods: ASSET, a 12-month, randomized, multicenter, open-label study enrolled *de novo* RTxR with deceased or living donors, cold ischemia time $<$30h and donor age 10–65 years. Within 24 hours after transplantation, 229 patients were randomized (1:1) to the low dose (LDTac) or very low dose (VLDTac) tacrolimus arm. For the first 3 months all patients received everolimus (1.5 mg bid, C0-h$\geq$3 to $\leq$8ng/mL) and tacrolimus (0.1 mg/kg/day, C0-h 4-7 ng/mL) therapy. The LDTac group continued the initial regimen unchanged whereas the VLDTac group received tacrolimus targeted to C0-h 1.5-3ng/mL until month 12. All patients received basiliximab and steroids.

Results: The study is ongoing at 36 centers worldwide and data will be available in May 2009. The primary objective is to compare renal function (cGFR [MDRD]) at month 12 between the treatment groups.

Conclusion: ASSET is a pivotal study to evaluate the efficacy of everolimus with low or very low-exposure of tacrolimus to prevent allograft rejection and preserve renal function in RTxR. The following results will be presented: primary endpoint and secondary endpoints (a composite of BPAR, graft loss, death or loss to follow-up; CrCl (Cockcroft-Gault), cGFR (MDRD and Nankivell) through the 12 months study period.

O-202 EFFICACY AND SAFETY OF AN EVEROLIMUS/ENTERIC-COATED MYCOPHENOLATE SODIUM REGIMEN AFTER CALCINEURIN INHIBITOR WITHDRAWAL IN DE NOVO RENAL TRANSPLANT PATIENTS: RESULTS OF THE ZEUS TRIAL

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Aim of the study: To investigate safety and efficacy of an everolimus/enteric-coated mycophenolate sodium (EC-MPS) regimen after Cyclosporine (CsA) withdrawal in *de novo* kidney allograft recipients at month 12 post transplantation.

Methods: In this 1-year, prospective, open-label, controlled, multi-center study 300 renal allograft recipients were randomized to an immunosuppressive regimen consisting of either Everolimus/EC-MPS or CsA/EC-MPS. All patients (pts) received induction therapy with Basiliximab and were treated with CsA, EC-MPS and corticosteroids for the first 4.5 months. Subsequently patients were randomized 1:1 to either a) continue CsA/EC-MPS (n=145) or b) convert to Everolimus/EC-MPS (n=155). CsA and Everolimus trough levels were 100-150ng/ml and 6-10ng/ml, respectively. Dosing for EC-MPS was 720mg BID.

Results: BPAR was reported in 23/155 (14.8%) Everolimus/EC-MPS-treated vs. 22/145 (15.2%) CsA/EC-MPS patients during the first year after transplantation. One death was observed in the CsA/EC-MPS group and no graft loss was reported in both groups. The number and proportion of patients withdrawn due to adverse events during 12 months were 37/155 (23.9%) in the Everolimus/EC-MPS and 28/145 (19.3%) in CsA/EC-MPS group. The table shows laboratory parameters and adverse events which are of interest after conversion from CsA to Everolimus.

Table 1

	CsA/EC-MPS	Everolimus/EC-MPS
Hyperlipidaemia [% of pts]	40	46
Total Cholesterol [mmol/L]	6.24	6.61
LDL [mmol/L]	3.48	3.87
HDL [mmol/L]	1.48	1.45
Triglycerides [mmol/L]	2.74	3.72
Blood pressure [mm Hg]	133/81	132/79
Anaemia [% of pts]	27.6	33.5
Leucopenia [% of pts]	15.9	15.5
Thrombocytopenia [% of pts]	3.4	11
Mouth ulceration [% of pts]	2.1	17.3
Diarrhoea [% of pts]	26.9	34.8
Serious infections [% of pts]	26.2	27.1
Oedema [% of pts]	42	47

Renal function expressed as calculated GFR (Nankivell method) improved from randomization to month 12 by 10.4 mL/min/1.73m^2 in favor of the Everolimus/EC-MPS regimen ($p<0.001$).

Conclusions: The introduction of Everolimus/EC-MPS in *de novo* renal transplant patients after CNI withdrawal reflects a novel therapeutic approach which significantly improves renal function without compromising efficacy and safety.

O-203 INITIATION OF EVEROLIMUS WITH CALCINEURIN INHIBITOR (CNI) REDUCTION IN THORACIC TRANSPLANT PATIENTS WITH IMPAIRED RENAL FUNCTION: A MULTICENTER, RANDOMIZED STUDY

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Purpose: CNI therapy is a major contributor to deteriorating renal function following thoracic transplantation. Initiating everolimus and reducing CNI exposure may improve renal function. The Nordic Certican Trial in Heart and Lung Transplantation (NOCTET) is the first randomized, comparator study to assess this strategy in thoracic transplantation.

Methods: A 12-month, open-label, multicenter study was undertaken in patients receiving a heart or lung transplant $>$12 months previously who had GFR 20-90ml/min/1.73m^2. Patients were randomized to continue their current

CNI-based immunosuppressive regimen unchanged or start everolimus with CNI reduction targeting cyclosporine C_0 <75ng/mL or tacrolimus C_0 <4ng/mL. Primary endpoint was change in measured GFR from baseline to month 12.
Results: 283 patients (191 heart, 92 lung) were randomized: everolimus 141, controls 142. Baseline characteristics were similar between groups except for recipient age (everolimus 59±10 years, controls 56±11 years; p=0.03) and time post-transplant (everolimus 62±45 months, controls 75±54 months; p=0.02). Based on preliminary data from 169 patients (77 everolimus, 92 controls), mean cyclosporine C_0 at randomization (n=244, 86.2%) was 136±50ng/mL and 138±62ng/mL in the everolimus and control groups, respectively, decreasing to ≤60ng/mL from week 4 onwards in the everolimus group. Mean tacrolimus C_0 (n=39, 13.8%) was similar at randomization (everolimus 10.3±2.7ng/mL, controls 9.6±2.7ng/mL), remaining in the range 4.4-5.6ng/mL after week 4.
Conclusion: Study results will be presented comprising the primary endpoint, secondary endpoints (including left ventricular function in heart patients and bronchiolitis obliterans syndrome in lung patients), and safety data. In contrast to other recent trials, the study protocol was followed closely, resulting in the lowest cyclosporine levels reported in this setting. The findings will be highly relevant for clinicians facing the challenge of progressive renal dysfunction in maintenance thoracic transplant recipients.

O-204 TACROLIMUS REDUCES THE RISK FOR BRONCHIOLITIS OBLITERANS SYNDROME 3 YEARS AFTER LUNG-TRANSPLANTATION BY 50% IN COMPARISON TO CYCLOSPORINE IN A PROSPECTIVE RANDOMIZED INTERNATIONAL TRIAL OF 248 PATIENTS

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Objective: We performed a prospective randomized study comparing the efficacy and safety of two immunosuppressive regimens (Tac, MMF, Steroids vs. CsA, MMF, Steroids) after Lung Transplantation. Primary objective was the incidence of bronchiolitis obliterans syndrome (BOS). Secondary objectives were incidence of acute rejection and infection, survival and adverse events. 248 patients with a complete 3 year follow-up were included in this analysis.
Methods: Patients were randomized to treatment group A: Tac (0.01-0.03 mg/kg/d iv – 0.05-0.3 mg/kg/d po) or B: CsA (1-3 mg/kg/d iv – 2-8 mg/kg/d po). MMF dose was1-4 mg/d. No induction therapy was given. Patients were stratified for cystic fibrosis. Intention to treat analysis was performed in switched patients.
Results: 3 of 123 Tac patients and 41 of 125 CsA patients were switched to another immunosuppressive regimen. Groups showed no difference in demographic data. Kaplan Meier analysis revealed significantly less BOS in Tac treated patients (p=0.033, log rank test, pooled over strata). Cox regression showed a 50% lower risk for BOS in the Tac group. Incidence of acute rejection was 67.5% (Tac) and 75.2% (CsA) (p=0.583). 1- and 3-year-survival-rates were not different (85.4% Tac vs. 88.8% CsA, and 80.5% Tac vs. 83.2% CsA, p=n.s.). Incidence of infections and renal failure was similar (p=n.s.).
Conclusion: Tac significantly reduced the risk for BOS after 3 years. Both regimens have a good immunosuppressive potential and offer a similar safety profile with excellent one and three year survival rates. Acute rejection rates were similar in both groups. Incidence of infections and renal failure showed no difference.

Session 25. Novel insights in perfusion & preservation

O-205 FOXP3+ REGULATORY T-CELLS PLAY A DOMINANT ROLE OVER RORγT+ TH17-CELLS IN DECEASED DONOR KIDNEYS

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Th17 cells and regulatory T-cells (Tregs) have an interactive relationship mediated by IL-6 and TGF-β. Both cell types are involved in the reactivity against alloantigens and in response to tissue injury. Here, we studied the Th17-Treg network in zero biopsies of kidneys from living donors with relatively short ischemia-reperfusion times and from deceased donors with brain death related inflammation and prolonged cold ischemia-reperfusion times
Biopsies from deceased (n=13, > 14 h cold ischemia) and living (n=14, < 2 h cold ischemia) donors were studied at the end of cold storage and after 20-30 min reperfusion. In deceased donor kidneys higher mRNA expression levels of CD3ε were measured compared to living donor kidneys demonstrating that more T-cells had infiltrated these allografts (p=0.04). Moreover, in deceased donor kidneys the expression levels of TGF-β, which acts as a differentiation factor for FOXP3+ regulatory T-cells, was abundantly present (p=0.005) compared to living donor kidneys. Likewise, FOXP3 mRNA was detected at significantly higher levels in deceased than in living donor kidneys (p=0.001). In contrast, in biopsies from deceased donor kidneys the mRNA levels of RORγt, the transcription factor that directs IL-17 transcription, was significantly lower than in samples from living donors (p<0.001). Concurrently, no induction of the RORγt inducing cytokines (IL-6, TNF-α, IL-21) and IL-17 was measured.
In conclusion, the high mRNA expression levels of CD3ε, TGF-β and FOXP3 in deceased vs living donor kidneys suggests that regulatory T-cells, but not Th17 cells play a role in controlling tissue injury resulting from the pathophysiologic events inherent to deceased donation.

O-206 PERITONEAL COOLING MAY PROVIDE ADDITIONAL ISCHAEMIC PROTECTION FOR UNCONTROLLED NON HEART BEATING KIDNEY DONORS

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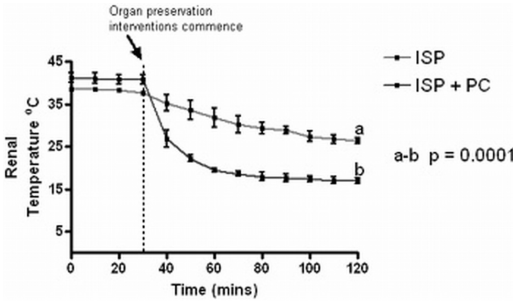
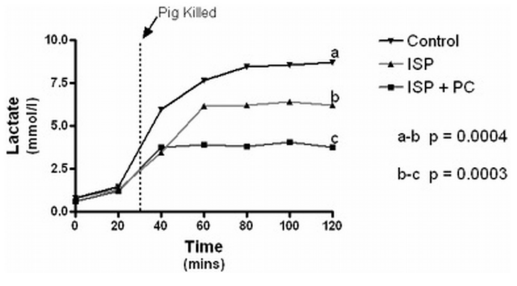
Purpose: Uncontrolled NHBD renal transplantation is made possible by interventions which ameliorate the effects of warm ischaemia. In most centres these techniques are focused on the timely introduction of cold in-situ perfusion to provide sufficient renal cooling and preservation. However, at laparotomy in our category II donors we typically see temperatures of 29-30°C.
In order to assess any potential benefit for contemporary uncontrolled NHBD programs we have developed a porcine model of the uncontrolled NHBD, comparing current in-situ perfusion (ISP) protocols with additional peritoneal cooling.
Materials and methods: Ten 30kg pigs were used; the in-situ perfusion (ISP) group modelled our current uncontrolled NHBD protocol. The peritoneal cooling (PC) group modelled current protocols with the addition of peritoneal cooling.
All pigs were anaesthetised prior to laparotomy, where two thermocouples and one microdialysis catheter were placed into each kidney. The abdomen was then closed. Following euthanasia, animals were subjected to 30 minutes warm ischaemia prior to application of the study group cryopreservation protocol.
Results: In the ISP group only 1/4 cases reached a mean renal temperature of 25C. In the PC group the mean time taken to reach 25C was 14.5 minutes. The final temperature after 90 minutes was 26.3±1.46C in the ISP group versus 16.9±1.17C in the PC group (p=0.0001).
Renal parenchymal microdialysis permitted measurement of biochemical markers of anaerobic metabolism (lactate) and cell injury/death (glycerol). At





120 minutes significantly superior results were seen in the PC vs ISP group; peak lactate 3.78±0.6 versus 6.23±0.26 mmol/l (p = 0.0003), and peak glycerol 284.5±45.9 versus 554.8±74.3 micromol/l (p = 0.0008).
Conclusions: This study has demonstrated superior renal cooling, and biochemical microdialysis evidence of improved ischaemic protection, with supplemental peritoneal cooling for uncontrolled NHBDs.

O-207 "TWO LAYER PRESERVATION METHOD" (TLM) IMPROVES POST-TRANSPLANT SURVIVAL AND EARLY KIDNEY FUNCTION FOLLOWING PROLONGED COLD ISCHEMIA

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Purpose: Oxygen solubility in perfluorocarbon (PFC) is about 25 times higher than in conventional solutions. TLM based on oxygenated PFC overlaid with UW solution has been successfully used especially in islet transplantation. The aim of the present study was to see whether TLM prevents tissue damage and improves early kidney function in a rat model following prolonged cold ischemia time.
Methods: Brown-Norway syngeneic rats were used as kidney donors and recipients. Kidneys were harvested and stored for 24 hours either in UW solution (Group 1, n=16), with TLM (Group 2, n=16) or transplanted immediately (Group 3, n=12). Kidney transplantation was performed after bilateral nephrectomy. In half of the animals in each group (8, 8 and 6, with random assignment) only survival was followed. In the other animals the grafts were procured for histological analysis 24 hours after transplantation. For tissue damage grading a blinded scoring method was applied. Apoptosis was assessed using a TUNEL assay.
Results: One-month survival in groups 1, 2 and 3 was 12.5%, 62.5% and 100%, respectively. There was significant difference in survival time (Group 1 vs 2, $p<0.01$). Median creatinine levels 24 hours after transplantation were 381 (292-443), 299 (255-374) and 121 (102-138 μmol/l, respectively, Group 1 vs 2, $p<0.02$). Histological scoring showed more severe tissue damage in Group 1 than in Group 2 ($p<0.01$). Apoptosis was not the main cause of tissue injury because of its rather low rate in all 3 groups. It was detected mainly in tubular cells and was more frequent in Group 1 than Group 2 ($p<0.01$).
Conclusion: Conservation with TLM significantly improves the outcome of kidney transplantation in a rat model and should be further studied in larger animals.
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O-208 ENISCHEMIC HYPOTHERMIC RECONDITIONING REVERSES PRESERVATION INDUCED LIVER INJURY BY MITOCHONDRIAL PROTECTION PRIOR TO REPERFUSION

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Background: Although the quality of cold-stored livers slowly declines beyond approximately 12 hours of ischemia and the risk of primary dys- or non-function steadily increases, up to 20% of all liver transplantations were done after cold ischemia times of more than 12 hours.We investigated the respective benefit and mechanistical background of two different techniques for resuscitating marginal liver grafts, unexpectedly subjected to long storage times.
Methods: Livers were harvested from male Wistar rats, flushed with 40 ml of Histidine Tryptophan Ketoglutarate (HTK) solution and cold-stored for 22h (CS22). Some grafts were subsequently subjected to 90 min of hypothermic reconditioning by venous systemic oxygen persufflation (VSOP) or oxygenated hypothermic machine perfusion (HMP) with HTK. Livers stored for only 6h (CS6) served as reference. Viability of all grafts was assessed thereafter by warm reperfusion in vitro.
Results: VSOP and HMP significantly increased endischemic tissue energy charge, and abrogated cellular enzyme loss upon reperfusion even significantly below control values. Ammonia clearance and bile production were more than 3-fold improved to similar values as CS6. Hypothermic reconditioning by both techniques induced mitochondrial chaperone expression (HSP70 family) and abrogated activation of caspase 9 and 3.
Conclusion: Viability of long preserved liver grafts can be augmented by transient hypothermic reconditioning using either machine perfusion or gaseous oxygen persufflation, both preventing initial mitochondrial dysfunction and subsequent tissue injury. Owing to the simplicity and ease of application, gaseous oxygen persufflation recommends itself as feasible alternative to the more cumbersome and cost-intensive perfusion protocol.

O-209 MITOCHONDRIAL PROTECTION BY OXYGENATED PERFUSION AFTER WARM ISCHAEMIA

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Introduction: We have previously shown that oxygenated perfusion at physiological temperature resuscitates non-heart-beating-donor (NHBD) livers after warm ischaemic injury. We have examined mitochondrial functional changes during ischaemia-reperfusion in NHBD livers and the relationship of these with hepatocellular injury.
Methods: Porcine livers were retrieved after cardiac arrest and divided into three groups: Group 1 (Control, n=5) no warm ischaemic injury; Group 2 (n= 5) 60 minutes of warm ischaemia; Group 3 (n= 5) 60 minutes of warm ischaemia followed by *in-situ* oxygenated perfusion. All livers were then cooled (60 minutes) during the bench work and then connected to an oxygenated normothermic extracorporeal perfusion circuit for 24 hours. Mitochondria were isolated from sequential liver biopsies and analysed for ATP content; mitochondrial function (respiratory control ratio (RCR); cytochrome *c* release and caspase activation. The perfusate was analysed for serum transaminase and base deficit.
Results: Group 1 livers maintained normal mitochondrial function during cold preservation and subsequent reperfusion. In Group 2 livers, cellular ATP levels reduced significantly during 60 minutes of warm ischaemia ($p<0.01$), with minimal change in mitochondrial function. However, subsequent cold preservation produced a significant decline in mitochondrial function (RCR 3.9±0.4 vs. 2.4±0.2 $p<0.001$) with parallel decline in mitochondrial ATP level (p= 0.001). Mitochondrial injury was associated with increased hepatocellular injury as evidenced by raised transaminase release ($p<0.05$). In Group 3 livers, in situ oxygenated perfusion improved mitochondrial RCR ($p<0.05$) and ATP levels significantly ($p< 0.01$) with greater functional recovery and bile production ($p<0.05$) compared to Group 2 livers.
Conclusions: These data suggest that mitochondria sustain progressive damage during sequential warm and cold ischaemia followed by reperfusion, leading to cell death in NHBD livers. In-situ oxygenated perfusion immediately following warm ischaemia confers mitochondrial resilience to ischaemia-reperfusion injury and may have therapeutic benefits in NHBD transplantation.

O-210 RELEASE OF AST AND LFABP FROM ISCHEMICALLY DAMAGED LIVERS DURING MACHINE PERFUSION: A NEW TOOL TO PREDICT VIABILITY AND PRIMARY-NON-FUNCTION

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Introduction: Increasing use of donor livers exposed to Warm Ischemia (WI) demands the development of criteria to assess viability *before* Transplantation (Tx). In analogy with the kidney, we hypothesized that analysis of specific biomarkers in perfusate of livers during Hypothermic Machine Perfusion (HMP) may provide viability criteria to predict risk of Primary-Non-Function (PNF).
Aim: To determine whether the cumulative release of Aspartate-aminotransferase (AST) and Liver-Fatty-Acid-Binding-Protein (L-FABP) in perfusate of ischemic livers during HMP correlates with liver viability and PNF.
Methods: Porcine livers (n=6/group) were exposed to incremental WI periods (0, 15, 30, 45, 60, 120'), procured and HMP preserved. We reported earlier (*Tx 2005*) a PNF risk of 0% if WI=0∼15';50% if WI=30∼45';100% if WI≥60'. AST and L-FABP release in perfusate was monitored during 240'. In individual livers, AST release could be represented by a logarithmic equation [$AST=\alpha + \beta_{AST}*\ln(time)$ during the initial 60' HMP; R^2=0.95-0.99; L-FABP release could be represented by a linear equation [$L\text{-}FABP=\alpha + \beta_{L\text{-}FABP} * time$] during the initial 30' HMP; R^2=0.85-0.99. In addition, the different WI groups were clustered according to the aforementioned risk of developing PNF. We analyzed whether β-coefficient could discriminate the various WI groups and PNF clusters.
Results: β-coefficient was different among the 6 WI groups (p=0.0006 for AST;0.018 for L-FABP), and for the 3 functional clusters (p=0.0001 for AST;0.0002 for L-FABP). There was a linear correlation between β_{AST} and $\beta_{L\text{-}FABP}$ (R^2=0.61, p=0.0001). β_{AST} and $\beta_{L\text{-}FABP}$ reflect WI damage and predict the risk of PNF.
Conclusion: β-coefficients calculated from initial AST or L-FABP release during HMP are promising clinical tools to predict viability of ischemic livers and subsequent risk of PNF. Based on our observations, Tx of HMP preserved porcine livers with a $\beta_{AST} < 0.006$ or $\beta_{LFABP} < 0.004$ is safe.

O-211 HYPOTHERMIC IN SITU MACHINE PERFUSION WITH UW DURING DECEASED CARDIAC DEATH DONATION IMPROVES EARLY RENAL FUNCTION

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In situ hypothermic machine perfusion (HMP) may optimize the quality of Deceased Cardiac Death (DCD) donor organs during donation by restoring circulation after cardiac arrest using extra corporeal membrane oxygenation techniques under hypothermic conditions.
Methods: Conventional organ procurement through hypothermic single flush with the HTK or UW solutions was compared with in situ perfusion applying the Extra Corporal Organ Procurement System (ECOPS) in a pig DCD donation (n=3 per group). After 20h cold storage, kidneys were transplanted in recipients followed by 4h blood reperfusion. In the donor, kidney temperature was monitored. After transplantation, urine production in the recipient during reperfusion were measured. Samples of blood, urine, perfusate and biopsies taken for biochemical evaluation and RT-PCR.
Results: At the end of the DCD donor procedure, mean temperature of the kidney using ECOPS was 15.3±0.6 °C (HTK-E) and 16.3±2.5 °C (UW-E) respectively compared to standard washout 23.8±3.2 °C (HTK-C) and 24.7±0.9 °C (UW-C) ($p<0.05$). Diuresis after transplantation was 29.7±6.4 ml (HTK-E) and 221±41 ml (UW-E) in experimental groups compared to 14±6.9 ml (HTK-C) and 43.7±16.0 ml (UW-C) in control groups ($p<0.05$). GFR was highest in the UW-E group (maximum 10 ml/min at t=3hrs) compared to almost absent in all other groups. mRNA levels of MCP-1 and TNF-alpha did not differ between treatments. VWF were higher in HTK-C compared to UW-C.
Conclusion: ECOPS improves cooling efficacy during DCD donation. In addition, the cooling of organs is different between HTK and UW. Renal function after transplantation is restored earlier with use of UW in the ECOPS system. No evident effects were shown on mRNA levels.

O-212 RENAL RESISTANCE DURING MACHINE PERFUSION IS A RISK FACTOR FOR DELAYED GRAFT FUNCTION AND POORER GRAFT SURVIVAL

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Renal Resistances (RR) during Machine-Perfusion (MP) are used to discard kidneys likely to fail post-Tx but threshold RR (above which kidneys are discarded) have been determined empirically.
Aims: We studied the prognostic value of RR on Delayed-Graft-Function (DGF), Primary-Non-Function (PNF), graft-survival.
Methods: An international/prospective trial (*NEJM-2009*) including kidney pairs of 336 consecutive Heart-Beating (HB)&Non-Heart-Beating (NHB)donors shows that MP leads to less DGF & prolonged graft-survival *vs* Cold Storage (CS). In this trial, recipient centres were blinded to preservation-method (MP/CS) and to MP parameters. Surgeon decision to accept/discard kidneys was solely based on traditional donor data. In MP arm, the RR (mmHg/mL/min-Real Time) on LifePort®Kidney-Transporter was recorded (30'/1h/2h/4h/MP end). Univariate/multivariate analyses were done to determine impact of RR on DGF/PNF/graft-survival.
Results: Higher RR at different time-points resulted in higher %DGF (17.3% *vs* 37.9% for RR: 0.28 at MP end). RR was associated with increased Odds Ratio for DGF (OR 2.69; p=0.03 for RR: 0.28 at MP end) independent of donor-type (HB *vs* NHB), -age, cold-ischemia-time, reTx *vs* first-Tx. Highest RR groups showed highest serum creatinine up to 3 months post-Tx. RR was linked to risk of 1year graft loss; in case of DGF, RR threshold of 0.28 at MP end resulted in a 17% poorer graft survival *vs* immediately functioning grafts. Only 7 MP kidneys developed PNF; no discriminative RR to discard PNF kidneys was found.
Conclusion: This study demonstrates (for the first time) the exact prognostic value of RR during MP. RR correlates with DGF&graft survival, not PNF. Many kidneys with elevated RR were probably erroneously discarded in the past. RR is an additional tool to increase the kidney pool. Pre-Tx knowledge of the risk of DGF may help clinicians to select recipients and/or adjust immunosuppression (nephron-sparing protocol in high risk grafts for DGF).

O-213 IGL-1 SOLUTION PROTECTS AGAINST LIVER ISCHEMIA REPERFUSION INJURY BY INHIBITION OF ENDOPLASMIC RETICULUM-STRESS

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Injury due to cold ischemia-reperfusion (IR) represents a major cause of primary graft non-function following liver transplantation. We postulated that IR-induced cellular damage might cause alterations of the secretary pathway, particularly at the level of endoplasmic reticulum (ER) function. Under these circumstances, the ER triggers an adaptive response named the 'unfolded protein response'. Here, we investigate the involvement of ER-stress in organ preservation, comparing cold storage in UW and in IGL-1 solution.
Sprague-Dawley Rats were flushed and preserved in UW solution for 8h at 4°C, and then orthotopic liver transplantation was performed according to the Kamada's cuff technique. In an additional experimental group, the same surgical procedures as described for group UW was carried out, but livers were flushed and preserved in IGL-1 solution.
Blood and liver samples were obtained 24h after liver transplantation. Hepatic injury was assessed by determination of AST/ALT. Mitochondrial damage and ATP depletion were evaluated in liver tissues after 24 h of liver transplantation. To evaluate RE stress when livers were preserved in UW and IGL-1 solution, the following markers of RE stress were evaluated: GRP78, ATF6, eIF2 α, and CHOP.
IGL-1 solution reduces liver injury and mitochondrial damage. Thus, at 24h of transplantation transaminases levels and GLDH were reduced significantly when livers were preserved in IGL-1 solution compared with liver preserved in UW solution. Here, we delineate a role for endoplasmic stress during preservation/reperfusion of pre-damaged liver grafts, which is aggravated by the use UW solution, and IGL-1 solution protect from RE stress, and attenuate significantly the expression of GRP78, CHOP, eIF2 α and ATF6 when compared with liver preserved in UW solution. Our results show that IGL-1 solution may be a useful means to circumvent excessive endoplasmic stress reactions associated with liver transplantation.

O-214 ANALYSIS OF MACHINE PERFUSION PARAMETERS OF KIDNEYS PROCURED FROM EXPANDED CRITERIA DONORS

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Using expanded criteria donor (ECD) organs is one of the strategies for making more transplants available. Machine perfusion has been proven to offer advantages in kidney storage. Parameters of machine perfusion can be used as a predictors of kidney function after transplantation.
Aim: The aim of this study was to analyse the differences in perfusion parameters of kidneys depending on donor criteria.
Patients and methods: One hundred and seventy two patients received cadaveric renal transplants between January 1, 2006 and August 31, 2008. Data on donors, recipients and preservation were collected. 88 kidneys were stored by machine perfusion. Parameters of perfusion such as renal flow, resistance, lactate dehydrogenase and lactates measured in the fourth hour of perfusion were analysed.
Results: Average 1-year graft survival was 86.9% for the whole group and mean creatinine concentration was 1.58 ml/dl. One case of primary non-function was observed (0.6%). More than 25% of transplanted kidneys were harvested from ECD.
Kidneys harvested from ECD had significantly lower total renal flow during perfusion (118±38 vs 161±48; $p < 0.001$), lower flow per gram of kidney tissue (0.5±0.15 vs 0.7±0.21; $p < 0.001$) and higher resistance (0.32±0.16 vs 0.21±0.09; $p < 0.001$) in comparison to kidneys harvested from standard criteria donors.

Perfusion parameters

	SCD (n=58)	ECD (n=30)	P
Renal flow (±SD) (ml)	161±48	118±38	<0.001
Renal flow (±SD) (ml/g)	0.7±0.21	0.5±0.15	<0.001
Resistance (±SD) (PRU)	0.21±0.09	0.32±0.16	<0.001
Lactate dehydrogenase (U/L)	473	540	NS
Lactates (mg/dl)	12.6	7.5	NS

There were no differences in lactates concentration and lactate dehydrogenase activity.

Conclusions: Selected perfusion parameters correlate with kidney donor criteria, with expanded criteria kidneys presenting poorer perfusion parameters and, possibly, inferior post-transplant function.

O-215 PREVENTION OF OXIDATIVE STRESS INDUCED ORGAN DAMAGE IN A PORCINE BRAIN DEAD DONOR MODEL

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Introduction: The "autonomic storm" initiated after brain death induces a cascade of chemokine and cytokine release which causes cell damage and diminishes organ quality. Recently published data on donor pre-treatment showed limited success. This study aimed to evaluate the impact of an antioxidative treatment after brain death induction on organ quality in a pig model.
Methods: Brain death was induced in 16 pigs by trepanation of the skull and increasing intracranial pressure until brain stem herniation occurred. 10 hours after brain death diagnosis, the pigs were randomized in two groups (n=8). Group 1 was infused 500 ml of a solution containing alpha-ketoglutaric acid and 5-MMF over 4 hours whereas group 2 received 500 ml NaCl. Interleukins and markers for oxidative stress are determined using FlowCytomic. 24 hours after brain death multiorgan donation was performed and tissue samples were taken immediately after organ retrieval. Histology and immunohistochemistry as well as PCR analysis were performed.
Results: Markers of oxidative stress as well as the concentration of the interleukins analysed were highest in all animals 8 hours after brain death induction. It was feasible to lower CP and MDA levels as well as chemokine and cytokine concentrations significantly in the experimental group 1. Histology and immunohistochemistry revealed significantly lower apoptotic cells as well as lower anti-nitrotyrosine positive cells in group 1 when compared to group 2 immediately after explanation and after CIT. ATP levels were highest in the control group, but significantly higher in group 1 when compared to group 2 at any time point.
Discussion: We could diminish oxidative stress induced cell damage and prevent the detrimental effects of the "autonomic storm" by applying a solution containing alpha-ketoglutaric acid and therefore achieved better organ quality after multiorgan donation in a pig brain death model.

Session 26. Impact of donor risk factors on kidney graft survival

O-216 THE CUMULATIVE NEGATIVE IMPACT OF DONOR RISK FACTORS ON KIDNEY GRAFT HISTOLOGY AND FUNCTION

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With increasing number of patients waiting for kidney transplantation, there is a worldwide tendency to accept donors with comorbities and older age. High donor age, hypertension and reduced GFR are known risk factors for post transplant graft function. The aim of our study was to analyse the cumulative influence of these and other donor and donation associated risk factors on donor kidney histology and transplant outcome.
Baseline biopsies of kidneys from 482 deceased donors and donor risk factors were examined. All biopsies were re-evaluated by one pathologist. Graft function and survival of the 833 renal transplantations from these donors were analysed.
The most frequent donor risk factors were cerebrovascular cause of death, smoking, age over 50 years, hypertension and unstable hemodynamics after brain death. Less common were resuscitation, arteriosclerosis, ischaemic heart disease and alcohol abuse. We found a significant association between the cumulative number of concurrent risk factors and histological lesions, measured by % glomerulosclerosis, arteriolar hyalinosis, vascular intimal sclerosis, tubular atrophy and the CADI score (Figs 1, 2).
The increase of concurrent risk factors from 0 to 5-8 resulted an increase of DGF rate from 7.4% to 49.3% (Chi-square, $p<0.0001$) and a reduced estimated 3 year GFR (Cockcroft-Gault) from 92.9 ml/min to 64.6 ml/min (Anova, $p<0.0001$). Transplantations from donors with >4 risk factors (N:67) had significantly decreased graft survival compared to those with ≤ 4 risk factors;

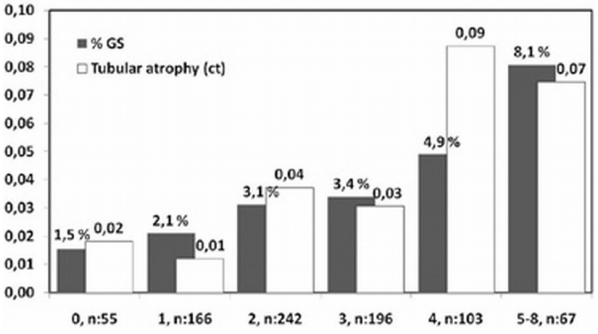

Figure 1. Mean score of tubular atrophy and mean glomeruosclerosis (%GS) by number of donor risk factors.

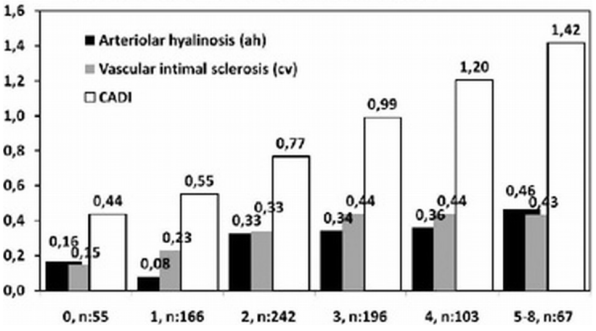

Figure 2. Mean arteriolar hyalinosis, vascular sclerosis and CADI scores by by number of donor risk factors.

the five year death censored graft survivals were 82.6% and 92.6%, respectively.
Conclusion: A thorough perusal of donor medical history can yield valuable information and predict donor kidney histology as well as post transplant graft function and survival.

O-217 ACCEPTABLE LONG-TERM RESULTS WITH KIDNEYS FROM OLD DONORS GIVEN TO OLD RECIPIENTS

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Purpose: To retrospectively assess long-term safety and efficacy of patients that received a cadaveric kidney transplant within the ET Senior program with special regard to recipients 70 years and older.
Methods/Materials: From 5/1999 to 1/2009 a total of 84 patients with a mean age of 67.8 (65-80) years, among them 21 recipients over 70 years received a kidney from a cadaveric donor over 65. The mean number of mismatches in AB/DR was 2.8/1.4, cold ischemia time 13:41 (05:04 – 24:54) hours. Initial CNI-free immunosuppression consisted of an IL-II-blocker (n=81), Campath or Belatacept (according to study protocols). Cyclosporine/Tacrolimus was started after stabilization of renal function at day 6.3/4.2. Observation time was 50.4 (1-116) months.
Results: Patient/graft survival of the entire cohort was 97.5%/94% at year one, and 83%/73% at year five. Mean serum creatinine levels at year 1/5/9 were 1.6/1.8/1.8 mg/dL. In the >70 years old population the patient/graft survival at year one was 89% each, at year five 66%/50%. Two rejections occurred, controlled with steroid boluses. The causes of death were cardiac failure (n=4), pneumonia (n=2), sepsis (n=1), cerebrovascular accident (n=1). Six out of them died with a functioning graft. Major complications were cancer in six patients, congestive heart failure (n=3), arrhythmias requiring pacemaker implantation (n=2), valve replacement (n=)1, multiple bone fractures (n=1), perforation of sigmoid colon (n=1). The mean serum creatinine levels at year 1/5/9 were 1.4/1.5/1.8 mg/dL.
Conclusion: Transplantation of kidneys from old cadaveric donors given to old recipients produces excellent short term and acceptable long-term results. Most complications are caused by underlying age-associated co-morbidities.

O-218 EVALUATION OF IMMUNOLOGICAL RISK IN RENAL TRANSPLANT RECIPIENT FROM EXTENDED CRITERIA DONORS

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Purpose: Whether recipients of marginal donors are more sensitive to elicit a strong and specific immune response associated with a higher risk of acute rejection remains uncertain but is a major question in the field of renal transplantation. Here, we sought to analyze in a large cohort of kidney transplant recipients (n=2121 patients) of extended criteria donors (ECD, n=656 patients) or optimal donors (OD, n=1465 patients), the influence of ECD on the incidence of biopsy proven AR after cadaver renal transplantation. We also aimed to determine the impact of immunological risk factor on ECD graft outcome.
Results: Biopsy AR was not statistically different between recipients from ECD donor group (16%) and from OD group (17%) (p=0.52). This result remains not significantly different after adjustment on immunological risk including panel reactive antibody and previous history of kidney transplantation (17.9% and 17.5% respectively in ECD and OD groups, p=0.92 in patients with a high immunological risk and 15.6% and 17% respectively in ECD and OD groups, p=0.47 in patients with a low immunological risk).
Conclusion: Our study suggest that incidence AR is not increased in recipient of ECD or donor aged $\geq$ 50 yr when compare with recipient of OD or donor aged $<$50 years and that graft outcome is not significantly influenced by immunological factors.

O-219 SURVIVAL BENEFIT OF KIDNEY TRANSPLANTATION FROM DONORS AFTER CARDIAC DEATH

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Background: The continuing shortage of kidneys for transplantation requires major efforts to expand the donor pool. Donation after cardiac death (DCD) has been shown to increase the number of available kidneys but remains underutilized. It is unknown whether patients who receive a DCD kidney live longer than patients who continue dialysis treatment with the option of later receiving a conventional kidney from a brain dead donor (DBD).
Methods: This observational cohort study included patients registered on the Dutch waiting list for a first kidney transplantation between 01/01/1999 and 12/31/2004 (N=2575). Patients were followed from listing until the earliest of death, living donor kidney transplantation or 12/31/2005. Patient survival between treatment groups was compared using sequential stratification (an extension of Cox regression).
Results: 459 patients underwent DCD kidney transplantation while 680 patients received a DBD transplant. Graft failure in the first 3 months after transplantation was twice as likely for DCD kidneys compared to DBD kidneys (12% vs. 6.3%, p=0.001). Standard criteria DCD kidney transplantation was associated with an overall mortality hazard ratio of 0.44 (95% CI: 0.24-0.80, p=0.007) compared to conventional therapy (dialysis treatment with the option of later receiving a standard criteria DBD kidney). The reduced mortality rate translated into 2.4 months additional expected lifetime during the first 4 years after transplantation for recipients of DCD kidneys compared to patients receiving conventional therapy.
Conclusions: Standard criteria DCD kidney transplantation is associated with increased survival of wait-listed patients with end-stage renal disease.

O-220 LONG-TERM OUTCOME OF KIDNEY TRANSPLANTATION FROM DONORS AFTER CARDIAC DEATH

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Background: The shortage of organ donors presents a major obstacle for adequate treatment of patients with end-stage renal disease. Donation after cardiac death (DCD) has been shown to increase the number of kidneys available for transplantation. However, the long-term outcome of DCD kidney transplantation remains to be established.
Methods: This observational cohort study included all DCD kidney transplantations recovered in our procurement area from 01/01/1981 until 12/31/2005 (N=297). Patients were followed until the earliest of death or 12/31/2006. Clinical outcomes were compared to matched kidney transplantations from brain dead donors (DBD, N=594), using multivariable regression models to adjust for potential confounders.
Results: DCD activity resulted in a 44% increase in the number of deceased donor kidneys from our organ procurement area. After adjustment for potential confounders, the odds of primary non-function and delayed graft function were 6.5 (95% CI: 3.5-11.8, p$<$0.001) and 10.3 (95% CI: 6.7-15.9, p$<$0.001) times greater, respectively, for DCD kidneys compared to DBD kidneys. Recipients of DCD kidneys had a 6.2 mL/min (95% CI: 3.0-9.4, p$<$0.001) lower glomerular filtration rate at 1 year after transplantation but a similar rate of subsequent decline in kidney function (p=0.87) as recipients of DBD kidneys. The hazard of death-censored graft loss (restricted to viable grafts) and of recipient death were similar for DCD and DBD kidney transplantations (HR=1.22, 95% CI: 0.79-1.86, p=0.37 and HR=1.16, 95% CI: 0.87-1.54, p=0.32, respectively).
Conclusions: The satisfactory long-term prognosis of viable DCD kidneys and their recipients highlight the need for more widespread use of DCD kidneys.

O-221 DECEASED AFTER CARDIAC DEATH DONATION: LONG TERM RESULTS IN A MATCHED SINGLE-CENTER STUDY

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Purpose: The gap between performed kidney transplantations and active organ needs has resulted in alternative ways to increase the donor pool such as deceased after cardiac death (DCD) donation, gaining more importance due to decreasing donor numbers within the last decade.
We performed a matched single-center study of kidney grafts from DCD-donors compared to grafts from heart-beating donors over a period of 25 years.
Material and methods: Between January 1984 and December 2008 4177 kidney transplantations have been performed at the transplant center in Vienna. Long-term outcomes were compared to 88 grafts obtained from our DCD-program. Data were collected prospectively in our database and recipients were matched on a one-to-one basis according to sex, donor age, cold ischemic time (CIT), number and year of transplantation.
Prognostic significance of cold ischemic time (CIT), first warm ischemic time (WIT), delayed graft function (DGF), donor age, HLA- mismatch and acute rejection were calculated by a Cox-model, graft survival being the endpoint.
Results: Despite we noted a significantly higher rate of DGF in the cohort that received their graft from DCD-donors (71,6% versus 35%) long-term outcomes concerning graft and patient survival were similar in both groups.
At 15 years graft survival for kidneys from heart-beating donors was 53,6% compared to 53,3% in other group.
Univariate analyses revealed donor age (p=0,03), DGF (p=0,0001) and CIT (p=0,0001) as risk factors, whereas only DGF (p=0,01) showed significance in multivariate analyses.
Conclusions: Despite a high DGF rate, outcomes from kidneys obtained from DCD-donors are similar to grafts from heart-beating donors. Due to a lack of large, matched studies our experience proves that grafts obtained from DCD-donors can be used successfully to increase the donor pool and offer good long-term results.

O-222 DUAL KIDNEY TRANSPLANTATION FROM ELDERLY DONORS: LONG TERM OUTCOME AND HISTOLOGICAL FEATURES

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Introduction: Dual kidney transplantation (DKT) has widened the use of organs from very elderly donors, however one of the major concerns about these grafts is their long term outcome. In this light, we have analyzed the histological changes in our DKT population who underwent a protocol biopsy and we have evaluated the renal function of those who have completed a 5 year follow up.
Patients and methods: Since 2000 to present, 130 DKT have been performed at our center. Mean donor age was 72$\pm$6 years. Mean recipient age was 61$\pm$5 years. Immunosuppression therapy was based either on CNI + MMF and steroids or PSI + MMF and steroids. 23 patients with T0 donor biopsies

underwent a protocol biopsy (T1) at a mean time of 22±16 months after transplant. The progression of interstitial fibrosis (IF) and tubular atrophy (TA) was assessed according to the 1997 Banff chronic/sclerosing score. The long term results (5 years) refer to 33 patients transplanted before 2003.
Results: There was no significant progression of IF/TA at protocol biopsies. The mean interstitial fibrosis scores were 1.0±0.4 at T0 and 1.4±0.7 at T1; the mean tubular atrophy scores were 1.3±0.3 at T0 and 1.3±0.6 at T1. After 5 years, 31/33 (94%) patients are alive and 27 grafts are functioning (81%). Only 1 graft loss occurred due to chronic allograft injury at 1.5 years after transplant. Mean serum creatinine at 5 years was 153±64 μmol/L, mean calculated creatinine clearance was 44±13 ml/min and mean proteinuria was 0.59 g/day.
Conclusion: The absence of significant histological worsening, the good graft survival and the satisfactory renal function at 5 years suggest that kidneys from very elderly donors are compatible with an optimal long term outcome.

O-223 KIDNEY ARTERIOLAR HYALINOSIS AND INTIMAL FIBROSIS INHERITED FROM DONOR IS A RISK FACTOR FOR LONG-TERM GRAFT DYSFUNCTION

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Background: Older and marginal donors, who often have a vascular cause of death, are increasingly used in order to limit the continuously growing waiting time for kidney transplantation. There is evidence that inherited vasculopathy frequently founded in these donors' kidneys is a risk factor that determine short and long-term graft outcome. The aim of this study is to evaluate arteriolar hyalinosis and arterial intimal fibrosis as an independent histological factor of long-term graft dysfunction.
Method: All kidney recipients between January 2003 and June 2006, who had a graft biopsy at the time of transplantation, were retrospectively included. Patients follow up range from 1 to 4 years. Double blind histological analysis was done according to the Banff 2005 classification.
Result: Medium age of donor and recipient was respectively 59.6 (31-82) and 52.5 (20-71) years. Glomerulosclerosis and interstitial fibrosis, but not arteriolar hyalinosis, were significantly correlated with donor age (p = 0.00019 and 0.043). Graft delayed function was significantly correlated with acute tubular necrosis (p=0.03) and arterial intimal fibrosis (p=0.025), but not arteriolar hyalinosis. In contrast, long-term graft dysfunction, was signicantly correlate with arterial intimal fibrosis at 4 year (p=0.002) and arteriolar hyalinosis at 1 year (p=0.034). Arterial intimal fibrosis seems to be an independent risk factor for delayed graft function and poor long-term outcome.
Conclusion: Inherited histological vasculopathy lesion is a risk factor of long-term graft dysfunction.

O-224 SYSTEMIC AND LOCAL RENAL COMPLEMENT ACTIVATION IN BRAIN-DEAD DONORS

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Introduction: Increased systemic complement activation is observed after traumatic brain injury or stroke. Inherent to systemic complement activation is generation of C3a and C5a which are known to provoke inflammation. In kidney transplantation, grafts derived from brain-dead donors show inferior transplant outcome compared to living donors. We hypothesized that during brain death (BD), systemic complement is activated, contributing to the renal injury observed in brain-dead donors.
Materials and methods: In human living (n=20) and brain-dead donors (n=30), complement pathway activity was analysed by a WIELISA assay in serum collected just before organ retrieval. In same donors, C3a receptor (C3aR), C5a receptor (C5aR) and decay accelerating factor (DAF) expression was analysed by Real-time PCR in kidney biopsies obtained at donation, after cold preservation and 45' after reperfusion.
Results: We found significant systemic complement activation after BD of mainly the alternative pathway ($P<0.05$). In renal tissue, C3aR was significantly upregulated at all three time points in brain-dead vs living donors, while C5aR reached statistical significance only after cold ischemia in brain-dead donors ($P<0.05$). Moreover, a positive regression was found between both C3aR and C5aR expression in brain-dead donor grafts after reperfusion and serum creatinine 14 days after transplantation ($P<0.01$). Gene expression rates of complement activation inhibitor DAF was not changed during transplantation of living or brain-dead donor kidneys.
Discussion: This study shows that systemic complement is highly activated in clinical and experimental BD and that renal C3aR and C5aR are significantly upregulated in brain-dead donors compared to living. Furthermore, a positive regression was found between C3aR and C5aR expression after reperfusion of BD kidney grafts and renal function early after transplantation. Complement activation inhibitor DAF was not upregulated during transplantation. Concluding, systemic and local complement activation may contribute to renal damage observed in brain-dead donors.

O-225 RISK FACTORS FOR DETERIORATION OF RENAL FUNCTION AFTER DONOR NEPHRECTOMY

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Introduction and objectives: There are very few recent studies investigating increased risks for adverse effects that lead to chronic kidney disease (CKD) of kidney donors. The aim of this study was to identify the factors to protect renal function in actual live kidney donors who had undergone live donor nephrectomy at Tokyo Women's University Hospital between the years 2004 and 2005.
Materials and methods: Sixty eight individuals who underwent donor nephrectomy were enrolled in this study. Donor age, body mass index (BMI), casual blood pressure, serum creatinine at pre-operation and at 3 months follow-up, uric acid at pre-operation and several other clinical parameters were assessed as risk factors. The severity of arteriosclerosis in renal arteries from the back table biopsy were semi-quantitatively evaluated and classified into four grades. The ratio of glomerular sclerosis of the 0 hr biopsy specimens were also determined. Impairment of the renal function after surgery was expressed by the difference of serum creatinine at pre-operation and at 3 months follow-up.
Results: The ratio of glomerular sclerosis (r=0.34, p=0.004), systolic blood pressure (r=0.31, p=0.01) and diastolic blood pressure (r=0.28, p=0.02) were positively correlated with donor age in simple regression analysis. Deterioration of renal function after donor nephrectomy was negatively correlated with BMI (r= -0.31, p=0.009) and positively correlated with severity of arteriosclerosis in interlobular artery (r=0.23, p=0.05). In a multiple regression analysis model respecting severity of arteriosclerosis in interlobular artery the influence of uric acid at pre-operation, systolic blood pressure and serum creatinine remained significant (r2=0.1479, p=0.0004, p=0.017, p=0.023).
Conclusions: Preventing progression of arteriosclerosis and selecting the optimal BMI before donor nephrectomy will help avoid the impairment of renal function in live kidney donors who have undergone live donor nephrectomy.

O-226 MEDIUM-TERM FOLLOW UP OF RENAL TRANSPLANT RECIPIENTS FROM A RANDOMISED CONTROLLED TRIAL OF LAPAROSCOPIC VERSUS OPEN LIVE DONOR NEPHRECTOMY

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Background: Laparoscopic live donor nephrectomy continues to gain in popularity but there are still some concerns that this technique reduces morbidity in the donor at the expense of increased morbidity in the recipient. The aim of this study was to evaluate recipient outcome at a median follow up of 6 years following a randomised controlled trial of laparoscopic versus short incision open donor nephrectomy.
Methods: Eighty-four live kidney donors were randomised in a 2:1 ratio to laparoscopic (LDN n=56) or short incision open donor nephrectomy without rib resection (ODN n=28). The two groups of transplant recipients were followed up for between 4 and 8 years and outcome data recorded prospectively. Particular attention was paid to rates of ureteric complications and renal function parameters.
Results: LDN operation time was longer (168±30 vs 145±27 min; P=0.0042) and LDN kidneys suffered longer first warm ischaemic times (3.8±1.1 vs 2.2±1.1 minutes; $P<0.0001$). There were no episodes of arterial or venous thrombosis but one kidney in each group suffered delayed graft function. At a median follow-up of 74 months, one ureteric stenosis requiring re-operation had occurred in each group (NS) and there were no differences in renal function or allograft survival between the ODN and LDN groups (Table 1).

Table 1

	LDN (n=56)	ODN (n=28)	P Value
Creatinine - Year 1	129±40	125±35	0.692
Creatinine - Year 2	168±176	156±162	0.761
Creatinine - Year 3	141±72	168±165	0.468

Conclusions: Laparoscopic nephrectomy does not lead to an increase in ureteric complications. Despite subjecting the donated kidney to a prolonged pneumoperitoneum and longer first warm ischaemic time, laparoscopic donor nephrectomy does not compromise long-term recipient renal function.

Session 27. Surgical challenges in liver transplantation

O-227 INTENTIONAL PORTAL PRESSURE CONTROL IS A KEY TO IMPROVE THE OUTCOME OF ADULT LIVING DONOR LIVER TRANSPLANTATION (A-LDLT) WITH SMALLER GRAFTS

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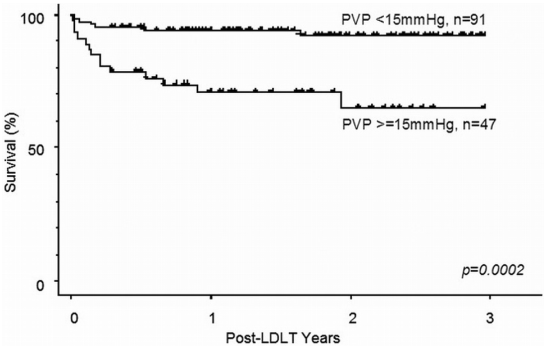
Introduction: Because of small-for-size (SFS) graft idea, larger size grafts, i.e. right lobe grafts, became the standard graft type in A-LDLT. However, graft selection has recently changed in our institute; i.e. smaller grafts can be utilized in a certain conditions.
Methods: A total of 621 A-LDLT were performed since 1998. Large series of portal vein pressure (PVP) control was introduced in 2006, and 138 cases were analyzed. PVP was controlled mainly by splenectomy, and by creating port-systemic shunt additionally if indicated. The optimal PVP is set <20mmHg after reflow of graft. Graft size, graft type and patient survival were analyzed with or without PVP control.
Results: Prior to PVP control, only 10.1% (47 out of 464 A-LDLTs) were graft/recipient weight ratio (GRWR) <0.8%, with 74.4% and 70.0% survival rate at 1- and 5-year after A-LDLT. In contrast, after 2006 (introduction of PVP control), the selection of SFS grafts increased up to 24.6% (34 out of 138 A-LDLTs), with 84.5% survival rate at 1-year. Although only 4.7% of A-LDLT used left lobe grafts before 2005, 31.2% of A-LDLT utilized left lobe grafts after 2006 with better outcomes. Not only the survival improvement in SFS grafts, but also those in GRWR>0.8% were observed 87.2% vs. 74.8% at 1-year after A-LDLT (after 2006 vs. before 2005).
Retrospectively, we analyzed the patient survival at 15mmHg of final PVP, and PVP <15mmHg demonstrated significantly better 1-year survival than ≥15mmHg (94.4% vs. 71.1%).



Conclusions: PVP control can improve patient survival in SFS grafts as well as in appropriate size grafts in A-LDLT. As PVP control can overcome size mismatching, it may be applied not only in A-LDLT, but also in DDLT or split-liver transplant when the graft size is considered smaller for recipient.

O-228 DUAL GRAFT LIVING DONOR LIVER TRANSPLANTATION WITH RIGHT AND LEFT LIVER GRAFT COMBINATION

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Background: In this study, we reviewed the outcome of DLDLT with combined right and left liver (RL/LL) grafts in the context of donor safety and recipient outcome.
Patients and methods: From 2000 March to 2007 June, 213 cases DLDLT were performed from in our institution. Of them, 37 cases (17.4%) were performed by using combined RL/LL grafts. We analyzed short-term and long-term outcome of RL/LL DLDLT. We compared outcomes including donor morbidity, graft and patient survival and recipient morbidity rate with those of single right lobe LDLTs and DLDLTs with another graft combination.
Results: In 37 cases, modified right lobe graft was used in 33 patients and right lobe (RL) graft in 4 patients. Mean GRWR of RL/LL DLDLT was 1.10% which was slightly higher than those of single RL LDLTs (0.94%) and DLDLT with another graft combination (1.02%), but there was no statistical significance. There was no donor mortality. And morbidity rate of RL donor was 3.5% and one of LL donor was 1.2%. Overall 1- and 3-year survival rate was 97.3% and 95.8% which were comparable to that of single RL LDLT. Of 37 cases RL/LL DLDLT, 3-year graft survival rate (92.3%) of RL graft was slightly lower than that of LL graft (97.8%). Morbidity rate of each graft was similar. And overall morbidity rate of RL/LL DLDLT was not different from single RL LDLT and DLTLT with another graft type.
Conclusion: RL/LL DLDLT is feasible option for overcoming small-for-size graft in adult LDLT. In the context of donor safety, it did not increase donor morbidity rate in right liver donor.

O-229 REAPPRAISAL OF EFFICACY AND SAFETY OF SELECTIVE HEMI-PORTOCAVAL SHUNT IN ADULT LIVING DONOR LIVER TRANSPLANTATION

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Aim: The purpose of this study is to evaluate the efficacy and safety of hemi-portocaval shunt (HPCS) as an inflow modulation in small-for-size graft liver transplantation.
Background: We have developed selective HPCS based on portal vein pressure and actual graft volume to overcome small-for-size syndrome. The number of the patients with HPCS reached to double what we reported before and 3-years mean follow-up was achieved.
Patients and methods: From July 2003 to January 2009, twenty patients (mean age 46.0 years old, mean body weight 73.2kg) underwent living donor liver transplantation (LDLT) with HPCS. All patients except one who had fulminant hepatic failure underwent LDLT due to liver cirrhosis (the mean MELD

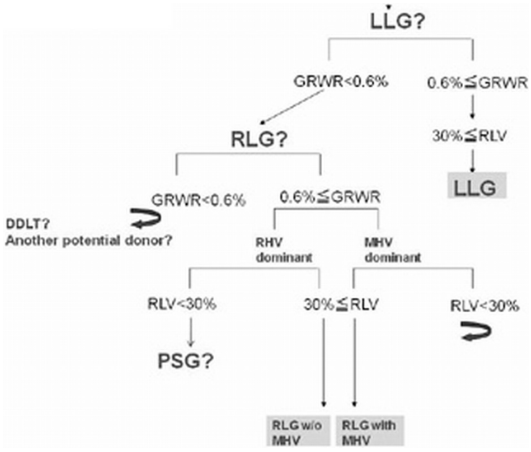

Figure 1. Graft selection.

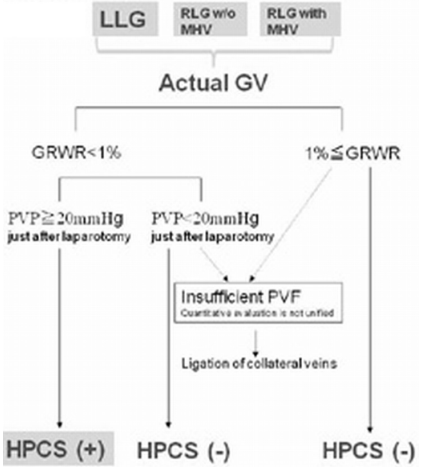

Figure 2. Selective HPCS.

score was 19.3 (6-43)). Graft selection was done by our algorithm under informed consent of the patient and his family.
We used a left lobe graft in 9 patients and a right lobe graft in 11 patients. The mean GRWR was 0.71% (0.49-0.98). All recipients were given a HPCS depend on the portal vein pressure and the actual graft volume.
Results: The mean follow-up period was 873 days (three-year cumulative survival was 90.0% (Kaplan-Meier). Eighteen patients are alive with normal liver function and good liver regeneration. One patient with co-infection of HIV and HCV died of fungal infection on 147POD and the other patient died of VOD on 16POD after liver transplantation. All shunts have not been closed operatively nor interventionally except one who had VOD.
Conclusion: In conclusion, HPCS is a safe and effective procedure if it is created by appreciating portal vein pressure and graft volume. Further more, our graft selection algorithm and the indication of creating HPCS would make it possible to address scheduled utilization of small-for-size graft.

O-230 LIVING LIVER DONOR MORTALITY AND MORBIDITY DEMAND GENUINE TRANSPARENCY: THE SITUATION IN EGYPT

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Background: The reluctance to publish a complete account of any death or serious complication among living-liver donors, although understandable in a fraught medicolegal environment, is not good for patient care or liver transplantation. A donor death or serious complication will have a devastating effect not only on the families of the donor and recipient but also on the clinical staff involved. The impact may spread to other potential donors and recipients, and lead to negative publicity and potential economic damage to the transplant center.
Objective: To discuss the mortality and morbidity within living liver donors in Egypt and to try answering the question: "What level of risk are the potential donors and the society willing to accept?"
Methods: By personal communication and reviewing all published data and reports from centers performing LDLT in Egypt, morbidity and mortality within the donors were analyzed.
Results: From August 2002 to January 2009, the number of living donor liver transplants performed in Egypt topped out to more than 740 procedures in 11 centers. There were two reported donor mortality (0.29%). The first was a 45 year-old male who donated a right lobe to his brother and died of sepsis from bile leak 1 month after donation. The second was a 22 year-old male who donated his right lobe to his father, suffered from massive intraoperative bleeding from the stump of the portal vein and died of multisystem organ failure after 10 days. Donor morbidity included: portal vein thrombosis (0.5%), biliary complications (2%), bleeding (3%), intraabdominal collections (6%), pneumonia (4%), pleural effusion (22%), and depression (12%).
Conclusion: Live liver donor morbidity and mortality remains underreported. LDLT programs are best served by collaborative effort between centers reporting accurate information characterized by genuine transparency.

O-231 LEFT LIVER LOBE TRANSPLANTATION IN ADULT RECIPIENTS FROM SPLIT LIVERS AND LIVING DONORS

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The use of left livers for transplantation in adult recipients remains contreversial and challenging. We present a single center experience of left lobe transplantation over a 12 year period.
Patients and methods: From march 1996 to november 2008, 27 adult patients, 13 males and 14 females with a mean age of 49 years (range: 18-67), a mean body weight of 59 kg (range: 40-84) received a left lobe transplant from 17 split livers and 10 living donors. Mean Graft-to-Recipient-Ratio (GRWR) and mean MELD score were 0.88% (range: 0.57%-1.28%) and 18 (range: 6-32) respectively. Main indications for liver transplantation (LT) were alcoholic (n=10) and viral-related (n=6) cirrhosis; 4 patients did not have cirrhosis and 3 had late retransplantation. In 12 patients, a venous splanchnic decompression (2 porta-caval shunts and 10 meso-caval shunts with porto-mesenteric disconnection) was performed at the end of the transplantant procedure in order to decrease portal pressure to the graft.
Results: After a mean follow-up period of 43 months, 18 (66.6%) out of the 27 patients are alive; 3 patients had early retransplantation for technical complication, small-for-size syndrome and venous portal steal syndrome. Six deaths (22.2%) occured in the peri-operative period from graft ischemia due to excessive portal decompression in the 2 patients with porto-caval shunt, sepsis after re-LT in 2 cases, small-for-size syndrome in 1 case and cardiac failure in 1 case. In univariate analysis, risk factors for early patient death were GRWR below 0.8% and portal décompression. When no portal decompression was performed, all 15 recipients survived without need for retransplantation.
Conclusions: When appropriate graft/recipient matching is performed, excellent outcome can be expected in adult patients following left liver lobe transplantation. Portal decompression should be used in very selected cases.

O-232 RECIPIENT OUTCOMES IN DOMINO LIVER TRANSPLANTATION WITH DONOR VENA CAVA PRESERVATION

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Introduction: Domino liver transplantation (LT) is an accepted resource to increase donor pool using whole livers from live donor with Familial amyloid polyneuropathy (FAP). Hepatectomy with vena cava preservation (VCP) is considered a safer procedure and hemodinamically more stable for the donor, but this requires vascular reconstruction of the graft's outflow. The aim of the study is to retrospectively evaluate complications and outcomes of FAP liver recipients with donor VCP.
Materials and methods: From Jan-01 to Dec-08, 30 patients received a LT from a FAP donor with VCP, one patient was excluded due to primary nonfunction of the graft. Venous outflow reconstruction of the FAP-graft was performed using a venous patch from the deceased donor, by 2 techniques: using suprahepatic VC (n=27) or the iliac bifurcation (n=3), both associated with venoplasty of the three hepatic veins. Persistence of ascitis postoperatively was defined by production of $>$500ml/day for more than 10 days. Patient demographics, ischemia time, vascular complications and outcomes were analyzed.
Results: No clinical or radiological signs of venous outflow obstruction were found in our series with a median follow up of 39.7 months (range 2.6-98.1). The main causes of liver disease were HCV (43.3%) and ETOH (36.7%); HCC was present a third of the patients. Recipient's means age was 62,5 years old with a mean MELD score of 18,4. The mean ischemia time was 9,5 hours (570 minutes). Of the 6 patients presenting with ascitis pre-LT, two persisted short term and were treated conservatively. The 1 year patient survival was 96,6% and the one year graft survival was 100%.
Conclusions: Domino LT is associated with good results. Donor VCP doesn't compromise recipient outcomes despite the increased dificulty related to the vascular reconstruction. In our series, there wasn't recurrence of FAP in the recipient.

O-233 AUXILIARY LIVER TRANSPLANTATION FOR ACUTE LIVER FAILURE: A REAPPRAISAL

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Background: Auxiliary liver transplantation (AxLT) is an attractive option in patients with acute liver failure since it allows delayed regeneration of the native liver and discontinuation of immunosuppression. However, use of AxLT remains controversial due to technical complexity, increased morbidity and insufficient regeneration of the native liver in some cases. The aim of our study was to assess the role of AxLT based on our experience in 27 patients.
Patients and methods: From 1992 to 2008, AxLT was performed in 27/154 (17%) patients who had emergency liver transplantation. There were 15 females, 12 males; mean age 32 years (range 16-62). Indications for AxLT were paracetamol overdose (3), HBV infection (8), drug-induced (9), mushroom poisoning (2), hepatitis of unknown origin (4) and others (1). Criteria for AxLT were: absence of multi-organ failure, a potential for regeneration and availability of an optimal allograft. 16 patients received a right graft, 3 received a whole graft, and 8 received a left graft. We considered 2 periods: before (first) and after (second) 2000.
Results: Mean duration of surgery was 10.9 hours; mean blood units transfused was 5.3. Mean follow up was 110 months. Early post-transplant mortality rate (within 3 months) was 44% in the first period and 22% in the second. Survivors who had complete regeneration and were free of immunosuppression were 4/10 (40%) in the first period and 4/6 (66%) in the second. Among these, all auxiliary grafts atrophied with progressive tapering of immunosuppression.
Conclusion: This study suggests that initial results of AxLT were hampered by a learning curve. In recent years, results of AxLT became comparable to conventional LT and most patients could be weaned off immunosuppression. Although applicable to a minority of patients, AxLT should be considered in those with a potential for regeneration.

O-234 LIVER TRANSPLANTATION (LT) FROM DONATION AFTER CARDIAC DEATH (DCD) DONORS: MULTICENTRE BELGIAN EXPERIENCE 2003-2007

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Introduction: We retrospectively reviewed the DCD-LT Belgian experience in terms of patient and graft survivals, and of biliary complications.
Patients and methods: From 2003 to 2007, 58 DCD-LT were performed in Belgium, 56 from Maastricht category III donors. Mean donor age was 44 years. Mean donor BMI was 24.5. All DCD procedures were performed in the OR. Mean delay between respiratory withdrawal and cardiac arrest was 14.7 min. Mean delay between respiratory withdrawal and aortic flush was 25 min.
Results: Mean cold ischemia was 451 min. Peak of transminases was 2,241±338 UI/mL. Global patient survival was 91.3%, 81.2% and 68.1% at 1 month, 1 year and 2 years, respectively. Graft survival was 84.4%, 70.3% and 49.7% at 1 month, 1 year and 2 years, respectively. Causes of early mortality were operative death (n=2), PNF, MOF and ARDS. Late deaths were due to accident (n=2), malignant tumour (n=5) and biliary sepsis. Two patients needed early reLT for PNF and HAT. Six patients needed later reLT for diffuse bile duct lesions. Eleven patients developed biliary stenoses requiring endoscopy and/or surgery. In univariate analysis, significant donor factors for death were delay between respiratory arrest and cardiac arrest of more than 15 min, and cold ischemia of more than 6 hours. In the recipient factors, HU status of the recipient was the only significant risk factor for early death. Censoring the graft losses within the first 3 months, the overall rate of symptomatic ischemic bile duct lesions was 38% (19/50).
Conclusion: DCD donors may be a source of viable liver grafts. However actual results are inferior to the results of DBD LT, and prognostic criteria should be evaluated to improve these results.

O-235 NEW NATURAL BANDING METHOD OF PORTO-CAVAL SHUNT BY USING A PARAUMBILICAL VEIN AS SHAPE MEMORY GRAFT IN SMALL-FOR-SIZE GRAFT LIVING DONOR LIVER TRANSPLANTATION

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In order to obviate a small-for-size graft syndrome (SFSGS), a portacaval (PC) shunt had been considered in a case of adult-to-adult living donor liver transplantation (AA-LDLT). However, the permanent PC shunt sometimes revealed the graft atrophy in the late period of LDLT, thereby resulting in liver dysfunction. Therefore, we have already reported the effect of a time-lag ligation of PC shunt after LDLT. But this procedure has a problem of management of long intra-abdominal stay of cathetel. In this study, we developed a new technique of PC shunt for both the prevent of SFSGS in the early period and the liver atrophy in the late period after LDLT.
Materials and methods: We have used a paraumbilical vein for interposed PC shunt after re-canalization by a surgical instrument in 4 adult to adult LDLTs who had a portal hypertension exceed 25cmH2O or SFS graft after the implantation. The patency of PC shunt using the paraumbilical vein had been evaluated by the dynamic abdominal CT.
Results: The PC shunt using the paraumbilical vein spontaneously and gradually closed within 6 months after LDLT. (Conclusion) The paraumbilical vein which is seen as a tubular structure arising in the fatty falciform ligament between the left lobe of the liver and usually has no blood flow. Our result paraumbilical vein might closed as shape memory graft with a reduction of portal flow after regeneration of graft liver. The PC shunt using the paraumbilical vein might be useful for the both SFSGS in the early period and the prevent of liver atrophy in the late period after LDLT

O-236 THE EFFECT OF DIFFERENT TECHNIQUES FOR BILE DUCT ANASTOMOSIS ON POST-TRANSPLANT BILIARY COMPLICATION IN LIVING DONOR LIVER TRANSPLANTATION

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Biliary complications remain a major cause of morbidity after liver transplantation. And, suboptimal blood supply to the bile duct by technical cause is one of the important pathogenesis of biliary complication. The objective of this study is to verify the effect of different techniques for bile duct anastomosis on post-transplant biliary complication. Among the 121 liver transplants done at our center from August, 2005 to August, 2008, 68 right lobe LDLT recipients were enrolled in this study. Different techniques for biliary anastomosis were done during different periods. The first 38 recipients used 'Classic dissection with size-matched anastomosis' (Group 1), the next 16 recipients used 'Hilar plate looping with size-matched anastomosis' (Group 2), and the last 14 recipients used 'Hilar plate looping with glissonian overlapping anastomosis' (Group 3) technique. 'Hilar plate looping' loops the complete hilar plate and glissonian sheath around the hepatic duct after full dissection of the right hepatic artery and portal vein. This thick cover of hilar plate around the graft duct preserves the peri-ductal arterial plexus, and prevents their retraction. Bilomas or strictures that required surgical or radiologic intervention and developed within 6 months after transplantation were defined as post-transplant biliary complications. There were 52 males and 16 females with a mean age of 50.6±7.9 years. Hepatocellular carcinoma with B-viral hepatitis was the most common underlying liver disease (31/68; 45.6%). The incidence of complications are shown in Table 1.

Post-transplant biliary complication (Developed within 6 month after transplantation)

	Group 1 (n=38)	Group 2 (n=16)	Group 3 (n=14)
Bile leak (Biloma)	2	3	–
Bile duct stricture	12	–	–
Both	1	–	–
Total	15 (39.5%)	3 (18.8%)	0 (0.0%)

There was significant difference in complication rates between group 1 and 3 (p=0.011), however, there was no significant difference between group 2 and 3 (p=0.310). This new technique of 'Hilar plate looping with glissonian overlapping' during donor surgery significantly reduces recipient biliary complications in LDLT.

O-237 POLYCYSTIC LIVER AND KIDNEY DISEASE: LIVER TRANSPLANTATION ALONE OR COMBINED LIVER KIDNEY TRANSPLANTATION?

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Polycystic liver disease (PLD) is frequently associated with autosomal dominant polycystic kidney disease (ADPKD). Clear indications for combined liver and kidney transplantation (CLKT) are dialysis-dependent status and overt terminal renal failure. If renal insufficiency is less pronounced, the indication for an associated kidney transplantation (KT) is controversial. In this study we review our experience with isolated LT and CLKT in patients with PLD.
Methods: Between 1995 and 2008, 37 patients underwent LT for PLD. 3 patients with isolated PLD received LT alone. 34 patients had combined PLD and ADPKD: 19 underwent isolated LT and 15 CLKT. Among the 15 CLKT patients, 10 were dialysis-dependent at time of transplantation whereas KT was performed preemptively in 5 (creatinine clearance (CrCl) 34.2, 32.1, 38.3, 27.5 and 15.4 mL/min respectively).
Results: The 1 and 5 year patient and liver graft survival are 95% and 90%, respectively. Of the 19 patients who underwent isolated LT for combined PLD and ADPKD: 3 received a KT 9, 9 and 8 years post-LT because of evolving ADPKD and calcineurin inhibitor (CNI) toxicity (*pre-LT* CrCl 106, 58,6 and 103,9 mL/min respectively); 2 developed terminal kidney failure 7 and 9,5 years post-LT (*pre-LT* CrCl 66,4 and 52,4 mL/min, respectively); 1 developed acute renal failure immediately post-LT, requiring permanent dialysis (*pre-LT* CrCl 47,6 mL/min).
Conclusions: This series (the largest reported so far) demonstrates that short and long term survival after LT and CLKT for PLD is excellent. Terminal kidney

failure after isolated LT is due to evolving renal polycystosis and CNI toxicity. In patients with preserved or mildly affected renal function and who receive isolated LT, strategies to spare the nephron mass are essential. In patients with evolving renal impairment pre-transplant, CLKT is the preferred option, anticipating the need for later KT.

Session 28. New organ donation challenge: from living donor to non heart beating donor

O-238 SUCCESSFUL EXPANSION OF THE LIVING DONOR POOL BY ALTERNATIVE LIVING DONOR PROGRAMS

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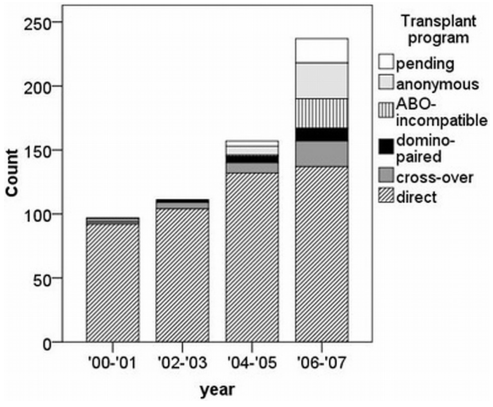
Introduction: The development of alternative living donor programs considerably increased the number of renal transplantations in our centre.
Method: This retrospective study includes all 786 patients and 1059 potential donors that attended our pre-transplant unit between January 1st 2000 and December 31st 2007, with the request for a living-donor renal transplantation.
Results: More than 50% of the potential donors were first degree family members. The recipients brought one potential donor in 77.2% of cases, 2 donors in 15.9%, 3 or more donors in 6.8% of cases. In the regular living donor program a compatible donor was found for 467 recipients. Without considering alternative donation 579 donors would have been refused, 13 donations pending. Alternative living donor kidney transplant programs led to an increase in the number of compatible combinations. The kidney-exchange program (35), ABO-incompatible donation (25), altruistic donation (37) and domino-paired altruistic donation (17) increased the number of compatible combinations with 114 (24.4%). Contrary to the direct donation program, donors in the alternative programs were primarily partners and other no family members ($p<0.001$). Eventually for 54.9% (581/1059) of our donors a compatible combination was found, 458 donors were definitely refused, 20 donations pending.
In 26.4% of cases the donor was refused because another donor was more compatible. Donor-recipient incompatibility comprised 19.4% in the final refused population. Without considering alternative donation 36.4% of the refused donors would have been declined on incompatibility. This means that 20% of the whole potential donor population was incompatible with their first choice recipient.



Conclusion: The implementation of alternative living donation programs led to a significant increase in the number of transplantations, while transplantations via the direct donation program steadily increased. This success compensates for the fact that almost two donor screenings had to be done for every transplantation. A major increase in the proportion of alternative living donations can be expected.

O-239 DONOR ETHNICITY AND PARTICIPATION TO (ALTERNATIVE) LIVING KIDNEY DONATION PROGRAMS

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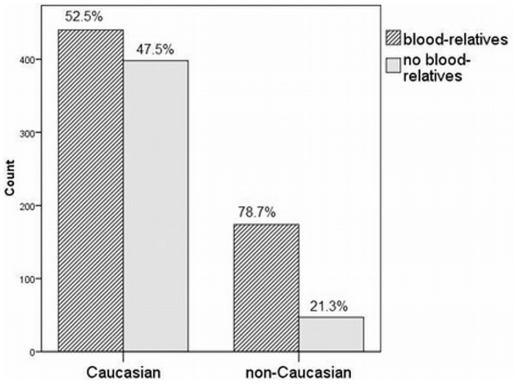
Introduction: In Rotterdam 30% of inhabitants are immigrants Immigrants represent 33% of the patients on the waiting-list for transplantation.
Method: This retrospective study includes all 1059 potential donors that attended our pre-transplant unit between January 1st 2000 and December 31st 2007, with the request for a living-donor renal transplant procedure. Ethnicity was classified as: Caucasian, African, Asian, Arabian or Turkish. Living donor programs in our center are: Direct, Kidney-exchange, Domino-paired, ABO-incompatible, and Altruistic donation.
Results: Predominantly Caucasian donors attended our out-patient clinic (79%). From all 1059 potential donors 581 eventually were coupled to a compatible recipient. In the population of actual donors, the preponderance of Caucasian donors was even more striking (85%). Only 39.4% of non-Caucasian potential donors actually donated, compared to 58.9% of the Caucasian potential donors ($p<0.001$). Among non-Caucasian ethnicities, Arabian potential donors were least likely to donate (23.5%) and Asian potential donors were most likely to donate (50.0%). Non-Caucasian donors significantly less often participated in the alternative living donor programs (3.6% respectively 12.6%, $p<0.001$). In the donor population Caucasians were 50.6 ± 12.8 years old and non-Caucasians were 41.6 ± 11.3 years old ($p<0.001$). In the non-Caucasian donor population first degree relatives predominated, whereas first degree relatives and "no family" were equally represented in the Caucasian donor population ($p<0.001$). In comparison to the Caucasian population, partners are under-represented in the non-Caucasian population ($p<0.001$).



Reasons for donor decline were not different.
Conclusion: Non-Caucasian recipients less often attend the pretransplant outpatient clinic with a living potential donor, and these potential donors are less likely to donate. Non-Caucasian couples less often participate in alternative living donor programs. Non-Caucasian donors are primarily represented by first-degree relatives, whereas only half of the Caucasian donors are first degree relatives. Partners are under-represented in the non-Caucasian donor population.

O-240 APPROACHES FOR LAPAROSCOPIC LIVE DONOR NEPHRECTOMY SHOULD BE SELECTED ACCORDING TO SURGEON'S SKILL AND DONOR'S PHYSICAL STATUS

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Objectives: Donor's safety, quality of life and the graft quality are the primary basis of live donor nephrectomy. Therefore, we have investigated the outcomes of different surgical approaches to find the best method for laparoscopic live donor nephrectomy.
Methods: A total of 259 donors who underwent laparoscopic live donor nephrectomy were included in this retrospective study. Three approach have been performed: transperitoneal hand-assisted (HALDN, n=104), pure retroperitoneal (RPLDN, n=131), retroperitoneal hand-assisted (HARPDN, n=24). Selection of the approach was based on the history of abdominal surgery, patient's choice or surgeon's preference. The followings were compared: age, gender, body mass index (BMI), laterality, operation time (OT), blood loss (BL), warm ischemic time (WIT), the number of analgesics use (NA), early graft function measured with radioisotope renal scan on day 1 (the peak uptake time, the 20 min to peak uptake ratio, effective renal plasma flow, and donor complication. Correlation between OT and the thickness of perinephric fat (TPNF) measured with CT scan was studied. The difference in the above outcomes among 5 surgeons was also compared.
Results: There was no difference in age, gender, BMI, laterarity or BL. OT and WIT were significantly longer in RPLDN or HAPRDN than in HALDN. NA was signifcantly larger in HALDN than in the other approaches. Early graft function was equivalent in all the approaches. The frequency of minor or major

complications and open conversion were significantly larger in HARPDN than in the others. OT, WIT, and the complication rates were significantly different depending on the surgeon. OT significantly correlated with TPNF in RPLDN but not in HALDN.
Conclusions: RPLDN is the first choice of approach based on less pain. However, HALDN should be choosen depending on surgeon's skill and TPNF.

O-241 COMPLICATION RATES IN 1019 CONSECUTIVE LIVING DONOR NEPHRECTOMIES

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Purpose: Living kidney donor nephrectomy (LDN) has been performed at our hospital since 1960. We wanted to assess early postoperative complication rates during the last decade.
Materials/Methods: We retrieved the files from live kidney donors in the period 1997-2008. Complications related to the donation were identified, registered and reviewed by a panel, based on the Clavien grading system for surgical complications (grade $\geq$3 = major complication).
Results: During the time period there were a total of 1023 LDN performed at our hospital. We were able to collect data on 1019 of the donors (593 female). Mean age was 47,7 years (SD 11,8) and mean BMI was 25,4 (SD 3,2).
There was no peri- or postoperative mortality. A total of 30 major (2,9%) and 184 (18,1%) minor complications were registered. In 329 (32,3%) donors the right kidney was removed. Kidney vessel anomalies were present in 238 (23.4%) donors. Laparoscopic nephrectomy was introduced in 1998 and 244 (23,9%) nephrectomies were done laparoscopically. Three of these needed urgent conversion. There was a higher frequency of major complications in the laparoscopic group (4,1% vs. 2,6%), but the difference was not statistically significant. There have been fewer major complications during the last years of laparoscopic nephrectomy indicating a "learning curve" for the procedure. In the postoperative period 17 donors underwent re-operation. Wound infection developed in 38 (3,7%) donors. Significant peri- and postoperative bleeding occurred in 16 (1,6%) patients, eight (0,8%) of these received blood transfusions. There were seven cases of renal artery lesion.
Conclusion: The risk of major complications after LDN is low (2,9%), but might represent a potential hazard to the donor. A vigilant surveilance of peri- and postoperative care is mandatory in living donor nephrectomies.

O-242 5-YEARS EVALUATION OF THE NATIONAL LIVING DONOR KIDNEY EXCHANGE PROGRAM IN THE NETHERLANDS

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Background: Living donor kidney exchange has become an efficient solution for recipients with a blood type or cross-match incompatible donor. However, no information is available on the practical problems inherent to these programs. Here we describe our 5 years experiences with 312 couples enrolled from the seven transplant centers.
Methods: Our protocol consists of five steps; registration, computerized matching, cross matching, donor acceptation, and transplantation. We prospectively collected data of each step of the procedure.
Results: Out of the 312 registered pairs we created 194 computer-matched combinations. However, 72/194 recipients proved to have a positive cross match with their new donor, which was not predicted by the screening results of the recipient centers. Alternative solutions were found for 47 couples, resulting in a total of 169 new combinations with negative cross matches. Thereafter, due to 24 individual clinical problems, the exchange procedure had to be discontinued for 59 couples while only for 21 of them alternative solutions were found. At the end of the day 131 patients (42%) had received exchange kidneys, 75 (24%) were transplanted outside the program, 67 (21%) are still on the crossover waitlist and 39 (13%) had left the program for medical or psychological reasons.
Conclusion: A living donor kidney exchange program is a dynamic process. Many clinical hurdles and barriers are encountered that for a large part were not foreseen but should be taken into account when programs are initiated based on computer simulations. Success is dependent on a flexible organization able to create alternative solutions when problems arise. Centralized allocation- and cross match procedures are instrumental in this respect.

O-243 LIVING KIDNEY DONORS INCREASE KIDNEY FUNCTION OVER LONG TIME

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Background: Kidney tranplantation with organs from living donors has increased. We wanted to find out more about kidney function and donor experiences with the focus on long-term follow-up.
Material and methods: All kidney donors from 1965-2005 undergoing surgery at our instituion were included in a survey study. Medical analyses of kidney function, microalbuminuria, blood pressure, parate hormone were made as well as a questionnaire.
Results: Of the 1112 donors 13% had died, 6% could not be identified and 4% were living abroad. Thus, 840 persons, i.e. 77% were available for the study. 661 accepted to participate. The study is still ongoing and we have test results from the majority.
The present mean age (SD) of the donors was 61 (13) years, range 24-93. Time since donation was 14 (9) years, range 2-42 years. The mean (SD) s-creatinine at follow-up was 94 (26), range 48-330 micromol/L. The number investigated was 496. The mean s-urea was 6.9 (1.8) mmol/L, range 1.8 – 20.2 (n= 470). S-Cystatin C gave a mean value of 1.1 (0.29) mg/L, range 0.6-4.2 (n= 398).
Measured GFR with iohexolclearance or CrEDTA clearance (n = 153) showed a mean (SD) value of 67 (15) mL/min/1.73m^2 body surface, range 23-111. The most interesting result is the following: We estimated the GFR that the donor would have had with two kidneys today and compared that with the actual kidney function. The ratio between the actual and expected GFR was calculated for each donor. Whatever method used for calculation or measuring the actual GFR the kidney function had increased with time since donation.
Conclusions: This large study of living kidney donors shows that the remnant kidney after donation has the capacity to increase kidney function for many years.

O-244 ELEVEN YEAR EXPERIENCE OF NON-DIRECTED LIVING RENAL DONATION IN CHRISTCHURCH, NEW ZEALAND

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Purpose: Christchurch Hospital provides renal transplantation for about one million people in New Zealand's South Island. Transplantation in NZ (pop. 4.1 million) is closely aligned with Australia (pop. 21 million) through the Transplantation Society of Australia and New Zealand and the ANZDATA registry – a complete registry of all renal transplants in Australia and NZ. The first non-directed living renal transplant reported to ANZDATA was in Christchurch on 24/07/1998. We describe Christchurch's non-directed living renal donation programme from 1998-2008.
Methods: Potential donor assessment was consistent with TSANZ guidelines for non-directed living renal donation. Kidney allocation used the New Zealand deceased donor National Kidney Allocation Scheme.
Results: There have been 33 assessments of potential non-directed donors resulting in 13 donations (Age 46.5 (22-63): 5-M, 8-F: 6-open, 7-laparoscopic). There was no significant donor morbidity and all except for one recipient were alive with a functioning graft at the end of 2008 – the 1998 recipient died with a functioning graft in 2008. Of the 20 remaining potential donors, 5 received information but took no further action, 1 withdrew after counselling, 9 offers were declined, 1 was referred to another unit and 4 remain active in the assessment process.
From 1998 to 2008 149 living donor transplants were performed in Christchurch: 87 (58.4%), 49 (32.9%) and 13 (8.7%) from genetically-related, emotionally-related and non-directed donation respectively. Nationally, during the same period, 21 of 509 (4.1%) living donor transplants were non-directed. In Australia only 16 of 2555 (0.6%) were non-directed. In 2008 national and Christchurch figures for non-directed donation transplants were 8 of 69 (11.6%) and 4 of 28 (14.3%) respectively.
Conclusions: Non-directed living renal donation has become an important component of the Christchurch and NZ national transplant programmes. A similar trend has not been seen in Australia.

O-245 VERY LONG-TERM DATA ON LIVING KIDNEY DONORS: A SINGLE CENTER EXPERIENCE SINCE 1959

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Background: Very long-term data on living kidney donors are important with regard to safety reasons. The aim of this study was to survey our entire experience with living kidney donors since its inception in 1959.
Methods: We retrospectively looked for all kidney donors from June 1959 until December 2007. Whenever these donors were living and located, we called them to ask if they were willing to fill a questionnaire and to perform serum creatinine and albuminuria dosage.
Results: Out of 397 living kidney donors, we were able to get informations in 297 cases (75%):42 were dead of whom one went on hemodialysis for 2 years and 255 were still alive. A questionnaire was sent: 5 refused to fill it and so far 177 answered. Mean age was 56 years (21-88). Mean current serum creatinine was 98 mol/l (53-153) and mean eGFR was 69 ml/min/1.73m^2 (22-125). Mean proteinuria was 0.06g/d (0-1). Two patients were on hemodialysis. Ninety seven % of donors never regret theirdonation.
We focused on 68 individuals who gave a kidney more than 30 years (mean 39 years). Mean current age was 72 years (57-77). Diabetes mellitus was absent in 86% of cases, present in 7% and unknown in 7%. Dyslipidemia was absent in 62% of cases, present in 27% and unknown in 11%. Hypertension was absent in 65% and present in 35%. Mean serum creatinine was 94 mol/l, mean eGFR was 63ml/min and mean proteinuria was 0.07g/l.
Conclusion: These data coming from one of the longest experience in the world bring important data regarding long term safety of living kidney donation. The prevalence of hypertension was not different from an age-matched population in our country nor was the incidence of end stage renal failure.

O-246 HOW FRANCE LAUNCHED ITS NHBD PROGRAM

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Non-heart-beating donor (NHBD) renal transplants have been introduced into clinical practice in France since 06/2006. After change of the French law, NHBD program was founded in an original and multicentric way with a national medical protocol. Only uncontrolled donors with an initial asystolic period <30mn and total warm ischemia time <150 minutes were considered. In situ kidneys perfusion must be realized by a double-balloon catheter and in situ cooling with a fourth generation liquid or a regional normothermic circulation; kidneys must be retrieved in less than 180 mn. All kidneys must be machine-perfused using the continuous-hypothermic pulsatile preservation system before transplantation. Methods used to assess the organ viability included perfusion parameters and morphologic assessment.
After 2 years of activity for 9 first pilot sites, first results show no in-hospital donor recruitment. Majority of donors were men (90%) with a mean age of 41 years and 70% belonged to the Maastricht class I. Organ's retrieval was done in 86 out of the 200 listed donors, procuring 98 kidneys which were grafted to 95 recipients and 73 harvested kidneys have been discarded because of morphological aspect, viability tests or positive serology. We observed a frequent procedure's failure (nearly 50%), because of too short delay, difficulties in initial medical evaluation, and canulation procedure or relative's opposition. Concerning recipients, they were mainly long waiting patients with a cold ischemia mean time around 14 hours. The overall graft survival was 83% with 90% of delayed graft function. NHBD kidneys are a valuable additional source of organs for transplantation. An improvement of organ quality perfusion after the warm ischemia period and a decrease of the DGF rate could be obtained with a preferential use of regional normothermic circulation as perfusion modality for this type of donor

O-247 PREDICTION OF DEATH IN POTENTIAL CONTROLLED NON-HEARTBEATING DONORS: PRELIMINARY RESULTS OF A PROSPECTIVE STUDY

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Introduction: In controlled non-heartbeating donors (NHBD), liver and lung donation is possible if the donor dies within 60 minutes after withdrawal of treatment. Kidney donation is not possible after 120 minutes. Donor efforts in this group are associated with unnecessary cost and are disappointing for the relatives and involved health care personnel. The objective of this study is to identify patients who do not die within 60 and 120 minutes.
Method: This is a prospective cohort study of potential controlled NHBD in the Netherlands from April 2007 until October 2008. Patient and treatment characteristics were analysed as potential risk factors for time of death. Also the prediction of the intensivist was registered. Univariate and multivariate logistic regression techniques were used.
Results: 142 potential donors were studied of whom 74% died within 60 minutes, 7% between 60 and 120 minutes and 19% thereafter. In the univariate analysis, controlled ventilation, use of norepinephrine, absence of reflexes, cardiac co-morbidity and a neurological diagnosis other than post-anoxic encephalopathy were associated with death within 60 minutes ($P<0.05$). Extubation and use of sedation were not associated with early death. In the multivariate analysis, controlled ventilation (OR 4.4, 95% CI: 2.0-10, $P<0.001$) and a neurological diagnosis other than post-anoxic encephalopathy (OR 2.9, 95% CI:1.4-8.0, P=0.007) remained independent risk factors for early death. The prediction of the intensivist was not accurate.
Conclusion: The vast majority of potential controlled NHBD die within one hour after withdrawal of treatment. Controlled ventilation and a neurological diagnosis other than post-anoxic encephalopathy were independent risk factors for early death. However, it was not possible to identify potential donors with a very low likelihood of early death in whom donation efforts are futile.

O-248 TRENDS IN ORGAN DONATION AND TRANSPLANTATION IN RUSSIA. ANALYSIS OF 2006-2008 NATIONAL REGISTRY DATA

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Background: Prior to 2006, organ donation and transplantation activity in the country (142,0 million inhabitants) has remained at the critically low level (kidney transplantation rate did not exceed 3,0 pmp). Due to the reasons of organizational, legal, economic, educational character positive trends have been observed from 2006. For the first time there's the data of the national registry provided by 34 kidney transplant (tx) centers, 6 liver tx centers, 4 heart tx centers, 2 pancreas tx centers.
Results: Results are presented in the table.

Organ transplants (tx)	2006	2007	2008	Increase %
Kidney tx (pmp)	556 (3,9)	666 (4,7)	782 (5,5)	40,7
Living kidney tx %	25,0	20,9	18,5	–
Liver tx	88	117	125	42,0
Living liver tx %	51,1	41,0	37,6	–
Heart tx	11	19	26	134,4
Lung tx	1	–	-	–
Pancreas tx	13	18	9	–
Living pancreas tx %	53,84	38,88	0,0	–
Total tx	669	820	942	40,8

Total number of kidney transplantations has increased almost 1.5 times, primarily through deceased donor organs, as a result of the increasing activity of local donation programs in some large regions (Moscow, St. Petersburg, Ekaterinburg, Novosibirsk). Practical application of transplant coordination and acceptance of brain death criteria is reflected in the increasing value of extrarenal transplantations performed in a few transplant centers. Up to now the problem of donor organ shortage is solved by using of living donor kidneys (18 centers) and liver (2 centers). During this period 19 kidney tx centers (55%) have increased their activity. 15 kidney tx centers perform more than 20 operation per year and only 6 kidney tx centers – more than 50 ones. Shumakov Institution is the largest center: in 2008 there were performed 164 solid organ transplantations (106 kidneys; 43 livers incl.8 split tx,; 15 hearts).
Conclusion: Up to now despite the increasing number of transplant operations population provision is still very unsatisfactory. Unrealized potential of de-

ceased donor donation creates good preconditions for the significant growth of solid organ transplants based on development of regional and national transplant coordination system. The main national challenge is to extend the donor hospital number and turn its staff attitude to organ donation.

Session 29. Pharmaco & immunological monitoring

O-249 IMPACT OF STEROID WITHDRAWAL ON THE IMMUNE RESPONSE OF RENAL TRANSPLANT RECIPIENTS

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Superior kidney graft outcome has been described after steroid withdrawal in a large prospective study within the Collaborative Transplant Study. To analyze effects of steroid withdrawal on clinically relevant immune parameters, we assessed CD4 helper activity, sCD30, immunoglobulin-secreting cell formation, neopterin and intracellular cytokine production in a prospective randomized study of 84 renal transplant recipients (CsA/Aza, CsA/MMF, Tacr/Aza; steroid tapering $\geq$6 months posttransplant) at 2 years posttransplant.

Two-year graft function was better in patients off steroids (creatinine clearance: 62$\pm$7 versus 47$\pm$4 ml/min, p=0.03). Lower steroid dosage was significantly related to lower serum lipid levels, systolic and diastolic blood pressure ($p \leq 0.002$).

Multivariate logistic regression showed that steroid-free therapy was independently associated with enhanced T cell proliferative capacity (p=0.004) and CD4 cell IL-4 responses (p=0.07; p=0.03, univariate), which was previously shown to predict a low risk of acute rejection. Enhanced CD4 cell IL-2 production on steroid-free treatment (p=0.02, univariate) could not be confirmed in the multivariate analysis. Logistic regression, however, confirmed a downregulated IL-2R (CD25; p=0.02, univariate; p=0.01; logistic regression) and CD40 expression on B cells (p=0.03, univariate and logistic regression) in steroid-free patients. Interestingly, patients on steroid treatment exhibited even higher CD25 expression on CD4 cells than healthy controls (p=0.02). MMF compared to Aza showed only a minor effect on B cell CD25 downregulation (p=0.05; logistic regression). Steroid-free treatment had no impact on monocyte activation, CD4 helper activity, sCD30 and immunoglobulin-secreting cell formation.

Our data show that steroid-free maintenance immunosuppression enhances T cell proliferation but provides graft protective immunological effects via enhanced CD4 cell IL-4 production and suppression of CD25 and CD40 expression. Upregulation of IL-2R and the B cell costimulatory pathway by steroids might result in B cell responses against the graft during periods of infection-induced IL-2 release.

O-250 THE ASSOCIATION OF EARLY SUBTHERAPEUTIC MPA EXPOSURE (<30 mg*h/L) AND ACUTE REJECTION: A COHORT ANALYSIS OF THE CLEAR STUDY

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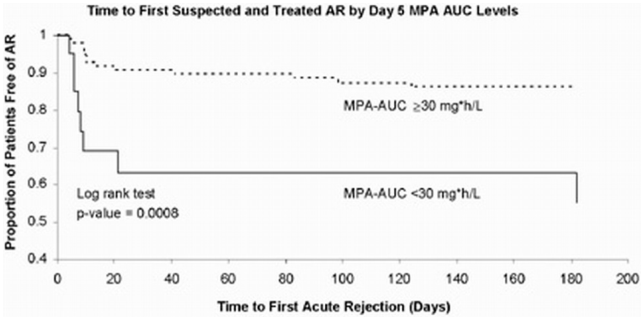
Adequate early mycophenolic acid (MPA) exposure may be associated with a decreased rate of acute rejection (AR). A greater proportion of patients may achieve higher MPA levels with the use of a post-transplant mycophenolate mofetil (MMF, CellCept®) loading dose.

Purpose: This cohort analysis of a randomized trial examines the efficacy and safety of a 5-day 3-g MMF loading dose to increase the proportion of renal transplant patients exceeding the MPA therapeutic level of 30 mg*h/L by Day 5 versus standard post-transplant 2-g daily dosing.

Methods: The loading-dose arm (n=68) received MMF 1.5 g BID days 1–5, then 1.0 g BID. The standard-dose arm (n=67) received MMF 1.0 g BID. Tacrolimus was adjusted to trough levels of 8–15 ng/mL. All patients received steroids and ~85% received an IL-2 receptor blocker. Full MPA AUCs were measured at Days 3 and 5. Results are reported for the modified ITT population who had measured AUCs.

Results: There were significantly more AR episodes (suspected and treated, biopsy-proven including and excluding borderline) in patients with MPA AUC <30 mg*h/L at Day 5 vs. those with levels $\geq$30 mg*h/L (*p*-values 0.0008 to <0.0001).



In patients with MPA AUC <30 mg*h/L at Day 5, 50.0% (8/16) had suspected and treated AR vs. 15.5% (13/84) in patients with MPA AUC $\geq$30 mg*h/L at Day 5 (*p*=0.0047). Higher MPA AUC at Day 5 was significantly associated with a decreased risk of AR in the univariate analysis. Only anemia was found to be significantly higher in patients MPA AUC >60 mg*h/L at Day 5.

Conclusions: Subtherapeutic MPA exposure (<30 mg*h/L) at Day 5 post-transplant is significantly associated with an increased incidence of AR in the first 6 months post renal transplant.

O-251 PHARMACOMETRICS OF VOCLOSPORIN IN A PHASE 2B RENAL TRANSPLANT TRIAL

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Purpose: Voclosporin (VCS) is a next generation calcineurin inhibitor (CNi) being developed for solid organ transplantation. Established therapeutic drug monitoring of CNi-based immunosuppression is based on trough concentration ranges that may not adequately determine clinical outcome. However, VCS has been developed using a pharmacometric approach which balances VCS concentration (PK), calcineurin inhibition (PD) and defined clinical outcomes–graft rejection (BPAR) and new onset diatetes (NODAT)–to determine an ideal therapeutic window.

Methods: PROMIS*E* was a 12 month, randomized, concentration-controlled study in *de novo* renal transplant patients comparing three oral voclosporin dosing groups (low, mid, and high dose) to tacrolimus. A total of 334 patients were enrolled in the Phase 2B study of which 248 were randomized to the VCS arms. VCS trough concentrations and calcineurin activity (CNa) were determined using an LC/MS based assay.

Results: PK/PD modelling of BPAR and NODAT versus trough concentration (C_0) of VCS predicted an optimal C_0 range of between 32-60 ng/mL.

In addition, Cox regression analyses of calcineurin activity (CNa) and BPAR suggested patients are 1.7 times more likely to reject if CNa_0 was above 1.3 pmol/min/mg.

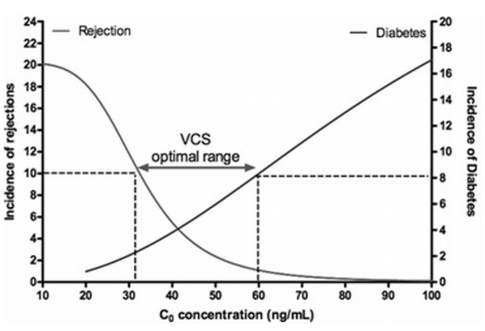

Figure 1. VCS C_0 vs incidence of rejection & diabetes.

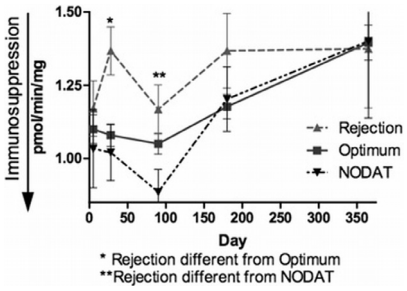

Figure 2. Calcineurin activity at time 0 (mean ± SEM).

Conclusion: VCS efficacy and toxicity can be characterized by a classic sigmoid relationship with blood concentration. Furthermore, CNa_0–based dosing offers the potential to determine VCS activity at the molecular site of action. A pharmacometric approach has enabled the quantification of a therapeutic window, unique for a CNi, which allows an enhanced ability for optimized and individualized dosing. This novel dosing paradigm for VCS will be explored in upcoming Phase 3 studies.

O-252 *CYP3A5* AND *ABCB1* POLYMORPHISMS INFLUENCE TACROLIMUS CONCENTRATIONS IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN RENAL TRANSPLANT PATIENTS

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Purpose: Peripheral blood mononuclear cells population (PBMCs) is expected to be a more specific biological matrix than whole blood in order to reflect the pharmacological efficacy of tacrolimus (Tac). The lymphocyte expression of P-glycoprotein (P-gp), an efflux transporter encoded by the *ABCB1* gene, might influence Tac intracellular concentration and therefore its immunosuppressive activity. This study investigated the effect of genetic polymorphisms in *CYP3A5* and *ABCB1* genes on Tac blood and intracellular concentrations seven days after renal transplantation.
Methods: 96 renal recipients were genotyped for three different *ABCB1* gene polymorphisms (1199G>A, 3435C>T, and 2677C>T/A) and the *CYP3A5*3* gene polymorphism. Trough blood and PBMCs Tac concentrations were monitored by immunoassay and LC-MS/MS respectively, and compared according to recipient genotypes.
Results: Dose-adjusted Tac PBMCs concentrations correlated significantly with dose-adjusted Tac blood concentrations (r^2=0.6138, *P*=0.0057). The *ABCB1 1199A* carriers presented a 1.4 fold increased Tac PBMCs levels (*P*=0.0014). The *ABCB1 3435T* and*2677T/A* carriers were both associated to a 1.3 fold increased Tac PBMCs levels (*P=0.0089* and *P=0.0122* for *3435T* and *2677T/A* carriers, respectively). Dose-adjusted Tac PBMCs levels were significantly lower in patients expressing CYP3A5 compared with patients who did not (*CYP3A5*3/*3*), (*P*=0.0021). Tac dose requirement, based on blood TDM, and dose-adjusted Tac blood levels were both lower in *CYP3A5*1* carriers (*P*=0.0005). The impact of *ABCB1* genetic polymorphisms on Tac blood concentrations was negligible.
Conclusions: Our results confirm the impact of *CYP3A5* polymorphism on Tac blood pharmacokinetics parameters. This study reports for the first time the influence of and *ABCB1* polymorphisms on Tac intracellular concentrations. As Tac PBMCs concentrations could be a better marker of Tac efficacy than whole blood, it might be interesting to genotype recipients for *ABCB1* in order to better individualize the Tac immunosuppressive therapy in renal transplantation.

O-253 FINAL RESULTS OF PRETRANSPLANT PHARMACOGENETIC ADAPTATION OF TACROLIMUS (TAC) TREATMENT AFTER RENAL TRANSPLANTATION

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CytochromeP450 3A5 (CYP3A5) polymorphisms may be associated with TAC dose requirements. CYP3A5 expressors (CYPA5*1 carriers) need a higher daily dose to achieve therapeutic trough drug (C0) level. The aim of this study was to assess the role of pharmacogenetic pretransplant adaptation for pharmacokinetic and clinical outcome after renal transplantation (RT).
This multicenter, prospective, randomized, open-label trial included 280 de novo RT recipients randomized at day 0 to receive TAC from day7 according to CYP3A5 genotype (group A, 0.30 mg/kg/d in CYP3A5*1 carriers or 0.15 mg/kg/d in CYP3A5*3/*3) vs. a control group with usual TAC daily dose (group B, 0.20 mg/kg/d). Patients received from day0 a biological induction, MMF and steroids. At the end of the follow-up, 111 and 115 patients were available for analysis.
No difference was shown between the 2 groups for baseline characteristics. Genotype frequency was 79.8% CYP3A5*3/*3, 15.8% CYP3A5*1/*3 and 4.4% CYP3A5*1/*1. The mean TAC daily dose was not different among the 2 groups during the follow-up. A significantly higher proportion of patients reached targeted C0 at day 10 (51% vs. 36%, p=0.03). Patient and graft survival were 99% vs. 100% and 99% vs. 98% for group A and group B respectively. In *1/*1 and *3/*3 genotype, the TAC C0 at day 10 and serum creatinine at M1 were better in groupe A.

	*1/*1		*1/*3		*3/*3	
	Group A	Group B	Group A	Group B	Group A	Group B
C0 @ D10	14.0±2.3	5.6±2.3*	12.3±1.4	10.1±1.6	12.0±0.9	16.6±0.8*
SeCr @ M1	146	153	118	133	131	141
SeCr @ M3	128	137	124	121	125	128

Conclusion: Prospective adaptation of TAC daily dose according to CYP3A5 polymorphisms is associated with a higher proportion of patients reaching the targeted C0 and a numerical better renal function at M1. Longer follow-up is being analyzed.

O-254 AN INTENSIFIED DOSING OF ENTERIC-COATED MYCOPHENOLATE SODIUM IN RENAL TRANSPLANT PATIENTS RESULTS IN IMPROVED EFFICACY WITHOUT COMPROMISING SAFETY

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In this study we examined influence of an intensified enteric-coated mycophenolate sodium (EC-MPS) dosing on mycophenolic acid (MPA) exposure, IMPDH activity, efficacy and safety 6 months after renal transplantation
Methods: 128 de-novo kidney transplant recipients were treated with basiliximab, steroids and cyclosporine and randomized to standard EC-MPS (SD: 1440mg/d; n=65) or to an intensified EC-MPS dosing regimen (ID: 2 weeks: 2880 mg/d; subsequent 4 weeks: 2160 mg/d; followed by 1440 mg/d; n=63). Efficacy and safety and. in a subgroup of 75 patients steady state pharmacokinetics of MPA and the inhibition of IMPDH-activity were evaluated.
Results: Analysis of 12-h MPA profiles of 60 patients demonstrated that MPA exposure was significantly higher in the intensified group on day 3 compared to standard group (MPA-AUC: 45.0±15.8 vs. 32.7±18.7mg*h/L; p=0.007) resulting in lower IMPDH-activity in the intensified compared to standard regimen (IMPDH-AEC: 33.6±15.6 vs. 44.4±19.1 (nmol/mg protein*h)*h; p=0.045). In 128 patients the incidence of BPAR was significantly lower in the intensified compared to the standard group (3.2% vs. 16.9%; p=0.017). Patient survival was 98.4% in the ID and 96.9% in the SD group, and 2 graft losses were observed in each groups. Interestingly, the ID regimen was not associated with a higher rate of hematological side effects (47.6% ID vs. 46.2% SD) nor with a higher risk of infections (63.5% ID vs. 75.4% SD). BK virus was detected in 5 vs. 2 patients and CMV in 5 vs. 8 patients respectively (ID vs. SD). Slightly more gastrointestinal symptoms were reported in the ID group (81% vs. 75.4%).
Conclusion: An intensified dosing regimen of EC-MPS leads to a significantly higher MPA exposure early post-transplantation resulting in a significantly lower rate of BPAR without compromising safety.

O-255 PROTON PUMP INHIBITORS REDUCE MYCOPHENOLATE EXPOSURE IN HEART TRANSPLANT RECIPIENTS – A PROSPECTIVE CASE CONTROLLED STUDY

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Purpose: This prospective study investigates the impact of proton pump inhibitors (PPI) on mycophenolic acid (MPA) pharmacokinetics in heart transplant recipients receiving mycophenolate mofetil (MMF) and tacrolimus.

Methods: MPA plasma concentrations at baseline (C0h), 30minutes (C0.5h), 1 (C1h) and 2hours (C2h) were obtained by high-performance-liquid-chromatography (HPLC) in 22patients treated with pantoprazole 40mg and MMF 2000mg. Measurements were repeated 1month after pantoprazole withdrawal. A 4-point limited-sampling-strategy was applied to calculate MPA area under the curve (MPA-AUC).
Results: Predose MPA concentrations with PPI were 2.6±1.6 mg/L vs. 3.4±2.7 mg/L without PPI (p=ns). Postdose MPA concentrations were lower with PPI at C0.5h (8.3±5.7 mg/L vs. 18.3±11.3 mg/L, p=0.001) and C1h (10±5.6 mg/L vs. 15.8±8.4 mg/L, p=0.004) without significant differences at C2h (8.3±6.5 mg/L vs. 7.6±3.9 mg/L). MPA-AUC was significantly lower with PPI medication (51.2±26.6 mgXh/L vs. 68.7±30.3 mgXh/L; p=0.003).
The maximum concentration of MPA (MPA-Cmax) was lower (12.2±7.5 mg/L vs. 20.6±9.3 mg/L; p=0.001) and the time to reach MPA-Cmax (tmax) was longer with PPI (60±27.8 min vs. 46.4±22.2 min; p=0.05).
Conclusion: This is the first study to document an important drug interaction between a widely used immunosuppressive agent and a class of drugs frequently used in transplant patients. This interaction results in a decreased MMF drug exposure which may lead to patients having a higher risk for acute rejection and transplant vasculopathy.

O-256 NOVEL T-CELL ASSAY TO ASSESS EFFECTS OF SOTRASTAURIN, A PROTEIN KINASE C-INHIBITOR, IN COMBINATION WITH TACROLIMUS

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Conventional pharmacokinetic monitoring is inadequate to accurately predict the response to immunosuppressants in individual recipients after organ transplantion, particularly in the context of multi-drug regimens. Therefore, we developed a T-cell function assay to monitor the effects of the novel early T-cell activation inhibitor sotrastaurin, which differs in its mode of action from calcineurin-inhibitors, in combination with tacrolimus.
Methods: Blood was obtained from healthy subjects (HS, n=20), who were treated with single doses of sotrastaurin (400mg) and tacrolimus (7mg) alone or in combination. Blood from renal transplant recipients (RTx, n=28), participants of a multi-center study, was obtained pre-transplant and on day 8 post-transplant before that day's dose. Whole-blood was stimulated ex-vivo via calcium-dependent signal for T-cell proliferation (^{3}H-thymidine uptake) or via calcium-independent signal for T-cell activation (IL2/TNFα+ T-cells by flow cytometry).
Results: In HS, tacrolimus inhibited calcium-dependent but not calcium-independent signaling (T-cell activation) whereas sotrastaurin inhibited T-cell proliferation and activation effectively. After combined drug administration, inhibition of both, T-cell activation (p=0.01) and proliferation (p<0.001), was pronounced compared to sotrastaurin alone (Table).

T-cell marker	Inhibition (mean ± SD %)		
	Tacrolimus	Sotrastaurin	Combination
IL2/TNFα$^+$ T-cells	16±12	75±22	90±9
^{3}H-thymidine uptake	76±11	82±9	96±2

In RTx treated with sotrastaurin (200mg bid) combined with standard- or reduced-exposure of tacrolimus, T-cell activation and proliferation were inhibited post-transplantation by 95±11% (p<0.001) and 73±32% (p<0.001), respectively, compared to pre-transplantation. Inhibition was similar in combination of sotrastaurin with tacrolimus standard- or reduced-exposure.
Conclusion: We developed a T-cell function assay which quantifies the effects of sotrastaurin in combination with tacrolimus. Future studies in larger cohorts are needed to show if this assay might help to tailor the sotrastaurin dose for individual transplant recipients to optimize its efficacy and safety.

O-257 The JAK-INHIBITOR CP-690,550 INHIBITS EFFECTOR T-CELLS BUT DOES NOT AFFECT THE FUNCTION OF HUMAN CD4$^+$CD25^{bright}FOXP3$^+$ REGULATORY T-CELLS

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Cytokines of the common γ-chain that activate the JAK-STAT pathway are critical factors for the growth, differentiation and function of both CD25$^{-/dim}$ T-cells and CD4$^+$CD25^{high} FoxP3$^+$T-cells. The JAK-inhibitor CP-690,550 is currently being investigated for prevention of allograft-rejection in patients. It remains unknown whether CP-690,550 has a differential effect on effector and regulatory T-cells. Here, we studied the *in vitro* and *in vivo* effects of CP-690,550 on both T-cell populations.
Blood from healthy individuals was stimulated with increasing concentrations (25-2000 U/mL) of IL-2. By phosphospecific flow cytometry, we observed a dose-dependent increase in the level of phosphorylated(P)-STAT5 in the CD4$^+$CD25^{high}FoxP3$^+$ and CD4$^+$CD25$^{-/dim}$T-cells. At 2000 U/mL, median P-STAT5 levels were more increased in CD4$^+$CD25^{high}FoxP3$^+$ T-cells (3 to 70%) than in CD4$^+$CD25$^{-/dim}$T-cells (1 to 43%), p=0.02. In the presence of a clinically relevant CP-690,550 dose of 200 ng/mL, the IL-2-induced P-STAT5 was partially inhibited in CD4$^+$CD25^{high}FoxP3$^+$T-cells, while almost completely blocked in the CD25$^{-/dim}$ T-cells (decreased by 63% vs. 90%, median, p=0.02). Analysis showed a higher IC_{50} CP-690,550 level for the CD4$^+$CD25^{high}FoxP3$^+$ T-cells (136 ng/mL) compared to the CD4$^+$CD25$^{-/dim}$T-cells (58 ng/mL), p=0.05. In the presence of CP-690,550 (100 ng/mL), co-culture of CD25$^{-/dim}$T-cells with CD4$^+$CD25^{high}T-cells at a 10:1 ratio inhibited the proliferative response by 48%(median, 39-51%), which was comparable to 54%(44-64%) in the absence of CP-690,550. Thus, the magnitude of regulatory function was the same in the presence as in the absence of the drug. These in vitro data were confirmed in kidney transplant patients (N=8) receiving 30 mg CP-690,550 BID, as their CD4$^+$CD25^{high}T-cells exhibit strong regulatory activities.
In conclusion, our findings show that the JAK-inhibitor CP-690,550 inhibits effector T-cell function but spares the suppressive activity of CD4$^+$CD25^{high}FoxP3$^+$T-cells. JAK/STAT inhibition provides a novel mechanism for modulation of anti-donor responses in transplant-patients.

O-258 IMPACT OF BASILIXIMAB THERAPY ON REGULATORY T-CELLS EARLY AFTER KIDNEY TRANSPLANTATION: CD25 DOWN-REGULATION BY RECEPTOR MODULATION

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Purpose: *Basiliximab* is a monoclonal anti-CD25 antibody successfully used to prevent acute graft rejection after organ transplantation (Tx). Its application might not only affect activated/effector T-cells, but also regulatory T-cells (T_{regs}) of the CD4$^+$CD25$^+$CD127^{low}FoxP3$^+$ phenotype. We investigated the influence of *Basiliximab* on the frequency of peripheral T_{regs} in patients early after kidney Tx.
Methods/Materials: Blood from *Basiliximab*-treated patients (n = 13; injection on days 0 and 4) and from a non-*Basiliximab* group (n = 6) was collected preoperatively and at defined points within 3 months after Tx. All patients received initial triple immunosuppression consisting of a calcineurin inhibitor, mycophenylate mofetil and steroids. The frequency of T_{regs} was determined by multi-color flow cytometry using monoclonal antibodies (mAb) to CD4, CD25, CD127 and FoxP3.
Results: Treatment of patients with *Basiliximab* resulted in a decrease in the percentage of CD4$^+$CD25$^+$FoxP3$^+$ T_{regs} which lasted for about three months. This decrease was accompanied by a rise in CD4$^+$CD25$^-$FoxP3$^+$ T-cells expressing the CD127^{low} phenotype. The frequency of CD4$^+$FoxP3$^+$ cells remained stable suggesting that the drop of CD4$^+$CD25$^+$FoxP3$^+$ T_{regs} may result from blocking of anti-CD25 mAb by *Basiliximab* or down-regulation of CD25 molecules rather than from elimination of the cells. *In vitro*, pre-incubation of CD4$^+$CD25$^+$FoxP3$^+$ cells with *Basiliximab* did not inhibit staining by anti-CD25 mAb. However, when CD4$^+$CD25$^+$Foxp3$^+$ cells were cultured at 37°C in the presence of *Basiliximab*, down-regulation of CD25 occurred within 48h, thus indicating receptor modulation.
Conclusion: *Basiliximab* therapy has a direct effect on CD4$^+$CD25$^+$FoxP3$^+$ T_{regs}. Although the functional consequences of CD25 internalization and/or shedding are not known, these observations raise questions about the use of *Basiliximab* in tolerance promoting protocols.

O-259 PREFERENTIAL INCREASE IN MEMORY AND REGULATORY SUBSETS DURING CD4+ T-CELL IMMUNE RECONSTITUTION AFTER THYMOGLOBULIN INDUCTION THERAPY IN RENAL TRANSPLANT PATIENTS RECEIVING SIROLIMUS VS CYCLOSPORINE

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Thymoglobulin induction with sirolimus maintenance therapy is effective but no study has compared immune reconstitution with sirolimus vs calcineurin inhibitors.

Methods: In a 12-month, randomized, open-label, single-center pilot study, peripheral lymphocyte reconstitution was compared in *de novo* kidney transplant patients receiving sirolimus (n=9) or CsA (n=10). All patients received Thymoglobulin (2.5mg/kg/day for 1 day, 1.25mg/kg/day for 3 days), MMF and corticosteroids. Lymphocyte count was recorded at day 0, during days 1-14, and at months 1, 2, 3, 6 and 12. Cell counts were compared between treatment groups using a Fishers test on a compacted data set, allowing a single comparison across all post-baseline timepoints.
Results: Total lymphocytes were profoundly depleted in both groups. Reconstitution was greater in the CsA arm vs sirolimus (p=0.004). $CD4^+$ T-cell count recovery in the CsA cohort was also higher (p=0.025). At baseline, naive T-lymphocytes ($CD4^+$ $CCR7^+$ $CD45RA^+$) were more numerous in the sirolimus cohort vs the CsA arm (p=0.028) but became less numerous vs CsA after Thymoglobulin therapy (p=0.019). In contrast, memory cells ($CD4^+$ CD45RO) were less frequent in the sirolimus group vs the CsA arm at baseline (p=0.006) but were more frequent after Thymoglobulin (p=0.05). The number of regulatory T cells ($CD4^+$ $CD25^{bright}$), similar at baseline in the two groups, was significantly increased after Thymoglobulin in the sirolimus cohort vs the CsA arm.
Conclusion: The pattern of homeostatic reconstitution after Thymoglobulin induction differs between sirolimus and CsA, with a disproportionately high recovery of memory and regulatory T-cell subsets on sirolimus. These data suggest that the beneficial effect of sirolimus that favour T-regulatory cells during immune reconstitution could be counterbalanced by a parallel increase of memory subsets, more resistant to immune regulation.

Session 30. New approaches to diagnostic prediction

O-260 HLA CLASS-I ALLOANTIGEN IMMUNOGENICITY CAN BE PREDICTED BY TERTIARY STRUCTURE AND ELECTROSTATIC CHARGE DISPARITY

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Knowledge of the physiochemical nature of HLA amino acid (aa) polymorphism and the tertiary protein structure may enable prediction of the relative immunogenicity of HLA alloantigens. We examined whether analysis of HLA tertiary protein conformation and the number and physiochemical properties of aa polymorphisms enables characterisation of immunogenic B-cell epitopes and prediction of humoral alloimmunity against donor/recipient HLA mismatches.
Homology modelling was used to predict the tertiary protein conformation of HLA class-I alleles using the Modeller program. The electrostatic charge on the molecular surface of each molecule was calculated using the DelPhi algorithm. A computer program was developed to allow comparison of mismatched HLA-A and -B specificities with the HLA class-I type of 32 highly sensitised patients (HSP) awaiting kidney transplantation, and determine the number and position of polymorphic aa and the overall electrostatic charge disparity. HSP sera were screened using Luminex/single-antigen beads to determine HLA-specific antibody levels against all possible mismatched HLA class-I specificities for each patient.
HLA-specific antibody was detected against 1,666 (85%) of 1,964 mismatched HLA specificities evaluated, with a close correlation between increasing number of aa polymorphisms and the presence and magnitude of the alloantibody response ($p<0.0001$). The electrostatic disparity score was an independent predictor of alloantibody production (adjusted p=0.0005). Mismatched specificities with electrostatic scores within the first decile of the scale led to weak alloantibody responses (median MFI 2,330) whereas those with scores above the sixth decile led to strong alloantibody production (median MFI $>10{,}000$). Serologically defined B-cell epitopes expressed on different HLA class-I molecules had similar surface electrostatic charge, explaining well-characterised HLA cross-reactivity.
Differences in tertiary structure conformation and electrostatic charge between HLA class-I specificities enable prediction of donor HLA types with low immunogenicity for a given recipient.

O-261 MICA ANTIBODIES ASSOCIATE WITH BIOPSY-PROVEN CELLULAR REJECTION IN RENAL TRANSPLANT RECIPIENTS

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Introduction: The human MHC class I chain-related genes, *MICA* and *MICB*, encode stress-related molecules recognised by NK cells. MIC molecules are also expressed in renal transplants and induce antibody (Ab) responses.
Materials and methods: We have analysed MICA Ab profiles in the post-transplant sera of 299 renal transplant patients, using high resolution Luminex-based screening and single antigen commercial kits (LABScreen). Sequenced-based MICA typing was also performed on 223 donor and recipient pairs.
Results: MICA Abs were detected in 62/299 (20.7%) patients. Post-transplant biopsies were performed on all patients and the incidence of cellular rejection was significantly increased in MICA Ab+ve patients (18/62, 29.0%) vs MICA Ab-ve patients (40/237, 16.9%) (Chi Square = 4.64, P = 0.03, OR = 2.0). Of those patients with MICA Abs and biopsy proven cellular rejection 12/18 (66.6%) did not have detectable Abs directed against HLA class I or II antigens. The production of detectable MICA Abs could also be attributed to certain mismatched amino-acid residues in the 2nd and 3rd extra-cellular MICA protein domains.
Conclusions: The production of MICA Abs in renal transplant recipients acts as a correlate of cellular rejection and donor-recipient mismatching for specific MICA epitopes may affect graft outcome.

O-262 HLA-DP ANTIBODY FORMATION BEFORE AND AFTER RENAL TRANSPLANTATION

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HLA-DP antigens are expressed on renal endothelial cells and supposed to present a target for humoral immune response in kidney transplantation, because they are usually described after failure of a transplant. Until the Luminex Single Antigen assay became available, anti-HLA-DP antibodies have been difficult to detect. This study analyses frequency, specificity and time of occurrence of DP ab by LSA.
410 transplant patients were tested during transplant screening for presence of HLA-DP ab. The pattern of ab specificities was correlated to particular motifs of 6 hyper variable regions (HVR A-F) in exon 2 of the HLA-DPB1 gene, which are shared between groups of DPB1 alleles.
HLA-DP ab were demonstrated in 48 (12%) patients: 30 were recipients of a first, 7 of a second graft. 11 showed DP ab before transplantation: 10 female, 1 male. All female patients had been pregnant and the male patient had received 64 transfusions with leukocyte depleted blood. Pre-transplant DP specificities were correlated to 2 particular motifs: C and F. In 8 patients, a “single motif” was sufficient to account for all DP specificities evidenced in the sera. In one, 2 motifs were present, in 2 there was no clear motif. Post-transplant DP ab were correlated to 4 motifs: B, C, E and F. In 28 patients a single motif accounted for all specificities detected, in one 2 motifs were present and in 8 the motif was unclear. The donor-specificity of the ab is presently being investigated.
This study showed that DP ab are regularly found in recipients before and after transplantation as a result of pregnancy, blood transfusion or previous grafting. They are correlated with particular motifs of HVR of the DPB1-gene. Clinical relevance of DP ab for transplantation is a subject of further study.

O-263 EFFICACY OF AN ACCEPTABLE MISMATCH PROGRAM (AM) FOR THE ACCESS TO RENAL TRANSPLANTATION OF HIGHLY SENSITIZED PATIENTS: THE FRENCH EXPERIENCE

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The AM program started in France on 04/2005, adapted from the AM program of EuroTransplant, to facilitate hyperimmunized patients to the access of a renal transplant and to achieve a good graft survival. Any eligible hyperimmunised patient (class I IgG>80% PRA), after determination of permissible Ags by Single Antigen Assay, has a national priority to receive any graft without

any HLA class I mismatch (MM) between the donor and the combination of the recipient own Ag and its acceptable Ags. A maximum of 1 DR MM is accepted. Between 01/04/2005 and 31/12/2008, 235 renal transplantations has been performed using this program. In the positive cross match (XM) patients as compared to the transplanted patients, patients are more likely to be waiting for a second graft (84% vs 75%) and are more highly immunized (62 versus 50% of anti HLA class II P.R.A.). Transplanted patients had on average 2.5 MM with their donor, only considering their own HLA antigens, and 36% had no DR MM.
After 44 months, we observed an improved 2 years access to transplantation for highly immunized incident patients, increasing from 42 to 51%, whereas this rate decreased for the non immunized or more slightly immunized patients.
Among the 424 XM performed, 185 XM were positive. The vast majority of positive XM are B cell XM, and half of the patients with a positive XM had a HLA class II P.R.A. over 71% and waited for retransplantation.1 year graft survival in " AM " patients is identical to that of non-sensitized recipients.
In conclusion, this program improved access to transplantation for hyper-immunized patients with no difference in graft survival. An improved program performance is expected using HD class II techniques.

O-264 ANTI-INFLAMMATORY EFFECTS AFTER STEROID PRETREATMENT OF BRAIN DEAD DONORS ARE REFLECTED IN ZERO-HOUR BIOPSIES–NO PROTECTIVE EFFECTS IN ELDERLY BRAIN DEAD DONORS

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Brain death (BD) of the donor and its complex pathophysiological changes have a significant influence on graft function. As zero-hour graft biopsies provide valuable diagnostic information of subtle inflammation, we aimed to evaluate the potential anti-inflammatory effect of steroid (methylprednisolone) treatment in deceased organ donors. We therefore studied mRNA gene expression of zero-hour kidney biopsies derived from 63 deceased donors. Among them 20 patients were included within the European Senior-Program (ESP; n=12 with steroid treatment, age=68.5$\pm$3.1); n=8 without steroid pretreatment, age=67$\pm$3.1). Remaining patients (normal collective) received either steroids (n=19, age=51.7$\pm$10.7) or remained untreated (n=24, age=41.6$\pm$11.1). Biopsies derived from untreated living donors (n=8, age=44.6$\pm$9.7) were also included in the study. Donor treatment consisted of 250 mg methylprednisolone i.v. at time of consent for organ donation and thereafter 100 mg/h i.v. Intraoperative biopsies were taken 30 min. after reperfusion and were immediately snap-frozen until analysis by real-time RT-PCR. In comparison to the normal collective, elderly deceased organ donors revealed a significant de novo gene expression of selected candidate markers including immunoproteasome subunits (PSMB8,9,10; $p<0.001$ respectively), chemokines such as CCL19/21 ($p<0.05$) or CD68 ($p<0.01$). Moreover, methylprednisolone treatment demonstrated to have no effect on marker gene expression in zero-hour biopsies in this patient group. In contrast, steroids resulted in a significant down-regulation of investigated markers in biopsy specimens within the normal collective. Especially the gene expression levels of PSMB8,9 and 10 were comparable to gene expression levels in biopsies derived from living donors ($p<0.05$, with steroid vs no steroid), suggesting a therapeutic effect of steroid pretreatment in this patient group. Our results clearly demonstrate the enhanced immunogenicity of transplants derived from elderly brain dead donors implicating the importance of age-adapted pretreatment strategies.

O-265 ALTERATIONS IN INTRAGRAFT microRNA EXPRESSION DURING ACUTE REJECTION OF THE RENAL ALLOGRAFT: IMPLICATION FOR DIAGNOSIS AND MECHANISM OF THE ALLO-IMMUNE INJURY

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Acute rejection (AR) is exemplified by alterations in the expression of protein encoding genes. Because the noncoding microRNAs (miRNAs) regulate the expression of a vast array of genes, we investigated whether AR is associated with alterations in intragraft miRNA expression. miRNA expression patterns of human renal allografts were ascertained using microfluidic cards (N=7 biopsies). A subset of 17 miRNAs were differentially expressed (P-value <0.01);10 miRNAs were expressed at a lower level and 7 miRNAs were expressed at a higher level in AR samples, and the presence or absence of AR could be predicted using miRNA expression profiles. Differentially expressed miRNAs were validated in an independent set of 26 biopsies. Levels of over-expressed miRNAs correlate with the intragraft levels of mRNA for CD3 and CD20, and levels of under-expressed miRNAs correlated with the mRNA for renal tubule proteins NKCC2 and USAG1, as well as with kidney allograft function. Further in vitro experiments in activated PBMCs and in human renal epithelial cells (HRECs) subjected to pro-inflammatory cytokines provided new insights in the regulation of miRNA in human cells and suggested that the over- or under-expression of miRNAs in AR samples not only reflected the variation in the proportion of immune cells and resident cells but also that several miRNAs were specifically regulated in response to the activated status of the cells. Upon PHA activation, miR-155 was upregulated whereas miR-223 and let-7c were down regulated in PBMCs, and pro-inflammatory cytokines induced a down regulation of miR-30a-3p in HRECs. Finally, several of the differentially expressed miRNAs could be quantified in urine samples from kidney transplant recipients, thus paving the way for the investigation of miRNAs as novel noninvasive biomarkers of allograft status.

O-266 POST TRANSPLANT HLA AND MICA IMMUNISATION AND CHRONIC REJECTION IN HEART/LUNG TRANSPLANTATION: ONE CENTER STUDY

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During the 15th IHIWS, we participated to the component studying the effects of post Transplant HLA and MICA antibodies (Ab) on long term Heart/Lung graft survival. This component was chaired by Prof. P.Terasaki and Dr M.Ozawa. We report here on data of our own center. We have included 101 patients (45 Heart, 21 Heart+Lung, 35 Lung)grafted between 1987 and 2007 with a follow up of at least 6 months with a good organ function. Clinical information was obtained and blood collection for HLA and MICA immunization study was collected during summer 2007. One year later, clinical outcome was requested. HLA and MICA Ab screening was performed with Luminex technology (LABScreen Mixed One Lambda) and the specificity analysis (DSA versus NDSA) was performed with LABScreen Single Antigen Assay. HLA Ab screening was positive in 17 patients. Seven out of these 17 patients (41%) displayed complications. When DSA class II and MICA Ab were both present, clinical complications were observed (3/3). HLA Ab screening was negative in 84 patients (83.2% of the cohort)whereas MICA Ab screening was positive in 5 cases. Height out of these 84 patients (9.5%) displayed complications. MICA Ab were not associated with complications. In summary, immunological complications occurred preferentially in Heart transplantation and are associated with HLA DSA class II. Moreover, HLA NDSA were preferentially anti class I and not associated with complications. The incidence of MICA immunization is low (9%) but when MICA Ab are associated with HLA DSA class II, clinical complications were observed.

O-267 INTRAGRAFT ECTOPIC LYMPHOID TISSUE DURING CHRONIC REJECTION: HIJACKING OF AN EMBRYONIC DEVELOPMENTAL PROGRAM PROMOTES THE DEVELOPMENT OF A LOCAL AGGRESSIVE ALLOIMMUNE RESPONSE

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Introduction: During chronic rejection, inflammatory infiltrates organize themselves into a functional ectopic lymphoid tissue within rejected organs.
In the present study we postulated that this process could rely on the recapitulation of the developmental program triggered in the embryo during the ontogeny of secondary lymphoid organs, i.e. lymphoid organogenesis.
Methods/Results: We prospectively collected 20 human renal grafts explanted for terminal chronic rejection and 12 controls (6 native kidneys and 6 renal grafts removed for non-immune failure).
Patchy nodular CD20+ B cell infiltrates were evidenced in all chronically rejected grafts but in none of the controls.
The level of expression of the genes involved in lymphoid organogenesis (LO) was measured by Q-PCR. LO genes were not expressed in the 12 control tissues. On the contrary, the 20 chronically rejected grafts were distributed into 3 clusters corresponding respectively to a stepwise increase in the number and the level of expression of LO genes. The samples in which the complete set of LO genes were expressed displayed a highly functional intragraft lymphoid tissue supporting: i) the local maturation of B cells from naive to memory or plasmacells; and ii) the local generation of alloantibodies. In contrast, in samples in which the LO recapitulation was incomplete B cell maturation was blocked. Accordingly, we observed that the time of transplantation (from trans-

plantation to dialysis) was significantly prolonged for the grafts in which the LO recapitulation was abortive.
Conclusion: During chronic rejection, the immune system hijacks the embryonic developmental program to build an intragraft functional ectopic lymphoid tissue supporting an aggressive local humoral alloimmune response. The identification of the molecular checkpoints critical for the completion of the program paves the way for innovative therapeutic strategies to control chronic rejection.

O-268 DONOR NATURAL KILLER CELLS DETERMINE LONG-TERM HUMAN KIDNEY TRANSPLANT OUTCOMES THROUGH HLA-C SUB-GROUP DEPENDENT RECIPIENT DENDRITIC-CELL MATURATION

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Introduction: Natural killer (NK) cells have a critical role in the maturation of the immune response. We hypothesised that NK cells are a major determinant of long-term kidney transplant outcome through interactions between NK-cell killer immunoglobulin receptors (KIR) and their ligand HLA-C. HLA-C has two subgroups, HLA-C1 and HLA-C2: based on KIR specificity, HLA-C2 is a more potent inhibitor of NK cell activation than HLA-C1.
Methods & results: (i) In 760 kidney transplant recipients, those with HLA-C2 genotype had better 10-year graft survival than those with HLA-C1 genotype (66% & 44% respectively; p=0.002, HR=1.51, 95%CI=1.16-1.97). A multivariable analysis confirms this association. Donor HLA-C genotype did not influence long-term graft survival. (ii) Isolated NK cells (by CD56 staining) were present in a peri-tubular distribution in kidneys (n=5) donated for transplantation (pre-perfusion). (iii) In an allogeneic (indirect) NK-Dendritic Cell (DC) in-vitro co-culture system, the possession of HLA-C2 by DC was associated with anti-inflammatory cytokine production (IL-1ra/IL-6), diminished DC maturation (CD86, HLA-DR), and absent CCR7 expression. In contrast, possession of HLA-C1 by DC was associated with pro-inflammatory cytokine synthesis (TNF-α, IL-12p40/p70), enhanced DC maturation and CCR7 expression. These responses were IL-15 dependent.
Conclusion: These data indicate that donor derived NK cells differentially interact in situ with recipient DC through KIR/HLA-C interactions in the presence of IL-15 (which is present in the kidney early after transplantation). HLA-C2 recipients sustain less priming for indirect allorecognition than HLA-C1 recipients and have better long-term outcomes. As the NK(KIR)/DC(HLA-C) synapse is not inhibited by current immunosuppressive protocols, it represents a potent new therapeutic target in human kidney transplantation.

O-269 THE IMMUNOREGULATORY MOLECULE HLA-G INHIBITS THE mTOR PATHWAY AND CELL CYCLE OF ACTIVATED T CELLS THROUGH SHP2

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Aim: HLA-G is involved in regulating T cell responses and is associated with lower rejection in human transplantation. We have shown that HLA-G regulate T cell regulation by inhibiting their cell cycle progression. We have analysed the pathway involved in the cell cycle inhibition.
Results: Soluble HLA-G (HLA-G5) inhibited both CD4 and CD8 T cell proliferation. This effect is due to the interaction of HLA-G and the inhibitory receptor ILT2 since siRNA to ILT2 allow activated T cells incubated with HLA-G to proliferate. ILT2 is a transmembrane receptor with an intracellular domain with ITIM motifs which can recruit phosphatases. Immunoprecipitation of ILT2 in presence of HLA-G co-precipitated the phosphatase SHP2. In addition, incubation of T cells with HLA-G is associated with the occurrence of the P-SHP2 which is the functional form. It also correlates with the dephosphorylation of mTOR but not with CD3zeta or ERK. Moreover, the inhibition of SHP2 with NFC87877 in this condition inhibits the dephosphorylation of mTOR as well as the transfection of siRNA to SHP2. In addition, siRNA to SHP2 inhibited the $p27^{kip}$ expression observed in presence of HLA-G. Altogether, these data indicates that SHP2 is implicated in the regulatory effect of HLA-G in T cells.
Conclusion: The immunoregulatory molecule HLA-G regulates the cell cycle of T cells by modulating the mTOR pathway through the activation of the phosphatase SHP2.

O-270 ADULT BONE MARROW AND FETAL LIVER MESENCHYMAL STEM CELLS (MSC) AFFECT LYMPHOCYTES PROLIFERATION BY PREVENTING ENTRY INTO THE CELLULAR CYCLE: DIRECT INVOLVING OF HLA-G

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Human Bone Marrow Mesenchymal Stem Cells (BM-MSC) are multipotent progenitor cells with transit immunomodulatory properties. These cells suppress NK cells cytotoxicity and inhibit DC activation as well as the initial differentiation of monocytes to DCs. MSC also induces T cells to become unresponsive, but the mechanism is not well known. In addition, this effect is limited to few days in culture and in vivo. The object of this study was to identify MSC from various origins and to investigate their effects on T cell proliferation, phenotype and function. MSC isolated from fetal liver (FL-MSC) exhibit the same phenotype than human bone marrow MSC. When cultured with IL 2/OKT3-stimulated T cells, both BM-MSC and FL-MSC affected T cell proliferation in a ratio-dependant manner but do not induced their apoptosis. The inhibitory effects of MSC on T cells were associated with the inhibition of the cell cycle, as confirmed by a strong down regulation of phospho-Rb, Cyclin A, D and E and an up regulation of p27kip1. The regulation of the cell cycle entry was modulated by the expression of HLA-G on MSC, since it was inhibited by using the neutralizing anti-HLA-G antibody. Both BM-MSC and FL-MSC expressed HLA-G protein. In contrast to BM-MSC, FL-MSC has sustained immunoregulatory properties and HLA-G expression even after passage 27 in vitro. In conclusion, we have isolated FL-MSC with sustained immunomodulatory properties through the expression of HLA-G and that could be considered for cellular therapy to prevent allograft rejection.

Session 31. Cardiovascular risk factors in kidney transplantation

O-271 REGIONAL VARIATION IN CARDIOVASCULAR RISK POST KIDNEY TRANSPLANT: THE PORT INTERNATIONAL DATA COLLABORATION

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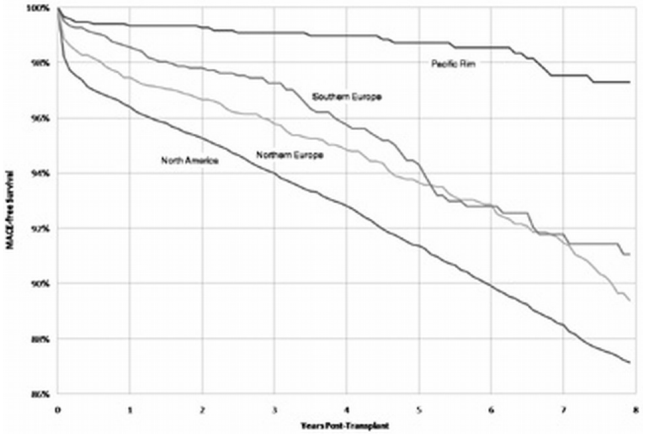
Cardiovascular disease remains the leading cause of death with functioning kidney transplant. The Patient Outcomes in Renal Transplantation (PORT) international data collaboration compiled data from 14 transplant centers from North America, Europe, Japan, and New Zealand with the primary goal of assessing risk of major adverse cardiac events (MACE), including non-fatal acute myocardial infarction, coronary revascularization, or sudden/cardiovascular death. Data were collected on 37,076 kidney transplant recipients. Analyses were limited to adult recipients transplanted in 1990 or later (N=23,575). The incidence of MACE was analyzed in 4 separate regions: North America, Northern Europe, Southern Europe, and the Pacific Rim. Kaplan-Meier analyses estimated MACE-free survival during the first 8 years by region as displayed in figure 1.



After adjustment for age, gender, diabetes, cardiovascular comorbid conditions

at transplant (history of AMI, CHF, coronary revascularization, CVA, peripheral arterial disease surgery), donor type, obesity, history of cancer, and time on ESRD therapy, the adjusted relative risk of MACE was 0.84 (95% CI: 0.72-0.98, p=0.02), 0.81 (0.65-1.01, p=0.06), and 0.32 (0.22-0.47, p<0.01) for Northern Europe, Southern Europe, and the Pacific Rim compared with North America, respectively. While regional variation in cardiovascular risk is well known in the general population, this study found similar variation in risk in this population of kidney transplant recipients.

O-272 THROMBOPHILIC FACTORS DO NOT PREDICT OUTCOMES IN PATIENTS RECEIVING A RENAL TRANSPLANTATION AFTER 2000

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Objective: We performed a prospective study to evaluate if a panel of 11 thrombophilic factors were associated with thrombo-embolic events or acute rejection at 1 year post- renal transplantation.
Methods: An incident cohort of patients transplanted between 2001 and 2007 (N=310) was prospectively screened on the day of renal transplantation for a large panel of 11 thrombophilic factors. All patients received aspirin, started before transplantation and continued *ad vitam*.
Results: The incidence risk of thrombo-embolic events or acute rejection episodes during the first post-transplant year (composite primary endpoint) was 17.2% in patients with 1 thrombophilic factor (N= 250) and 16.7% in patients free of thrombophilic factor (N=60) (P>0.99). None of the individual thrombophilic factor was associated with the composite endpoint. The incidence of the primary endpoint was similar among patients free of thrombophilia and those with 2 (N=135) or 3 (N=53) thrombophilic factors (16.7%, 16.3%, and 15.1% respectively, P=NS). The incidence of thrombo-embolic events was 3.2% and 5.0% in patients with and without thrombophilic factor respectively (P=0.46). Acute rejection occurred in 14.0% and 13.3% of patients with and without thrombophilic factor respectively (P>0.99). The incidence of cardiovascular events during the first post-transplant year, serum creatinine level at 1-year post- transplantation, 4-year actuarial graft and patient survival were not influenced by the presence of 1 thrombophilic factor (P=NS).
Conclusion: The presence of thrombophilic factors does not influence thrombo-embolic events, acute rejection, graft or patient survival among patients transplanted after the year 2000 and receiving prophylactic aspirin.

O-273 OBSTRUCTIVE SLEEP APNEA IS HIGHLY PREVALENT AMONG KIDNEY TRANSPLANTED PATIENTS AND IS ASSOCIATED WITH INCREASED CARDIOVASCULAR RISK

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Background: The prevalence of obstructive sleep apnea (OSA) is much higher in patients on chronic dialysis than in the general population. Here we used a large, randomly selected sample of kidney transplanted patients to assess for the first time the prevalence of OSA and its clinical correlates. We also compared the prevalence of the disorder between waitlisted dialysis patients (WL) and kidney transplanted patients (Tx).
Methods: Data from 100 kidney Tx and 50 WL obtained in a cross-sectional survey were analyzed. Socio-demographic data, history of renal disease, medication, co-morbidity and laboratory parameters were collected at enrolment. Patients completed a battery of self-administered questionnaires and underwent one-night polysomnography. Definition of moderate and severe OSA was an apnea-hypopnea index (AHI) higher than 15/hour.
Results: The prevalence of mild (5/h≤AHI<15/h), moderate (15/h≤AHI<30/h) and severe OSA (AHI≥30/h) in the Tx group was 18%, 11% and 14% versus 28%, 16% and 10%, respectively, among WL. The AHI was significantly correlated with age (rho=0.34), body mass index (rho=0.45), neck- (rho=0.4) and abdominal circumference (rho=0.51) and hemoglobin (rho=0.24) in the transplanted group. The proportion of males was significantly higher among OSA patients versus those without OSA (80% vs 49%; p<0.01). A significantly higher proportion of patients used three or more antihypertensive drugs in the OSA group versus the non-OSA group (56% vs 31%; p<0.05). The ten-year Framingham coronary heart disease risk (median; IQR: 14.5; 13.2 versus 7; 9; p<0.01) and ten-year Framingham risk for stroke (median; IQR: 10; 11.2 versus 5; 5; p<0.05) were twice as high in OSA versus non OSA patients.
Conclusions: The prevalence of OSA is similarly high in transplanted and waitlisted patients. OSA may contribute to increased cardio-cerebro-vascular risk in transplanted patients.

O-274 USE OF CARDIOVASCULAR MEDICATIONS AFTER KIDNEY TRANSPLANTATION: ASSOCIATIONS WITH CARDIOVASCULAR RISK PROFILE

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Cardiovascular (CV) disease is the most common cause of mortality and morbidity following kidney transplant (KTx). Despite the high rates of CV events in KTx recipients, there are few studies examining the effects of CV medications; these data have largely been limited to the use of statins. PORT is the largest multi-center, international collection of non-immunosuppressive medication data in existence in the KTx population. This study examined the use of CV medications after KTx.The study population included all adult KTx recipients with graft function 30 days post-transplant from a subset of the 14 participating transplant centers. 9 of 14 centers provided data on use of beta-blockers, ACEIs/ARBs, calcium channel blockers, antiplatelets, diuretics, and other antihypertensive drugs (N=12,150). One additional center provided data on use of statins and other lipid-lowering agents (LLA). Medication use was defined as using the medication at any time during each 30-day period post-transplant. There was a significant increase in the use of all CV medications from 1990–2006 (Figure 1). This was most marked in patients transplanted from 2000–2006 compared to 1990–1994 (OR 13.89).

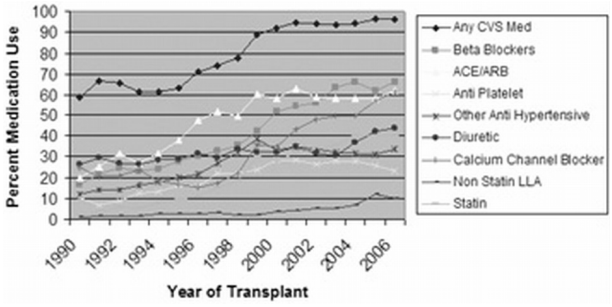

Figure 1

Despite increased use of both statins and LLA in patients with CV co-morbidity prior to transplant, use is still lower than expected at 3 months post-transplant (Figure 2). Less than 50% of patients with a history of DM or MI used statins. Similarly, the use of Aspirin and Beta Blockers, while increased in those with CV risk factors, was lower than expected. The use of ACE/ARBs was low in all groups with no increase in use in patients at high CV risk.

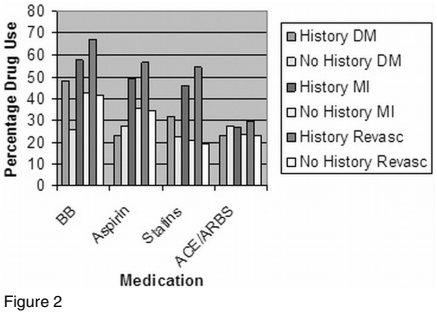

Figure 2

This is the first study to describe the use of CV medications in a large multicenter international group of renal transplant recipients.

O-275 HOW TO PREDICT THE CARDIOVASCULAR (CV) RISK AFTER RENAL TRANSPLANTATION

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Aims of this study in 359 renal transplant recipients, with a graft functioning for at least 1 year (KTX), were: 1) to evaluate the incidence of post-tx CV events; 2) to identify current main CV risk factors; 3) to assess the predictive role of existing CV risk scores.
Methods: Major Acute Clinical Events (MACE: angina, AMI, ictus cerebri, cardiac death) and routine biochemistry were prospectively yearly analyzed in 359 KTX who received a renal transplant in a single center between January 1997 and December 2007, median follow up time was 70 months. All transplant candidates with positive cardiac history or age over 50 years were pre-tx evaluated with pharmacological ecocardiostress; positive pts underwent then coronary angiography followed by PTCA or CABG, as indicated.
Results: The incidence of MACE increased over post-tx time: MACE affected 0.27%, 2.41% and 8.94% of KTX within the first 6 months, 5 years and 10 years post-tx, respectively. At univariate analysis, risk factors associated with MACE were male gender (P=0.0051), age $>$ 55 y (P=0.033), BMI $>$ 27 (p=0.046), pre-tx positive CAD history (p=0.0001), pre-tx total cholesterol $>$204 mg/dl (p=0.003), pre-tx systolic blood pressure $>$ 142 mmHg (p=0.002), presence of left ventricular hypertrophy before tx (P=0.0003), post-tx diabetes on therapy (P=0.0002), post-tx serum creatinine $>$ 1.7 mg/dl (P=0.05). Evaluating the Framinghan and the INDANA CV risk score indexes, only INDANA could significantly ($p<0.05$) predict the MACE observed in our population, as this CV score index is also including renal function.
Conclusions: MACE after renal tx relates to traditional pre and post-tx CV risk factors. The significative increase of MACE 5 years after tx indicate the need for an aggressive cardiac re-evaluation: INDANA index may help to select the population at high CV risk.

O-276 METABOLIC SYNDROME AFTER RENAL TRANSPLANTATION: CHANGES IN MARKERS OF INFLAMMATION AND ADHESION MOLECULES

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Purpose: In kidney transplant recipients (KTR) the high incidence of metabolic syndrome (MS) is related to a clustering of cardiovascular risk factors or metabolic abnormalities and has been reported to have adverse effects on patient and graft survival. This study was focused on the incidence and characteristics of MS in a population of KTR, evaluating the levels of circulating markers of cardiovascular risk with or without a diagnosis of post-transplant MS.
Methods: The study recruited 565 KTR transplanted between 1996 and 2004, without pre-transplant diabetes, stable renal function at 1 year post-transplant and at least 4 years follow-up. MS was diagnosed in the presence of $\geq$3 of the following risk factors: obesity, dyslipidemia (raised triglicerydes level, reduced HDL cholesterol), hypertension, impaired glucose tolerance. The serum levels of the following biomarkers of cardiovascular risk were compared across patients with or without MS: CRP, Lp(a), IL-6, IL-10, TGF-beta, TNF-alpha, IFN-gamma, MCP-1, P-selectin, sCD40L, t-PA, VCAM-1.
Results: Ninety-seven patients (17.2%) had MS at 1 year post-transplant, 70 of them with 3 and 27 with 4 risk factors. Three patients with MS died of cardiovascular disease.
In univariate analysis, MS patients showed significantly higher levels of CRP, Lp(a), IL-6, VCAM and P-selectin and lower levels of IL-10 and TGF-beta (Table 1). Using multivariate regression analysis to determine the independent association of the aforementioned parameters with the risk of MS, CRP, sVCAM and P-selectin remained independent predictors of MS, while IL-10 resulted as protective factor for the development of MS.
Conclusions: Our study suggests an association in KTR of MS with elevated levels of adhesion molecules and altered balance between pro-inflammatory and anti-inflammatory molecules. The predictive value of these biomarkers in relation to post-transplant MS needs to be better assessed in further longitudinal studies.

Serum levels of circulating biomarkers of cardiovascular risk in renal transplant recipients with or without a diagnosis of Metabolic Syndrome

Parameter	MS group (n= 97)	No-MS group (n= 468)	p
CRP (mg/dL)	0.9±0.3	0.6±0.2	0.001
Lp(a) (mg/dL)	48.7±43.1	28.8±46.2	0.02
IL-6 (pg/mL)	30.4±48.3	20.3±33.3	0.043
IL-10 (pg/mL)	4.8±3.5	12.1±23.2	0.013
TGF-beta (ng/mL)	758.5±225.3	826.7±222.8	0.026
TNF-alpha (pg/mL)	13.3±2.2	11.9±37.3	n.s.
IFN-gamma (pg/mL)	30.4±6.6	32.4±52.3	n.s.
MCP-1 (pg/mL)	249.3±246.5	227.4±479.5	n.s.
sP-selectin (ng/mL)	279.2±453.8	107.3±127.1	<0.001
sCD40L (ng/mL)	18.6±6.4	19.7±29.3	n.s.
t-PA (pg/mL)	7460.1±6110.6	6553.2±4058.8	n.s.
sVCAM-1 (ng/mL)	1866.5±859.8	1402.6±1259.9	0.005

Session 32. Composite tissues & xenotransplantation

O-277 FIRST FACE ALLOGRAFT: A THREE YEARS FOLLOW UP

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The first human face allotransplantation was performed in Amiens (France) on November 2005. We report outcomes up to three years after transplantation.
The recipient, a 38 years old woman, was mutilated by a dog bite, received a facial allograft (nose-lip-chin) from a brain dead woman. A vascularized sentinel donor skin graft was also performed. Donor bone marrow was infused on days 4 and 11 post transplantation. Initial immunosuppression protocol included Thymoglobulins, tacrolimus, prednisone and mycophenolate mofetil (MMF). At 3 years the maintenance treatment includes prednisone (5 mg/d), MMF (1500 mg/d) and sirolimus (through level 8-10 ng/ml).
Results: Functional and aesthetical results: the patient has a complete recovery of sensibility and a motor recovery which allowed a complete mouth closure, with a possibility to drink and to eat and a normal phonation. Aesthetic results allowed the patient to live a normal social life.
Immunological follow-up: i) two episodes of acute rejection, which occurred at day 18 and 212, regressed successfully after 3 boluses of steroid. ii) Macroscopic aspect and histology of the biopsies from mucosa and sentinel flap skin graft didn't show any sign rejection. iii) anti-HLA antibodies have remained negative. iiii) Study of peripheral blood T lymphocyte subsets showed an increase in CD8+DR+ and CD4+CD25+CD127- T reg during the rejection episodes. Then, T reg decreased while CD8+DR+ remained at high level despite the absence of chronic rejection.
Side effects of immunosuppression: i) the patient started a degradation of renal function at 6 months, which improved after the switch from tacrolimus to sirolimus. At 3 years GFR (MDRD) is 79 ml/min/1.73m^2. ii) The patient developed mild hypertension, and alteration of lipid status related to sirolimus, easily controlled by statins.
Conclusion: Three years after transplantation the balance between the results and the complications is satisfactory.

O-278 CHRONIC REJECTION IN COMPOSITE TISSUE ALLOGRAFT

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Composite tissue allograft (CTA) showed little evidence of chronic rejection and it was reported only in non compliant recipients. The course of chronic rejection on bones, muscles, nerves, tendons and vessels may have yet undescribed implications.
For this reason we have studied all these structures in four bilateral hand grafted patients (9, 6, 2 years and 6 months of follow-up respectively) without macroscopic and histological signs of chronic rejection in the skin.
Bone quantitative parameters and architecture were studied by quantitative computed tomography and dual-energy-X-ray absorptiometry. Magnetic resonance imaging (MRI) allowed for visualization of bones, muscles, tendons and nerves, and magnetic resonance angiography for vessels, which were also assessed by Doppler ultrasounds. Nerves were also investigated by ultrasonography and electromiography. Microcirculation was studied by naifold capillary microscopy.
Bone quantitative parameters at radius and tibia distal level did not show any

significant reduction in graft bone density, and bone architecture was preserved in all patients. Nerves and tendons did not show any structural modification. Angio MRI and Doppler ultrasounds showed patency of all examined brachial and radial arteries; a small reduction in vessel size was reported in two patients which seems correlated to the surgery as it was present in all time points of the follow-up.
No signs of microvascular damage were reported but only minor alterations such as venular stasis. In the present study we evidenced only fatty degeneration of some intrinsec muscles in the majority of patients which seems to be clearly correlated to the period of muscular denervation.
Although the different times of follow-up the results were similar for all the recipients.
These data confirm that when there are no signs of chronic rejection in the skin there are neither in the other components of CTA.

O-279 VASCULARIZED BONE MARROW TRANSPLANTATION: AN ALTERNATIVE TO CONVENTIONAL CELLULAR BONE MARROW TRANSPLANTATION

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Current protocols for bone marrow transplantation (BMT) involve removing the bone marrow component directly from its donor microenvironment and then injecting such components into the circulatory system of the recipient. This procedure is usually preceded by conditioning protocols (body irradiation, immunosuppression, or both). Vascularized bone marrow transplantation (VBMT), in comparison with conventional marrow transplants, has the advantage of providing a microenvironment and immediate engraftment of both mature and progenitor hemopoietic cells at the time of transplantation in the absence of immunomodulation or irradiation. The aim of the study was to follow the development of microchimerism after allogeneic VBMT vs conventional BMT. In one group a VBMT model consisted of a donor Brown Norway (BN) rat hind limb heterotopic transplanted on recipient Lewis rats was used. An intravenous infusion of donor bone marrow cells in suspension equivalent to that grafted in the vascularized femur limb was administrated i.v. on recipient rats in the second group. Cellular microchimerism was investigated in recipients of VBMT vs BMT. Donor-derived cells could be detected in VBMT recipients at 30 and 60 days but not in recipients of i.v. suspension BMC grafting. VBMT provides a theoretical alternative to conventional cellular bone marrow transplantation by addressing crucial clinical problems such as failure of engraftment or graft versus host disease. It may be possible to develop a new approach for bone marrow transplantation based primarily on a microsurgical procedure (transplantation of vascularized bone marrow flaps).

O-280 TRANSPLANTATION OF hCD46 TRANSGENIC PORCINE ISLETS INTO DIABETIC NONHUMAN PRIMATES RESULTS IN LONG-TERM NORMOGLYCEMIA UNDER LIMITED IMMUNOSUPPRESSION

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Purpose: Intraportal xenotransplantation of porcine islets is characterized by an inflammatory graft loss and the need for intensive immunosuppression to avoid rejection. We investigated if the transgenic expression of a human complement regulatory protein (hCD46+) on porcine islets would improve islet xenotransplantation.
Methods/Materials: In 9 cynomolgus monkeys, diabetes was induced by i.v. streptozotocin (1500mg/m^2). Four (Group A) were transplanted with nontransgenic porcine islets, and five (Group B) with hCD46+ islets. Both groups received equal numbers of islets (85,000-100,000IEQ/kg) and limited immunosuppression (ATG, anti-CD154 monoclonal antibody, MMF). Follow up was for 3 months, except for 1 Group B animal that was followed >1yr to verify the durability of the normoglycemic status.
Results: Insulin-independent normoglycemia was achieved in 3 of 4 Group A monkeys for 5, 17, and 36 days, respectively, compared to 4 of 5 Group B monkeys for 87, 91, 92, and 396 days (P=0.004) (figure1). Fasting blood glucose values were well-controlled (<120mg/dL). In the fifth Group B monkey, exogenous insulin needs were reduced >50% for 3 months with detectable porcine C-peptide. Post-transplant fasting porcine C-peptide levels were 1.10+0.41ng/mL (Group A), vs. 0.90+0.51ng/mL (Group B) (P=0.546). After an i.v. glucose challenge these levels failed to increase in Group A (1.02±0.32ng/mL), while in Group B they markedly increased to 4.07+1.46ng/mL (P=0.02). There was no response by monkey beta cells. Animals stayed healthy and gained weight. Post-mortem liver histology showed many viable islets free from complement deposition in Group B, in contrast to marked C4d staining in Group A.

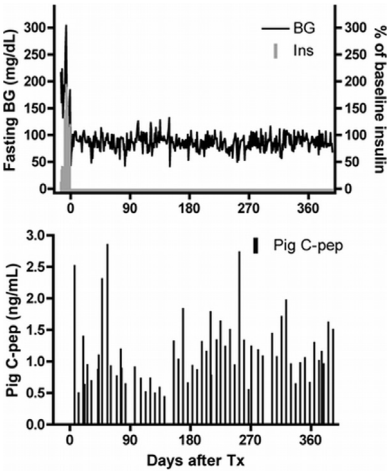

Figure 1. 1 year follow-up after islet xeno-Tx.

Conclusion: hCD46 expression on porcine islets significantly prolonged normoglycemia in monkey recipients (up to >1yr) and allowed for limited immunosuppression with minimal adverse events, thus advancing islet xenotransplantation toward clinical application.

O-281 IMPROVEMENT IN CARDIAC FUNCTION AFTER PRECLINICAL ORTHOTOPIC CARDIAC XENOTRANSPLANTATION

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Purpose: Preclinical 90-day median survival of pig-to-primate orthotopic cardiac xenotransplants is a likely standard for clinical application. In this report we examine the recovery of cardiac function after successful pig-to-primate cardiac xenotransplantation.
Methods: Five successful (CD46,n=3; GalKO/CD55,n=2) orthotopic pig-to-baboon heart transplants were performed. Immunosuppression consisted of ATG induction, tacrolimus, sirolimus, tapering steroids and αGal therapy in the CD46 transplants. Heart function was monitored biochemically, echocardiographically, and by intramyocardial electrocardiography.
Results: The five recipients survived 57, 40, 34, 22 and 14 days in a healthy condition. Mortality resulted from bowel infarction, pneumonitis, respiratory failure a surgical bleed and sudden death due to unknown cause, respectively. Autopsy revealed minimal or mild rejection in 4 recipients and mild to moderate in the 5th. All recipients exhibited a transient spike in serum troponin C after transplant. An improvement in LV ejection fraction was noted in 4 of 5 recipients within 7 to 14 days of transplant. Ejection fractions were normal at the time of death in 3 recipients and 40% and 45% in the remaining two.
Conclusions: These orthotopic recipients represent the longest survivors to date. In successful transplants early perioperative ischemia/reperfusion injury was shown to be completely recoverable indicating that normal cardiac reparative processes function across the xenotransplant barrier. Cardiac xenograft rejection was controlled in survivors who remained healthy on clinically used immunosuppressants. The model is challenging but these results support the potential viability of orthotopic cardiac xenotransplantation with its attendant advantages of complete implantability, intrinsic power supply and no anticoagulation. These early results along with the high impact that successful cardiac xenotransplantation would have justify continued preclinical studies.

O-282 LIVER XENOTRANSPLANTATION USING α1,3-GALACTOSYLTRANSFERASE GENE KNOCK-OUT (GTKO) PIGS

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Purpose: We have explored orthotopic transplantation (Tx) in baboons using livers from genetically-engineered pigs to determine whether these livers could 'bridge' a patient to alloTx.
Methods: *Group 1:* AlloTx in wild-type (WT) pigs (n=2) or baboons (n=1). *Group 2:* XenoTx in baboons using livers from GTKO (n=1) or GTKO transgenic for CD46 (GTKO/CD46, n=3) pigs. Immunosuppression consisted of thymoglobulin induction and tacrolimus, mycophenolate mofetil, and steroids maintenance.
Results: In *Group 1*, the two non-immunosuppressed WT pigs were electively euthanized at 3 days; liver function tests (LFTs) were normal and liver histology showed minimal acute cellular rejection. The immunosuppressed baboon was electively euthanized at 30 days; LFTs and histology were normal. In *Group 2*, the baboons survived for 4, 6, 6, and 7 days. Within 24h, albumin fell to the normal pig level (2.2±0.7g/dl), but was maintained at the normal baboon level (3.7±0.7g/dl) by i.v. human albumin. LFTs remained normal. Western blot demonstrated that pig proteins (albumin, plasminogen, fibrinogen, haptoglobulin) were produced by the liver. Complement activity (CH50 test), PT, PTT, and INR were normal. Production of numerous pig coagulation factors was confirmed. However, severe thrombocytopenia (platelets $<$20,000/mm^3) developed within 5h, with subsequent spontaneous internal hemorrhage, necessitating euthanasia. At necropsy, liver histology showed patches of hemorrhagic necrosis, platelet-fibrin thrombi, monocyte/macrophage margination, and vascular endothelial cell hypertrophy. *In vitro* studies demonstrated activation of baboon platelets leading to platelet/monocyte aggregates.
Conclusions: GTKO/CD46 pig livers function adequately in baboons for up to 7 days, but severe thrombocytopenia, results in internal bleeding. Activation of pig vascular endothelial cells and/or baboon platelets, with increased tissue factor activity, may result in platelet/WBC-aggregation and sequestration in the liver. Further genetic modifications of the pig, e.g., adding the TFPI gene, may help overcome the current limitations.

Session 33. How can we make the lungs breathing better?

O-283 INCIDENCE AND OUTCOME OF ABDOMINAL SURGICAL INTERVENTIONS FOLLOWING THORACIC TRANSPLANTATION

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Purpose: Abdominal complications after heart (HTx) or lung (LuTx) transplantation are associated with high risk of mortality. Aim of the present study was to analyze the frequency and outcome of abdominal interventions following HTx or LuTx.
Methods: Retrospective analysis was performed on 281 patients after HTx and 754 patients after LuTx (total n = 1035) undergoing abdominal surgery at the Hannover Medical School, Germany, between January 2000 and December 2008.
Results: In the course of transplantation 71 patients (6.9%) were in need of surgical interventions due to abdominal complications. The incidence was comparable in both groups of patients (5.7% after HTx vs. 7.3% after LuTx). Following HTx 3 individuals received emergency surgery due to bowel perforation, appendicitis and ileus. No patient died in relation to the disease. Elective operations (n=17) without incidence of mortality were performed based on varying diagnoses. Following LuTx 35 individuals were operated in 43 cases of emergency indication. Leading diagnosis was bowel perforation (n=10) with surgery performed 10.4 months after LuTx, although 7 of 10 patients were operated within the first four weeks post transplantation (time between LuTx and operations in general: 15.2 months). In recipients of LuTx emergency intervention were associated with a mortality of 25.6%, thereof 45.5% after bowel perforation. Elective surgical treatments (n=31) after LuTx were diverse and had no influence to mortality.
Conclusions: Early abdominal complications after LuTx correlate with a high mortality. Perforation of the bowel was the leading diagnosis with severe impact on the patient's outcome. In findings of an acute abdomen after HTx and LuTx we propose a broad indication for further diagnostics and a low barrier to force an early explorative laparotomy.

O-284 INCIDENCE OF DE NOVO MALIGNANCIES IN LUNG TRANSPLANT RECIPIENTS IN ITALY: A SINGLE-INSTITUTION EXPERIENCE, 1991-2008

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Background: Patients who underwent transplantation present an increased cancer risk. The present study aimed to define the cancer spectrum and to quantify the incidence rate (IR) and the excess risk of de novo-malignancies (excluding non-melanoma skin cancers) in patients who received lung transplantation (LTx).
Methods: We collected data (on baseline demographics, transplantation, last follow-up and eventual cancer) on 261 patients (68.2% males) who underwent LTx (28 combined heart-lung, 114 single- and 119 double-LTx procedures) at Policlinico "San Matteo" of Pavia (Northern Italy) (1991-2008). Period at risk of developing cancer (person-years, PY) was computed from 30 days post-LTx to date of cancer diagnosis, death, or last follow-up. Observed and expected cancer were compared through sex- and age-standardized incidence-ratios (SIRs) and 95% confidence intervals (CIs) using Italian Cancer Registries data as baseline IR
Overall, 1,079 PYs were accumulated (median follow-up, 3.4 years). 26 patients (24 males) developed at least one confirmed de novo-malignancy (29 single diagnoses). Among those 7 Non Hodgkin-NHL, 7 Kaposi's Sarcoma-KS, lung and colorectal cancers (4 diagnoses each) and 1 Hodgkin lymphoma-HL. Interestingly 10/16 malignancies occurred $<$2 years post LTx were viral related cancers: 5/7 KS and 5/7 NHL. Overall a significant increased SIR (4.1, 95% CI: 2.7-6.0) was observed. Increased SIRs were found for KS (331.5), NHL (27.9), colorectal (7.2) and overall solid cancers (2.0); furthermore significant increases were observed in males for HL (44.1), lung (3.5) and prostatic cancer (6.0). SIR were higher in younger patients (41.7 in $<$30 vs. 3.0 in $>$60 years old) and in early post-transplanted period (8.6 $<$2 years vs. 2.2 $>$2 years post-LTx).
Conclusions: LTx patients are at higher risk for cancer (mainly viral-related malignancies). Further investigation is needed to highlight the relationship between immunosuppression and cancer risk in LTx.

O-285 PROTOCOL-DRIVEN RECIPROCAL OUTCOMES OF MALIGNANCY AND CHRONIC REJECTION AFTER LUNG TRANSPLANTATION

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Background: The incidence of malignancy after solid organ transplantation is high, generally regarded as a consequence of immunosuppressive drugs given. We evaluated the cancer incidence and possible risk factors in lung transplantation patients, with emphasis on two different drug protocols.
Methods: The histopathological results of all lung transplant patients (1990-2007) were screened for malignancies using a national pathology registration. Risk factors for malignancy were evaluated in univariate and multivariate analyses. Immunosuppression from 1990 – 2001 was ATG, followed by ciclosporine, azathioprine and prednisolone (protocol 1). In case BOS in protocol 1, ciclosporin was switched to tacrolimus. In 2001 the protocol was changed into anti-CD25 (induction), tacrolimus, azathioprine and prednisolone. In addition patients received CMV prophylaxis and EBVguided tapering of azathioprine.
Results: Of the recipients, 25.5% developed a malignancy. These were mainly NMSC (12.4%) and PTLD (8.5%), but also malignancies in solid organs (6.6%). The standardized incidence ratio was 30.01. Both protocol 2 and older recipient age were significant independent risk factors for the development of a non-PTLD malignancy. Protocol 2 resulted in a significantly improved outcome

with regard to overall 1- and 5-years graft survival, freedom from BOS and development of PTLD. Remarkably, the percentage of malignancies in solid organs was higher in patients receiving tacrolimus, as standard and rescue therapy (8.0%) compared those not receiving tacrolimus (3.6%). This difference was not statistically significant.
Conclusion: The risk of developing a malignancy in our lung transplantation program is 30-fold of that of the normal population. A protocol change in 2001 resulted in a favourable outcome with respect to survival, BOS and PTLD. This was counterbalanced by a dramatic increase of non-PTLD malignancies. The switch to tacrolimus alone could not be identified as an independent risk factor.

O-286 STABILIZATION OF KIDNEY FUNCTION IN A CALCINEURININHIBITOR-EVEROLIMUS BASED IMMUNOSUPPRESSION AFTER LUNG TRANSPLANTATION

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Objective: Nephrotoxicity is a limitation in Calcineurininhibitor (CNI) based immunosuppression. Protective effects of Everolimus on kidney function were achieved in heart and renal recipients, but no data concerning renal function in high immunosuppressed patients after lung transplantation (LUTX) are published.
Aim of this study was to evaluate the impact of a CNI reduced, everolimus (EV), mycofenolat mofetil (MMF) and steroid based immunosuppression in LUTX with chronic renal failure.
Methods: In 42 LUTX (23 m/19 f; age: 51.9±12.0 yrs) with deterioration in renal function CNI (CsA: 15 pts/Tacrolimus: 27 pts) was stepwise halfed and EV was titrated to an average trough level between 5-8 ng/ml (CNI-EV), median 1.9 yrs after LUTX. Routine laboratory values, GFR, CNI-EV trough levels were monitored monthly before CNI-EV, at time of conversion and 3-12 months after CNI-EV switch. We evaluated safety, side effects and biopsy profen rejections retrospectively.
Results: A stabilizing effect was achieved in all 42 LUTX (GFR pre switch: 37.9±15.2 ml/min; GFR 12 months post switch: 37.9±15.0 ml/min). The greatest benefit was seen in LUTX GFR $>$ 40 ml/min at time of onset of CNI-EV therapy. In LUTX with GFR $<$ 40 ml/min renal function deteriorated significantly within 12 months after switch to CNI-EV (p=0.001). 3 LUTX (7.3%) died (2 acute renal failure, 1 HUS) and 1 LUTX underwent dialysis. Withdrawal of EV was indicated in 6 LUTX (14.3%). There were no significant changes in cholesterol and blood counts, but a significant increase of triglycides (p=0.05) within 12 months. No clinical relevant rejection episodes were detected.
Conclusion: CNI-EV immunosuppressive regimen is a safe feasible therapy (rejection/side effects) to stabilize renal function. The best protective renal effect can be achieved in patients with GFR greater than 40 ml/min.

O-287 SUBLINGUAL TACROLIMUS AS AN ALTERNATIVE TO INTRAVENOUS ROUTE IN THORACIC ORGAN TRANSPLANTATION

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Purpose: Tacrolimus (TRL), currently used in transplantation is characterized by narrow therapeutic index, low bioavailability (10–25%) and pharmacokinetic variability. Intravenous (IV) TRL is needed whenever oral route is strictly unavailable. The low amount (0.01-0.03 mg/kg/d) of the infusion formulation (5mg/mL), resulting in high dilution and careful infusion technical management, increased variability and overdose risk. Sublingual (SL) TRL administration was proposed occasionally as IV alternative. This study addressed the feasibility to provide SL TRL in transplanted patients.
Methods: Retrospective study conducted during 2005-2008 in 16 transplanted patients as 13 lung (10 cystic fibrosis), 3 heart, receiving SL TRL controlled by regular therapeutic drug monitoring as trough blood levels (C_0) analyzed by MEIA. Four full AUC were determined. Patients received SL TRL on a dose-to-dose basis from the oral formulation powder content and asked not to swallow for at least 15 minutes after intake.
Results: Mean age was 35.3±15.6 yrs in 14M/3F. The 146 C_0 samples collected during SL period (duration 15.8 days [2-46]) showed 90.4% conformity rate [5-15 ng/mL], 5.5% supratherapeutic and 4.5% subtherapeutic levels. Mean dose, C_0 and AUC were respectively 0.116±0.096 mg/kg/d, 12.9±5 ng/mL and 230±74 ng.h/mL, with1h average peak time concentration. These results were consistent with oral references and SL literature. Neither acute rejection nor renal toxicity or drug interaction management difficulties were notified, except some unpleasant taste reports. Whatever the contribution of TRL passive swallowed oral absorption, SL route was effective to replace IV delivery, even in one cystic fibrosis patient with digestive interruption due to oesophagectomy.
Conclusion: This study supported the convenience of TRL SL administration, even in unconscious patients. Limited short-term IV infusion resulted in potential clinical improvement and cost savings. Further investigations are needed to confirm SL dose ranging (0.1mg/kg/day) and critical intensive intestinal drug interactions evaluation during oral-SL switch.

O-288 TRANSPLANTATION OF TISSUE-ENGINEERED TRACHEAS -DECELLULARIZATION BY ULTRA-HIGH PRESSURE METHOD AND SEEDING OF MESENCHYMAL STEM CELLS-

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Background: Tissue-engineered tracheal grafts are expected to be applied for the treatment of extensive tracheal defects. Here, we tried to elucidate the feasibility of a novel airway replacement using tracheal bioscaffolds processed by ultra-high pressure method (*UHP)* and mesenchymal stem cells.
Methods: *Rat study-1:* B-N rat tracheas were decellularized by i) cryopreservation (*CR*), ii) Triton X-100 (*TX*) or iii) *UHP* (980 MPa for 10 min. at 4 °C). Fresh (*FR*) or treated each trachea was transplanted in the subcutaneous space of the Lewis rat. *CR* and *UHP* tracheas were also orthotopically engrafted to Lewis rats. *Rat study-2:* Syngeneic mesenchymal stem cells (1.0×10^6) suspended in thrombin solution were sprayed with fibrinogen solution on *UHP* tracheal grafts at the time of allogeneic orthotopic transplantation. *Pig study:* Decellularized porcine tracheas by *CR*, *TX* or *UHP* were served for the pathological study, compression test or PCR assay for porcine endogenous retrovirus (PERV) DNA.
Results: In both rat and pig studies, cellular contents in *TX* and *UHP* tracheas were clearly excluded except in deep cartilage. Rat *CR, TX and UHP* tracheas showed minimum allo-rejection 4 weeks after subcutaneous transplantation, although severe wall thickness with marked cell infiltration was observed in rat *FR* tracheas. Structural strength of porcine *TX* tracheas declined by about 60% compared with those of porcine *CR* or *UHP* tracheas. Orthotopic *CR* and *UHP* grafts were reepithelialized by 4 weeks after transplantation. Furthermore, *UHP* grafts seeded mesenchymal stem cells showed recellularization of the cartilaginous region within 4 post-transplant weeks. PERV-DNA was undetected only in porcine *UHP* tracheas.
Conclusions: UHP could be an option for the preparation of allogeneic or xenogeneic tracheal bioscaffolds. Seeding of syngeneic mesenchymal stem cells seems to accelerate regeneration of tracheal cartilage of decellularized grafts.

Session 34. Pancreas transplantation: clinical aspects

O-289 A RANDOMIZED, PROSPECTIVE TRIAL OF ALEMTUZUMAB VERSUS RABBIT ANTI-THYMOCYTE GLOBULIN INDUCTION IN KIDNEY-PANCREAS TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

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Objective: To review our single center experience with alemtuzumab (Alem) versus rATG induction in simultaneous kidney-pancreas transplantation (SKPT).
Methods: From 2/05 thru 10/08, 46 SKPTs (45 with portal-enteric drainage) were entered into a prospective, randomized trial of single dose Alem vs multiple dose rATG antibody induction therapy in combination with tacrolimus, MMF and early steroid elimination.
Results: 28 patients (pts, 61%) received Alem and 18 (39%) received rATG induction. There were no significant differences between the 2 groups in 1 year (92% Alem vs 100% rATG) or overall (92% Alem vs 92% rATG) pt survival; 1 year (91% Alem vs 92% rATG) or overal1 (87% Alem vs 85% rATG) kidney graft survival; or 1 year (87% Alem vs 92% rATG) or overall (83% Alem vs 92% rATG) pancreas graft survival rates (all p=NS). The 1st year and overall acute rejection (AR) rates (both 17% Alem vs 39% rATG, p=.10) and infection rates (36% Alem vs 67% rATG, p=.09) were slightly lower in the Alem

group. CMV infections were significantly lower (0 Alem vs 15% rATG, p=.04), and bacterial and fungal infections were slightly lower in the Alem group. Post-operative bleeding (9% Alem vs 0% rATG, p=.30) was slightly greater in the Alem group.There were no differences in surgical complications, readmissions or reoperations between groups. The one year mean serum creatinine (1.1 vs. 1.2 mg/dl), calculated MDRD GFR (5716 vs 5514 ml/min) and glycohemoglobin (5.2% vs 5.1%) levels were similar in the Alem and rATG groups, respectively.
Conclusion: Excellent results can be achieved with either Alem or rATG induction in SKPT, although Alem may be associated with fewer AR episodes and infections, and more bleeding complications.

O-290 COMPARING RISK FACTORS AND INCIDENCE OF CANCER IN KIDNEY-PANCREAS AND KIDNEY TRANSPLANT RECIPIENTS REPORTED BY UNITED NETWORK FOR ORGAN SHARING (UNOS) BETWEEN 1988-2006

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Kidney-Pancreas (KP) transplantation is the treatment of choice in type-1 diabetic patients with end-stage renal failure. However, recipients are at increased risk of infections and cancer. Our aim was to identify risk factors and incidence of cancer in KP transplant recipients and compare it with renal transplant (RT) recipients.
UNOS data as of 25/02/2008 were used *. The data included 14152 KP transplant recipients and 234145 RT recipients transplanted between 1988-2006. Relative Risk (RR)/Odds Ratio (OR) for cancer were calculated for each risk factor.
In total, 691 (4.9%) KP recipients (M=404) and 12120 (5.2%) RT recipients (M=8087) developed cancer during the study period. Female KP recipients and male RT recipients had higher risk of developing cancer (OR=1.05, 1.36 respectively) compared to their opposite sex recipients. Skin cancers were the most common types of post-transplantation cancer. In KP recipients, 159 (23%) patients had cancer within 3 years and 335 (48.5%) within 6 years post-transplantation but in RT recipients, cancer was diagnosed in 4079 (33.7%) patients who were up to 3 years post-transplantation and in 4650 (38.4%) recipients who were 6 or more years post-transplantation.
Age ≥65 years (OR: KP=1.77, RT=1.96), white-ethnicity (OR: KP=3.05, RT=2.98) and previous history of cancer (RR: KP=1.74, RT=3.38) were associated with increased risk of cancer in the recipients. While different immunosupression regimens using Ciclosporin (RR: KP=2.11, RT=1.45), Muromonab (RR: KP=1.87,RT=1.23), Steroid (RR: KP=1.35,RT=1.73) and Azathioprine (RR: KP=2.3,RT=1.63) were associated with increased risk of cancer, other regimens using Tacrolimus (RR: KP=0.45,RT=0.63), Sirolimus (RR: KP=0.71,RT=0.66) and Mycophenolate-mofetil (RR: KP=0.55,RT=0.77) were associated with a reduction in the risk of cancer. Incompatible donor-recipient ABO had a RR of 1.41 and 0.89 in the KP and RT recipients.
*Supported by Health Resources & Services Administration contract 234-2005-370011C.

O-291 PATIENTS AGED 50 YEARS OR MORE AS RECIPIENTS FOR PANCREAS TRANSPLANTATION

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Background: Ageing of western population has increased the number of patients aged 50 years or more seeking for PTx that traditionally has been reserved to younger recipients.
Material and methods: Between May 1996 and February 2009 288 PTx were performed. Twenty-seven PTx were in recipients aged 50 years or more (≥50); 261 PTx were in recipients aged less than 50 (<50). Most pancreas grafts were implanted according to the technique of portal-enteric drainage (<50: 145 vs. ≥50: 16). A significant proportion of grafts was transplanted with systemic-enteric drainage (<50: 81 vs. ≥50: 7), while systemic-bladder drainage was used in a minority (<50: 35 vs. ≥50: 4). Excluding recipient age (p =0.0001), there were no differences in baseline donor and recipient characteristics. Similar quadruple immunosuppressive regimen was used in all recipients.
Results: Delayed graft function occurred in 2.7% pancreas and 8.3% kidney grafts in <50 as compared with 7.4% and 5.9%, respectively, in ≥50 (p=NS). The rate of relaparotomy was 16.9% in <50 vs. 11.1% in ≥50 (P=NS). The rate of pancreas graft failure due to thrombosis was 3.4% (n= 9) in <50 and 3.7% (n= 1) in ≥50; non-occlusive thrombosis developed in 16 (6.1%) additional pancreata in <50 and in 4 (14.8%) in ≥50 (p=NS). There was no difference in incidence of infection and early rejection (17.2% in <50 vs 7.4% in ≥50). One-year patient, kidney and pancreas survival rates were: 95%, 91% and 84% and in <50 vs. 96%, 93% and 92% in ≥50 (P=NS); five-year figures were: 92%, 84% and 76% in <50 vs 96%, 93% and 87% in ≥50.
Conclusion: Our experience shows that PTx can safely and effectively be performed in properly selected patients aged 50 years or more.

O-292 IMPACT OF DONOR AGE IN PANCREAS TRANSPLANTATION. A UK SINGLE CENTRE EXPERIENCE

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Background: The shortage of cadaveric donors for pancreas transplantation has prompted to use organs from donors previously regarded as suboptimal. Organs from pediatric (age <18 years) and older (age> 45 years) donors are used more extensively worldwide.
Objective: The aim of our study was to compare the outcome and complications in pancreas transplant recipients transplanted with organs from different donor age groups.
Material and method: 166 pancreas transplants were performed in our unit between 2001 to December 2008. 128 simultaneous pancreas kidney (SPK), 30 pancreas after kidney (PAK) and 8 pancreas transplantation alone (PTA). Clinical data was collected prospectively into an electronic database (Microsoft Excel). Patients were grouped according the donor age and analysed. Group I (n=25): donor age< 18, Group II (n=116): donor age 18-45 and Group III: (n=25) donor age >45 years old. Clinical outcomes including early and long term surgical morbidity (e.g. bleed, thrombosis, infections, etc), graft, patient survival and hospital stay were compared between all groups.
Results: The one year patient survival rate in Group I was 100%, 89% in Group II and 88% in Group III. The one year pancreas graft survival rate was 84%, 76% and 68% respectively.

Major surgical complications (%)	Group I	Group II	Group III
Graft thrombosis	8	15	20
Bleed/Haematoma	12	12	20
Wound infection	0	21	16
Radiological collection drainage	0	15	16
Major fistula	8	11	8
Peritonitis/Intrabd. Abscess	4	22	24

The median HDU/ITU stay was shorter in group I (3.5days) and II (2.5days) compare to group III (5.5 days), the median hospital stay was similar (18, 15.5 and 17.5 days respectively).
Summary: Both patient and graft survival rate was higher in the group receiving transplant from pediatric donors. Similarly the rate of major surgical complication tended to be lower. Recipients with organs received from younger donor have shorter HDU/ITU stays.

O-293 LONG FUNCTIONING PANCREAS AND/OR KIDNEY GRAFT PREVENTS CARDIOVASCULAR DEATH IN SPKTX RECIPIENTS

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This study evaluates long term survival and cardiovascular death incidence among spktx recipients in relation to function of their grafts.
Between 1988 and 2008 101 spktx were performed. Recipients who had follow – up longer than 18 month were included (n=62). There were three groups: group I (n=33) with good function of both grafts, group II (n=19) who had lost transplanted pancreas while having good functioning kidney graft and group III (n=10) who lost both transplanted organs. Survival rates and incidence of cardiovascular death between groups were compared.
The cumulative survival rates for group I, II, III after 5, 10, 15 years were: 100%, 87%, 58% vs 100%, 83%, 41% vs 58%, 43%, 0%. The survival rate was significantly higher in group I and II than in group III (log-rank test; p<0,01). There were no significant difference in survival rates between group I and II. In group I deaths due to cardiovascular event and leukemia were noted. In group

II deaths for cardiovascular event and sepsis were observed. In group III all eight deaths were due to cardiovascular events.
Preserved function of kidney graft is sufficient to prevent cardiovascular death of spktx recipients.

O-294 THE IMPACT OF PANCREAS ONLY TRANSPLANTATION ON EXISTING RENAL FUNCTION: DOES IT DETERIORATE?

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Introduction: The long-term effects of pancreas only transplantation on kidney function among transplanted patients is still debatable.
Aim: To examine trends in renal function and factors which may contribute during early post-transplant years.
Method: From 2001 to 2008 we performed 36 pancreas only transplants in 31 patients (28 PAK & 8 PTA). Serum creatinine at given time points (pre-transplant, one week, 1, 3, 6, 12 and 24 months) were evaluated and estimated glomerular filtration rate (MDRD) was calculated. Several potential risk factors effecting renal function were analysed. Induction immunosuppression was the same in all patients.
Results: The median eGFR remained unchanged throughout (48, 56, 47, 47, 47, 47 and 48 at pre, 7, 30, 90,120,360 and 720 days of operation respectively). The pattern was similar for median serum creatinine (138, 118, 136, 139, 138, 138 and 144) at above time points. Analysing PAK and PTA separately showed that: In PAK subgroup the median eGFR remained unchanged (44 and 48 at pre-operative and at 24 months respectively). This was mirrored in the functioning PAK group where the eGFR increased from 47 to 50. In the PTA group however there was a marked drop at one year (84 to 66) of overall PTAs compare to functioning PTAs which showed no changes in eGFR at one year (81 to 76). A large reduction of eGFR (>20%) was seen in 2 patients whom had received PAK (baseline eGFR of 26 and 42) and one PTA (baseline eGFR of 62).
Summary: In our series the renal function does not deteriorate after solitary pancreas transplantation. In the PTA subgroup only the loss of graft seems to be detrimental to native renal function. Reduction in eGFR following pancreas transplantation was pronounced in patients with low baseline eGFR.

Session 35. Clinical immunosuppression: renal function

O-295 IMPROVEMENT IN RENAL FUNCTION FOLLOWING CONVERSION FROM LOW-DOSE MYCOPHENOLATE ACID TO HIGHER-DOSE ENTERIC-COATED MYCOPHENOLATE SODIUM (EC-MPS) WITH CONCOMITANT TACROLIMUS REDUCTION IN MAINTENANCE KIDNEY TRANSPLANT RECIPIENTS

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Due to pharmacokinetic interactions, mycophenolic acid (MPA) dose is frequently reduced in tacrolimus-treated recipients. An alternative could be increasing EC-MPS dose and reducing tacrolimus, but the impact on renal function is unknown.
Methods: A multicenter, randomized, open-label study was undertaken in maintenance (>12 months) kidney transplant recipients with stage 3 eGFR (aMDRD, 30-59mL/min/1.73m^2) receiving MMF 1g/day or EC-MPS 720mg/day with tacrolimus (C_0 ≥5ng/mL) ± corticosteroids. Patients were randomized to unchanged treatment (converting from MMF to equimolar EC-MPS if required) (standard) or switch to high-dose EC-MPS (1440mg/day) with reduced tacrolimus (2ng/mL≤C_0≤4.5ng/mL).
Results: 94 patients were randomized (high EC-MPS 46, standard 48), with similar time post-transplant in both groups. Mean EC-MPS dose was 1406-1440mg/day and 711-720mg/day in the high-EC-MPS and standard groups, respectively. Mean tacrolimus C_0 in the high-EC-MPS group was 4.9±1.6ng/mL, 4.5±1.7ng/mL and 4.1±1.7ng/mL at months 1, 3 and 6 post-conversion (all $p<0.001$ vs standard group). Mean tacrolimus C_0 was ≥5ng/mL in the standard group throughout (range 6.9-8.1ng/mL). Mean eGFR was 46.4±11.2 at baseline and 49.1±11.1mL/min/1.73m^2 at month 6 with high-EC-MPS vs 45.3±9.5 and 44.7±11.5mL/min/1.73m^2 in the standard arm. The primary endpoint, adjusted change in eGFR from baseline to month 6 was -0.48±0.93mL/min/1.73m^2 in the standard arm vs 2.48±0.95mL/min/1.73m^2 in the high-EC-MPS patients (-2.96mL/min/1.73m^2, 95% CI -5.60 to -0.32; p=0.028, ANCOVA). There were no deaths, graft losses or biopsy-proven acute rejections. A similar incidence of adverse events suspected to be related to the study drugs occurred with high EC-MPS (17.8%) vs standard treatment (17.0%); rates of infections were 20.0% vs 29.8% respectively.
Conclusions: Conversion to high-dose EC-MPS with concomitant reduction in tacrolimus exposure can improve renal function without compromising efficacy.

O-296 RENAL FUNCTION, EFFICACY AND SAFETY OF SIROLIMUS AND MYCOPHENOLAT MOFETIL THERAPY AFTER EARLY CALCINEURIN-INHIBITOR WITHDRAWAL IN DE NOVO RENAL TRANSPLANT PATIENTS: ONE-YEAR ANALYSIS OF A RANDOMIZED MULTICENTER TRIAL

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This prospective, randomized, multicenter study was designed to examine, whether an early conversion approach from a CNI-based immunosuppression with cyclosporine, mycophenolate mofetil (MMF) and corticosteroids (ST) to a CNI-free immunosuppression regime with sirolimus, MMF and ST avoids the long-term detrimental effects of cyclosporine on renal function, while simultaneously providing adequate efficacy and safety.
141 patients were randomized to receive either sirolimus or low-dose cyclosporine in conjunction with MMF and ST after induction with ATG-F and a short course of standard-dose cyclosporine/MMF. The primary end point was eGFR at 12 months. Secondary end-points included patient/allograft survival, acute rejection and safety parameters.
The mean calculated GFR (Nankivell) was higher in patients receiving sirolimus (64.5±25.2 ml/min) than in patients receiving cyclosporine (53.4±18.0 ml/min). There was no difference in patient and death-censored graft survival (98.6% for both groups). The rate of biopsy-proven acute rejection after conversion was similar in both groups (17% vs. 16%). A higher rate of discontinuations was noted for the sirolimus group 35.7% vs. 19.7%. However, infectious complications after conversion (43.5% vs. 54.9%) and CMV viraemia (5.8% vs. 26.8%) were found to be higher in the cyclosporine group. A regimen of delayed sirolimus, MMF and ST is beneficial for maintenance of renal function and reduction in infectious complications, as compared to a cyclosporine-based immunosuppression.

O-297 RENAL FUNCTION IN EVEROLIMUS/ENTERIC-COATED MYCOPHENOLATE SODIUM TREATED DE NOVO RENAL TRANSPLANT RECIPIENTS AFTER CALCINEURIN INHIBITOR WITHDRAWAL: THE ZEUS STUDY

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Aim of study: Assessment of an Everolimus/Enteric-coated mycophenolate sodium (EC-MPS) regimen on renal function after Cyclosporine (CsA) withdrawal in renal allograft recipients at month 12 post transplantation.
Methods: In this study 300 renal allograft recipients were randomized to an immunosuppressive regimen consisting of either Everolimus/EC-MPS or CsA/EC-MPS. After induction therapy with Basiliximab all patients (pts) received CsA, EC-MPS and corticosteroids for the first 4.5 months post transplantation when pts were randomized 1:1 to either a) continue CsA/EC-MPS

therapy (n=145) or b) convert to Everolimus/EC-MPS (n=155). Dosing for EC-MPS was 720mg BID. Everolimus and CsA trough levels were 6-10ng/ml and 100-150ng/ml, respectively. As primary endpoint renal function was assessed by the calculated Glomerular Filtration Rate (cGFR; Nankivell-method). In addition, renal function was determined by cGFR according to Cockcroft-Gault and MDRD method, serum creatinine and slope of creatinine.
Results: At randomization renal function was comparable in both groups. At month 12 cGFR (Nankivell formula) was 72±18 for the Everolimus/EC-MPS and 62±17mL/min/1.73m^2 for the CsA/EC-MPS treatment group, respectively. The observed GFR slope from month 4.5 to month 12 was +8.6 [95%CI: +5.6;+11.6] for Everolimus/EC-MPS pts and -1.8 [95%CI: -4.9;+1.3] mL/min/1.73m^2 for CsA/EC-MPS pts.

ANCOVA model		CsA/EC-MPS [mL/min/1.73m^2]	Everolimus/ EC-MPS	Difference	p-value
Nankivell formula					
Unadjusted	Baseline	63.0±15.5	64.2±17.4	1.1	n.s.
mean ± SD	Month 12	61.1±17.4	72.5±18.2	11.4	<0.0001
LS-Mean	Baseline	63.6	63.6	–	–
[95% CI, 2-tail]	Month 12	61.9 [58.8, 65.0]	72.3 [69.3, 75.3]	10.4 [7.9,12.9]	<0.0001

At baseline no significant differences in proteinuria were observed between the Everolimus/EC-MPS (351±259 mg/d) and the CsA/EC-MPS (366±774 mg/d) treatment group. At month 12 proteinuria slightly increased in the Everolimus/EC-MPS (455±510 mg/d) and slightly decreased in the CsA/EC-MPS treatment group (284±472 mg/d), respectively. Proteinuria was reported by the investigator in 16% of Everolimus treated pts and 17% in CsA treated pts.
Conclusion: Our results confirm the expected improvement of renal function after CNI withdrawal after introduction of the non-nephrotoxic Everolimus/EC-MPS regimen in *de novo* renal transplant patients.

O-298 SLOW RECOVERY OF GRAFT FUNCTION IS ASSOCIATED WITH POOR GRAFT AND PATIENTS SURVIVAL IN LIVING DONOR TRANSPLANTION

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Background: The definition and clinical outcome of slow recovery of graft function (SGF) in living donor kidney transplantation are unclear. This study was performed to evaluate the clinical characteristics and pathologic findings in patients with SGF.
Methods: 310 recipients were included. According to estimated GFR at day 14 after transplantation, recipients were categorized into immediate graft function (IGF group, eGFR >60 mL/min) and SGF (eGFR <60 mL/min). Clinical characteristics, pathologic findings, and clinical course were compared between groups.
Results: Mean age in the IGF group was younger than the SGF group in recipient and donor (p <0.05). BMI ratio of donor to recipient was higher in the IGF than the SGF group (p = 0.000). The portion of unrelated donor in the SGF group was higher than the IGF group (35.2% vs. 26.7%, p = 0.038). Protocol biopsy was performed at day 14 after transplantation revealed that IGF group showed normal finding in 138 (71.1%) patients, borderline or acute rejection in 38 (19.6%) patientz. Of the 64 SGF group, 27 (42.2%) patients showed normal, 24 (37.5%) patients showed borderline or acute rejection (p = 0.000). Mean serum creatinine levels in the IGF group were continuously lower than the SGF group during first one year. (p < 0.000) Ten-year graft survival rate were not significantly different between groups, but occurrence of acute rejection within one year significantly decreased the long-term graft survival rate in the SGF group compared with the IGF group (74% vs. 97%, p=0.001). Ten-year patient survival rate in the SGF group was also significantly lower than the SGF group (94.2%, vs. 97.3% p = 0.017)
Conclusion: SGF observed in early posttransplant period is responsible for decreased long-term grafts and patients survival.

O-299 COMPARISON OF HISTOLOGICAL LESIONS ON TEN YEAR PROTOCOL BIOPSIES IN KIDNEY TRANSPLANT RECIPIENTS WITH OR WITHOUT CYCLOSPORINE

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Purpose: Very few studies have compared long-term lesions on protocol biopsies from kidney transplant recipients who either received cysclosporine or not.
Methods: Two pathologists, unaware of treatment group, retrospectively analyzed histological lesions present on protocol biopsies at 10 years in patients who received cyclosporine (n= 53) or not (n= 93).
Results: Mean 10-year serum creatinine was significantly higher in the cyclosporine (CyA) group: 173 versus 113 μmol/L. The glomerulosclerosis percentage was higher in the CyA group (30 versus 17%, p=0.026), as was the mean Banff fibrosis score (1.6 versus 0.9, p=0.0003). In the control group, 49% of patients had interstitial fibrosis versus 77% in the CyA group (p<0.002). The mean fibrointimal thickening score (cv) was similar in the two groups: 1.7 (CyA) versus 1.4 (control). The arteriolar hyalinosis (AH) score was higher in the CyA group (2.0 versus 1.1, p<0.0001). In the CyA group 91% of patients displayed AH versus 64% in the other group (p<0.0001). In these patients, deposits were only sub-endothelial in 55% of cases, and sub-endothelial and muscular in 45% of cases, whereas in cyclosporine-treated patients, deposits were sub-endothelial and muscular in 71% of cases (p=0.007). If only muscular deposits are considered, the proportion of patients displaying CyA arteriolopathy was surprisingly 28% in the control group and 64% in the CyA group (p=0.0001). This pattern of arteriolopathy was more frequent in patients treated for hypertension (49% versus 31%, p=0.04).
Conclusion: This unique long-term comparative study shows that 1) lesions suggestive of CyA nephrotoxicity are not universally encountered ten years after transplantation, 2) the specificity of arteriolopathy must be questioned since a significant proportion of patients who never received any cyclosporine display muscular arteriolar hyaline deposits.

O-300 INTERSTITIAL FIBROSIS AND FIBROUS INTIMAL THICKENING IN *DE NOVO* RENAL ALLOGRAFTS UNDER SIROLIMUS OR CYCLOSPORINE: RESULTS OF A RANDOMISED, CONTROLLED TRIAL (FIBRASIC)

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Calcineurin inhibitors are a major cause of chronic allograft nephropathy and long-term graft failure. In a prospective, randomised trial of sirolimus (SRL)-versus cyclosporine (CsA)-based immunosuppression in *de novo* renal allografts, we morphometrically determined the fractional interstitial volume (VvInt) and the arterial intima/media ratio (I/M) in implantation and protocol biopsies at 6 months. The concomitant immunosuppression, including daclizumab, steroids and mycophenolate mofetil, was similar in 24 SRL and 21 CsA treated patients. Graft function (eGFR) was evaluated at 6 and 12 months with the MDRD formula (Jeliffe). At implantation VvInt (SRL: 25±8.4% vs. CsA: 27.2±11%) and I/M (SRL: 38.8±14.6% vs. CsA: 50.3±40.7%) were comparable in SRL and CsA treated grafts. In contrast, at 6 months VvInt (SRL: 20.9±7% vs. CsA: 27.5±9%; p = 0.055) and I/M (SRL: 27. 5±11.3% vs. CsA: 53.3±30%; p = 0.02) were lower in the SRL treated grafts. Graft function (eGFR) was comparable in the SRL and CsA treated patients at 6 months (SRL: 50±17 ml/min vs. CsA: 50±20 ml/min) and at 12 months (SRL: 49±13 ml/min vs. CsA: 53±21 ml/min). Thus, Sirolimus appears to protect the renal allograft against the development of interstitial fibrosis and arterial vessel intimal hyperplasia in the early phase after transplantation. However, this beneficial effect was not associated with a superior graft function at 6 months and 1 year. Longer follow-up may be needed to translate the histological improvement into better graft function.

Session 36. New immunosuppressive strategies in kidney transplantation

O-301 IMMEDIATE VERSUS DELAYED EVEROLIMUS: COMPARABLE RENAL FUNCTION AND WOUND HEALING COMPLICATIONS IN KIDNEY TRANSPLANT RECIPIENTS AT RISK OF DELAYED GRAFT FUNCTION

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Increased incidences of delayed graft function (DGF) and wound healing complications (WHC) were associated with the proliferation signal inhibitor (PSI) Sirolimus. Everolimus is also a PSI but with a different pharmacokinetic profile. The CALLISTO study reports 12 month data for WHC, DGF and renal function in *de novo* deceased-donor renal transplant recipients (RTxR) at risk of DGF with either immediate initiation of everolimus 1.5mg/day (IE) post-RTx or delayed everolimus (DE) after 4 weeks of treatment with mycophenolic acid.

Methods: 139 RTxR were randomized to IE (n=65) or DE (n=74) with cyclosporine+steroids+anti-IL-2R-antibody. Everolimus target C_0 levels were 3-8ng/mL.
Results: Both groups showed similar incidences of DGF (IE 24.6%; DE 24.3%). Median CrCl (Cockcroft-Gault) was comparable at Month 12 (IE 39.9mL/min [7.8–98.4], DE 43.1mL/min [6.5–92.2]). Maximum creatinine clearance was stable by Week 2 through to Month 12. Median nadir serum creatinine of 138μmol/L (57-637) and 133μmol/L (51-695) was reached within 90 (IE) and 85 (DE) days (n.s.). Proteinuria/creatinuria ratio (g/mmol) was similar at Month 12 for IE 0.2 (0-1.3) and DE 0.3 (0-4.5) with 24h-proteinuria of 0.2g/L for both. Sixteen IE and 24 DE patients underwent at least 1 dialysis session (excluding D1). Mean dialysis sessions/patient (5 [1-26] IE vs 3 [1-12] DE) and median duration of dialysis (11.5 days [1-28] IE vs 5.5 days [1-29] DE) were comparable. WHC were balanced at Month 3 and Month 12 for both arms and almost unchanged from Month 3 onward (Table).

	Month 3		Month 12	
	IE n (%)	DE n (%)	IE n (%)	DE n (%)
WHC (related to initial transplant)	24 (36.9)	28 (37.8)	26 (40.0)	28 (37.8)
Fluid collection	24 (36.9)	25 (33.8)	24 (36.9)	25 (33.8)
Wound dehiscence	0 (0.0)	2 (2.7)	0 (0.0)	2 (2.7)
Urine leak	2 (3.1)	2 (2.7)	2 (3.1)	2 (2.7)

Conclusion: In renal transplant recipients at risk of delayed graft function the 12-month analysis of the immediate everolimus regimen showed comparable incidences of delayed graft function, wound healing complications and renal function.

O-302 EC-MPS IS ASSOCIATED WITH SUPERIOR EFFICACY OUTCOMES COMPARED TO MMF IN *DE NOVO* KIDNEY TRANSPLANT PATIENTS: A POOLED ANALYSIS

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Dose adjustment of mycophenolic acid (MPA) is associated with increased risk of kidney allograft rejection and graft loss. Enteric-coated mycophenolate sodium (EC-MPS) delays release of MPA vs MMF and may permit higher dosing in *de novo* patients, with consequent efficacy benefits.
Methods: A pooled data analysis was undertaken based on 1891 *de novo* kidney transplant patients receiving EC-MPS (n=1289) or MMF (n=602) with CsA and steroids in multicenter studies ERL B301 (n=423), ERL B2405 (n=1076), RAD B201 (n=196) and RAD B251 (n=196). Entry criteria were consistent between trials. Starting dose was bioequivalent for MMF (2000mg/day) and EC-MPS (1440mg/day). Induction was permitted in ERL B301 as per center practice. Multivariate logistic regression analysis including treatment type was performed to identify other potential explanatory variables (recipient age, gender and race, induction therapy, diabetes at baseline and all variables by treatment interaction).
Results: Using MMF equivalents, mean MPA dose during months 0-12 was similar with EC-MPS (1.82±0.37mg/day) or MMF (1.86±0.29mg/day). On univariate analysis, graft loss, biopsy-proven acute rejection (BPAR) and a composite of death, graft loss or BPAR were each significantly less frequent with EC-MPS at month 12 post-transplant (Table). Multivariate analysis demonstrated a significantly lower rate of all efficacy endpoints with EC-MPS vs MMF (Table). Similar results were observed at month 6 post-transplant. Age by treatment interaction was significant for death, BPAR and the composite endpoint. The incidence of serious adverse events was similar with EC-MPS (56%) versus MMF (53%).

Event at month 12	EC-MPS (n=1289)	MMF (n=602)	Univariate analysis P-value	Multivariate analysis P-value
BPAR	260 (20.2%)	147 (24.4%)	0.037	0.011
Graft loss	45 (3.5%)	37 (6.1%)	0.009	0.006
Death	16 (1.2%)	14 (2.3%)	0.084	0.008
Death, graft loss or BPAR	308 (23.9%)	174 (28.9%)	0.020	0.018

Conclusions: Results from this 12-month pooled analysis of >1,800 patients document a significant improvement in efficacy outcomes in *de novo* kidney transplant patients receiving EC-MPS vs MMF when receiving concomitant CsA and steroids.

O-303 EXCELLENT EFFICACY AND SAFETY WITH ONCE-DAILY PROLONGED-RELEASE TACROLIMUS (ADVAGRAF®) IN KIDNEY TRANSPLANT PATIENTS

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Purpose: Advagraf®, a once-daily prolonged-release formulation of tacrolimus, may improve post-transplant adherence and, thus, long-term graft protection. In this follow-up study, we explored the efficacy and safety of Advagraf as long-term treatment in kidney recipients.
Methods: This multicentre, open, single-arm additional 1-year follow-up study included adult kidney transplant patients who had previously received Advagraf in an EU Phase III study for a duration of 12–26 months (mean time post-transplant approximately 18 months). Patients were maintained on their original immunosuppressive regimen, unless medical needs necessitated otherwise, and were assessed at baseline and quarterly. Primary endpoints were patient and graft survival. Other endpoints included incidence and severity of biopsy-proven acute rejection (BPAR), adverse events (AEs) and renal function.
Results: 177/191 (92.7%) patients completed this 1-year follow-up (61.3% male; mean age 46.9 years); 14 patients withdrew: AEs (6), withdrawal of consent (2), pregnancy (2), switch to other immunosuppression (1), prohibited co-medication (1), and other (2). Tacrolimus dose and trough levels remained stable throughout (table 1).

Table 1. Tacrolimus mean (SD) daily dose and whole blood trough levels over time

Follow-up (months)	1–3	4–6	7–9	10–12
N	191	188	181	171
Daily dose (mg/kg)	0.084 (0.052)	0.081 (0.049)	0.078 (0.048)	0.076 (0.047)
N	180	168	154	138
Trough level (ng/mL)	7.8 (2.5)	7.7 (2.5)	7.5 (2.5)	7.8 (2.8)

At 12-month follow-up, 30.9% of patients were on 'dual-therapy' Advagraf regimens (5.2% corticosteroids; 25.7% MMF). Kaplan–Meier graft and patient survival and freedom from BPAR were high (98.9%, 100%, and 99.5%, respectively), and corticosteroid-resistant BPAR occurred in only 1 (0.5%) patient. The most common causally related AEs were gastrointestinal disorders (11.5%), hypertension (8.4%), increased creatinine (7.3%), and bacterial urinary tract infections, non-insulin dependent diabetes mellitus and hyperlipidaemia (all 5.2%). Creatinine clearance (Cockcroft–Gault) and serum creatinine remained stable throughout (table 2).

Table 2. Renal function over time

Follow-up	Day 1 (n=188)	Month 3 (n=149)	Month 6 (n=184)	Month 9 (n=137)	Month 12 (n=164)
Mean (SD) creatinine clearance (mL/min)	66.1 (22.0)	66.4 (23.4)	66.8 (25.7)	68.1 (27.9)	69.8 (26.6)
Mean (SD) serum creatinine (μmol/L)	131.6 (50.3)	132.7 (50.2)	144.9 (118.2)	141.2 (108.8)	135.3 (93.2)

Conclusions: These data continue to support the efficacy and safety of once-daily prolonged-release Advagraf in kidney recipients, with excellent graft and patient survival and renal function.

O-304 BASILIXIMAB VERSUS DACLIZUMAB COMBINED WITH TRIPLE IMMUNOSUPPRESSION IN DECEASED DONOR RENAL TRANSPLANTATION: A PROSPECTIVE RANDOMIZED STUDY

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Introduction: In this prospective, randomized, single-center study, we compared the efficacy and safety of two anti-interleukin-2 receptor monoclonal antibodies combined with triple immunosuppression in adult recipients of at least 1 HLA-mismatched deceased donor renal transplant.
Methods: Patients taking cyclosporine microemulsion (CsA-Neoral), mycophenolate mofetil and methylprednisolone were randomly assigned to induction with either basiliximab or daclizumab, given in standard doses. An intent-to-treat analysis of 1-year data assessed incidence of acute rejections, graft function, safety of this therapy, and patient and graft survival.
Results: Two hundred and twelve patients were studied. At 12 months, eleven (10.3%) patients in the basiliximab group and ten (9.5%) patients in the daclizumab group experienced biopsy-confirmed acute rejection. Mean serum creatinine was 104±32 μmol/L in the basiliximab and 107±37 μmol/L in the daclizumab group. The basiliximab and the daclizumab group had similar incidences of graft loss (5.6% and 9.5%, respectively) and patient death (2.8% and 2.9%). Incidences of infections that required hospital treatment were sim-

ilar between the basiliximab and daclizumab groups (34.9% and 42.3%, respectively) apart for a lower incidence of cytomegalovirus disease in patients receiving basiliximab (13.2% vs. 21.6%). One renal-cell carcinoma in native kidney and one basal cell carcinoma of the skin was detected in the basiliximab, and one malignant melanoma in the daclizumab group. Patient survival at 1 year was 97.2% for the basiliximab and 97.1% for the daclizumab group; graft survival was 94.4% vs. 90.5%, respectively. No significant differences were observed between the two groups.
Conclusions: Basiliximab or daclizumab combined with triple therapy was an efficient and safe immunosuppression strategy, demonstrated with low incidence of acute rejections, an acceptable adverse event profile, excellent graft function, and high survival rates in adult recipients within the first year after deceased donor renal transplantation.

O-305 EFFECT OF DONOR BONE MARROW CELLS INFUSION ON ALLOIMMUNIZATION IN KIDNEY ALLOGRAFT PATIENTS

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Objectives: The aim of this study was to investigate the role of donor bone marrow cells infusion in post-transplantation anti-HLA antibody induction and outcome of kidney allograft patients.
Methods: Between June 2006 and May 2007, a total of 40 living donor kidney transplants; 20 recipients with Donor Bone Marrow Cells (DBMC) infusion ($2.1\times10^9\pm1.3\times10^9$ MNCs/body including $3.5\times10^7\pm1.6\times10^7$ CD34+ progenitor cells) and 20 without infusion as control, were entered into study and followed prospectively for one year. Both groups received the same baseline immunosuppressant consisting triple drug regimen. WBC cross match, Panel Reactive Antibody (PRA) and HLA-DNA typing were performed for all patients. Pre and post-transplant (days 14, 30, 90) sera samples were screened for the presence of anti-HLA antibodies, and subsequently antibody identification was determined for positive patients.
Results: Incidence of acute rejection (AR) was 30% in controls versus 15% in DBMI patients. All patients with AR had a pre-transplant anti-HLA antibody in both groups. 35% in DBMI and 30% in controls had pre-transplant antibodies but without acute rejection. In controls, 2 patients with AR and 2 without AR were positive for both Donor Specific Antibody (DSA) and non DSA. All 3 patients with AR in DBMI showed non DSA post operatively, but with a lower strength to HLA antigens. Mean percentages of post-transplant PRA was 16.5% vs. 38.5% in controls. The lower titer of antibodies and lower average serum creatinine were found for patients with AR in DBMI compared to controls.
Conclusion: Infusion of DBM mononuclear cells was perfectly tolerated, but the descending rate of creatinine level was slower than control group. The absence of GVHD and lower percentages of PRA in DBMI group are possible manifestations of functional immune modulation achieved by the DBMC infusion protocol.

O-306 FACTORS ASSOCIATED WITH GRAFT LOSS IN CHRONIC KIDNEY TRANSPLANT RECIPIENTS CONVERTED TO MMF (CellCept®)

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Introduction: In renal transplantation, there is evidence for the benefit of introducing MMF into the immunosuppressive regimen even some years post-transplant, in particular in association with CNI reduction and in patients requiring intervention due to progressive renal function decline. Here we explore factors associated with a failure of MMF introduction to save the graft, defined as graft loss or death up to 4 years after intervention.
Methods: TranCept is a prospective, multicenter observational study of patients switched to MMF more than 6 months after transplantation with the objective to document outcomes up to 4 years after switch. In an analysis of 1710 evaluable patients we studied factors leading to graft loss by multivariate Cox regression. Backwards variable selection based on Akaike's Information Criterion was used to optimize the regression model.
Results: The yearly graft loss rate was 4% in Kaplan-Meier estimates. In the Cox regression (table 1), proteinuria at the time of switch has a strong and significant association with graft loss, whereas chronic allograft nephropathy did not reach significance (p=0.069). A deteriorating renal function before MMF introduction and a low mean eGFR value were also independently graft loss predictors, as well as time from transplantation to switch (which ranged from 0.5 to about 20 years).

Table 1

	Hazard ratio	p-value
CNI: Tacrolimus (vs. CsA)	0.5346327	0.20
Time from Tx to switch [years]	1.0609374	0.017
Pre-switch eGFR slope [ml/min/year]	0.9478793	0.040
Mean pre-switch eGFR [ml/min]	0.9755857	0.014
Donor type: Living (vs. deceased)	0.8232826	0.46
Age at switch [years]	1.0008968	0.92
Donor age [years]	1.0112781	0.15
Gender: male (vs. female)	1.4133710	0.11
Renal function decline as switch reason (vs. others)	1.0519674	0.82
Hb at switch [g/dl]	0.8181374	<0.001
Proteinuria at switch (vs. no proteinuria or unknown)	2.3577330	<0.001
Pre-switch BPAR (vs. none)	0.6888665	0.22
Biopsy-proven chronic allograft nephropathy (vs. none)	1.6190356	0.069
Interaction CNI: time to switch	1.0553180	0.30
Interaction pre-switch eGFR slope: mean value	0.9999677	0.94

Low hemoglobin has also a significant association with graft loss. The CNI type at switch (cyclosporine or tacrolimus), pre-switch biopsy proven acute rejection (BPAR) were not significant predictors (BPAR was significant in an analysis of death-censored graft loss). The explanatory variables that were significant in this analysis remained significant after the variable selection procedure.
Conclusion: In this observational study, proteinuria and unfavorable renal function evolution prior to switch to MMF-based regimens appear to be the most relevant risk factors for graft loss.